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*Review*

# Plant protection: Paramount to food security in India

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**India carries the heavy burden of feeding a billion plus population. While it is a challenge in itself, the task is compounded further by limited resources and crop losses due to pest and diseases. Though beating the odds of diminishing land and water resources remain bleak, pest and disease inflicted crop losses can be managed with suitable crop protection techniques. Pesticide, integrated pest management (IPM), biopesticdes, Bt technology among many others have been in use for pest and disease management. Unfortunately in India, the presence of these crop saving measures and techniques have not been able to tame the mounting crop losses. The future of India's agriculture depends a lot on developing a suitable and economically feasible plant protection exercise.**

**Key words:** Plant protection, pesticides, food security, integrated pest management (IPM), biopesticides, India.

## INTRODUCTION

Ever-growing population, climatic changes and unprecedented losses due to pests and diseases pose serious threat to food security. Precisely food security implies availability of adequate food to everyone in all times to come. Food is one of the three basic needs of man, without which his survival is at stake. Plants constitute the basic source of food. Healthy plants not only guarantee bountiful harvest but ensure nutritional essence as well. Plants are to be nurtured like a child from the very beginning to prevent invasion from biotic and abiotic agents by employing integrated crop management. Plant health management is crucial to food security, which is jeopardized due to unprecedented threat by large number of insect-pests, diseases, weeds and several edaphic and environmental stresses. Every year in India, pests and diseases eat away, on an average, 20 to 30% of food, worth about Rs. 50,000 crore, produced by the farmers. Due to unabated rise in population, reduction in arable land will be an ongoing process, hence we may have to strive hard to grow more food from limited land, employing innovative strategies and more importantly adopting multipronged initiative and timely diagnostic and management strategies to combat attack from pests and environmental stress, and manage plant health to mitigate losses. This review presents: (1) Protection through pesticides, (2) benefits of pesticide use, (4) nano and spurious pesticides, (5) modern pesticide application technologies, (6) pesticide residue, (7) promotion of biocontrol, IPM and organic farming, (8) GM crops and (9) future of plant protection.

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## PLANT PROTECTION

Plant protection is the science and practice of managing pests, plant diseases, weeds and other pest organisms that damage agricultural crops and forestry. Agricultural crops include field crops (maize, wheat, rice, etc.), vegetable crops (potatoes, cabbages, etc.) and fruit and horticultural crops.

### Major thrust areas of plant protection

The major thrust areas of plant protection are:

1. Promoting integrated pest management (IPM).
2. Ensuring availability of safe and quality pesticides for sustaining crop production from the ravages of pests and diseases.
3. Coordinating the quarantine measures for reporting, detecting and eliminating exotic pests.
4. Fulfilling the requirements set out in the WTO/SPS agreement and protocols including the preparation of a country pest list, conducting pest risk analyses and surveillance for economically important pests and diseases.
5. Testing bio-pesticides (bio-rationals) and other chemicals generally regarded as safe (GRAS) to ensure that they afford the farmer the protection needed and to put in place the necessary regulations for the importation of these bio-pesticides.
6. Ensuring that the country is well served with respect to being able to respond, identify and manage new pest incursions. This involves the continued close relationship with the Plant Quarantine section and focuses on strengthening existing laws and regulations as well as providing technical expertise and training to the quarantine staff.
7. Assisting in the education of farmers, schools and other tertiary institutions and the public in general about pests and diseases of plants.

### Protection through pesticides

Plant diseases and other pests cause 40% reduction in yield worldwide (Oerke et al., 1994). As per recent estimates every year in India, pests and diseases eat away, on an average, 20 to 30% of food, worth about Rs. 50,000 crore, produced by the farmers (Agriculture Today, 2014). Pesticides play an important role in plant protection. Pesticides are the basic tools for managing plant pests. Man in his endeavor to provide protection to plants and ensure food availability to ever growing population has greatly depended on pesticides, which have been used since 19th century. I earnestly believe that pesticide would still continue to be used as the most important arsenals of controlling plant pests to ensure food security. However, their use has to be made judiciously, which unfortunately is not, and their reckless and indiscriminate use has created more problems. Pesticides came under serious criticism with the publication of 'Silent Spring' in 1962 by Carson (1962), who apprehended threat to man and environment. Another publication, 'Silent Spring Revisited' by Marco et al. (1987) also corroborates the concerns of Rachel Carson. Today, indiscriminate use of pesticides has undoubtedly adversely affected ecological balance resulting in pest resurgence, aggravation of minor pests, pesticide resistance, and environmental pollution and more importantly their residues in food and feed, posing serious health hazards to man and animal. The use of endosulfan in Kerala has created furor due to unprecedented health hazards leading to neurological problem in children which led to the ban of the pesticide in Kerala (Srivastava, 2013). Now the big question before us is – whether pesticides are really responsible for hazards or its man's folly which has created these problems? This paper will now probe and see what best can be done for the society.

In India, pesticide use, which is approximately 500 g/ha is too small as compared to those in developed nations such as UK, USA, China and Japan (Table 1) and therefore I leave it to you to think and ponder whether such low amount can pose threat to the environment. In India, 229 pesticides are registered as against 5487 in China. Amongst registered pesticides in India, fungicides are hardly 39 in number (Srivastava, 2013). Indigenous production of pesticides is more than the domestic consumption and a large quantity of pesticides is being exported to foreign countries. However, the consumption of chemical pesticides has declined from 75,033 metric tones in terms of technical grade in 1990-91 to 50583 metric tones in 2011-12 (Bhardwaj and Sharma, 2013). Managing the pest problem with less number of pesticides is a good sign and efforts are being made to encourage introduction of more low volume and high efficiency pesticides. The major benefits of pesticides are:

1. Use of pesticides improves crop yields.
2. It helps to keep food prices in check for the consumer.
3. Pesticides allow consumers to consume high-quality produce that is free of insect blemishes and insect contamination.
4. Pesticide products are used to control household pests like termites, roaches, ants, rats and other pests.
5. Herbicides are used to control vegetation that clogs navigable and other waterways or threatens to obstruct highway, utility and railroad rights of way.
6. Pesticides are used to protect and enhance lawns, gardens, public parks, playing fields, lakes and ponds for public enjoyment.

There are wide inter-state disparities in the consumption of pesticides. While states like Andhra Pradesh, Uttar

**Table 1.** Pesticides consumption in some of the major countries.

| Name of country | Pesticide consumption (Kg/ha) |
|---|---|
| USA | 7.0 |
| Europe | 2.5 |
| Taiwan | 17 |
| Japan | 12 |
| Korea | 6.6 |
| India | 0.5 |

Pradesh and Maharashtra recorded high consumption of pesticides during the year 2011-12, states like Jharkhand, Odissa, Himachal Pradesh, Uttaranchal and the north eastern states recorded very low consumption of the same during the said period. Though the consumption figures for states like Jharkhand and Uttaranchal showed improvement in recent times, the total quantity of pesticides consumed is still very less. Further, there are wide variations in consumption of pesticides in respect of certain states from year to year. For example, while Andhra Pradesh recorded a consumption of 1541 metric tons of technical grade pesticides during 2007-08, the same declined to 1015 MT during 2009-10. The State recorded a huge rise in consumption during 2010-11 at 8869 MT but declined a bit to 8529 MT during 2011-12. Rajasthan's consumption figure of 3804 MT during 2007-08 came down to 1652 MT for 2011-12. Similarly, states like Odisha witnessed a steady decline of consumption of pesticides over the years, from a peak of 1588 MT in 2009-10 to 491 MT in 2011-12. So, there is a need to devise an effective mechanism to assess the demand and availability of pesticides in the states in terms of formulations.

Information provided by State Governments reveal that around 90 million hectares of cropped area is within the ambit of pesticides usage, leaving out significant swathes of agricultural land in the country where pesticides are not being applied to crops. Different estimates show that more than 50% of consumption of pesticides is garnered by insecticides, whereas herbicides and fungicides together contribute about 30 to 40% of total pesticide consumption. Among the crops, cotton, rice, vegetables and fruits account for the largest share of pesticide consumption in the country. In fact most of the hazards faced by the society are due to indiscriminate use of insecticides and therefore a course correction is required. Pesticides are poisonous entities and hence need to be used with utmost care. $LD_{50}$ values are indicative of their toxicity, which for common man is reflected by colored triangles – green denoting less toxic while red, is extremely toxic. Pesticides most frequently responsible for hazards are organochlorine, organophosphates and carbamates insecticides. Fungicides, conversely do not pose much hazards except organo-mercurials. It may however be clearly understood that there are many commodities, including beverages and drugs, whose $LD_{50}$ is comparable with pesticides. Should we then blame pesticides for hazards or users for reckless use?

## Pesticide residue

The consumption of pesticide in India is one of the lowest in the world (Table 2). However, despite the low consumption of pesticides, India has more problem of pesticide residues *vis-a-vis* other countries and these have entered into food products and underground water because of non-prescribed use of chemical pesticides, wrong advice and supply of pesticides to farmers by vested interests, non-observance of prescribed waiting period, pre-marketing pesticide treatments during storage and transport, use of sub-standard pesticides, effluents from pesticide manufacturing units, continued use of persistent pesticides for public health programmes, lack of awareness and lack of aggressive educational programmes for farmers/consumers. Presence of pesticide residues in agricultural produce – particularly fruits and vegetables, milk and eggs, has become a matter of concern. There is sufficient evidence that it is a widespread phenomenon and that too with insecticides but again a man-made problem for his selfish gain, ignoring the principle of 'safe use of pesticides. There were instances when Indian products were banned from export owing to the presence of the pesticides over the prescribed limit. Recently, the domestic market has also been plagued by similar incidents.

Most recently, Saudi Arabia has banned Chilli from India due to the presence of pesticide residue. India's basmati trade has also been rocked by problems of pesticide residue. Several US-bound consignments were rejected due to the presence of traces of pesticides such as Bavistin, Isoprothiolane and Tricycalzole that have not been registered with the US food and Drug Administration (USFDA). Without USFDA approval, the chemical present in rice consignement have been considered as illegal and not safe for human consumption. In June, 2010, a Humberg based lab issued reports to buyers objecting that organic Basmati rice imported from India had elevated levels (0.03%) of Carbendazim and Isoprothiolane. This stalled the export of 20,000 tonnes of organic rice from India. In 2010, the Europion Union (EU) rejected three consignments of bhindi from India because of the same reason. Higher level of monocrotophos, acephate and triazaphos residues were found in these consignments. The EU has a tolerance limit of 0.05 mg/kg for moncrotophos residue in bhindi, while for acephate and trizophos, the maximum residue limit is 0.02 and 0.01 mg/kg, respectively. In the consignment that was rejected, the monocrotophos residue level was 0.13 mg/kg and that of acephate 0.13 mg/kg.

**Table 2.** Consumption of pesticides in states of India.

| S/N | States/UTs | 2007 - 08 | 2008 - 09 | 2009 - 10 | 2010 - 11 | 2011 - 12 |
|---|---|---|---|---|---|---|
| 1 | Andaman & Nicobar | - | 6.24 | 14.00 | - | - |
| 2 | Andhra Pradesh | 1541.00 | 1381.00 | 1015.00 | 8869.00 | 8529.00 |
| 3 | Arunachal Pradesh | 16.00 | 10.00 | 10.00 | 10.00 | 17.45 |
| 4 | Assam | 158.00 | 150.00 | 19.00 | 150.00 | 160.00 |
| 5 | Bihar | 870.00 | 915.00 | 828.00 | 675.00 | 655.00 |
| 6 | Chandighar | - | - | - | - | - |
| 7 | Chhattisghar | 570.00 | 270.00 | 205.00 | 570.00 | 510 |
| 8 | Dadra & Nagar Haveli | - | - | - | - | - |
| 9 | Daman & Diu | - | - | - | - | - |
| 10 | Delhi | 57.00 | 57.00 | 49.00 | 48.00 | 46.00 |
| 11 | Goa | 2.30 | 8.90 | 10.30 | 8.90 | 8.40 |
| 12 | Gujarat | 2660.00 | 2650.00 | 2750.00 | 2600.00 | 2540.00 |
| 13 | Haryana | 4390.00 | 4288.00 | 4070.00 | 4060.00 | 4050.00 |
| 14 | Himachal Pradesh | 296.00 | 322.00 | 328.00 | 328.00 | 315.00 |
| 15 | Jammu & Kashmir | 1248.00 | 2679.00 | 1640.00 | 1817.75 | 1711.13 |
| 16 | Jharkhand | 81.00 | 85.00 | 88.50 | 84.30 | 151.37 |
| 17 | Karnataka | 1588.00 | 1675.00 | 1647.00 | 1858.00 | 1272.00 |
| 18 | Kerala | 780.00 | 272.00 | 631.00 | 657.32 | 629.46 |
| 19 | Lakshadweep | - | - | - | - | - |
| 20 | Madhya Pradesh | 696.00 | 663.00 | 645.00 | 633.00 | 850.00 |
| 21 | Maharashtra | 3050.00 | 2400.00 | 4639.00 | 8317.00 | 6723.00 |
| 22 | Manipur | 26.00 | 30.36 | 30.36 | 29.81 | 29.81 |
| 23 | Meghalaya | 6.00 | - | 6.10 | 10.33 | 9.42 |
| 24 | Mizoram | 44.00 | 44.25 | 39.05 | 3.91 | 0.39 |
| 25 | Nagaland | 5.00 | 17.83 | 13.58 | - | 15.00 |
| 26 | Odisha | - | 1155.75 | 1588.00 | 870.50 | 491.00 |
| 27 | Pondicherry | 41.00 | 39.00 | 39.29 | 39.29 | 39.78 |
| 28 | Punjab | 6080.00 | 5760.00 | 5810.00 | 5730.00 | 5690.00 |
| 29 | Rajasthan | 3804.00 | 3333.00 | 3527.00 | 3623.00 | 1652.00 |
| 30 | Sikkim | 6.00 | 2.68 | 4.22 | - | - |
| 31 | Tamil Nadu | 2048.00 | 2317.00 | 2335.00 | 2361.00 | 1968.00 |
| 32 | Tripura | 27.00 | 38.00 | 55.00 | 12.00 | 30.06 |
| 33 | Uttar Pradesh | 7332.00 | 8968.00 | 9563.00 | 8460.00 | 8527.00 |
| 34 | Uttaranchal | 270.00 | 221.10 | 222.00 | 198.54 | 233.20 |
| 35 | West Bengal | 3945.00 | 4100.00 | N/A | 3515.00 | 3730.00 |
| | **Grand Total** | **41637.3** | **43860.07** | **41821.4** | **55539.65** | **50583.47** |

The Delhi High Court has recently directed the Delhi government to set up a Pesticide Residue Management Cell (PRMC) under the control of food commissioner of the state. The high court had acted suo moto on a report of NGO Consumer Voice, which had in 2010, found that 35 varieties of vegetables and fruits, picked from Delhi markets and tested for pesticide content, had toxins beyond permissible limits. The court's order came after it was informed that 5.3 percent of vegetables and 0.5 percent of fruits sold in Delhi had pesticide residue above the prescribed maximum residue limit (MRL). The bench noted that by one calculation the entire population of Delhi was consuming food items with pesticide residue beyond permissible limits. The report also claimed that pesticide components such as Chlordane, Endrin, Heptachlor, Ethyl and Parathion are used in growing a number of vegetables, which have the potential to cause serious neurological problems, kidney damage, skin diseases, cancer and other diseases.

## Plans to counteract the problem of pesticides residue

Highest contamination in food occurs due to abuse of pesticides in fields. Good agricultural practices are key to contain the residues level below MRL. The Department of

Agriculture and Cooperation, Ministry of Agriculture is regularly monitoring the pesticides residues under the central sector scheme, 'Monitoring of Pesticide Residue at National Level'. The scheme monitors the pesticides residue in agricultural commodities and environment so that corrective measures can be taken, as required. The scheme involved monitoring and analysis of pesticides residues in agricultural commodities in different agro ecological regions of the country to address the concerns of food safety and impact of pesticides on India's food and agricultural trade. 22 laboratories of different department and ministries are part of this network. These laboratories are equipped with latest equipments and manpower to carry out residue related work. There are more than 29 private pesticides residue laboratories recognized by the Agriculture and Processed Food Products Export Development Authority (APEDA) for pesticide residue analysis in agri-export commodities. Besides this, National Institute of Plant Health Management (NIPHM), Hyderabad imparts training on pesticides residues through an identified course programme.

Food Safety and Standards Authority of India (FSSAI) lays down standards for articles of food and regulate their manufacturer, storage, distribution, sale and import to ensure availability of safe and wholesome food for human consumption. Besides following scientific method of crop production, various physical methods like washing dislodge pesticide residues present on the surface of the produce. For residues present inside the matrix of the produce, safe waiting period and cooking would serve to degrade the molecules to non toxic levels. Farmers, stake holders and officials involved in marketing of the produce must be apprised of the permissible residue limits for each commodity and the waiting periods before the beginning of the season, harvesting of the produce and before marketing for domestic as well as export.

India cannot out rightly reject the use of pesticides in its crops but instead choose to be cautious. The best way to tackle this is to adopt chemical which is recommended to the specific crop and the right dose. There is a need to sensitize growers and bring a change in their attitude towards mankind and have to take a balanced approach and in any case, total reliance on pesticides has to be abandoned. Simultaneously pesticide industry has to take cognizance of widespread hazards of certain pesticides in certain areas, and therefore such pesticides need not be marketed/promoted in such areas, and relief needs to be provided and use of such deadly pesticides should be totally stopped in such areas.

**Curbing spurious pesticides**

Farmers currently put lesser emphasis on brand whereas they are highly price sensitive. This essentially brings us back to the problem of spurious pesticides in the market. In the pursuit of cheaper fix for crop problems, farmers get duped by fake pesticides. Agrochemicals Policy Group (APG), the industrial body representing the crop protection companies, including the pesticides manufacturers and formulators, has estimated the crop loss at Rs. 6,000 crore annually due to use of spurious pesticides. The fake pesticides are inferior formulations, which not only fail to kill pests but also inflict damage to the crops. Poor and marginal farmers fall prey to such cheap products and end up with low crop yield (Kumar and Gupta, 2012). About 30% of the sugarcane crops in the second largest sugar producing state of Uttar Pradesh are lost due to fake pesticide products. The most recent to fall prey to the fake products is Jammu's Rs 4,000 crore apple industry.

Under the present arrangement, there is no mandatory requirement for checking of pesticides for their spurious content or 'misbranding' at the factory or manufacturers' level. Normally the process to test and inspect pesticides is initiated on the basis of complaints from farmers, by which time the pesticides are already in the market. The very fact that a sizeable number of samples were found spurious during testing, clearly shows that the existing system is either grossly inadequate or is not strong enough to monitor and check the quality control of pesticides effectively at every level till it reaches the farmers for usage. In fact, there is absence of quality checking mechanism even for major manufacturers of pesticides and that they should have looked into it being responsible for production of pesticides. Since safe use of pesticides is crucial not only for the farmers in view of increased soil stress due to over use of chemical pesticides but for the safety of human beings, animals and environmental sustainability. Therefore, the existing mechanism needs to be strengthened and made effective and more vigilant to check any spread of spurious and ineffective pesticide. The Government should work out a mechanism to make testing of pesticides mandatory at every possible level right from the factory level till it reaches the farmers, since this would require adequate number of testing laboratories and well trained Inspectors.

There are a total of 71 pesticides testing laboratories in the country which includes 68 State Pesticides Laboratories in 21 States and one Union Territory, two Regional Pesticides Testing Laboratories at Chandigarh and Kanpur and one Central Insecticides Laboratory in Faridabad. While the State like Tamil Nadu have 15 laboratories, other States like Assam, Bihar, Kerala, Odisha, Madhya Pradesh, West Bengal have only one laboratory each and the States like Jharkhand and Meghalaya do not have any such facility at all. There are 10,757 insecticide inspectors under state government that is, Department of Plant Protection, Quarantine and Storage. These inspectors have drawn 49,013 pesticides samples during 2013-14 to control the flow of spurious pesticides into market. Only 1073 (2.19%) pesticides

**Table 3.** Bio-pesticides usage.

| Year | Bio-pesticides |
|---|---|
| 2007-08 | 1873.00 |
| 2008-09 | 1459.00 |
| 2009-10 | 3366.00 |
| 2010-11 | 5151.00 |
| 2011-12 | 6506.00 |
| **Total** | **18355.00** |

Source: Directorate of Plant Protection, Quarantine and Storage.

samples were found misbranded and 469 misbranded cases have been prosecuted. During 2013-14, 124 pesticides samples (including biopesticides) have been drawn by the Central Insecticides inspectors and 23 samples were found to be misbranded. The prosecution is being launched against the dealers and manufacturers of these pesticides. Four accused have been convicted by the different courts for manufacturing misbranded pesticides during 2012-13.

A number of small player play a very important role in respect of formulation and production of pesticides in the country. While that brings down the cost of pesticides, the same increases the incidents of usage of spurious and ineffective pesticides in the country. In fact, a large scale usage of spurious pesticides is the main issue of concern, which happens primarily due to inadequate number of accredited pesticides testing laboratories. The existing Pesticides Laboratories at the State and Central levels are highly inadequate and there is an imperative need to establish well equipped pesticide testing laboratories in adequate numbers in each State across the country, keeping in view the needs of the crops grown in the region and manned by well trained staff. So far, only 4 out of 68 State Pesticides Testing Laboratories have been accredited by the National Accreditation Board for Testing and Calibration Laboratories (NABL). Both Regional Pesticides Testing Laboratories are accredited by NABL, whereas the Chemistry and Bio Assay Divisions of the Central Insecticides Laboratories have been accredited for testing of chemical pesticides and bio-pesticides, respectively. Efforts are being made to improve standard of infrastructure and laboratory practices in remaining labs for them to be accredited in the near future. Lack of accredited and well-equipped laboratories in the States is an important issue which has a direct bearing on the quality of pesticides available to farmers. Infrastructure for testing quality and composition of bio-pesticides, particularly to investigate presence of chemical pesticides, is deficient in States. In cases of misbranding of pesticides, prosecutions in States also tend to take a long time. The Department should initiate appropriate action to get establish well equipped pesticide testing laboratories in adequate numbers in each State across the country.

## Promotion of biopesticides

Bio-pesticides are typically microbial biological pest control agents that are applied in a manner similar to chemical pesticides. Most beneficial advantages of bio-pesticides are that they are the harmful residues are not detected. They can be cheaper than chemical pesticides when locally produced. They can be more effective than chemical pesticides in the long-term. They are biodegradable (Kumar, 2014c; Kumar et al., 2014). Biopesticide formed only a meager percentage of total pesticides used in the country. Bio-pesticides usage has displayed a resolutely upward trend during the previous plan period as is evident from Table 3.

The government of India has adopted measure to promote biological control of pests in India through establishment of 31 Central IPM centres in 28 states and one UT. The biological control laboratories have also been established in these centers. There are 352 bio control labs including bio control laboratories established by Indian Council of Agricultural Research (ICAR), State Agricultural Universities (SAUs), private sector lab, and private sector lab funded by GOI through grant in aid. These bio-control labs are engaged for multiplication, release and sale of different bio-control agents against different pests and weeds. A total 47,489 million biocontrol agents have been released by ICPMCs since 1919-92. The biocontrol agents are being released through inundative and inoculative methods. These releases were able to manage the pests like sugarcane pyrilla and sugar cane borers, cotton bollworms, apple wooly aphid and San Jose scale, different lepidepterous pest of vegetable cropsrice etc. Different types of bio control agents like insect parasitoids, insect predators, spiders, insect pathogens like nuclear polyhedrosis virus (NPV) and biopesticides like antagonistic fungi such as *Trichoderma viride* and *Trichoderma harzianum* and entomogenic pathogenic fungi like *Beauveria bassiana* and *Metarhizium spp*. are being produced in these biocontrol laboratories. The consumption of biopesticides is increasing every year. The annual consumption of bio-pesticides was 1,873 MT in 2007-08 which has increased to 8,110.35 MT in 2011-12- barely sufficient to cover less than 2% area of the 328 million ha under cultivation while the present requirement of quality bio- pesticides is to the tune of 1,00,000 tones. During 2011-12, a total of 7.96 lakh hectares was covered for pest monitoring activity. Similarly, the area coverage for augmentation and conservation of ‘friendly insects’ during 2011-12 was 7.60 lakhs hectares. Global awareness against use of synthetic pesticides and pesticide residues in food materials are a concern and may become international trade barriers. Therefore the eco friendly methods of crop protection and bio-control based IPM system are the need of the day.

The concerted efforts at the central and state level to popularize IPM approach among the farmers have created significant awareness in favour of biopesticides/bioagents. The steps taken to encourage the use of biopesticides/bioagents are summarized as under:

1. The guidelines for registration of biopesticides have been simplified.
2. Farmers, local entrepreneurs, NGOs have been encouraged for production of the same with assistance of ICAR (KVK) and Department of Biotechnology (DBT).
3. Central assistance as grants-in-aid provided to PDBC (ICAR) for research, development and production of bio-control agents.
4. Grants-in-aid provided to the States/UTs for infrastructural development for production of biocontrol agents and biopesticides by establishing SBCLs.
5. The Farmer's Field Schools (FFSs), training-cum-demonstration are playing major role in the promotion and popularization of biopesticides and biocontrol agents among the users.
6. Commercialization of biopesticides is allowed during the validity of provisional registration for 2 years which is also extendable for another 2 years when the applicants have made efforts to generate data to obtain regular registration under Section 9(3).
7. The Government is also promoting organic farming in the country which emphasizes enhanced use of bio-fertilizers and biopesticides, besides advocating greater use of organic manures, compost and vermi compost as substitutes for chemical pesticides and fertilizers.

It is evident that the use of bio-control agents in combating pests is on the rise, yet significant challenges posed by short shelf life, standardization and quality, storage and transportation need to be addressed by agricultural research institutions in the near future (Kumar and Upadhyay, 2003; Kumar et al., 2009). Quality of bio-pesticides, particularly in relation to threat of lacing with chemical pesticides is an emerging problem in different parts of the country. Infrastructure for laboratory analysis of bio-pesticides remains inadequate in the country (Kumar, 2013b).

## NANOPESTICIDES: A NEW HOPE

Nanotechnology visualized as a rapidly evolving field has potential to revolutionize agriculture and food systems. Conversion of macro-materials into nano size particles (1 to 100 nm) gives birth to new characteristics and the material behaves differently. Nanoparticles can be produced by different methods, chemical and biological, the former is commercially used. Nano-materials can be potentially used in the crop protection, especially in the plant disease management. Nanoparticles may act upon pathogens in a way similar to chemical pesticides or the nano-materials can be used as carrier of active ingredients of pesticides, host defense inducing chemicals, etc. to the target pathogens. Because of ultra small size, nanoparticles may hit/target virus particles and may open a new field of virus control in plants. The disease diagnosis, pathogen detection and residual analysis may become much more precise and quick with the use of nanosensors.

We utilized nano-technological interventions for developing nano-pesticides with potent fungicidal and miticidal properties. Nanosulphur and nano-hexaconazole were prepared using encapsulation technique (Gopal et al., 2011a, b; Chaudhary et al., 2010). Nano-hexaconazole was characterized by scanning electron microscopy (SEM), transmission electron microscopy (TEM), and Fourier transform infrared spectroscopy (FT-IR) etc. and it was found to be less than 100 nm in size. Nano-hexaconazole is five times more effective in controlling pathogens and nano-sulphur is ten times more effective for controlling mites as compared to its water dispersible powder (WDP) formulations (Gopal et al., 2012). Therefore, nanotechnology has potential to provide green and efficient alternatives for the management of pests in agriculture without harming nature.

### Modernization of pesticide application technologies

Application of pesticides for the management of diseases, pests and weeds has great role in maintaining crop health. Scientific research efforts focused on the improvement of pesticide application in agriculture is essential for crop protection and environmental safety as well. Basic fungicide application strategy should be focused on application system development, drift management, efficacy enhancement and remote sensing. Future research on application system should include sensor controlled hooded sprayers, new approaches to direct chemical injection and aerial electrostatic sprayers, new approaches to direct chemical injection and aerial electrostatic sprayers. For the accurate field application, on broad flow controllers should be used. Aircraft parameters such as boom position and spray release height can be suitably altered to determine their effect on the drift. The basic drift management research should be focused on testing of low dirft nozzles, evaluation of pulsed spray technologies and evaluation of drift control adjuvant. With the changing agricultural practices, pesticide formulations have also been changed. For the control release of fungicides in the target areas, micro-encapsules of fungicide play an important role (Tsuji, 2001). Microencapsulation of pesticides has considerably improved handling safety due to hazards and exposure reduction.

Recent advances in electronics, remote sensing and computer application have resulted in the precision application of pesticides. Advances in electronics has played an important role in the developments related to the controlled use of pesticide by better matching applications to the target requirements. Specified infected crop patches should be identified and selectively sprayed with the effective fungicides. Patch spraying can considerably reduce the excessive use of pesticide in the target environment. In widely spaced row crops like vegetables, fully automated detection system based on image analysis can be developed which in turn will guide the application of pesticides only in crop rows.

Remote sensing technique is being used in many countries to identify disease infected areas in the field so that spray can be directed only to those areas. In India too, this can be employed specifically in those crops where fungicide are used over a wide area like apple scab or potato late blight for specific application of fungicides in the infected patches.

Pesticide transport models are being used as a tool to develop effective pesticide management studies by the use of Root Zone Water Quality Model (RZWQM) as discussed by Malone et al. (2004). It has been show that if key input parameters are calibrated, RZWQM models can adequately simulate the process involved in pesticide in different agro climatic zones of India for effective and specific use of fungicide in different crops.

With the increased emphasis on protected cultivation in Indian, management of diseases in the green house environments could be tackled by using specific fungicide application. A new approach to fungicide application in green houses has been named as ‘Envirosol technology’. This technology uses carbon dioxide to deliver pesticides as aerosol droplets into enclosed spaces like green houses. Envirosol products such as Permigas, Pestigas, and Insectgas are commercially available. For instance, Floragas and Hortigas have been developed as postharvest fumigants for the treatment of cut flowers and asparagus (Carpenter and Stocker, 1992). Growers use high volume of spraying with motorized pumps and reduced volume spraying with thermal pulse jet foggers in controlling greenhouse diseases and pests. These applications too have runoff of excessive pesticide. Hence, specific users friendly greenhouse fungicide application technologies have to be developed in the future.

So, improved methods of application and judicious use of pesticide can greatly reduce the environmental and health hazards. Chemigation, direct injection, closed system handling and fertilizer impregnation technology would be of great use in this endeavor. Besides, pesticide should be used as an important component of IPM for reduced use of chemicals. An effective education programme about the specific and judicious use of pesticides can play an important role in future programmes on pest management and increased agricultural production

### Host resistance

Host resistance is one of the most important means of fighting against plant diseases/pests and therefore greater reliance should be given to host resistance in agrarian system, rather than resorting to use of pesticides, which poses health hazard problem to applicator, consumer and pollute environment and affect biodiversity by their impact on ecosystem. It is therefore desirable to use as far as possible resistant or tolerant cultivars in consultation with agriculture department or agricultural university.

## INTEGRATED PEST MANAGEMENT

To educate the farmers about ill-effects of the pesticides, need-based use of chemical pesticides and correct application techniques, an integrated pest management programme has also been started by the Government. Integrated Pest Management (IPM) is an eco-friendly approach which uses cultural, mechanical and biological tools and techniques for keeping pest population below economic threshold levels. This approach attaches a high premium on the efficacy of bio-control agents and bio-pesticides. However, need based and judicious use of chemical pesticides is permitted (Kumar, 2010a). With this objective, IPM helps in maximizing crop protection with minimum input costs, minimizing pollution in soil, water and air reducing occupational health hazards, conserving ecological equilibrium and reducing pesticide residue loads in food. Farmers of India do follow the IPM wherever they have been given proper technical guidance and awareness about the judicious use of chemical pesticides viz., Jind (Haryana), Ananad (Gujarat), Hoogly (WB), Astha (Maharashtra), Bambawad (UP), Gulbarga and Bidar (Karnataka), etc. However, our recent data indicates extreme lack of awareness about integrated pest management not only among farmers across the nation but also in the ranks of field functionaries. Availability of appropriate inputs of IPM also impedes following principles of the same.

### Impact of IPM

The impact of IPM is reported to have presumably led to reduction in consumption of chemical pesticides from 65,462 MT during 1994-95 to 47,020 MT during 2001-02. There is a marginal increase in the trend towards use of bio-pesticides from 8,110.35 MT in 2011-12 to 902 MT during 2001-02.

### Steps taken by government to popularize IPM

National Centre for Integrated Pest Management (NCIPM) was set up in 1988 under the Indian Council of Agricultural Research. There are 31 Central Integrated Pest Management Centres (CIPMCs) located in 28 States and one Union Territory. CIPMCs undertake following activities:

1. Surveillance and monitoring of insect pests and diseases.
2. Augmentation and conservation of natural enemies.
3. Production and releases of bio-control agents.
4. Human resources development through Farmers Field Schools (FFSs), season long training programmes etc.

NCIPM has achieved successes in validating and harmonizing IPM technologies in different crops. To facilitate popularizing IPM approach among farming community under Central Scheme 'Promotion of Integrated Pest Management' of Department of Agriculture and Cooperation, Government of India, information system for IPM has been created, which helps in efficient reporting and dissemination of information on pests surveillance, rearing of host culture, production and release of biological control agents in the field, conservation of naturally occurring biological control agents for control of crop pests and transfer of innovative IPM skills/methods/techniques to extension workers and farmers through conduct of training and farmers Field schools in all states.

Implementation of Integrated Pest Management in India needs a unified strategy in terms of development and country wide IPM programme, which ought to include improved planning, co-ordination, funding communication, networking at the central level with a concrete policy by the Government of India for the same. Agriculture is a state subject. Central agencies put their best efforts to provide necessary help and technical backstopping to state agencies. Better coordination between state and central bodies can improve lab to land transfer of technologies. Due to different initiates taken by the ICAR, the transfer of technology from research labs to farms has picked up and hopes to improve further in days to come. As of now, 77 crop specific package of practices have been prepared to help farmers and extension functionaries adopt IPM approach to combat pests and diseases in an environmentally friendly manner. However, the challenge is in periodic updating and improvement of these packages of practices so that prescription to farmers and extension functionaries are in tune with new knowledge and innovation. An important issue that confronts the sector relates to devising ways and means to enhance relevance and acceptability of package of practices among farmers in different agro-climate zones characterized by regional variations in farming traditions, practices and crop cycles. There is also a need to assure farmers that management solutions offered for control of pests and diseases conform to principles and standards of good agricultural practices.

### *Plant quarantine*

Plant quarantine regulatory measures are operative through the "Destructive Insects & Pests Act, 1914 in the country. The purpose and intent of this Act is to prevent the introduction of any insect, fungus or other pest, which is or may be destructive to crops. The import of agricultural commodities is presently regulated through the Plant Quarantine (Regulation of Import into India) Order, 2003 issued under DIP Act, 1914 incorporating the provisions of New Policy on Seed Development, 1988. Further, the significance of Plant Quarantine has increased in view of globalization and liberalization in international trade of plants and plant material in the wake of Sanitary and Phytosanitary (SPS) Agreement under World Trade Organization (WTO). The phytosanitary certification of agricultural commodities being exported is also undertaken through the scheme as per International Plant Protection Convention (IPPC) (1951). The primary objectives of the Scheme are:

1. To prevent the introduction and spread of exotic pests that are destructive to crops by regulating/restricting the import of plants/plant products and,
2. To facilitate safe global trade in agriculture by assisting the producers and exporters by providing a technically competent and reliable phytosanitary certificate system to meet the requirements of trading partners.

The Directorate of Plant Protection, Quarantine and Storage, Faridabad under the Department of Agriculture and Cooperation is the nodal agency for plant quarantine and export certification. To facilitate exports and imports of agricultural commodities, an important e-governance initiative has been undertaken with the launch of the Plant Quarantine Information System (PQIS) in April, 2011. The PQIS aims to reduce lead time in processing of import permits, release orders for import consignments and phytosanitary certificates for exports. Since April, 2011, 61,175 import permits, 160,628 import release orders and 452,395 phytosanitary certificates have been issued online using the PQIS. The Department of Agriculture and Cooperation is coordinating with the Customs Authorities in the integration of PQIS with the Electronic Data Interchange system being currently in use in the Customs Department to further facilitate imports and exports of agricultural commodities through a single window clearance system. There are 35 plant quarantine stations at different airports, seaports and land frontiers implementing the plant quarantine regulations. Qualitative and quantitative improvements in infrastructure and manpower is imperative at plant quarantine stations in the country to curb possibility of detection of quarantine pests and pesticides residues in Indian agriculture export consignments and wood packaging material. The NPQS, New Delhi and RPQSs at Chennai, Kolkata, Amritsar and Mumbai have been strengthened with modern equipment for plant quarantine testing, etc., to facilitate speedy clearance of imports and exports under the Food and Agricultural Organization - United Nations Development Programme (FAO-UNDP) Project.

***Promotion of organic farming***

Organic farming is another option to cut down pesticide use. Fashioned by environmentalists, organic farming is aimed at producing commodities without using pesticides and other agrochemicals. Although produce with organic certificates may fetch higher prices it cannot feed ever-increasing population and more so bearing the higher cost in times of inflation. Further reliance on certification needs reassurance. It appears more to be an illusion. It may, however, be practiced in Western world where population has stabilized and their kitty may allow them to bear the higher cost, but certainly not India for obvious reasons (Srivastava, 2005a). However, incentives of higher return has motivated farmers in producing organic food – especially in and around metros, where organic food has become the buzz word when it comes to healthy diet free from pesticide residues. Incidentally, in the European Union, Member States shall ensure by 1 January, 2014, adoption of IPM for sustainable agricultural production. One should not have blinkered vision towards pesticides, and it must be clearly understood that in the event of outbreak of diseases and pests, pesticides can only offer respite, and therefore their importance cannot be ruled out. What is important is the rational use of pesticides with right intention keeping in view mankind, environment and eco-system. Meanwhile repeated use of systemic insecticides and fungicides has to be avoided, and use of novel fungicides, bio-pesticides need to be promoted. Simultaneously the government has to exercise implementation of Central Insecticide Act (1967) in all earnest.

***Green molecules (GM) - An alternative***

It is aimed at developing transgenic or genetically modified crops. GM could be another route by which pesticide usage could considerably be reduced and simultaneously realizing higher yield. India commercia-lized its first transgenic crop, Bt cotton in March, 2002 (Bambawale et al., 2004) and now Bt cotton has completed its 12 years journey with remarkable success. Bt cotton covers 90% of India's cotton growing area. In 2011-12, the productivity of Bt cotton is 485 kg lint per ha, with 560 kg lint per ha in 2007. With Bt, India became a global exporter of cotton; since 2005, exports have been between 600,000 to 1.5 million tons each year. The Biotech industry credits the technology for bumper harvest, though farmers and activists blame the seed for failing the crops and debt traps. In fact biotech could be considered crucial to second green revolution since Bt crops have ability to fight against pests and require little or no pesticides. Bt cotton is the only transgenic crop currently approved for cultivation in India. Bt brinjal is under moratorium for commercial release. However, there are valid health and environment concerns regarding adoption of GM crops. To date, countries where genetically modified organisms (GMOs) have been introduced in fields, have reported no significant health damage or environmental harm. Some of the concern related to gene flow and pest resistance has been addressed by techniques of genetic engineering. However, the lack of observed negative effects does not mean that they cannot occur. Scientist call for a cautious case by case assessment of each product or process prior to its release in order to address legitimate safety concerns. Science cannot declare any technology completely risk free. Genetically engineered crops can reduce some environmental risks associated with conventional agriculture, but will also introduce new challenges that must be addressed. Society will have to decide when and where genetic engineering is safe enough.

**Pant health clinic**

Failure in timely diagnosis of diseases and other pests has often been responsible for devastating losses. Reducing crop losses by keeping pests at bay is crucial to food security. Plant clinic is an innovative paradigm which plays a vital role in assuring food security and ushering prosperity by providing timely diagnosis and rendering necessary advice to the growers, gardeners and other stakeholders for managing pest problem in India. Plant clinics are all about plant health. Though the major role of plant clinic lies in diagnostics and advisory, the activities of plant clinic extend beyond plant clinic, with emphasis on extension, working more closely with farmers and organizations involved in promoting food production. Srivastava (2005b, 2008, 2009) has redefined the role of plant clinics beyond diagnostics and advisory. These are:

1. Training and teaching to students.
2. Training farmers and extension personnel on field diagnosis of pests and diseases.
3. Producing fleet of plant doctors, keeping a vigil on bio-terrorism/invasive pathogen,
4. Promoting integrated pest management, monitoring pest/diseases distribution and their outbreak.
5. Issuing pest alerts.
6. Organizing plant health camps for creating awareness regarding likely appearance of pests/diseases.
7. Strengthening mobile clinic approach during disease/pest outbreak.
8. Reaching farmers through internet, mobile leaflets, handouts, hand bills etc.
9. Collaboration of development agencies/input dealers and media.

In India, plant health clinics need to be equipped with diagnostic facility with excellent communication skill and facility/ICT, and trained manpower since diagnosis is experience driven process, organizing trainings, maintaining linkages with development agencies/

electronic/print media. With monitoring and surveillance of diseases and pests, issuing pest alerts and their faster dissemination through electronic media, SMSs should be on the card. Plant health camps, training to farmers on field diagnosis of pests and diseases should be organized once or twice during the season. Clinic on wheels must be ready to face epiphytotics/pest outbreak by rushing to the affected area with a team of experts. Enrichment circulars, bulletins, leaflets should be brought out on regular basis for updating growers' knowledge to face the challenges. To improve the working of clinic with respect to the feedback from farmers, agencies must go into inspection and self-evaluation and monitoring and look at the future for the well-being of farmers producing food for us. Creation of well-organized clinics modeled on human clinic would not only boost food security but would help the image of the plant doctors, commanding same respect the human doctors or veterinarians enjoy. Let us rediscover Plant Health Clinic, which can heal the wound of farmers by providing unstinted plant health care support by extending timely diagnosis and recommendation to save the crops from ravages of diseases and pests. Let all governments rise to the occasion to save huge crop losses by supporting creation of plant clinics.

### Future strategies

1. Identification and use of biopesticides.
2. Studying the bio-ecological factors affecting sustainability of bioagents.
3. Identification and development of stress resilient bio control strains.
4. Simplification of process of registration for biopesticides with strict and adequate quality check from Govt. Departments.
5. Increased support to biopesticide industry for scaling up of production as a matter of Govt. policy which shall also enable generation of employment for small /micro industries at village level in line with concepts of model bio village.
6. Perfection of bio control based IPM system.
7. Improving awareness levels of field functionaries and farmers in IPM apart from fast tracking of crop protection advisories through KVKs, NGO etc.
8. Development of an Integrated Decision Support System for Crop Protection Services to monitor the pest dynamics through e-pest surveillance, analyze pest risks, provide pest forecasts along with mobile based dissemination of advisories keeping in view prevailing weather and change in climate.
9. The farmers should be educated on the merits and demerits of the pesticides. They should be educated to distinguish between use and over use.
10. Dealers and retailers should be made accountable to the over use of pesticides.
11. The price of the pesticides can be a deterrent for farmers in selecting the needed pesticide and instead they make seek the service of cheaper and unsuitable pesticide.
12. Ensure E-surveillance for effective pest monitoring, forecasting and better implementation of IPM.
13. Cases of pesticide residue should not be treated as a local issue and a national programme should be developed. Regular monitoring, testing and stringent laws should be developed to manage this.
13. Good agricultural practices (GAP) for sustainable agriculture.
14. Discovery of green molecules.
15. Use of nano- formulations for targeted delivery of pesticides.
16. Marker assisted selection and breeding for biotic stress tolerance.
17. Pest resistant transgenic crops.
18. Novel approaches of pest management (Gene therapy, RNai-mediated gene silencing).

## CONCLUSIONS

To feed the ever-growing global population, we need to produce more food and livelihood opportunities from less per capita arable land and available water. Providing ample food is only the first part of the challenge, the second and more important challenge is to produce this in a safe and sustainable manner. Most of the cultivated crops/varieties have reached their yield plateau, hence protection of crops to harvest maximum is one of the ways to meet the increasing demands of food and to attain National food security on sustainable basis. Chemical pesticides will continue to play a role in pest management because environmental compatibility of products is increasing and competitive alternatives are not universally available. Pesticides provide economic benefits to producers and by extension to consumers. One of the major benefits of pesticides is protection of crop quality and yield. Pesticides can prevent large crop losses, thus raising agricultural output and farm income. The benefits of pesticide use are high-relative to risks.

Non-target effects of exposure of humans and the environment to pesticide residues are a continuing concern. Side effects of pesticides can be reduced by improving application technologies. Innovations in pesticide-delivery systems in plants promise to reduce adverse environmental impacts even further but are not expected to eliminate them. The correct use of pesticides can deliver significant socio-economic and environmental benefits. Genetically engineered organisms that reduce pest pressure constitute a “new generation” of pest-management tools. This change in production system has made additional positive economic contributions to farmers and delivered important environmental benefits. But genetically engineered crops that express a control chemical can exert strong selection for resistance in pests. Thus, the use of transgenic crops will even increase the need for effective resistance management programmes. The national sale of biopesticides is very little compared to the pesticide market (Kumar, 2013b). However, the market share of biopesticides is growing faster than that of conventional chemicals. Many biocontrol agents are not considered acceptable by farmers because they are evaluated for their immediate impact on pests. Evaluation of the effectiveness of biocontrol agents should involve consideration of long-term impacts rather than only short-term yield, as is typically done for conventional practices. A concerted effort in research and policy should be made to increase the competitiveness of alternatives to chemical pesticides for diversifying the pest-management “toolbox”. But availability of alternative pest-management tools will be vital to meet the production standards and stiff competition is expected in these niche markets.

New scientific knowledge and modern technologies provide considerable opportunities, even for India, to further reduce current yield losses and minimize the future effects of climate change on plant health. Finding continuously new cost-effective and environmentally sound solutions to improve control of pest and disease problems is critical to improving the health and livelihoods of the poor. The need for a more holistic and modernized IPM approach in India is now more important than ever before. The IPM strategy should be implemented strictly. To achieve this, interactions between public sector research systems, farmers, private companies that conduct research in the field of plant protection and NGO’s should be strengthened to assure relevance of research and appropriate distribution of responsibilities. Rising food output is vital along with slowing of population growth and maintaining the ecological balance which will ultimately enhance our purchasing power. Indian Council of Agricultural Research (ICAR), Central Agricultural Universities (CAU), Central Universities (CUs), State Agricultural Universities (SAUs), State Universities (SUs) and Deemed Universities (DUs) have to plan and should immediately transfer the recently developed scientific protection technology in form of modules to the farmers field which can increase the yield significantly. The welfare of farmers is our top priority.

## Conflict of interest

The authors have not declared any conflict of interest.

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*Full Length Research Paper*

# Variation in nectar volume and sugar content in male flowers of *Musa* cultivars grown in Rwanda and their non-effect on the numbers of visiting key diurnal insect vectors of banana Xanthomonas wilt

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**Insects are a major mode of banana Xanthomonas wilt (XW) spread. High insect activity has been blamed for the high XW incidence in 'Kayinja' (ABB-genome) dominated banana landscapes across east and central Africa. 'Kayinja' male bud nectar composition reportedly contributes to high insect activity. The variation in nectar composition with agro-ecological zones and banana cultivars and its influence on the number of visiting insects in Rwanda were assessed. Three male buds were collected per cultivar for nectar extraction and analysis using a high performance liquid chromatography. Nectar volume and sugar concentrations varied (P<0.001) across 27 banana cultivars, annual seasons and agro-ecological zone. The highest nectar volume was recorded among the East African highland cooking cultivars (AAA-genome) in the high altitude site and the short-heavy rainy season. Nectar contained three sugars: glucose, fructose and sucrose, though hexose (glucose and fructose) was dominant. The three sugars varied significantly (P<0.001) within each cultivar. The total nectar-sugar concentration ranged from 2.3–32%, with the highest among dessert cultivars 'Kamaramasenge' (AAB-genome) and 'Gisukari' (AAA-genome). No strong correlation occurred between insect population and total nectar sugar concentration or nectar volume. Insect populations were rather influenced by the weather conditions, the long rainy season characterized by moderate well distributed rainfall recording the highest insect populations as compared to the short rainy season (with heavy rainfall) and the dry seasons.**

**Key words:** Banana, insects, nectar, vectors, *Xanthomonas campestris* pv. *musacearum*

## INTRODUCTION

Insects play an important role in the pollination of many plant species. In many plant-insect pollination systems, the plant produces a 'reward', usually in the form of nectar (Willson et al., 1996). Though parthenocarpic, bananas attract a diversity of insect species that obtain nectar and collect pollen from the male flowers. This relationship has been reported as a major mechanism for the spread of bacterial diseases in banana plants (Harrison, 1980). The role of insect vectors, mainly bees, flies and fruit flies (drosophilids) in the spread of Xanthomonas wilt of banana (XW) (causal agent *Xanthomonas campestris* pv. *musacearum* (*Xcm*)) has been confirmed (Tinzaara et al., 2006; Shimelash et al., 2008; Rutikanga et al., 2015).

Nectar sugar concentration may also affect visitor preference or association (Hainsworth and Wolf, 1976; Bolten and Feinsinger, 1978; Schondube and Martinez, 2003); for example, 'Kayinja' (*Musa* ABB) is reported to attract more insects than east African highland banana cultivars (*Musa* AAA), owing to its nectar sugars (Karamura et al., 2008). The observed field susceptibility of *Musa* cultivar 'Kayinja' to infection by *Xcm* has been attributed to its susceptibility to insect vector spread (Blomme et al., 2005; Ocimati et al., 2013). This banana cultivar has not only been decimated in central Uganda, but has also been blamed for the rapid spread of XW disease in central Uganda during 2002-2006 (Njeri, 2008).

Measuring the volume of nectar and its sugar concentration is common in the study of many ecological processes (Dungan et al., 2004), in particular, the study of plant-animal interactions (Bolten and Feinsinger, 1978; Kearns and Inouye, 1993) and can permit the calculation of carrying capacity for nectarivores (Petit and Pors, 1996). However, studies to assess the variability of nectar volume and sugar concentration of major banana cultivars has not yet been carried out in east and central Africa, where XW is now endemic.

Nectar concentration is highly influenced by environmental factors, especially temperature and humidity (Nicolson and Nepi, 2005). The differences in mean nectar concentration between plants can also be explained by contrasting environmental particularities of regions (Forcone et al., 1997; Bernardello et al., 1999). For example, the relatively low mean nectar concentration of some plant species could be related to the lower mean maximum temperatures and higher precipitation characteristics of their environment (Barros et al., 1983).

The environmental differences of the different agro-ecologies in east and central Africa could therefore have influences on nectar concentrations of *Musa* cultivars and therefore the prevalence of insect-vector mediated XW in the cultivars in different agro-ecologies and could influence decisions regarding deployment of cultivars in the different agro-ecologies in the region. However, the potential effect of the variable environment in east and central Africa on nectar concentrations and volumes in *Musa* cultivars is not known.

This study therefore sought to improve knowledge of the interaction of insect vectors (of *Xcm*) and agro-ecologies through determining: (i) male flower nectar variability in terms of volumes and sugar concentration across banana cultivars, seasons and altitudes in Rwanda and (ii) the influence of the variation in male flower nectar volume and sugar content on the population of insect vectors of *Xcm*.

## MATERIALS AND METHODS

### Description and selection of the study sites

This study was conducted in four banana growing agro-ecological zones of Rwanda, categorized into three altitude ranges: low 800-1,400 m above sea level (masl), medium (1,450-1,650 masl) and high (1,700-2,200 masl) (Table 1). The districts in the Lake Kivu border region, with a medium altitude (1,410-1,642 masl) were given special attention due to the high mean rainfall (compared with other medium altitude sites) and incidence of XW that has devastated bananas in the area (Table 1). Detailed information on the biophysical conditions of the four agro-ecologies is documented in Table 1. This study was conducted in 2012 at the middle of each of the four annual seasons prevailing in Rwanda (that is, (1) the short dry season (SdS) from January to February, (2) the short rainy season (SrS) characterized by heavy rains from March to May, (3) the long dry season (LdS) from June to August and (4) the long rainy season (LrS) from September to December). In each agro-ecological zone, three districts (administrative divisions) with XW were purposively selected and a highly infected sector (administrative division under district) chosen for data collection through interaction with the agricultural staff (Table 1). In each sector, three villages with the highest XW incidence (based on sector records on the disease incidence) were purposively sampled, from which a XW-infected banana field was selected for data collection.

### Nectar sampling and laboratory analysis

Three plants per banana cultivar were selected in each field, their male buds were harvested and flowers kept intact until analysis in the laboratory. Male buds of more or less the same age, with at least one open male bract exposed below the last female hand, were sampled. To avoid nectar fermentation, collected flowers were carried in an ice-cold container and kept under refrigeration at -8°C in the laboratory (Petit and Freeman, 1997).

Laboratory analysis was performed in the analytical chemistry laboratory of the Kigali Institute of Science and Technology. The male flowers were allowed to thaw at room temperature while still attached to the male buds. Nectar extraction was performed by rinsing each male flower (under the most recently open male bud bract) four times with 0.5 mL of distilled water using a pipette and this was repeated for seven male flowers from three male buds of the same cultivar (Nunez, 1977; Mallick, 2000).

With the help of the pipette, efforts were made to recover all the nectar-water mixture from the flowers. The nectar and distilled water solutions were thoroughly mixed using a vortex mixer. The volume of nectar for the banana cultivars was calculated as the

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**Table 1.** Description of the study areas.

| Category of altitude | District | Sector | Altitude (masl) | Type of soil | Mean rainfall (mm) | | | | Mean temperature (°C) | | | | Mean relative humidity (%) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | SdS | SrS | LdS | LrS | SdS | SrS | LdS | LrS | SdS | SrS | LdS | LrS |
| Low | 1.Kayonza | Mukarange | 1,300 | Ferralsols | 34.0 | 175.0 | 35.6 | 117.0 | 20.6 | 19.4 | 19.4 | 19.0 | 70.9 | 81.0 | 70.7 | 81.2 |
| | 2.Gatsibo | Kiziguro | 1,400 | Ferralsols | 14.0 | 107.0 | 9.2 | 111.0 | 20.3 | 17.5 | 21.9 | 20.5 | 69.6 | 79.3 | 60.4 | 78.7 |
| | 3.Nyagatare | Tabarwe | 1,380 | Regosols | 21.6 | 119.0 | 17.0 | 109.0 | 21.4 | 21.4 | 20.8 | 21.1 | 71.3 | 80.0 | 70.5 | 80.2 |
| | Averages | | | | 23.2 | 133.7 | 20.6 | 112.3 | | | | | | | | |
| Medium | 1.Ruhango | Bunyogombe | 1,600 | Lexisols | 43.3 | 174.0 | 25.8 | 108.0 | 19.8 | 18.9 | 18.3 | 19.2 | 68.4 | 79.9 | 70.0 | 78.1 |
| | 2.Huye | Mukura | 1,650 | Ferralsols | 84.9 | 197.0 | 10.5 | 171.0 | 20.0 | 19.1 | 19.3 | 19.1 | 71.0 | 80.0 | 60.0 | 85.0 |
| | 3.Gisagara | Save | 1,600 | Acrisols | 7.6 | 62.2 | 30.9 | 57.4 | 19.9 | 19.0 | 18.8 | 19.2 | 75.0 | 82.0 | 65.0 | 88.0 |
| | Averages | | | | 45.3 | 144.4 | 22.4 | 112.1 | | | | | | | | |
| High | 1.Rulindo | Rusiga | 1,887 | Cambisols | 48.9 | 155.0 | 44.9 | 189.0 | 17.5 | 17.0 | 17.4 | 17.4 | 80.0 | 85.0 | 76.0 | 89.0 |
| | 2.Gakenke | Nemba | 2,112 | Cambisols | 67.6 | 187.0 | 15.5 | 253.0 | 18.5 | 18.1 | 18.1 | 18.4 | 82.0 | 86.0 | 77.0 | 90.0 |
| | 3.Burera | Kinoni | 2,167 | Luvisols | 69.3 | 166.0 | 27.2 | 164.0 | 18.0 | 17.1 | 19.0 | 16.3 | 70.1 | 87.0 | 67.4 | 90.0 |
| | Averages | | | | 61.9 | 169.3 | 29.2 | 202.0 | | | | | | | | |
| Lake Kivu border (medium) | 1.Rubavu | Rugerero | 1,600 | Andosols | 36.1 | 164.0 | 54.2 | 158.0 | 20.7 | 20.2 | 19.9 | 20.2 | 75.0 | 80.0 | 68.1 | 75.3 |
| | 2.Rutsiro | Mushyonyi | 1,642 | Alisols | 78.1 | 139.0 | 27.3 | 119.0 | 20.3 | 20.1 | 19.7 | 20.1 | 70.0 | 80.0 | 65.0 | 82.0 |
| | 3.Karongi | Bwishyura | 1,596 | Ferralsols | 75.3 | 166.0 | 40.5 | 113.0 | 19.8 | 19.9 | 19.4 | 20.0 | 73.2 | 82.0 | 68.0 | 83.7 |
| | Averages | | | | 63.2 | 156.3 | 40.7 | 130.0 | | | | | | | | |

Source: Climatic data: Rwanda Meteorology Agency (2012) and soil information: Carte Pédologique du Rwanda. SdS, SrS, LdS and LrS, respectively, denote: short dry season (from January to February), short rainy season (March to May), long dry season (June to August) and long rainy season (September to December).

difference between the total volume of liquid within 3 mL test tubes (51 x 12 mm) and the volume of distilled water added during rinsing. Smaller volumes (0.01-0.7 mL) were measured using manual injection syringes from Waters U6K injection valve. High performance liquid chromatography (HPLC) was used to quantify the specific sugars in a nectar sample (Kearns and Inouye, 1993).

Acetonitrile (HPLC grade) was used as a solvent and the mobile phase was made with a solution of acetonitrile and deionized water in the ratio 75:25 at a flow rate of 1.25 mL/min. The column used was $NH_2$ (25 cm x 4 mm). A UV detector set at 193 nm and a recorder of 50 mV with a flow of 5 mm per minute were also used to perform the analysis .A mixed stock standard (100 mL) of fructose (10 mg/mL), glucose (100 mg/mL) and sucrose (100 mg/mL) was prepared using deionized water. An injection of 20 µl of the sample was made using an injector loop (full loading loop technique).

Each analysis was run for 45 min. Sugar ratios appeared in the form of curves with peaks. Curves were integrated to quantify and convert the sugar ratios (in %) using a computer-assisted digital microprocessor, the Baseline 815 software from Waters.

### Insect collection from banana male buds

To determine the effect of nectar concentration and volumes on insect vector abundance, insects on the male buds of different cultivars were captured at the different altitudes and in the middle of each of the four seasons. Insects were captured as described by Rutikanga et al. (2012), Tinzaara et al. (2006) and Fiaboe et al. (2008). Flying insects were captured using separate sweep nets.

Insects were captured in the morning (7-9 am), between 10 am and 12 noon, in the afternoon (1-3 pm) and in the evening (4-6 pm). These insects were sorted into different broad taxa, separately enumerated and put into labeled vials containing 70% ethanol for further identification to family and species level. Identification to family level was performed in the entomology laboratory of the University of Rwanda, College of Agriculture. The identification exercise was supported by use of an electron microscope and a data base for insect species available on the internet (Castner, 2000; Iowa State University, 2014). Only the major insects groups reported by Tinzaara et al. (2006), Fiaboe et al. (2008) and Rutikanga et al. (2015) were focused on in this study.

### Data analysis

Data were subjected to analysis of variance using GenStat, 5$^{th}$ edition, and the means separated using Fishers least significance difference at 5%. Correlations of nectar sugar content with nectar volume and of nectar sugar content and volume with insect populations across the four seasons were obtained using MS Excel.

## RESULTS

### Nectar volume

Significant differences (P< 0.05) in nectar volume were observed between the 27 banana cultivars (Table 2, Figures 1 to 4). Generally, higher nectar volumes were recorded among the east African highland cooking banana cultivars (*Musa* AAA-EA), with mean highest volumes recorded in 'Injagi' (0.49 mL/male flower in the low altitudes), 'Incakara' (0.59 mL in the Lake Kivu border area) and 'Barabeshya'(0.53 and 0.68 mL in the medium and high altitudes) (Table 2, Figures 1 to 4). In contrast, lower nectar volumes (0.26 mL) were recorded in the brewing banana cultivars (AAA-EA and ABB genotypes) and the least (0.13 mL) in the dessert banana cultivars (Table 2, Figures 1 to 4).

Nectar volumes significantly increased ($R^2$ >05) with the total amount of rainfall in all the banana cultivars (Figure 5). The lowest volumes were recorded during LDS (40.7 mm of rainfall) while the highest was in the SRS (156.3 mm) that is characterized by heavier rainfall (Figures 1 to 5). For example, nectar volumes of 0.01-0.26 mL in the LDS and 0.03-0.37 mL in the SDS (63.3 mm of rainfall), when compared with 0.07-0.42 mL in LRS (130 mm) and 0.13-0.49 mL in the SRS were recorded in the low altitude zone. Similarly, 0.07-0.48 and 0.12-0.55 mL, when compared with 0.17-0.61 and 0.21-0.68 mL, respectively were recorded in the high altitude zone. This was a general trend for all *Musa* groups investigated and other altitudes (agro-ecologies) in this study (Figures 1 to 5).

Nectar volumes generally increased with altitude in this study (Figure 6). For example, nectar yields during the SRS varied from 0.13 – 0.49 mL in the low altitude zones, 0.14 – 0.53 mL in the medium altitudes, 0.16 to 0.59 mL in the Lake Kivu Border region with a medium altitude and 0.21 to 0.68 mL (mean = 0.45 mL) in the high altitude (Figures 1 to 4).

### Nectar sugar concentration

Laboratory nectar analysis revealed the presence of three sugars: glucose, fructose and sucrose. Nectar from the different banana genotypes/cultivars was hexose-dominant (glucose and fructose which is dominant) (Figures 7 to 10). The percentage of the three sugars varied significantly (P<0.001) within each banana cultivar. For example, in the cultivar, 'Kamaramasenge' the level of glucose was 13.60%, fructose: 9.51% and sucrose: 7.73% in the low altitude zone during the long dry season. The total nectar sugar concentration was in the range of 3 and 32% across all banana cultivars and agro-ecological zones. The total nectar sugar concentration also varied significantly (P <0.001) among banana cultivars and cultivar groups (Table 2 and Figure 11). A case in point is the situation in low altitudes during the long dry season where higher total nectar sugar concentration (mean = 30.2%) was noted in dessert cultivars when compared with 24.8% in brewing cultivars and 16.8% in cooking cultivars (Figure 11). The dessert banana cultivars 'Kamaramasenge' (*Musa* AAB), 'Igisukari' (*Musa* AAA), 'Gros Michel' (*Musa* AAA) and 'Poyo' (*Musa* AAA) had the highest nectar sugar concentrations and this was consistent for all the four agro-ecological zones and cropping seasons (Figures 7 to 10). Brewing banana cultivars 'Ingumba' (*Musa* AAA), 'Nyiramabuye' (*Musa* AAA) and 'Kayinja' (*Musa* ABB) (Figures 7 to 10) contained sugars in almost the same concentration range as dessert bananas.

Nectar sugar concentration significantly ($R^2$ >5) declined with an increase in the total amount of rainfall (mm) and an increase in altitude (Figure 12) in all the banana cultivars. It should be noted that the precipitation also increased with an increase in altitude in the study sites. For example, the mean total sugar concentration across the altitudes declined significantly ($R^2$ = 0.788) from 25% in the cultivar 'Igisukari' at a low rainfall of 28 mm to 12% at 150 mm rainfall (Figures 12). Similarly, the mean sugar concentration across seasons for 'Igisukari' significantly ($R^2$ = 0.85) declined from 23% at 1350 masl to 14% at 2027 masl. Similar trends were observed for cultivars when disaggregated by seasons and/or altitudes.

Nectar sugar concentration was observed to decline with an increase in the nectar volume and this was consistent across all altitudes and seasons. For example, a negative linear relationship ($R^2$ = 0.5923) was observed between banana nectar volume and the concentration of sugars at high altitude and in the LRS (Figure 13). Similarly, the east African highland *Musa* cultivars having the highest nectar volume had the lowest sugar concentration, while the dessert types with the least nectar volumes had the highest sugar content.

### Variation in the number of insects per male bud with nectar volume and sugar concentration

Fruit flies in the family of Drosophilidae and Tephritidae followed by bees (Hymenoptera, Apidae) were the most dominant insects collected from banana male flowers in this study (Table 2). Other insects captured in smaller numbers included different species in the families of Lonchaedae, Muscidae, Neriidae and Sarcophagidae, Vespidae, Formicidae, Nitidulidae, Tenebrionida and Staphylinidae.

Some banana cultivars such as the beer cultivars 'Intuntu' (AAA-EA), 'Ingame' (AAA-EA) and 'Kayinja' (ABB) and the cooking cultivars 'Barabeshya' (AAA_EA), 'Incakara' (AAA-EA) and 'Injagi' (AAA-EA) (Table 2)

**Table 2.** Nectar volume and sugar concentration, and the respective insect populations for different cultivars averaged across all seasons and altitudes. The letter 'D' denotes: dessert type; 'B', beer; 'Ck', cooking and 'M', multiple use.

| Cultivar | Nectar features (Average) | | Major Captured Insect Population (Average) | | |
|---|---|---|---|---|---|
| | Volume [ml] | Sugar concentration (%) | Fruit flies (Diptera: Drosophilidae & Tephritidae) | Bees (Hymenoptera, Apidae) | Other flies (Diptera: Lonchaedae, Muscidae, Neriidae, Sarcophagidae) |
| 'Gros Michel'(D) | 0.2±0.02 | 16.4±4.9 | 9.6±3.2 | 6.1±1.0 | 1.5±0.6 |
| 'Igisukari'(D) | 0.1±0.01 | 17.7±6.2 | 9.9±3.2 | 6.2±1.6 | 1.6±0.6 |
| 'Kamaramasenge'(D) | 0.1±0.01 | 17.6±6.2 | 9.9±2.9 | 5.7±2.4 | 1.5±0.5 |
| 'Poyo'(D) | 0.2±0.04 | 16.1±5.2 | 9.2±2.1 | 5.8±1.2 | 1.7±0.6 |
| 'Barabeshya'(Ck) | 0.3±0.01 | 9.6±3.9 | 12.8±4.6 | 6.9±1.3 | 1.8±0.6 |
| 'Incakara'(Ck) | 0.3±0.01 | 9.7±4.0 | 12.9±4.7 | 6.9±1.4 | 1.8±0.6 |
| 'Ingagara'(Ck) | 0.4±0.07 | 10.0±3.5 | 10.3±3.2 | 5.6±1.1 | 1.6±1.1 |
| 'Ingaju'(Ck) | 0.3±0.07 | 13.4±6.5 | 12.5±2.9 | 6.6±1.4 | 1.3±0.6 |
| 'Ingenge'(Ck) | 0.3±0.09 | 11.8±5.1 | 11.1±2.8 | 6.1±0.9 | 1.4±0.7 |
| 'Injagi'(Ck) | 0.3±0.01 | 9.9±4.1 | 13.1±4.8 | 7.0±1.3 | 2.0±0.6 |
| 'Intokatoke'(Ck) | 0.5±0.02 | 8.4±3.8 | 10.9±3.3 | 6.3±1.5 | 2.0±0.5 |
| 'Inzirabahima'(Ck) | 0.4±0.1 | 8.9±4.8 | 10..8±2.2 | 5.5±0.7 | 1.7±0.8 |
| 'Inzirabushera'(Ck) | 0.4±0.09 | 8.8±5.0 | 11.3±2.6 | 6.6±0.6 | 1.9±0.7 |
| 'Kibuzi' (Ck) | 0.4±0.08 | 9.3±5.0 | 11.4±3.2 | 6.6±0.6 | 2.1±0.9 |
| 'Nkazikamwe' (Ck) | 0.3±0.02 | 9.3±4.0 | 3.8±2.0 | 0.4±0.3 | 0.5±0.4 |
| 'Intutsi' (Ck) | 0.4±0.09 | 13.3±6.5 | 11.0±5.2 | 2.3±3.5 | 0.8±1.3 |
| 'Impura' (B) | 0.3±0.1 | 9.2±2.3 | 2.8±1.6 | 0.4±0.3 | 0.7±0.6 |
| 'Ingame'(B) | 0.3±0.02 | 9.6±2.8 | 11.8±3.1 | 7.4±1.1 | 2.1±0.5 |
| 'Ingenge'(B) | 0.3±0.07 | 12.1±5.9 | 12.1±3.2 | 6.7±1.4 | 2.0±0.5 |
| 'Ingumba'(B) | 0.3±0.05 | 20.0±10.1 | 11.1±4.2 | 7.0±2.3 | 1.3±0.6 |
| 'Intuntu'(B) | 0.3±0.08 | 12.0±4.4 | 12.7±4.0 | 7.2±1.9 | 1.7±0.9 |
| 'Kayinja'(B) | 0.3±0.02 | 14.9±5.8 | 12.7±4.3 | 7.4±2.1 | 2.2±0.6 |
| 'Nyiramabuye' (B) | 0.3±0.04 | 15.6±6.4 | 11.8±4.3 | 7.5±1.9 | 2.0±0.6 |
| 'Umuzibo' (B) | 0.3±0.01 | 15.7±5.8 | 10.4±4.4 | 6.1±1.2 | 2.2±0.9 |
| 'FHIA17'(M) | 0.3±0.02 | 9.2±3.4 | 9.8±2.2 | 5.8±1.0 | 1.3±0.6 |
| 'FHIA25'(M) | 0.3±0.02 | 9.9±3.3 | 9.3±2.5 | 5.4±1.2 | 1.8±0.6 |
| CV% | 10.97 | 24.4 | 22.8 | 19.8 | 21.9 |

attracted more insects across all the agro-ecologies and especially during the LRS. 'Kayinja' a model cultivar in this study, had total nectar sugar and insect populations in levels comparable to those of AAA-EA beer and cooking types. However, no strong positive or negative correlation ($R^2$ = 0.0004 – 0.4) was observed between insect population and either the total nectar sugar concentration or total nectar volume, at the insect family group level, across all the altitudes and rainy seasons (Figures 14 to 17). For instance, dessert cultivars, though with the highest levels of total nectar sugar, generally attracted fewer insects than the beer (both ABB and AAA-EA) and cooking AAA-EA banana cultivars (Table 2).

More insects were generally observed in the long rainy season, characterized by moderate rainfall spread over a longer period of time.

## DISCUSSION

This study assessed the variation in the population of insect vectors of Xcm with nectar volume and sugar content across banana cultivars, at different altitudes and seasons in Rwanda. Nectar features (volume and sugar content) were influenced by the banana genotype/cultivar, characteristics of the seasons and altitude. A negative linear relationship was observed between nectar volume and sugar content i.e. the higher the nectar volume, the lower was the sugar content.

### Variation in nectar volume and sugar content across the study sites

Higher nectar volumes were recorded from the high

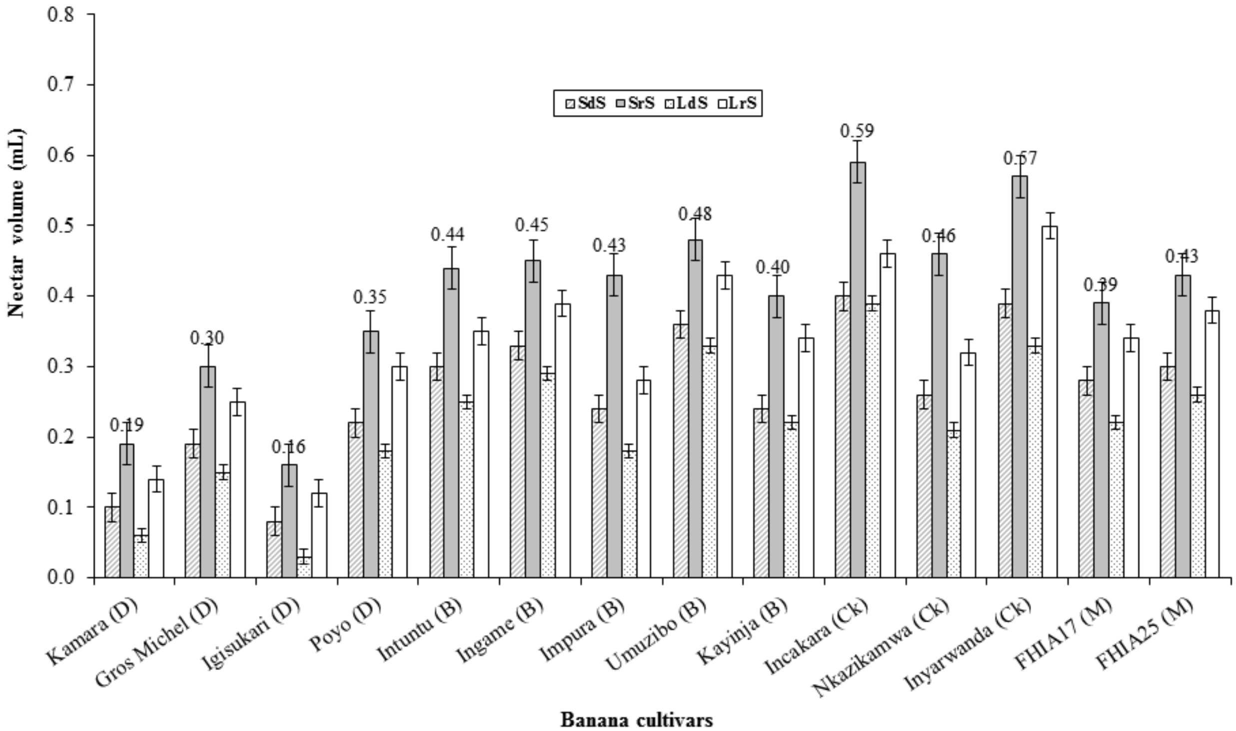


**Figure 1.** Nectar volume across banana cultivars in the Lake Kivu border region (with a medium altitude). Letters in brackets stand for D: dessert, B: beer, Ck: cooking and M: multipurpose. SdS, SrS, LdS and LrS respectively, denote short dry season (63.3 mm of rainfall), short rainy season (156.3 mm), long dry season (40.7 mm) and long rainy season, (130.0 mm). Error bars indicate the standard error of the mean.

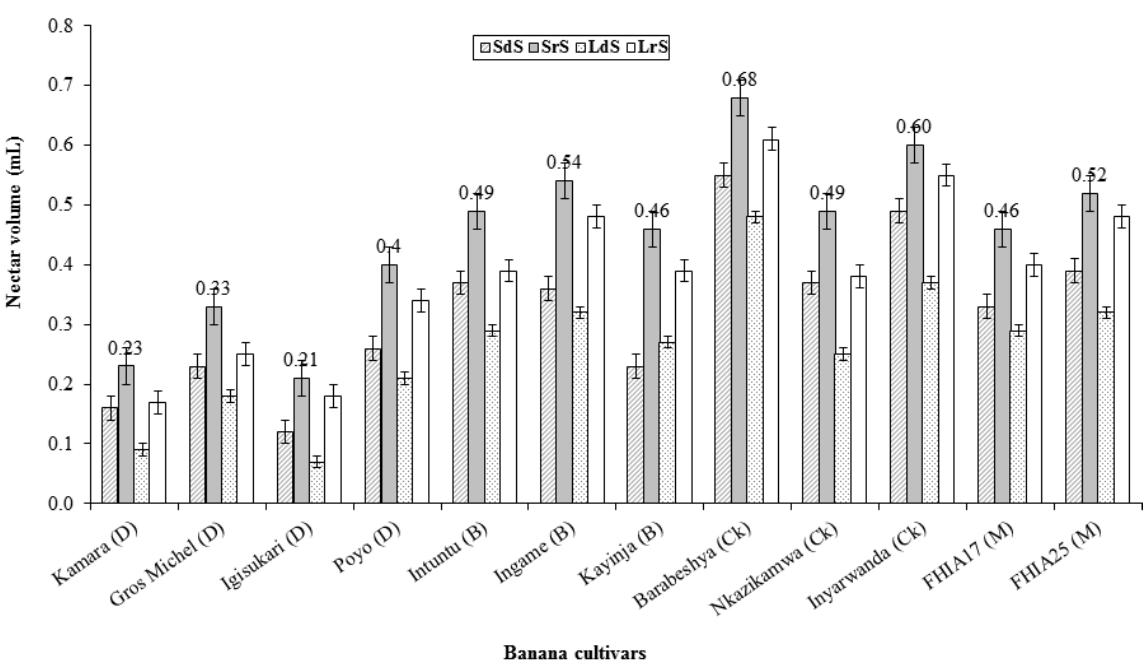


**Figure 2.** Nectar volume across banana cultivars in the high altitude areas. Letters between brackets stand for D: dessert, B: beer, Ck: cooking and M: multipurpose. SdS, SrS, LdS and LrS respectively, denote short dray season (61.9 mm of rainfall), short rainy season (169.3 mm), long dry season (29.2 mm) and long rainy season (202 mm). Error bars indicate the standard error of the mean.

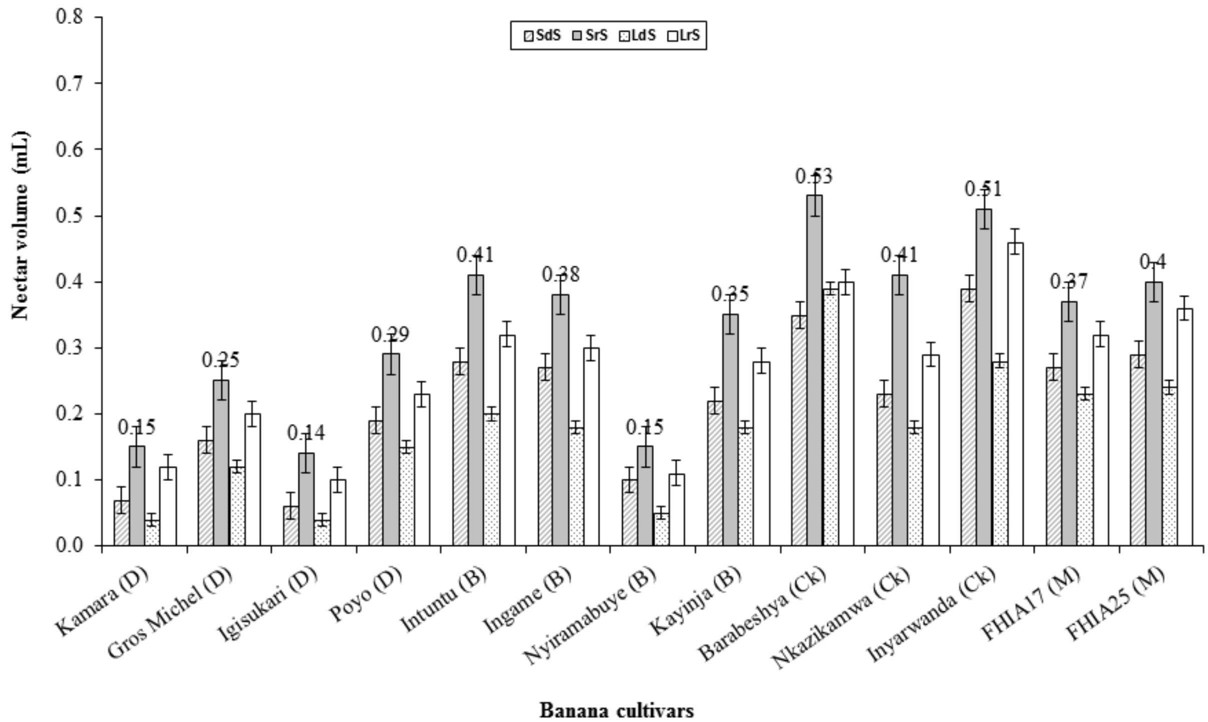


**Figure 3.** Nectar volume across banana cultivars in the medium altitude areas. Letters between brackets stand for D: dessert, B: beer, Ck: cooking and M: multipurpose. SdS, SrS, LdS and LrS, respectively, denote short dry season (45.3 mm of rainfall), short rainy season (144.4 mm), long dry season (22.4 mm) and long rainy season (112.1 mm). Error bars indicate the standard error of the mean.

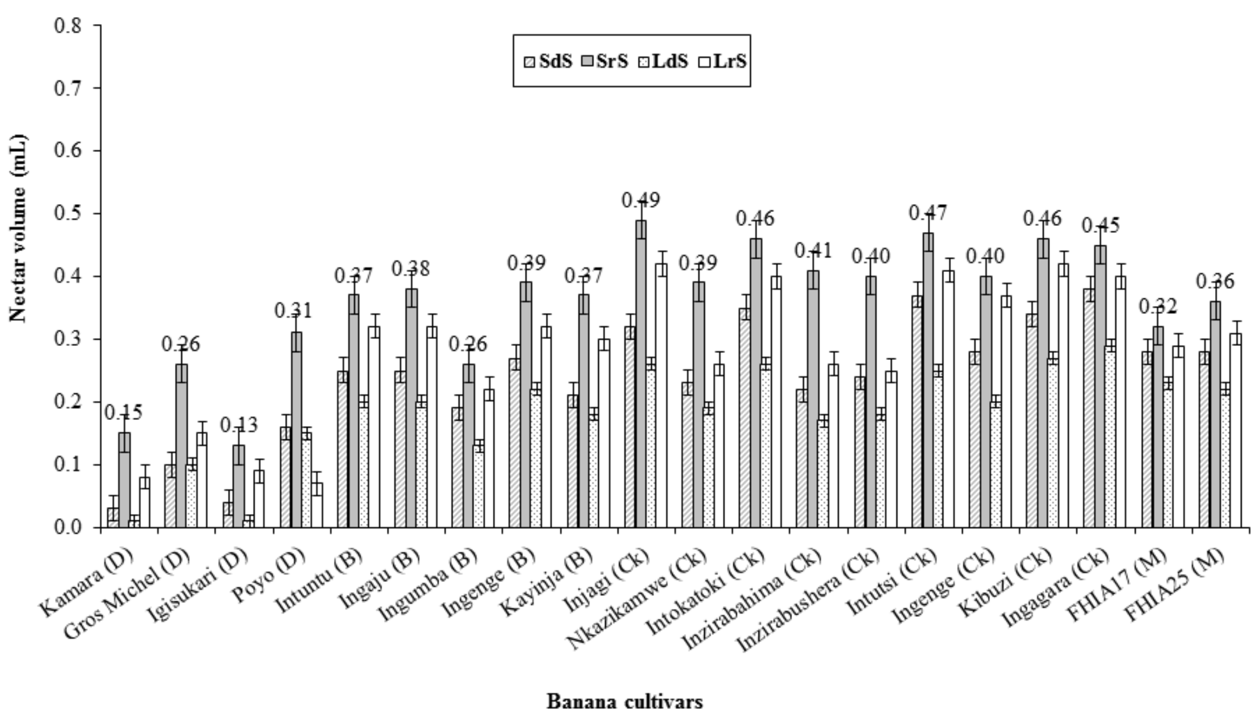


**Figure 4.** Nectar volume across banana cultivars in the low altitude areas. Letters between brackets stand for D: dessert, B: beer, Ck: cooking and M: multipurpose. SdS, SrS, LdS and LrS, respectively, denote short dry season (23.2 mm of rainfall), short rainy season (133.7 mm), long dry season (20.6 mm) and long rainy season (112.3 mm). Error bars indicate the standard error of the mean.

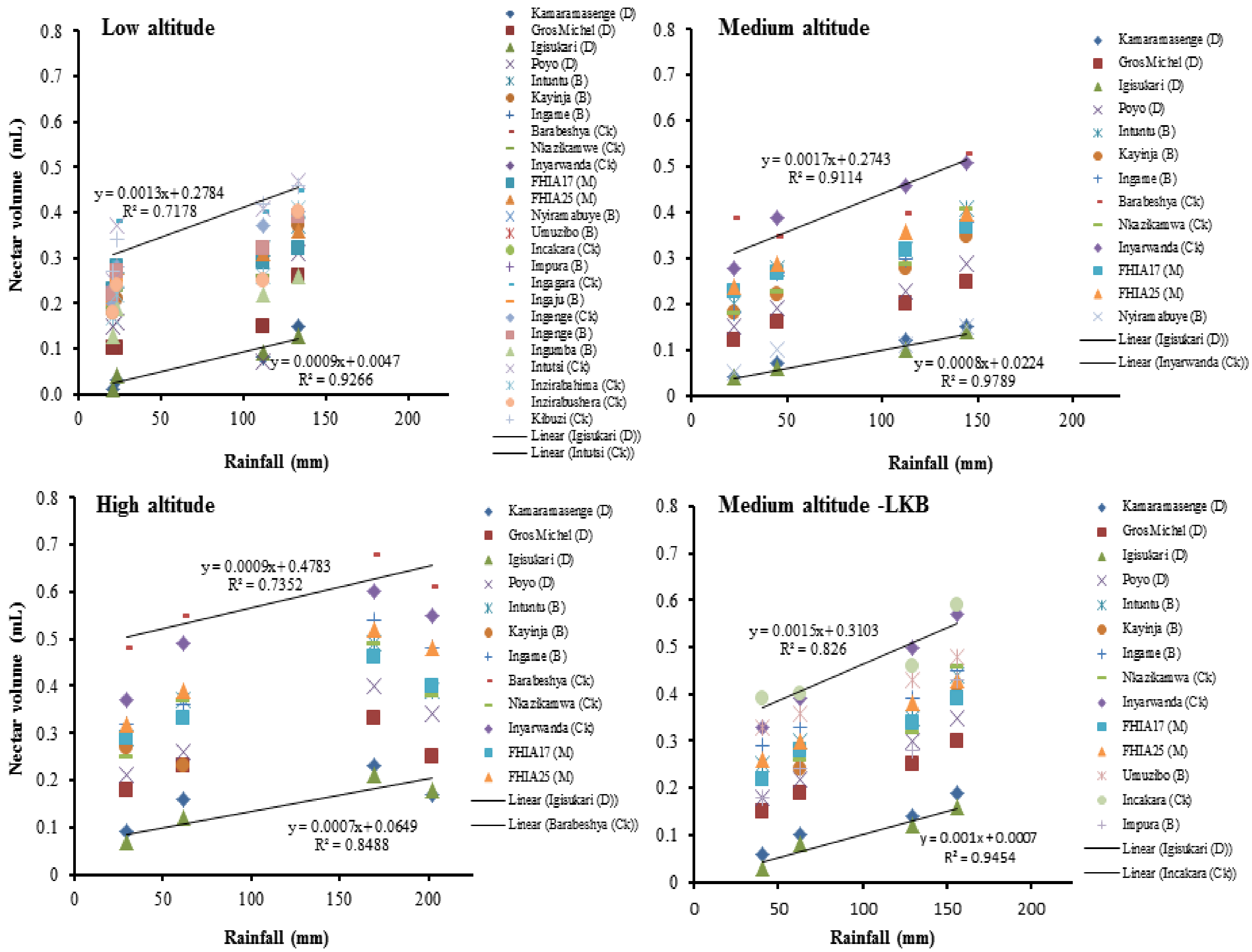


**Figure 5.** Variation in nectar volume (mL) in male flowers of different banana cultivars with amount of rainfall (mm) at four altitudes of 1300 - 1400 masl (low), 1596 - 1642 (Medium, Lake Kivu Border region), 1600 - 1650 (medium) and 1887 -2167 (high altitude).

altitude areas and the Lake Kivu border regions that are characterized by heavy rains and low mean temperatures (Atlapedia online, 2013). The good water supply to the roots, coupled with the low evapo-transpiration, probably influenced the amount of water in the nectaries. This justifies the higher nectar volumes recorded during the rainy seasons when compared with the dry seasons. Higher nectar sugar content was also noted in the low altitude sites characterized by lower rainfall levels. Low mean nectar concentration of some plant species has been reported to be related to lower mean maximum temperatures and higher precipitation (Barros et al., 1983).

Similarly, Nicolson and Nepi (2005) reported a decrease in nectar volume and increased concentration due to high temperatures. Nicolson and Nepi (2005) also reported that substantial amounts of genetic variation can be swamped in the field due to the environmental factors surrounding the plant.

## Variation in nectar volume and sugar content across banana cultivars

East African highland cooking banana cultivars (AAA genome) generally contained a higher nectar volume as compared to the dessert and beer types. In contrast, nectar sugar content in AAA-EA cooking banana cultivars was relatively low when compared with the dessert and beer (ABB and AAA) types. These observations suggest that nectar volumes and sugar content in bananas were influenced by the interaction between the genotypes of

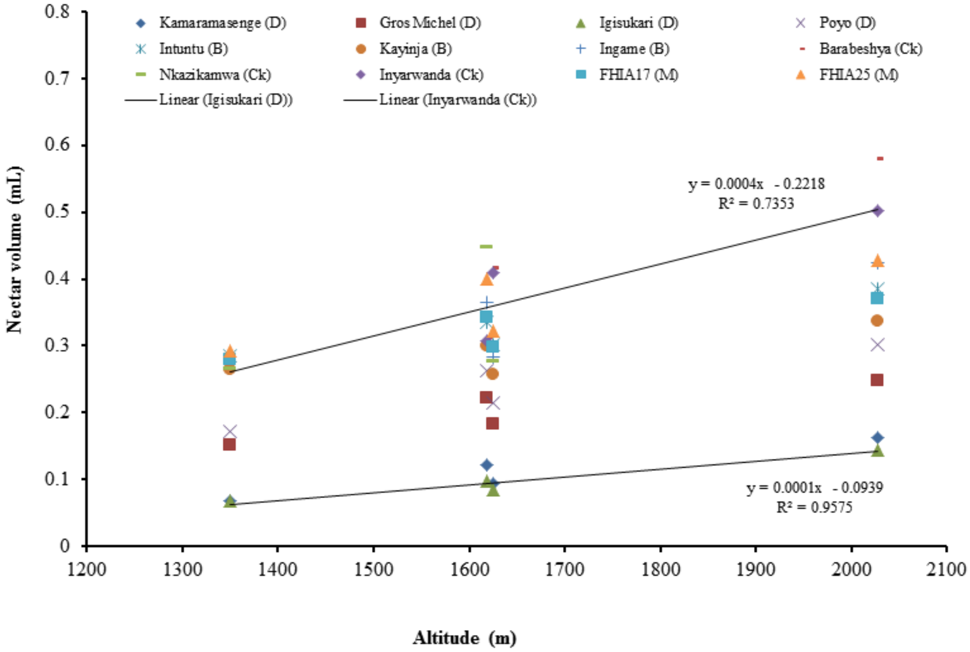


**Figure 6.** Variation in nectar volume (mL) in male flowers of different banana cultivars with altitude (masl) across all the rainfall seasons.

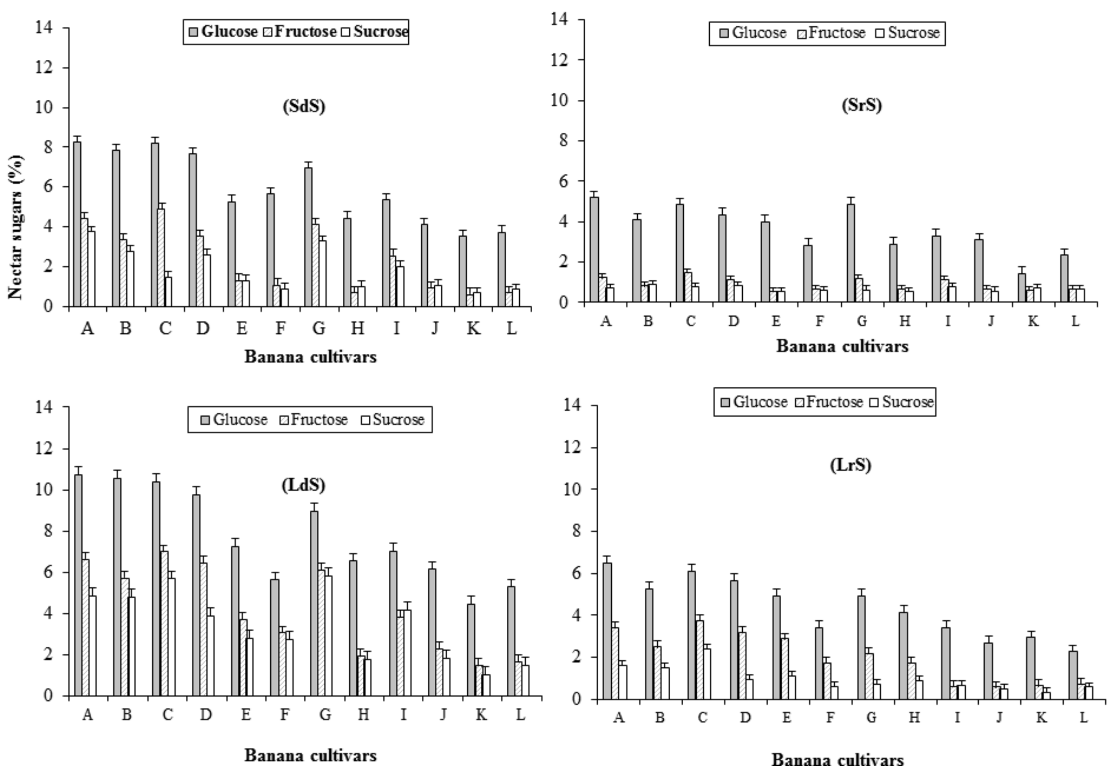


**Figure 7.** Nectar sugar content (%) in banana cultivars grown in the high altitude areas during the short dry season (SdS, 62 mm of rainfall, January to February), short rainy season (SrS, 169 mm, March to May), long dry season (LdS, 29 mm, June to August ) and the long rainy season (LrS, 202 mm, September to December). Error bars indicate the standard error of the mean. Letters on the X axis stand for: (A) Kamara, (B) Gros Michel, (C) Igisukari, (D) Poyo, (E) Intuntu, (F) Ingame, (G) Kayinja, (H) Incakara, (I) Nkazikamwe, (J) Inyarwanda, (K) FHIA17, (L) FHIA25.

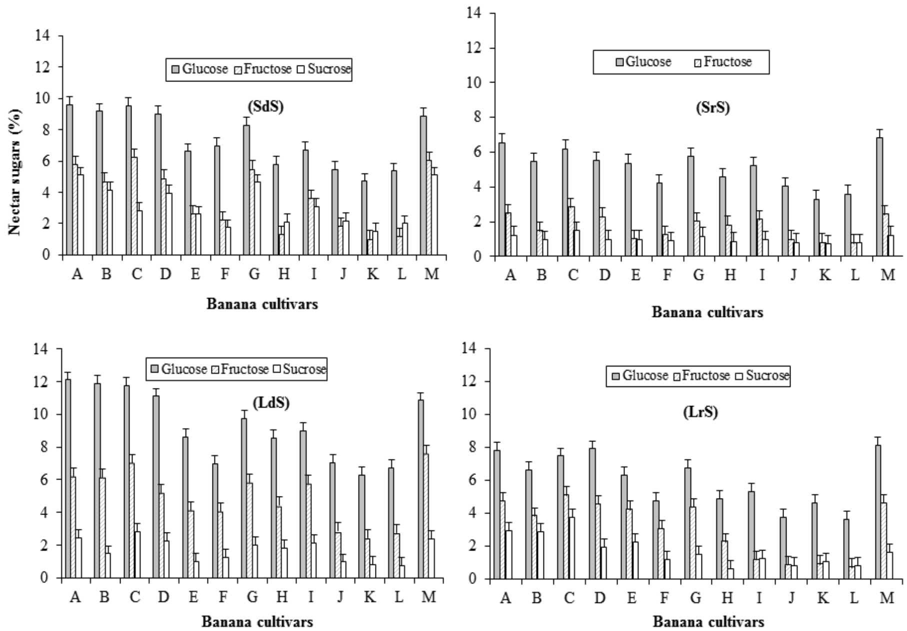


**Figure 8.** Nectar sugar content (%) in banana cultivars grown in the Medium Altitude areas during the short dry season (SdS, 45 mm of rainfall, January to February), short rainy season (SrS, 144 mm, March to May), long dry season (LdS, 22 mm, June to August) and the long rainy season (LrS, 112 mm, September to December). Error bars indicate the standard error of the mean. Letters at the 'X' axis stand for: (A) Kamaramasenge, (B) Gros Michel, (C) Igisukari, (D) Poyo, (E) Intuntu, (F) Ingame, (G) Kayinja, (H) Incakara, (I) Nkazikamwe, (J) Inyarwanda, (K) FHIA17, (L) FHIA25, (M) Nyiramabuye.

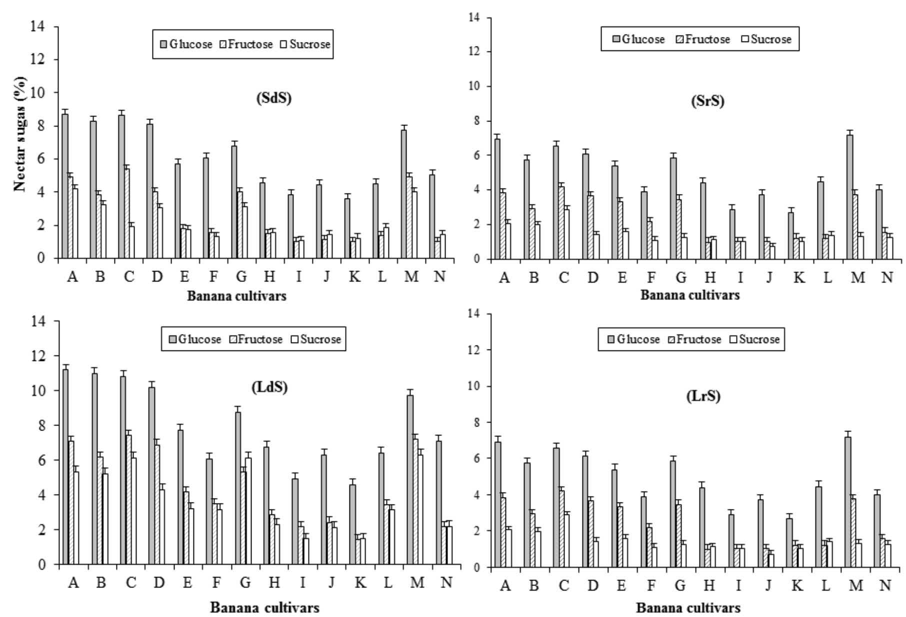


**Figure 9.** Nectar sugar content (%) in banana cultivars grown in the Kivu Lake Border (medium altitude) during the short dry season (SdS, 63 mm of rainfall, January to February), short rainy season (SrS, 156 mm, March to May), long dry season (LdS, 41 mm, June to August) and the long rainy season (LrS, 130mm, September to December). Error bars indicate the standard error of the mean. Letters at the 'X' axis stand for: (A) Kamaramasenge, (B) Gros Michel, (C) Igisukari, (D) Poyo, (E) Intuntu, (F) Ingame, (G) Kayinja, (H) Incakara, (I) Nkazikamwe, (J) Inyarwanda, (K) FHIA17, (L) FHIA25, (M) Umuzibo and (N) Impura.

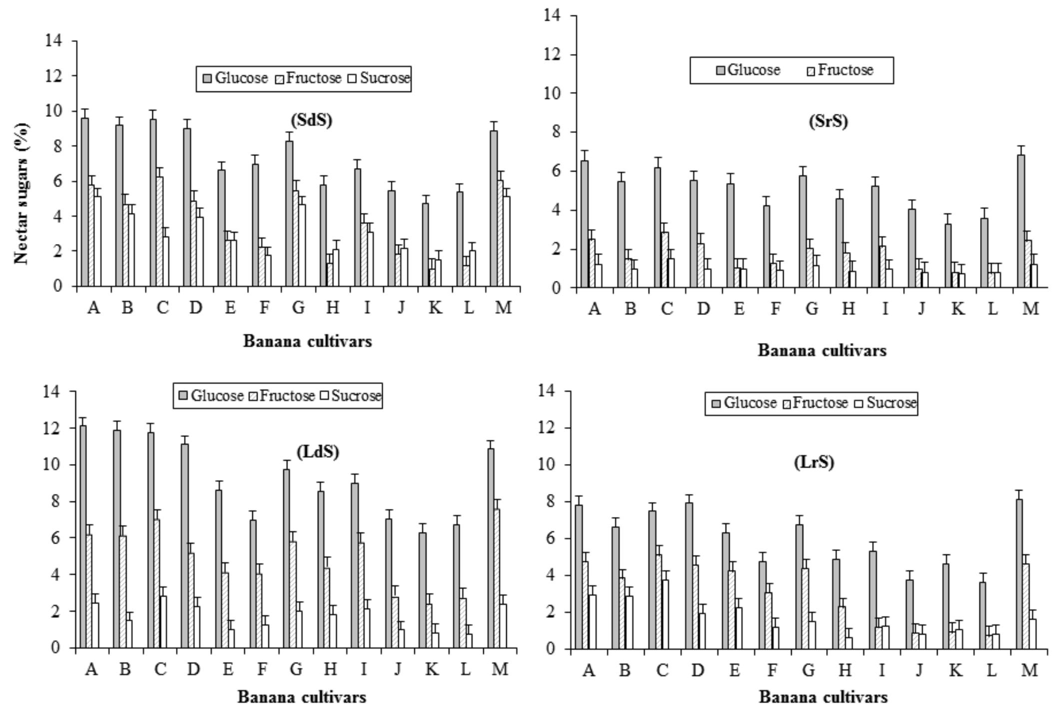


**Figure 10.** Nectar sugar content (%) in banana cultivars grown in the low altitude during the short dry season (SdS, 23 mm of rainfall, January to February), short rainy season (SrS, 134 mm, March to May), long dry season (LdS, 21 mm, June to August) and the long rainy season (LrS, 112 mm, September to December). Error bars indicate the standard error of the mean. Letters at the 'X' axis stand for: (A) Kamaramasenge, (B) Gros Michel, (C) Igisukari, (D) Poyo, (E) Intuntu, (F) Ingame, (G) Kayinja, (H) Incakara, (I) Nkazikamwe, (J) Inyarwanda, (K) FHIA17, (L) FHIA25, (P) Ingaju, (Q) Ingenge-cooking, (R) Ingenge-beer, (S) Ingumba, (T) Intutsi, (U) Inzirabahima, (V) Inzirabushera, (W) Kibuzi.

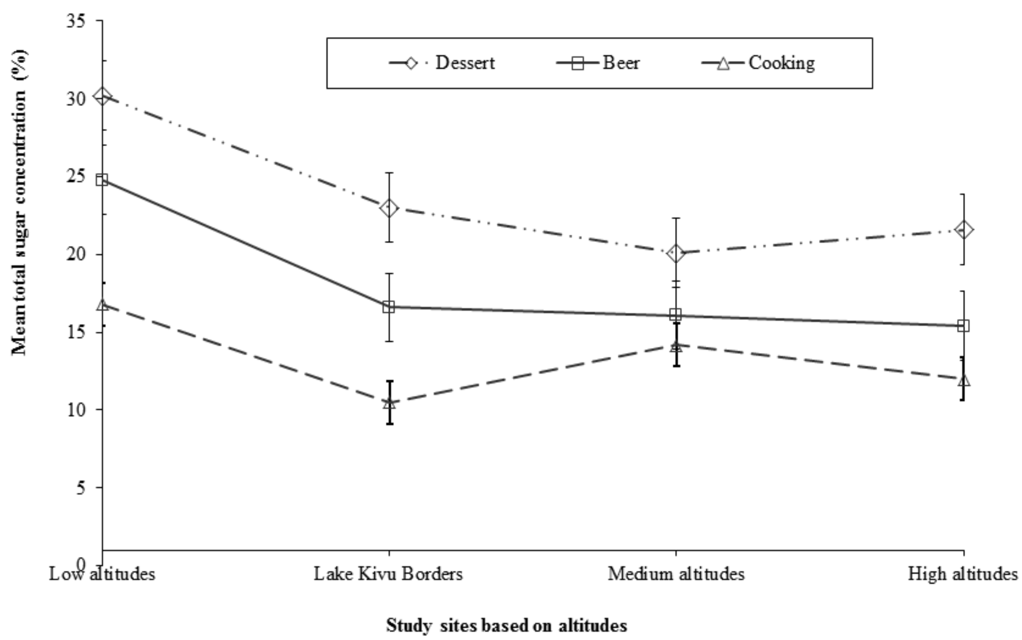


**Figure 11.** Mean total sugar concentration (%) for the three groups of banana cultivars across the study sites selected based on altitudes during the long dry season. Low altitude varied between 1300 and 1400 masl; Lake Kivu Borders -1596 to 1642; medium altitude – 1600 to 1650; and the high altitude site – 1887 and 2167 masl. Error bars indicate the standard error of the means.

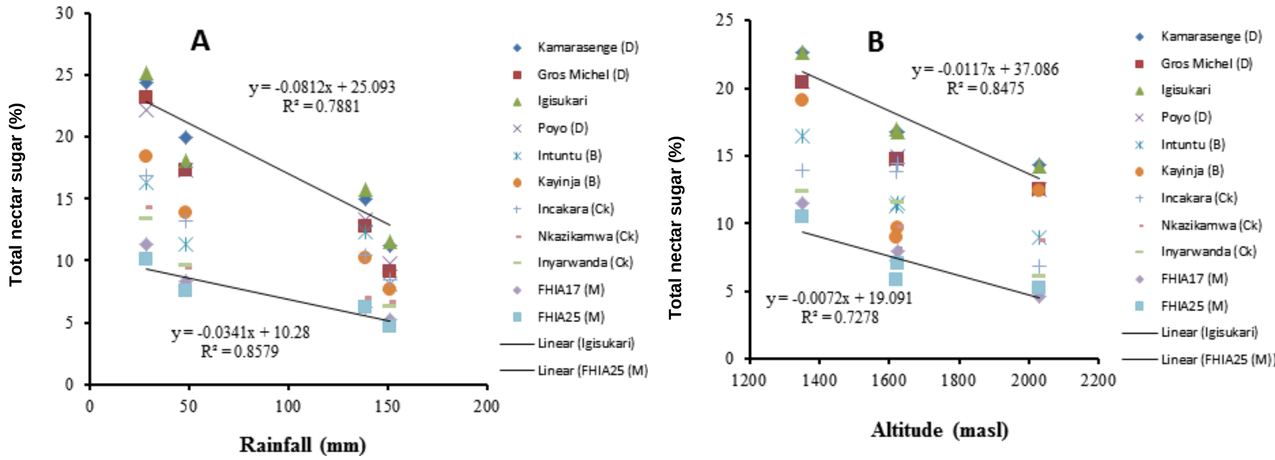


**Figure 12.** Variation in mean total nectar sugar (%) with rainfall (A) and altitude (B) for different banana cultivars in four agro-ecologies of Rwanda.

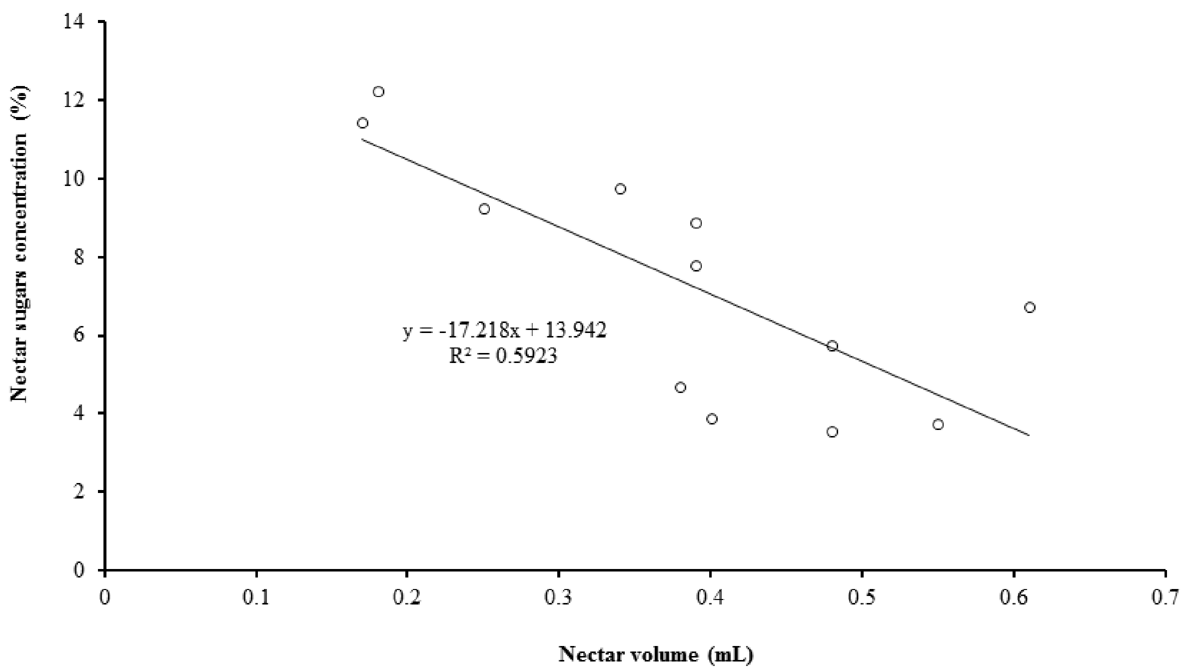


**Figure 13.** Variation of the total nectar sugars (%) with nectar volume for the major banana cultivars grown in the high altitude areas during the long rainy season.

the cultivars and their environmental conditions. Nectar is a complex mixture of substances dissolved in water, with water as the most abundant component (~30 to 90%) (Nicolson, 2002; Nicolson and Fleming, 2003).

## Nectar sugar composition and proportion for the banana cultivars identified in the study areas

The nectar in banana is chemically a very specific secretion made of 68% water and 32% dry weight (mainly sugars that make up about 90%) (Luttege, 1977). Three types of sugars, glucose, fructose and sucrose were detected in the banana cultivars. Hexose sugar was the predominant type of sugar in all the banana cultivars. Percival (1961) reported nectar to contain basically a sugar solution composed of one disaccharide (sucrose) and one monosaccharide-hexose (glucose and fructose), the amount and relative concentrations of which vary among species. The total amount of sugar content and

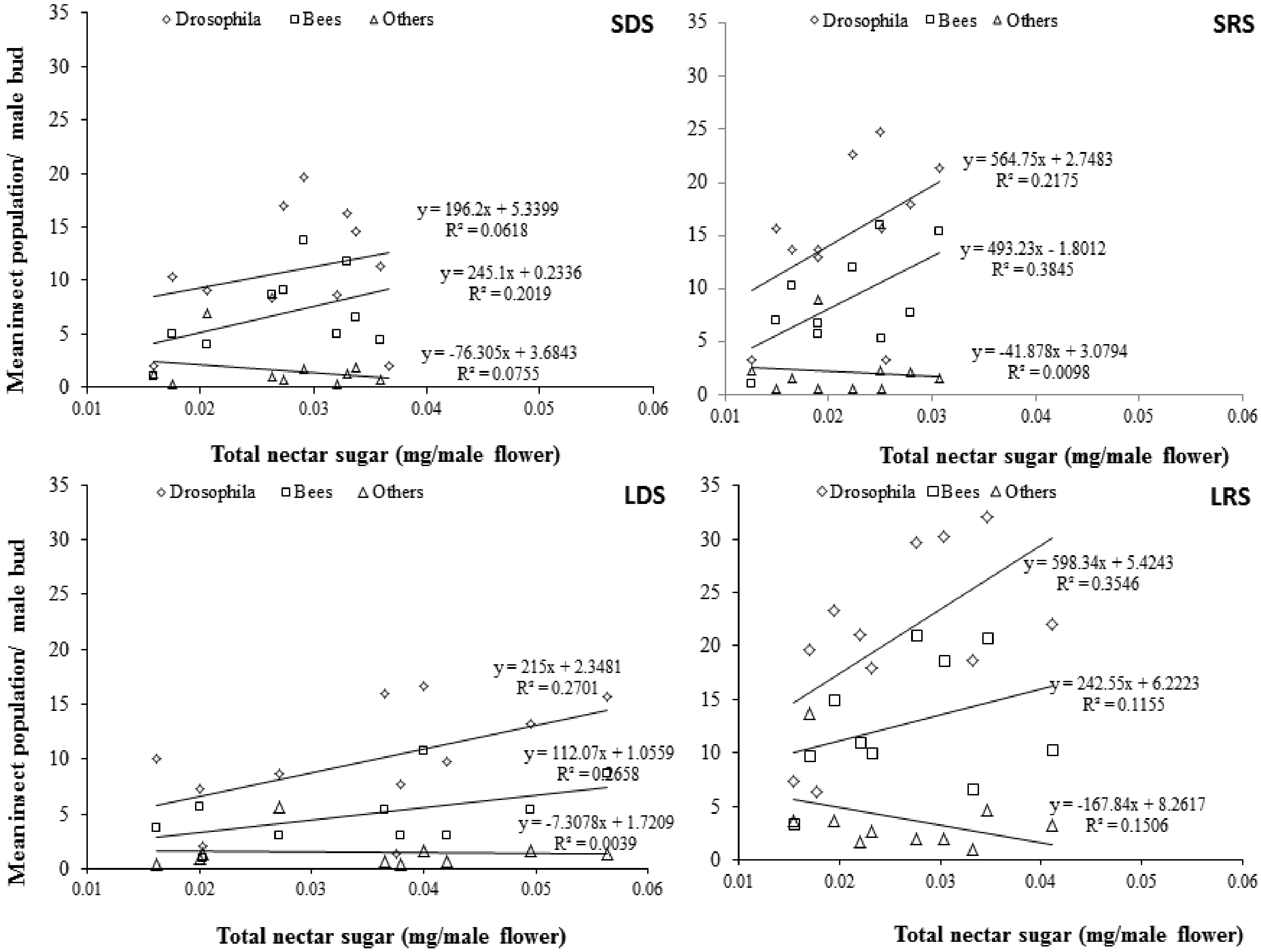


**Figure 14.** Variation of the total number of insects with total nectar sugar concentration for different banana cultivars at the high altitude areas during the short dry season (SDS), short rainy season (SRS), long dry season (LDS) and long rainy season (LRS).

the proportion of the sugars varied among banana cultivars. Within a cultivar, the proportion of the sugars in the nectar was observed to be consistent irrespective of the altitude and seasons. Luttege (1977) reported that sugar proportions in nectar tend to be constant within a species and in other cases within a genus or a family, revealing a clear phylogenetic influence on nectar sugar profiles. However, divergent nectar features have also been reported in plant taxa from the same lineage that has maintained a close relationship with different pollinator guilds (Temeles and Kress, 2003). Cawoy et al. (2008) reported minor changes in sugar proportions due to differences in age, variations among inflorescences and plants when compared with the distinct differences observed among species. The age of the male buds is therefore unlikely to have affected the results of this study, though efforts were also made to obtain male buds that were closely of the same age.

## Prevailing insect species on banana male flowers and their association with nectar sugars

In the current study, insects collected from banana male flowers were mostly fruit flies and bees. This is consistent with previous literature (Tinzaara et al., 2006; Rutikanga et al., 2015). These insects have been confirmed to be vectors of *Xcm* that causes XW disease in bananas (Tinzaara et al., 2006; Rutikanga et al., 2015). The proportions of the three sugars (glucose, fructose and sucrose) have been linked with different classes of pollinators. Sucrose-rich nectar has been found mostly in flowers pollinated by insects with long mouth parts (long-tongued bees, moths and butterflies), whereas hexose-rich nectar has been found in flowers pollinated by short-tongued bees, bats, perching birds and flies (Baker and Baker, 1983; Elisens and Freeman, 1988; Baker and Baker, 1990; Baker et al., 1998). Bats and birds have

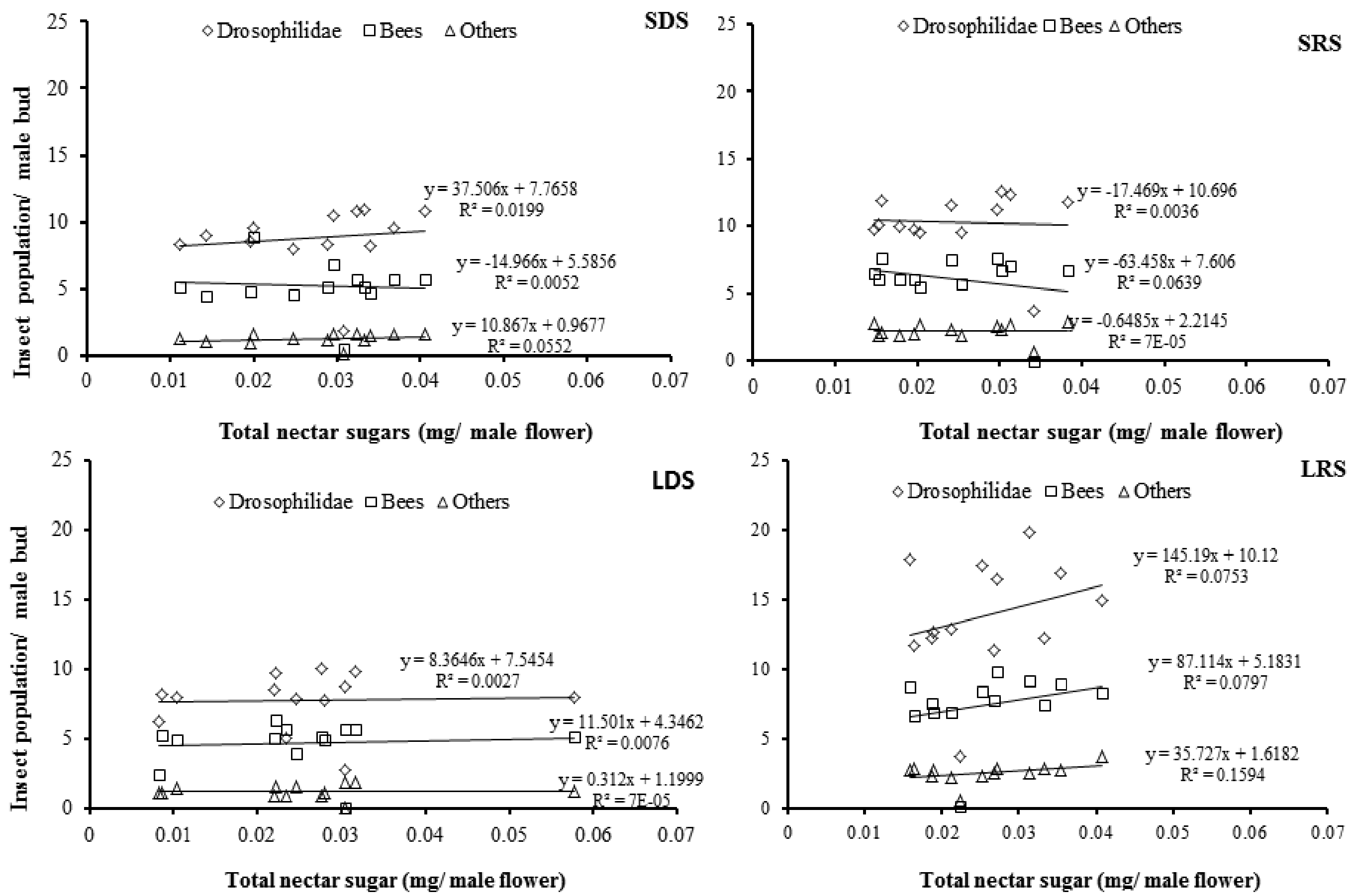


**Figure 15.** Variation of the total number of insects with nectar volume for different banana cultivars at the high altitude areas during the short dry season (SDS), short rainy season (SRS), long dry season (LDS) and long rainy season (LRS).

also been observed to suck nectar from banana male flowers (Buregyeya et al., 2014).

High nectar sugar content has been hypothesized to be responsible for a high prevalence of insects on the male inflorescence of some cultivars and thus the high XW incidence in them. For example, the ABB cultivar, 'Kayinja', which is amongst the cultivars with high nectar sugar content in this study, has previously been reported to be more susceptible to insect vector-mediated transmission (Blomme et al., 2005; Rutikanga et al., 2015). However, in this study, no strong positive correlation was observed between insect population and the nectar sugar concentration or the nectar volume. The dessert types with the highest sugar concentrations attracted the least number of insects.

## Prevalence of insect vectors of *Xcm* versus cropping seasons

More insects were noted in the long rainy season to have moderate and well distributed rainfall. During the rainy seasons, nectar volumes are high but with lower concentrations when compared with the dry seasons. These observations suggest that, rather than the concentration of nectar sugars, the prevalence of insects was influenced by the prevailing weather and environmental conditions that could have supported insect survival and activity, and possibly other stimuli other than nectar concentration.

According to Altieri and Letourneau (1984), the visual and chemical stimuli from host plants affect insect colonization and behaviour. In addition, the presence of other plants (not investigated in this study) that are often cultivated in the rainy seasons could also have influenced the number of insects.

## CONCLUSION AND RECOMMENDATIONS

The findings of the current study revealed that the attractiveness of a banana cultivar to insect vectors was not influenced by the nectar concentration and volume. AAA dessert cultivars generally had a higher content of nectar sugars across the altitudes and seasons though sugar content did not correlate to insect activity in the

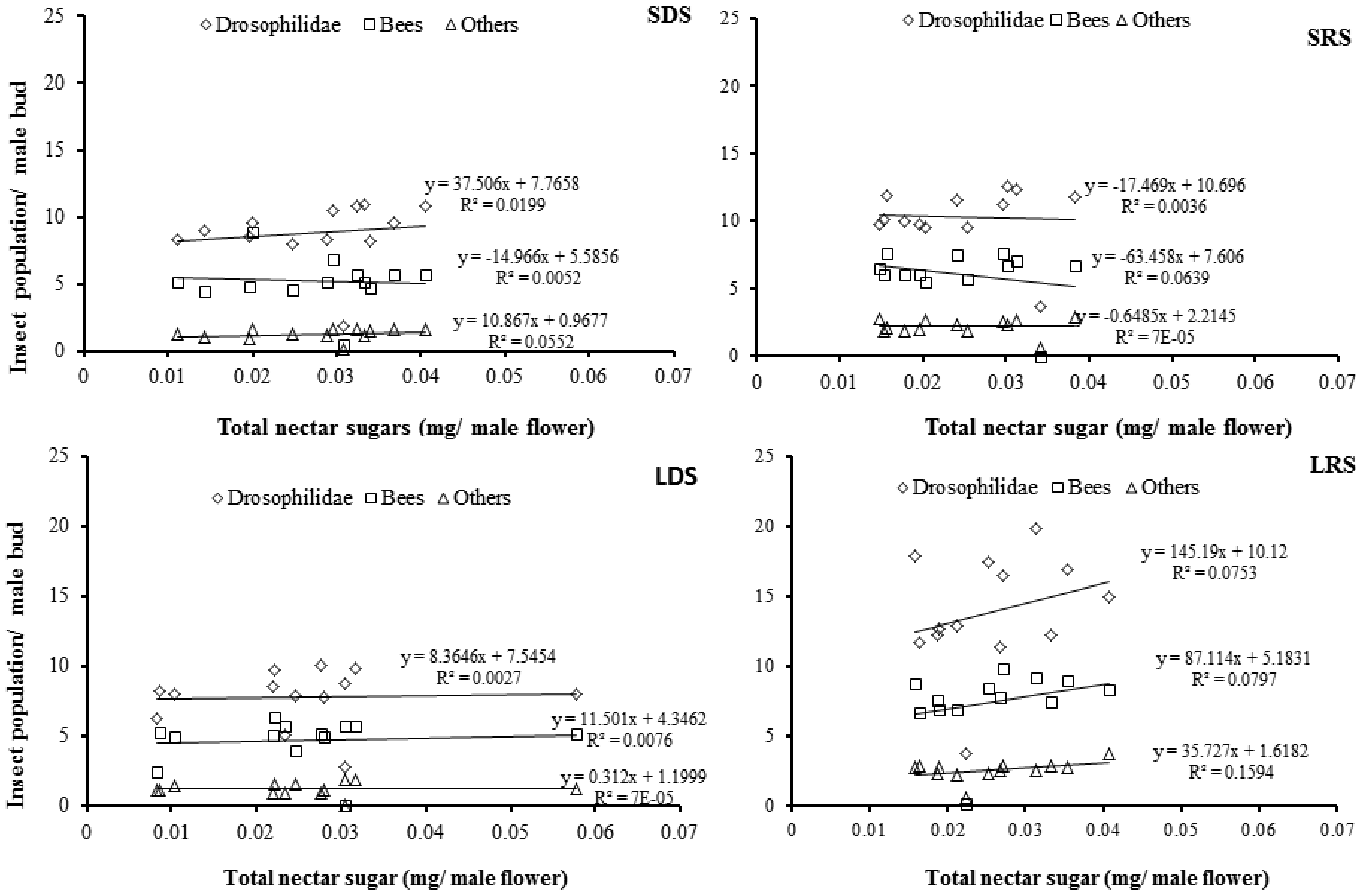


**Figure 16.** Variation of the total number of insects with nectar sugar concentration for different banana cultivars at the low altitude areas during the short dry season (SDS), short rainy season (SRS), long dry season (LDS) and long rainy season (LRS).

different study sites. Insect population and activity was mainly influenced by the prevailing weather conditions (dry vs wet season). It has been hypothesized that the high nectar sugar concentration in 'Kayinja', a highly susceptible cultivar to insect-mediated XW infections contributed to its attractiveness to insects and thus the observed susceptibility. On the basis of the above findings, this study rejects this hypothesis. In addition to the weather conditions, other factors not investigated in this study such as male inflorescence behavior (e.g. persistent vs. non-persistent bracts), appearance (e.g. flower shape and colour), smell and shape; and presence of other crops within or around the banana plantations in the rainy seasons could have also influenced insect activity. For example, cultivars with morphological forms of resistance to insect transmission, such as persistent male bracts and neuter flowers have been reported to escape insect mediated infections. The contribution of other stimuli such as inflorescence color and semio-chemicals to the attraction of insect vectors of *X cm* needs to be investigated. The number of flowering plants in the studied farms/seasons and other crops in the vicinity of the farms or in the agro-ecologies could have also influenced insect activity across fields or farms, yet this aspect was not evaluated in this current study.

## Conflict of interests

The authors have not declared any conflict of interests.

## ACKNOWLEDGEMENTS

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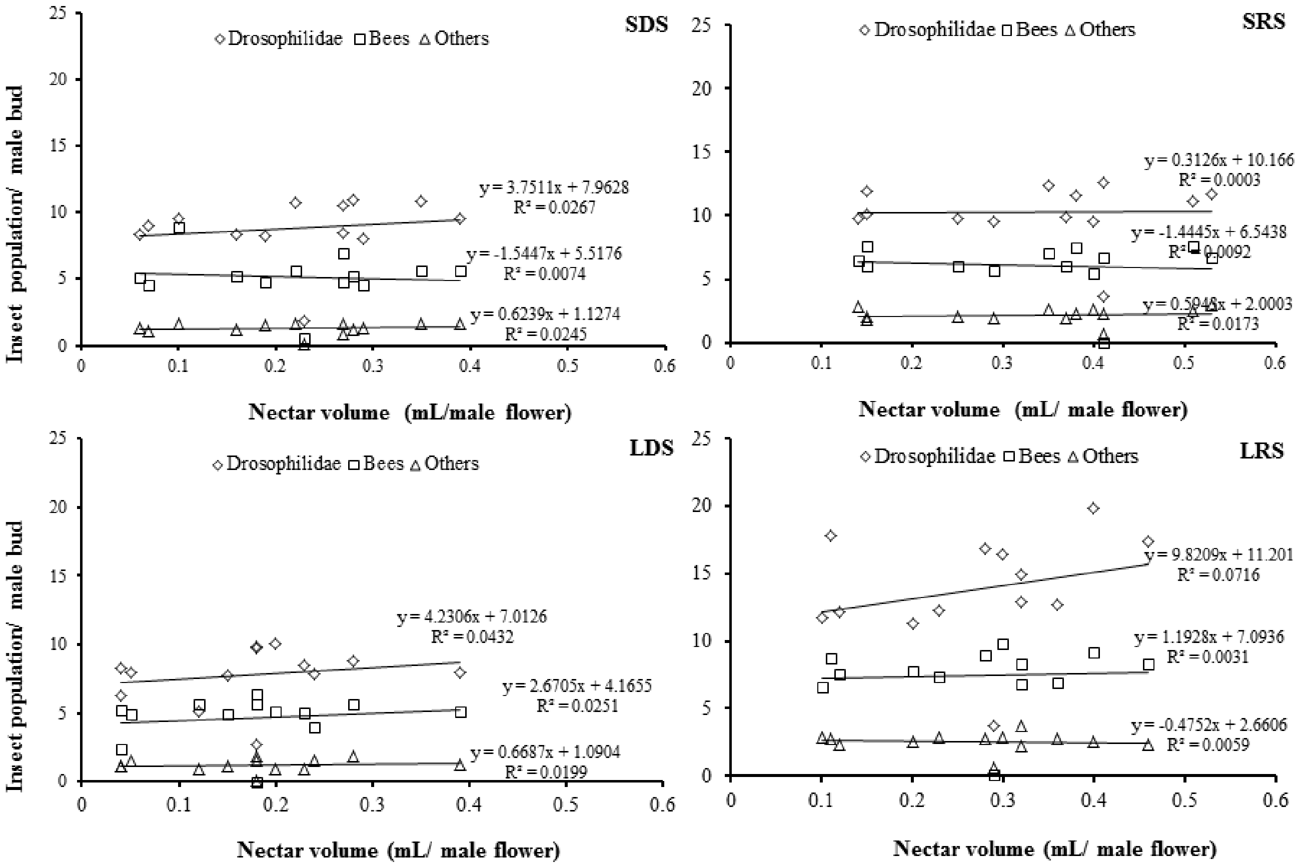


**Figure 17.** Variation of the total number of insects with nectar volume for different banana cultivars at the low altitude areas during the short dry season (SDS), short rainy season (SRS), long dry season (LDS) and long rainy season (LRS).

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*Full Length Research Paper*

# Salinization and pollution of water table with wastewater and its impact on oasis crops

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**The ancestral canal of Oued Righ at 150 km evacuates leachate water into the Chott Melghir, which has 50 oases. Excess water from discharges of sewage and drainage causes upwelling of groundwater that leads to an imbalance in the valley. The physico-chemical analysis of canal waters and water table has shown that the quality of these waters has very high salinity (class C5), with electrical conductivity of 26.30 ms/cm, and SAR> 28 (S4 class). Water hardness, the values of organic matter (O.M) and total solids (T.S) are very important. Thus, canal water has very poor quality, charged with mineral salts, and is a brackish water containing sodium chloride facies.**

**Key words:** Canal, degradation, water, palm, water table, sol, Oued Righ.

## INTRODUCTION

Oued Righ valley, located in the East of the septentrional Sahara, is a broad asymmetrical syncline pit. This region has sandy soil, mainly siliceous and forms of pure quartz; therefore, insoluble (Benhaddya, 2007). The water table is in the clay-evaporite sandy quaternary levels. The main system of agricultural production in this region is essentially phóeniculture. If the valley escapes the phenomenon of recovered water, it is due to the great collector, Wadi channel Righ, which is 136 km long and which transits the water around 5 $m^3$/s, and 120 to 160 million $m^3$/year (Khadraoui, 2005). However, over the years, the entire oasis shows a progressive fall in quantity and quality. This phenomenon has resulted in increased rates of discharge of sewerage and drainage water (Figure 1). A portion of the wastewater lacking pre-treatment joins the main collecting duct. The absence of natural outlets for receipt of waste, adequacy and effectiveness has caused an imbalance in the valley. There is flooding caused by the back water in the oasis, and the depletion of groundwater due to salinization problem. The oasis of the valley of Oued Righ can rightly be called sick oasis with too much water (Cote, 1998). Many palms are flooded in winter (Palm Tinedla, Djemaa, Ferdjaouenne and El Goug). There is secondary salinization after irrigation with highly mineralized water, and permanent hardness results in the suffocation of palms of Oued Righ (Sogreah 1970) (Figure 2). In this perspective, this work aims to analyze the water that

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**Figure 1.** Discharge point of wastewater.

**Figure 2.** Degradation of palm.

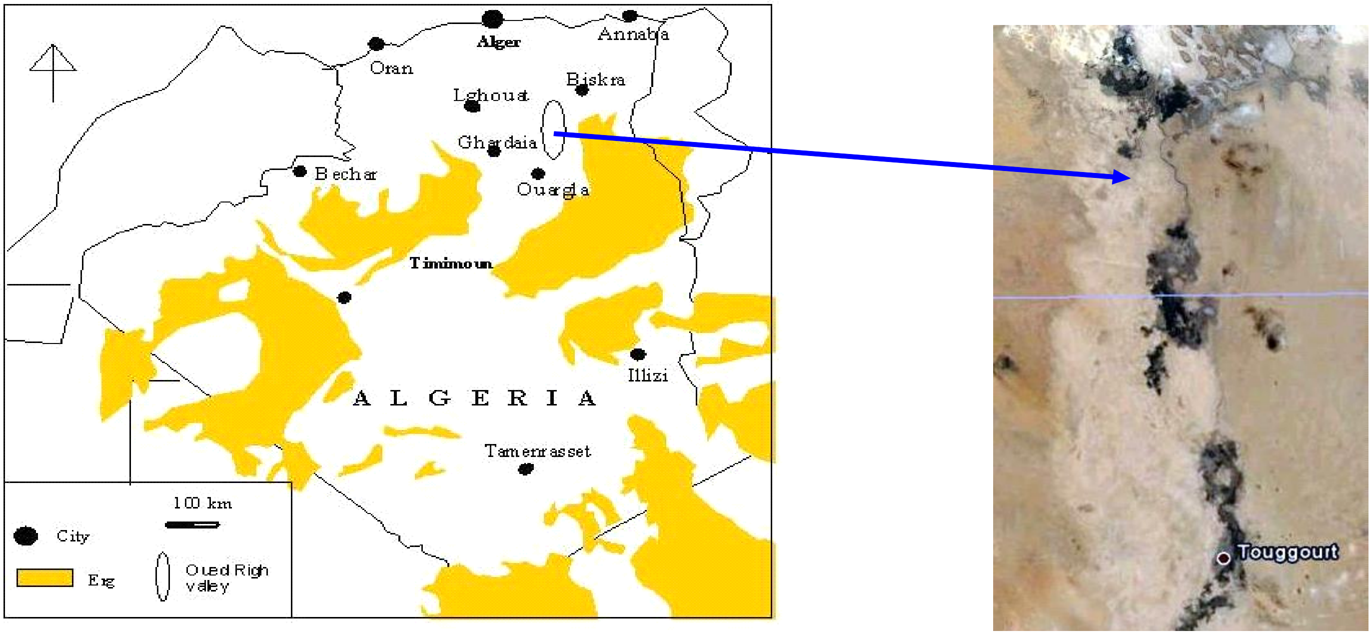



**Figure 3.** Location of the Oued Righ valley.

discharges at different points in the collecting duct which communicates with the water table, and the impact of these waters on the degradation of palm cultivation as well as the relationship between the channel and water table.

## Presentation of the area studied

Oued Righ is located in South eastern Algeria (Figure 3). It spreads over 150 km long. It is located in two wilaya: Ouargla and El Oued. It is bordered in the north by the Plateau Still; east by the Great Erg Oriental; South by the extension of the Grand Erg Oriental and west by the Sandstone Plateau. This region is characterized by an elongated depression from south to north. Highest coast is 100 m in El Goug upstream and 30 m in Chott Merouane downstream. The slope is generally very low (1‰). This slope allows excess water to flow to the north. Region of Oued Righ has nearly 50 oases and covers around 25,000 ha of palm (Dubost, 1991). These oases are aligned on a North-south axis.

## MATERIALS AND METHODS

In order to assess the impact of urban effluents on the canal, and subsequently its impact on date palm cultivation, three rounds of sampling were performed over three months of the year (February, 2009 and May October, 2010). They were nine stations discharging into the canal, at about 30 km; they cross the canal in the West (St: 1) and East (St: 9). The distance between the Kerdeche station and Rannou station is 19 km, and between Kerdeche and Temacine it is about 7 km. From Kerdeche station to Zaouia El Abidia station is about 30 km (Figure 4).

The research focuses on the waters of the groundwater by means of piezometers. For the samples collection, our choice was based on five stations, of about 46 km, from Kerdeche station to Sidi Slimane station. The samples were collected manually in plastic bottles, with identification of each point. The sampling stations chosen for the spatial and temporal variation of the water table and canal water composition is based on finding a possible contamination of these two levels and their impact on the growth of date palm tree, and also the impact of the upwelling water table on the date palm cultivation. In this study we tried to give some solutions to these problems.

The water samples' physico-chemical and pollution analyses were carried out in Water Treatment Laboratory of National Agency of Hydraulic Resource (NAHR). Measurement procedures are deducted from the standard analytical methods. The pH is determined using a pH meter (WTW), and the conductivity is determined using an electrical conductivity meter (DELTA OHM) which gives directly the sample conductivity in mmhos/cm or ds/m. The total and calcium hardness is determined by complexometric titration (EDTA). Finally, a variety of analytical methods were used for the various experimental tests: titrimetric, electrochemical and spectroscopic methods.

## RESULTS AND DISCUSSION

### Study of physico-chemical parameters and pollution in the canal

Temperature affects the degree of evapotranspiration and therefore it acts on the salinity of the water. In this study, the temperature is generally variable with an

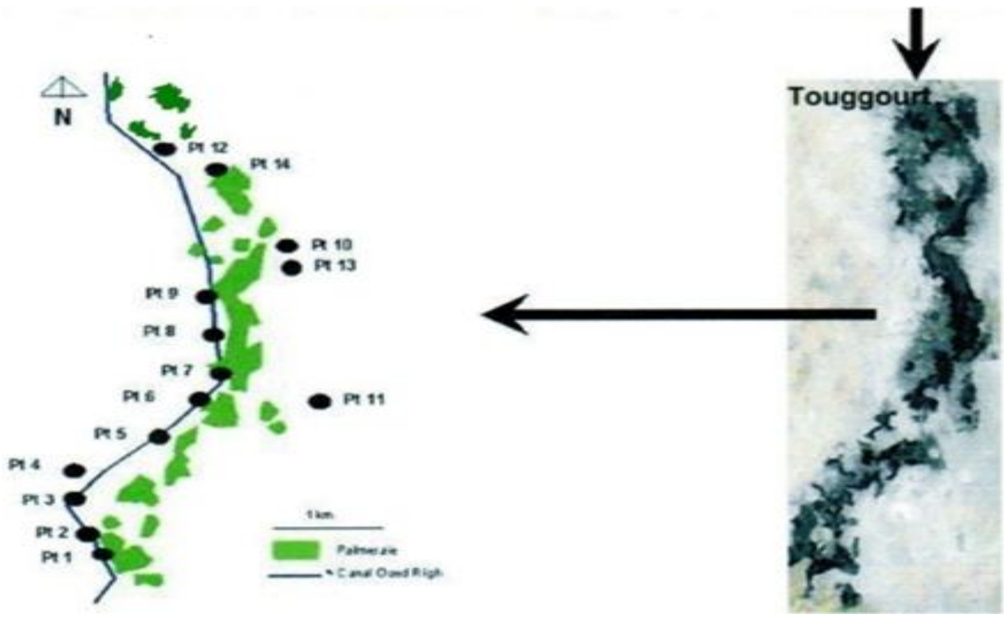



**Figure 4.** Different sampling points.

**Table 1.** Parameters of water quality of Oued Righ channel.

| Parameter | Value |
|---|---|
| pH | 7.3 - 8.3 |
| E.C (ms/cm) | 1500 - 26300 |
| T.H (°f)(total hardness) | 73 - 582 |
| T.S (mg/l) | 2249 - 16528 |
| O.M (mg/l) | 44.5 - 111 |

**Table 2.** Parameters of water quality groundwater.

| Parameter | Value |
|---|---|
| pH | 7 - 7.71 |
| E.C (ms/cm) | 6.04 - 17.90 |
| T.H (°f) | 219 - 408 |
| T.S (mg/l) | 5141 - 14920 |
| O.M (mg/l) | 7.93 - 52.86 |

average of 22.5°C. The results obtained for the canal waters and the waters of the groundwater Table during the period (2009-2010) are shown in Tables 1 and 2.

The pH and electrical conductivity (EC) are very high in the canal (Figure 5). However, these values were maximum at Station 12 in the water table. The canal water is very hard. The waters of Station 12 at the groundwater have a maximum hardness (Benguergoura and Remini, 2013). The results obtained for the total solids are very important for the majority of canal water (May 2010 and October 2010) due to the evaporation of water (Figure 6). Indeed content of salts can exceed 12 g/L of total solids in most solutions discharged into the canal. It should be noted that the upper limit allowed is 10 g/L for sustainable agriculture (Djennane, 1990). One can notice that the values of the total solids can reach 14920 mg/l at Station 12 or the water becomes unpleasant (Figure 7).

Sewage water with a level of organic materials (OM) > 15 mg/l is classified as highly polluted. In Figure 8, the canal's rate of organic materials is > 50 mg/l. In spatio-temporal evolution, the rate of organic matter ranges from 46 mg/l (Station 2) to 111 mg/l (Station 9) in October 2010. However, this wastewater contaminates the groundwater, which was confirmed at Station 12 (Figure 9). In this case, all the benefits of organic matter to the soil, such as better porosity, good permeability, good ventilation, better soil warming and good water retention (Jones and Jacobsen, 2001) will be absent (Mustin, 1987). Organic matter releases minerals which are essential to the nutrition and development of cultures (Bollag, 1998). However, salinity exists in the canal waters and groundwater is the main cause of low palms (Rietz and Haynes, 2003). Increasing salinity inhibits microbiological activity of organic matter. This leads to a decrease in crop yields.

## Hydrochemistry of canal water and groundwater

Agricultural practices, including the establishment of irrigation systems, have an effect on water quality. The

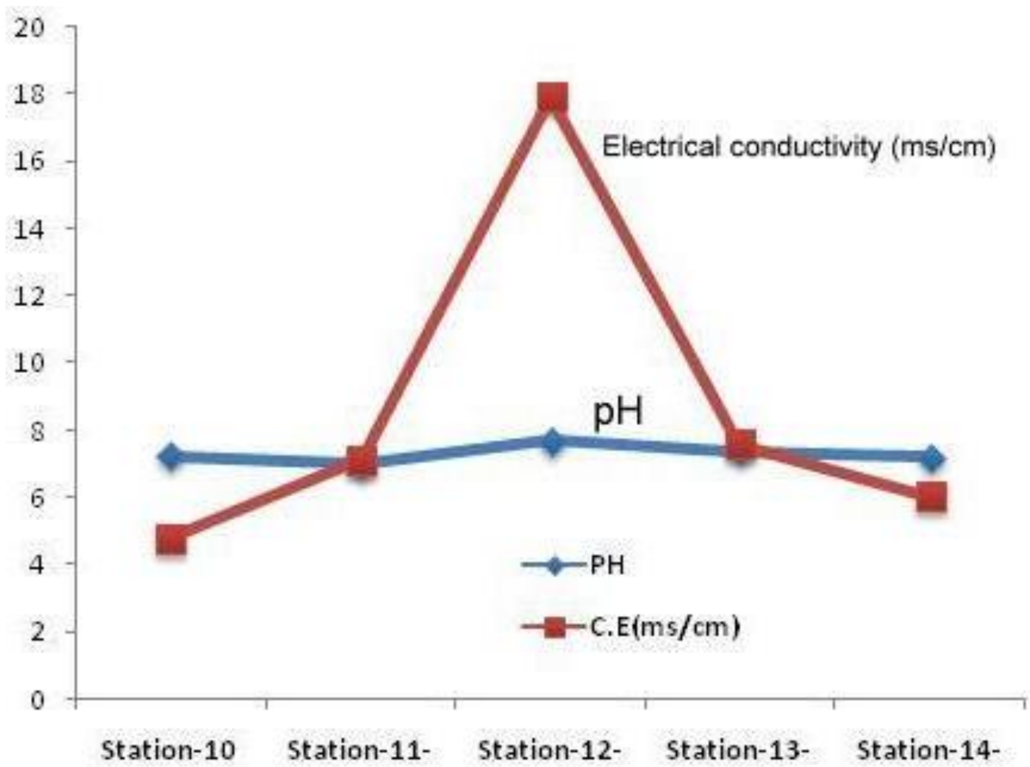



**Figure 5.** Evolution of pH and EC of groundwater.

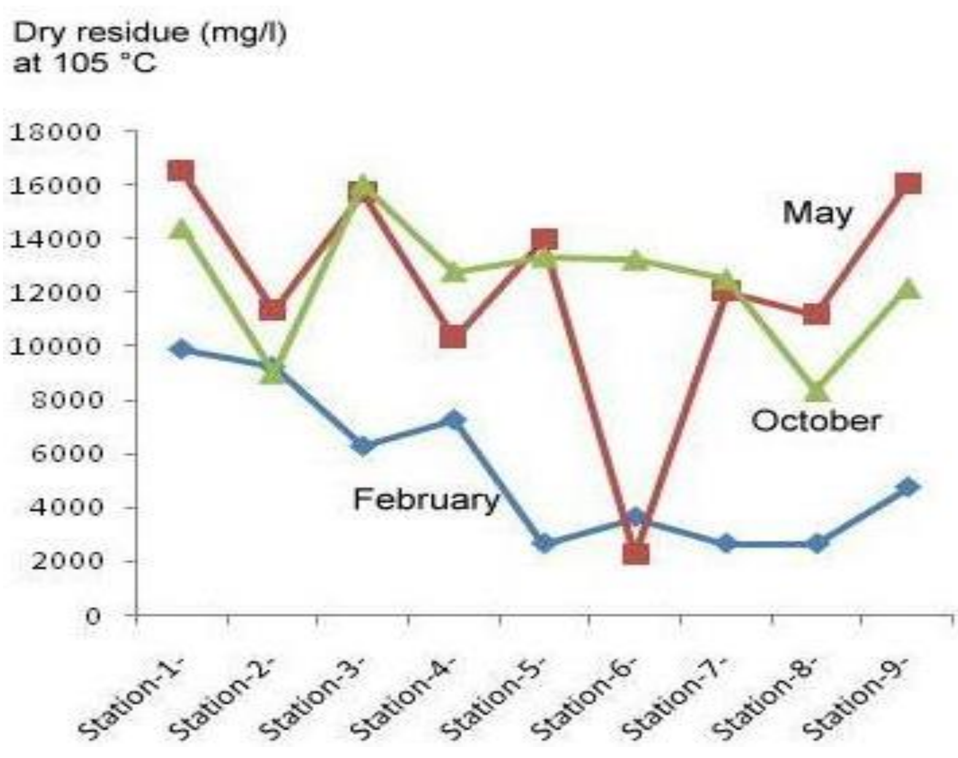



**Figure 6.** Spatio-temporal evolution of the dry residue of water channel.

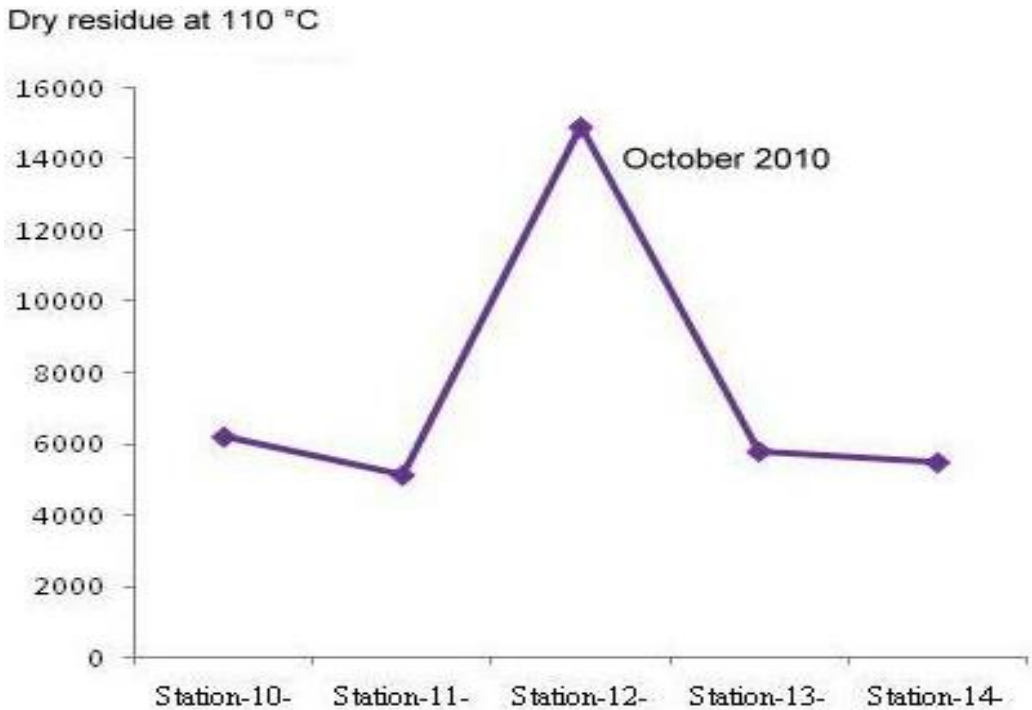



**Figure 7.** Evolution of total solids in water groundwater.

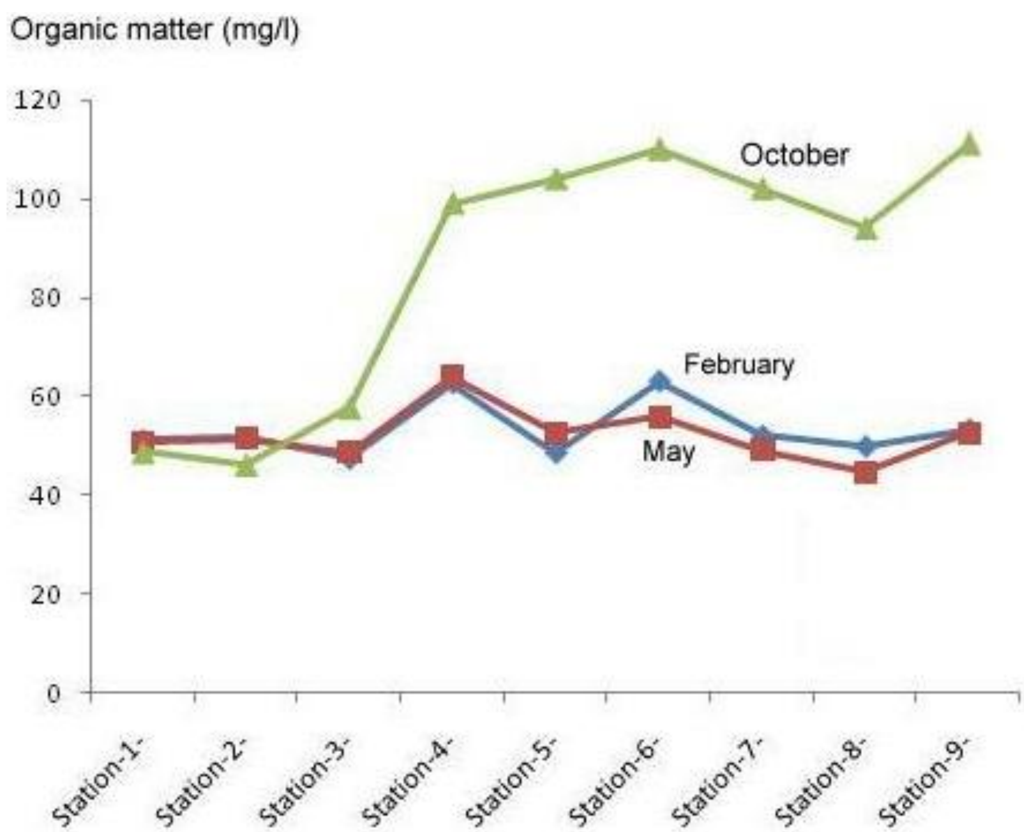



**Figure 8.** Spatio-temporal evolution of organic matter of water channel.

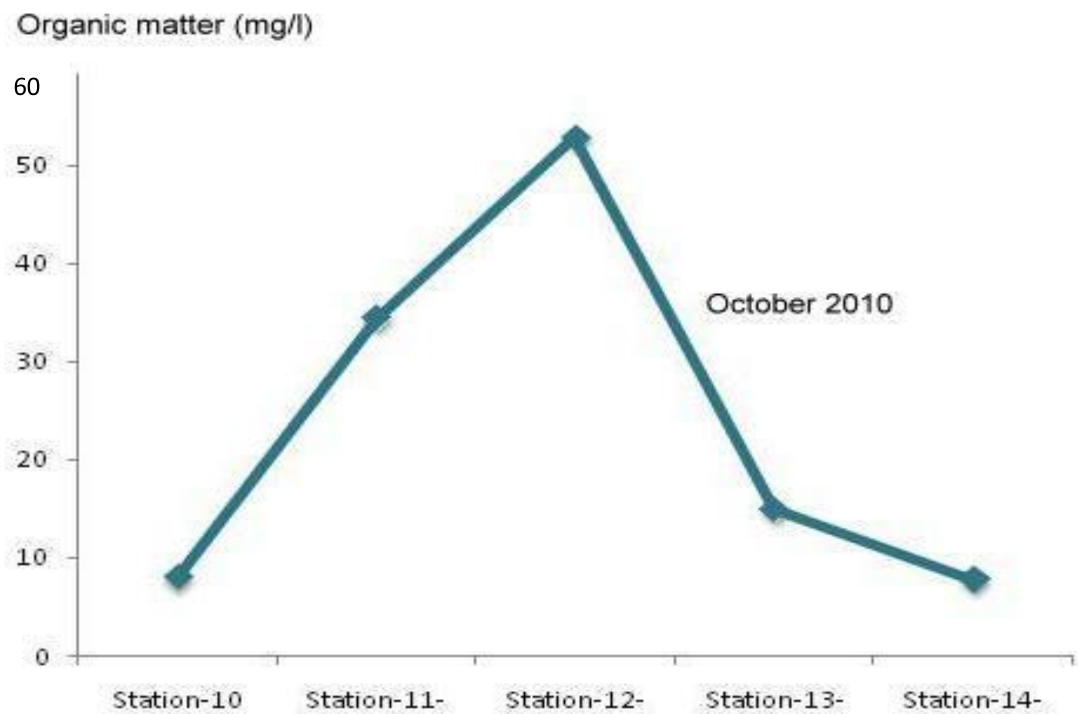


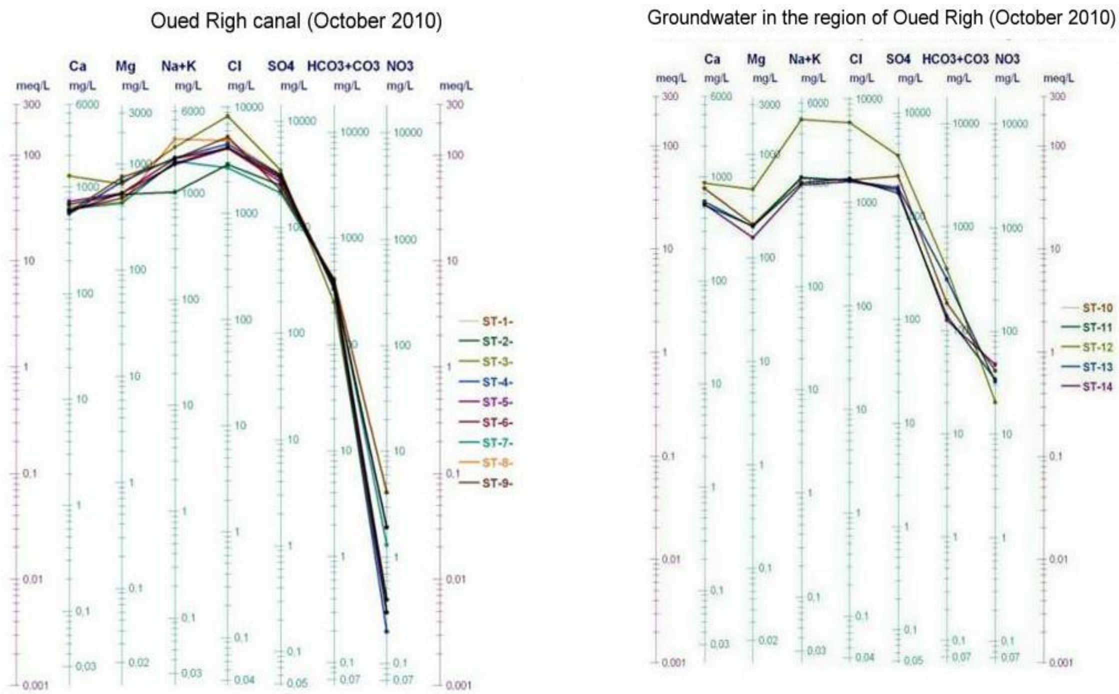

**Figure 9.** Evolution of organic matter in water groundwater.

mineral salts in irrigation water have an impact on the soil and plants (Ratner, 1935). They can cause changes in soil structure and disrupt the development of vegetation (Person, 1978). The distribution of palm and associated crops in the ground root system gives an overview of the degradation of phoeinicicole heritage due to the contamination of groundwater by salinity and wastewater channel. To assess the water quality of the Oued Righ channel and groundwater, we used Schoeller Berkaloff diagram to represent the chemical facies of several water samples. Each sample is represented by a broken line. The concentration of each chemical element is represented by a vertical line on a logarithmic scale. When the lines are growing, a chemical change of facies is demonstrated (Roland, 2012). The mineralization $Cl^-$ and $Na^+$ is dominant almost at 9 stations along the canal, followed by mineralization ions $SO_4^{2-}$ and $Ca^{2+}$, or even a high concentration of $Mg^{2+}$ ions. Sodium chloride facies were more (Figure 10a and b), with absence of bicarbonate facies. However, there is a clear dominance of sodium ion, followed by chloride and sulfate ions, at Station 12 groundwater which is grown all around the canal area. This confirms that the dominant hydrochemical facies is a chloride-sodium. So mineralization water of the web is linked to $Cl^-$ and $Na^+$. The line of Station 10 which has a higher concentration of sodium ions than sulfate ions gives sulfated-sodium facies. The bicarbonate facies groundwater is absent.

**Water-soil relationship and its impact on the culture of the date palm**

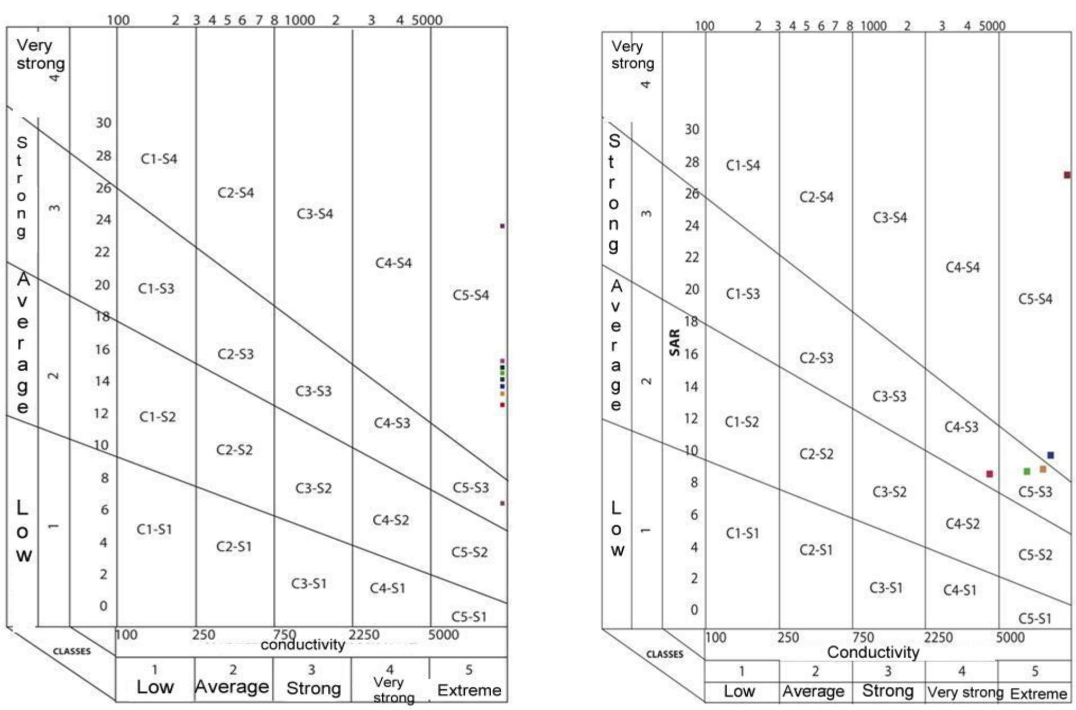
Oued Righ region is characterized by low rainfall and high evaporation. Some researchers estimate that it takes 1 $m^3$ of water to make 1 kg of dates (Simonneau, 1961). However, the most frequently used of 25.000 $m^3$/ha gives a water consumption of about 4 $m^3$ per kg of dates, for all the palm groves of Oued Righ. This amount needed for the development of 1 kg of dates amounts to 6 or 4 $m^3$. It is obvious that in different cases, a significant proportion of the water is intended for the fight against saline. Its underground water is too loaded with chlorides and sulfates or the existence of the risk of soil salinization (Mashali, 1996). A study requires a good estimate of some parameters of salinity in relation to agricultural use in the canal waters and waters of aquifer. This risk is determined using the value of Sodium absorbent sodium absorption ratio (SAR). For the same conductivity, the risk is even greater than the coefficient is higher. Richards' classification is very useful to characterize reliable irrigation water.

The results obtained by the Richards' diagram of the different stations water channel (Figure 11a and b) show that they are class C5S3. In this case, the water is highly mineralized. They are used only in exceptional circumstances. The C5S4 class affects virtually the majority of canal water. The waters are strongly




***a) Canal*** **b) Ground water**

**Figure 10.** Schoeller Berkaloff diagram of the canal and water groundwater (October, 2010).




***a) Canal*** ***b) Ground water***

**Figure 11.** Richards diagram of canal water and groundwater.

mineralized. This is not recommended for irrigation (Magistrad and Reitemeir, 1943). For the water table, C4S3 and C5S3 classes for Stations 10, 11 and 14 are used only in exceptional circumstances (Unesco, 1957). C5S4 class at Stations 12 and 13 is not advised for irrigation. Water quality and water channel of the web have very poor quality. So, there is a possible contamination of the two.

## Conclusion

Oued Righ is characterized by the presence of sandy soil mainly siliceous and insoluble pure quartz. The water table contains clay-evaporite sandy soil. The water table is in this relatively flat region, with regular fluctuations. The static level of shallow groundwater, fed by sewage and drainage, steadily increases, upon arrival on the soil surface. The concentrations of the chemical elements of the water channel have shown the dominance of saliferous gypsiferous ions and acquired carbonate in salinity. The canal is excessively salty, very hard, slightly basic, and hyper-chlorinated global chemical sodium facies. Irrigation water has very poor quality and mostly belongs to C5S4 class. As for the environmental aspect of the sources of pollution of the channel, values in Sec residue and organic matter are higher than the national and international standard.

Pollution is felt at Kardéche station upstream channel where the flow velocity is low. It may be noted that pollution also increases at Zaouia El Abidia station or the width and depth of the channel decreases. So the speed of the flow of water also decreases.

The monitoring of the evolution of the quality of the water table surrounding the channel shows that the contamination of the water is very pronounced at Station 12 (Sidi Slimane station). Degraded water channels are routed in areas that favor the percolation of groundwater contamination. The combined action of a climate is characterized by intense evapotranspiration and the presence of a shallow water table makes most soils undergo secondary salinization. There is degradation of palm trees surrounding the canal. The solution to this problem is to find a means of pretreating the water before discharging in the canal. A treatment plant should be installed with an aerated lagoon where adjacent wastewater undergoes treatment before being returned to the main collecting canal.

1. There should be increased frequency of irrigation, increased water supply to plants by considering the needs of leaching and/or association of different water sources.
2. The exploitation of deep groundwater should be controlled, to prevent the underground water upwelling, and the flooding of agricultural land.
3. Finally, public awareness should be raised to save water.

## Conflict of interest

The authors have not declared any conflict of interest.

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# Effect of water replacement and nitrogen fertilization on productivity variables of sugar cane

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**Although sugarcane crop has moderate tolerance to water stress, it shows high response to irrigation. Thus, the improvement of irrigation techniques becomes necessary to achieve maximum water use efficiency. Several studies in Brazil and worldwide on different sugarcane varieties have shown the effect of irrigation on productivity. Moreover, nitrogen fertilization stands out as one of the cultural practices of higher research demand, since studies on nitrogen show very variable results and sometimes even contradictory. The aim of this study was to evaluate the biometric indices of sugarcane in different growing stages, stalk productivity, and water use efficiency at different levels of water replacement by subsurface drip system with and without nitrogen application during cultivation. The experiment was carried out in the experimental field of the Federal Institute of Goias - Campus Rio Verde, GO. The experimental design was randomized blocks, with 5 × 2 factorial design and four replications. Treatments consisted of five levels of water replacement (100, 75, 50, 25, and 0% soil moisture at field capacity) combined or not with nitrogen fertilization (0 and 100 kg urea ha$^{-1}$). Plant height, stalk diameter, and leaf area of three plants were determined in the floor space of each plot in nine steps each month, from 90 days after planting (DAP), corresponding to the following periods: 90, 120, 150, 180, 210, 240, 270, 300, and 330 DAP. The results were subjected to analysis of variance by F test at 5% probability, and in cases of significance, linear and quadratic polynomial regression analysis was performed to the levels of water replacement. For nitrogen fertilization, means were compared by Tukey's test at 5% probability. The subsurface drip irrigation provided suitable conditions for accelerating sugarcane growth. The levels of water replacement contributed linearly on the development parameters and stalk yield. The nitrogen fertilizer contributed to the development of biometric variables in the latter stages of crop development.**

**Key words:** Subsurface drip irrigation, water deficit, growth, sugarcane.

## INTRODUCTION

Currently, sugarcane crops have played an important role in economic development, once sugarcane is considered one of the major agricultural commodities in terms of productivity (Devos, 2010), cultivated in tropical or subtropical climate, mainly used to produce sugar and biofuel (Kajihara et al., 2012).

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**Table 1.** Hydro-physical and chemical characteristics of the experimental soil at the depths of 0-0.20, and 0.20-0.40 m.

| Layer | Hydro-physical characteristics | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Grain size (g kg$^{-1}$) | | | $\theta_{CC}$ | $\theta_{PMP}$ | Ds | PT | Texture classification |
| m | Sand | Silt | Clay | m$^3$ m$^{-3}$ | | g cm$^{-3}$ | cm$^3$ cm$^{-3}$ | |
| 0-0.20 | 458.3 | 150.2 | 391.5 | 51.83 | 30.5 | 1.27 | 0.55 | Frank argillaceous |
| 0.20-0.40 | 374.9 | 158.3 | 466.8 | 55 | 31.33 | 1.28 | 0.51 | Clay |

| m | Chemical characteristics | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | pH | MO | P | K | Ca | Mg | Al | H+Al | S | CTC | V |
| | in $H_2O$ | g kg$^{-1}$ | mmol dm$^{-3}$ | | | | | | | | % |
| 0.00-0.20 | 6.2 | 63.42 | 0.23 | 2.04 | 20.40 | 16.80 | 0.0 | 57.75 | 41.80 | 99.55 | 41.99 |
| 0.20-0.40 | 6.6 | 44.47 | 0.08 | 4.09 | 14.40 | 13.20 | 0.0 | 44.55 | 31.69 | 76.24 | 41.57 |

$\theta_{CC}$, Field capacity (10KPa); $\theta_{PMP}$, permanent wilting point (1.500 KPa); Ds, bulk density; PT, total porosity; pH in distilled water. P and K, Mehlich$^{-1}$ extractor. MO, organic matter; V, base saturation.

Although sugarcane has some tolerance to water stress, it also shows high response to irrigation (Singh et al., 2007). It is assumed that the irrigation increases sugarcane productivity (Wiedenfeld and Enciso, 2008; Gava et al., 2011); however, the improvement of management techniques becomes necessary to achieve maximum efficiency in use of water resources, focusing on maximum productivity with lower volumes of water. According to Gava et al. (2011), plant growth and development are affected either by a lack of or excessive water supply.

Although, the irrigated farming may improve the production environment (Carr and Knox, 2011), for water use efficiency by the sugarcane crop, it is essential to identify the water requirement, responsible for the maximum yields (Wiedenfeld and Enciso, 2008).

Several studies in Brazil and worldwide on different sugarcane varieties have shown the effect of irrigation on productivity. Moreover, nitrogen fertilization stands out as one of the cultural practices of higher research demand, since studies on N show very variable results and sometimes even contradictory (Korndörfer et al., 2002). However, there are many studies on the importance of N in sugarcane crops (Oliveira et al., 2013; Franco et al., 2011).

Drip irrigation stands out among the irrigation methods used to meet the water requirement for sugarcane crop. This method allows precise control of water supplied in small quantities at high frequency to the root zone, allowing the maintenance of favorable conditions to root proliferation in the soil partly moistened (Souza et al., 2009). In addition, subsurface drip irrigation allows nutrient application directly to the root zone, without damaging the crop, allowing topdressing applications rationally and parceled out, according to the crop needs at various plant development stages.

Studies on sugarcane development have been used as an important tool to assess the performance of varieties, once initial fast and uniform growth leads to a good stand of sugarcane crop (Silva et al., 2007). Thus, the aim of this study was to evaluate the biometric indices of sugarcane growth in various development stages, stalk productivity, and water use efficiency at different levels of water replacement by subsurface drip irrigation and without nitrogen fertilization during the cultivation.

## MATERIALS AND METHODS

The experiment was carried in the experimental area of the Federal Institute of Goias - Campus Rio Verde, Goias, latitude 17° 48'28 "S and longitude 50° 53'57" O, with an average altitude of 720 m and slightly rolling slope (6%). The climate is classified as Köppen and Aw (tropical), with a rainfall season from October to May, and the dry months from June to September. The annual average temperature ranges from 20 to 35°C and precipitation range of 1500 to 1800 mm per year. The soil was classified as Red Latosol (LVdf) of medium texture. Table 1 shows the hydro-physical and chemical soil characteristics.

The experimental design was randomized blocks, 5 × 2 factorial design, with four replications. The treatments consisted of five levels of water replacement (100, 75, 50, 25, and 0% soil moisture at field capacity) combined with or without nitrogen fertilization (0 and 100 kg urea ha$^{-1}$).

Sugarcane planting was performed in March 2012, using the variety RB 85-5453, as it has high sugar content and precocity as main characteristics. The experimental plots consisted of three double-furrow openings ("W" planting) with a spacing of 1.40 × 0.4 m between rows and 8 m in length, totaling 35.2 m$^2$ of the total area per plot.

For treatments using water replacement, the subsurface drip irrigation method was used. The drip line was installed at 0.20 m above ground in the center of the double row and has the following characteristics: 16150 PC Dripnet model with thin wall, working pressure of 1 bar, nominal flow rate of 1.0 L h$^{-1}$, and drip spacing of 0.50 m.

Irrigation was carried out based on digital tensiometer puncture with a sensitivity of 0.1 kPa, with probes placed at depths of 0.20, 0.40, 0.60 and 0.80 m and distances of 0.15, 0.30, 0.45, and 0.60 m from the drip line, with daily readings of the soil matrix potential (Ψm). A critical tension of 50 kPa was used to determine irrigation water needs. The physical and hydraulic characteristics of the soil were determined by the soil water retention curve (Oliveira et al., 2014).

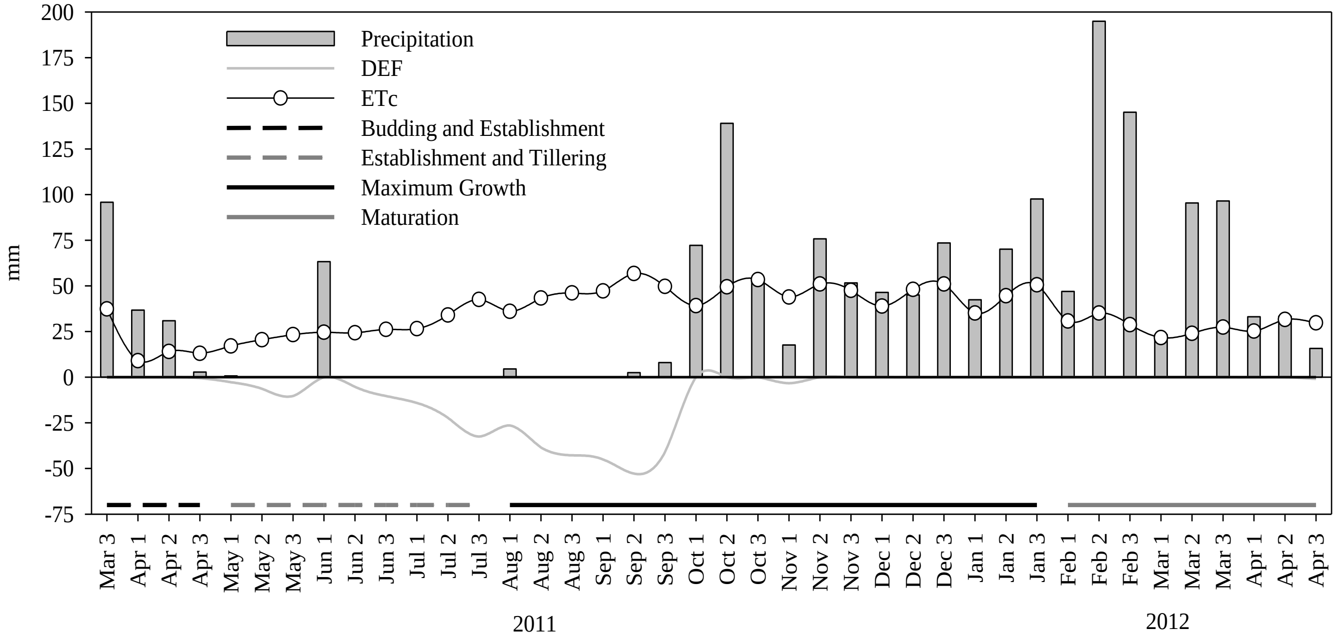


**Figure 1.** Water balance of sugarcane grown under rain fed management. DEF, Water deficit; ETc, crop evapotranspiration. Crop stages (adapted from Doorenbos and Kassam, 1994); budding and establishment (Kc = 0.6); establishment and tillering (Kc = 0.9 to 1.1); maximum growth (Kc = 1.3); and maturation (Kc = 0.7 to 0.9). Source: Normal INMET Station - Rio Verde - GO.

From the daily results of soil moisture, the volume of water used for each replacement level was determined and the increase of soil moisture to field capacity was considered for the treatments with 100% water replacement. For the other treatments, water laminas were applied according to the percentage of the predicted water replacement. At the end of the experiment, the total volume of water applied for irrigation (VTA) was 0, 126, 252, 378, and 504 mm of water for 0, 25, 50, 75, and 100% water replacement, respectively.

According to the results of soil analysis, the experimental area was fertilized, with application of 30 kg N $ha^{-1}$ (urea), 120 kg $P_2O_5$ $ha^{-1}$ (superphosphate), and 80 kg $K_2O$ $ha^{-1}$ (potassium chloride). In the plots where nitrogen application was predicted, it was fully applied through irrigation water (fertigation) parceled out in ten applications during the crop cycle. Potassium fertilization was partially performed to the furrow lines, representing 30% of total, and the remaining fertilizer was applied through irrigation water. For the treatment with 0% water replacement, the application of nutrients was performed by a fractioned way.

From the climatological data of the experimental period, the ten-day water balance was estimated for sugarcane grown under rain fed management, as described by Thornthwaite and Mather (1955), and the reference evapotranspiration (ET0) was calculated according to Allen et al. (1988) (Figure 1). The total rainfall during the crop cycle was 1479.6 mm and after discounting the volume of percolated water, an effective precipitation (PE) of 1019.2 mm was computed. The crop evapotranspiration (ETc) totaled 1817.5 mm.

The analysis of the biometric variables of sugarcane was performed once a month beginning at 90 days after planting (DAP), corresponding to 9 periods as follows: 90, 120, 150, 180, 210, 240, 270, 300, and 330 DAP. Plant height (AP, cm), stalk diameter (DC, mm), and leaf area (LA, $m^2$) of three plants were determined in the floor space of each plot (linear meter in the center of the double row). The AP was measured with the aid of a measuring tape from the ground to the collar leaf +1 and the DC was measured using a digital caliper placed at the stalk base. The AF was calculated by measuring the leaf length and width +1, and counting the number of green leaves, using the following equation: AF = (C × L) × (N + 2) × 0.7, where C is the leaf length+1; L is the leaf width +1; N is the number of green leaves; and 0.7 is the correction factor.

At harvest, the stalk yield (PC, Mg $ha^{-1}$), water use efficiency (EUA mm $Mg^{-1}$ $ha^{-1}$), and number of industrially tillers (NPI) were determined. The PC was determined by weighing the stalks within each plot using a digital hand scale, extrapolating to 1 ha. The EUA was calculated as the ratio between the total receiving water (calculated by the sum of PE and VTA) and the PC. The NPI was determined by counting plants in floor space.

The results of both the biometric variables obtained at each development stage and productivity indices were submitted to analysis of variance by F test at 5% probability, and in cases of significance, linear and quadratic polynomial regression analysis was performed to the levels of water replacement. For nitrogen fertilization, the means were compared by Tukey test at 5% probability using the statistical program SISVAR® (Ferreira, 2011).

## RESULTS

No significant interaction between the water replacement (RH) and nitrogen (N) was observed for the sources of variation evaluated (Table 2).

When analyzed separately, the response of the variable plant height (AP) for the factor RH was significant at 1% probability at all stages of crop development, as well as for the variable leaf area (LA), except at 90 DAP, where the significance level was 5% by the F test. For the stalk diameter (DC), no significant difference was observed at 90 DAP, since in general in

**Table 2.** Summary of the analysis of variance of plant height and stalk diameter of sugarcane subjected to different levels of water replacement, grown with and without nitrogen fertilization, at different development stages.

| Variable | Mean square value for the sources of variation | | | | | | Nitrogen | |
|---|---|---|---|---|---|---|---|---|
| | Water replacement (RH) | Nitrogen (N) | Interaction RH × N | Plot | Residue | CV (%) | | |
| DAP | Height of Plant (AP) | | | | | | AP (cm) | |
| | | | | | | | With | Without |
| 90 | 58.37** | 0.02$^{ns}$ | 11.25$^{ns}$ | 4.53$^{ns}$ | 12.67 | 10.03 | 35.52$^{a}$ | 35.47$^{a}$ |
| 120 | 153.06** | 14.08$^{ns}$ | 14.83$^{ns}$ | 12.96$^{ns}$ | 13.16 | 7.19 | 51.07$^{a}$ | 49.88$^{a}$ |
| 150 | 680.38** | 116.04$^{ns}$ | 33.96$^{ns}$ | 6.30$^{ns}$ | 44.19 | 9.37 | 72.69$^{a}$ | 69.28$^{a}$ |
| 180 | 470.66** | 12.58$^{ns}$ | 67.37$^{ns}$ | 29.03$^{ns}$ | 59.26 | 7.86 | 98.51$^{a}$ | 97.39$^{a}$ |
| 210 | 524.87** | 2.83$^{ns}$ | 85.98$^{ns}$ | 12.58$^{ns}$ | 41.37 | 5.52 | 116.81$^{a}$ | 116.29$^{a}$ |
| 240 | 1042.63** | 102.84$^{ns}$ | 50.40$^{ns}$ | 30.49$^{ns}$ | 56.57 | 5.20 | 146.26$^{a}$ | 143.04$^{a}$ |
| 270 | 770.19** | 181.01$^{ns}$ | 15.16$^{ns}$ | 46.69$^{ns}$ | 51.06 | 3.64 | 194.32$^{a}$ | 198.57$^{a}$ |
| 300 | 1785.58** | 310.52** | 7.01$^{ns}$ | 59.28$^{ns}$ | 34.15 | 2.08 | 284.18$^{a}$ | 278.60$^{b}$ |
| 330 | 1085.06** | 248.25** | 33.75$^{ns}$ | 22.30$^{ns}$ | 20.30 | 1.45 | 312.23$^{a}$ | 307.26$^{b}$ |
| DAP | Stalk diameter (DC) | | | | | | DC (mm) | |
| | | | | | | | With | Without |
| 90 | 7.57$^{ns}$ | 1.64$^{ns}$ | 1.45$^{ns}$ | 2.26$^{ns}$ | 3.30 | 8.48 | 21.63$^{a}$ | 21.23$^{a}$ |
| 120 | 17.39** | 5.10$^{ns}$ | 1.04$^{ns}$ | 0.25$^{ns}$ | 2.27 | 5.93 | 25.79$^{a}$ | 25.07$^{a}$ |
| 150 | 18.14** | 15.67* | 0.59$^{ns}$ | 3.19$^{ns}$ | 3.23 | 6.45 | 28.52$^{a}$ | 27.27$^{b}$ |
| 180 | 15.31** | 7.89* | 0.29$^{ns}$ | 4.59$^{ns}$ | 1.94 | 4.63 | 30.60$^{a}$ | 29.71$^{b}$ |
| 210 | 14.66** | 7.43* | 0.45$^{ns}$ | 4.36$^{ns}$ | 1.67 | 4.05 | 32.34$^{a}$ | 31.48$^{b}$ |
| 240 | 14.29** | 6.43* | 0.46$^{ns}$ | 3.79$^{ns}$ | 1.61 | 3.86 | 33.39$^{a}$ | 32.59$^{b}$ |
| 270 | 14.01** | 7.62* | 0.38$^{ns}$ | 3.78$^{ns}$ | 1.26 | 3.33 | 34.25$^{a}$ | 33.38$^{b}$ |
| 300 | 18.56** | 5.97* | 0.54$^{ns}$ | 3.14$^{ns}$ | 1.31 | 3.32 | 34.97$^{a}$ | 34.20$^{b}$ |
| 330 | 21.66** | 6.01* | 0.65$^{ns}$ | 2.95$^{ns}$ | 1.35 | 3.30 | 35.58$^{a}$ | 34.80$^{b}$ |
| DAP | Leaf area (AF) | | | | | | AF ($m^2$) | |
| | | | | | | | With | Without |
| 90 | 77.45* | 7.81$^{ns}$ | 3.69$^{ns}$ | 0.19$^{ns}$ | 5.07 | 11.86 | 19.43$^{a}$ | 18.55$^{a}$ |
| 120 | 123.64** | 23.82$^{ns}$ | 5.60$^{ns}$ | 2.61$^{ns}$ | 11.90 | 12.29 | 28.83$^{a}$ | 27.29$^{a}$ |
| 150 | 203.78** | 27.98$^{ns}$ | 4.39$^{ns}$ | 4.75$^{ns}$ | 7.39 | 8.32 | 33.52$^{a}$ | 31.85$^{a}$ |
| 180 | 236.95** | 33.74$^{ns}$ | 6.72$^{ns}$ | 12.39$^{ns}$ | 16.83 | 10.66 | 39.39$^{a}$ | 37.55$^{a}$ |
| 210 | 341.33** | 33.26$^{ns}$ | 12.54$^{ns}$ | 13.16$^{ns}$ | 17.75 | 9.19 | 46.77$^{a}$ | 44.94$^{a}$ |
| 240 | 573.40** | 164.55* | 7.28$^{ns}$ | 12.34$^{ns}$ | 23.29 | 8.40 | 59.48$^{a}$ | 55.42$^{b}$ |
| 270 | 1262.34** | 168.26* | 15.09$^{ns}$ | 1.64$^{ns}$ | 27.32 | 7.75 | 69.52$^{a}$ | 65.42$^{b}$ |
| 300 | 2132.63** | 194.87* | 23.54$^{ns}$ | 19.20$^{ns}$ | 31.90 | 7.27 | 79.89$^{a}$ | 75.47$^{b}$ |
| 330 | 2380.89** | 456.70** | 36.47$^{ns}$ | 11.64$^{ns}$ | 15.72 | 5.22 | 79.41$^{a}$ | 72.65$^{b}$ |

** and * Significant at 5 and 1% probability, $^{ns}$Insignificant at 5% probability by F test. Means followed by the same letter in the column do not differ at 5% probability by Tukey's test.

the early stages of plant development, no differentiation is observed with respect to the RH levels. However, at 120 DAP, a significant difference was observed at 1% probability level for all development stages. The factor N caused no significant effect on the biometric variables at the beginning of the cycle. In contrast, a significant interference at 1% probability was observed only at 300 and 330 DAP for the variable AP as a function of N, according to the F test (Table 2). For the DC, a significant (5%) difference was observed in the development stages evaluated from 150 DAP. For the AF, 5% significance was observed only from 240 DAP, which exhibited 1% significance in the last assessment (330 DAP).

When the variable AP was analyzed facing the presence and absence of N in the development stages with significant differences by Tukey' test, the sugarcane suffered an increase of 5.58 and 4.97 cm after N fertilization at 300 and 330 DAP, respectively, corresponding to an increase of 1.96 and 1.59% (Table 2).

The increase in DC was more pronounced with the N supply, since a significant difference was observed from the 150 DAP, with an increase of 4.38% (Table 2). In the subsequent development stages, the increase in DC of sugarcane grown with N was lower with values of 2.91, 2.66, 2.40, 2.54, 2.20, and 2.19% at 180, 210, 240, 270, 300, and 330 DAP, respectively. Thus, it is clear that the use of N by plant cane to provide an increase in the DC occurred in the early stages of maximal growth, and the growth rate was similar in the other stages, concluding that N was responsible for initiating the DC increment.

The N supply affected the AF development only from the 240 DAP, with an increase of 4.06 $m^2$, which corresponded to an increment of 6.83%. At 270 and 300 DAP, the increase in AF provided by N was less expressive, 5.90 and 5.53%, respectively. However, the maximum AF development due to the N supply occurred at 330 DAP, with an increase of 6.76 $m^2$, representing an increment of 8.51%, faced with the end of N application (Table 2).

The direct nitrogen application into the root zone may have contributed to the availability of this nutrient in the later development stages of sugarcane, by providing a direct contribution to the rapid N dispersion in the environment, allowing these plants to absorb a higher N level for a longer period of time.

The mean AP values as a function of RH behaved in linear models for all development stages (Figure 2). During the establishment and tillering, an increase of 1.60 cm at 90 DAP (Figure 2A) and 2.57 cm at 120 DAP (Figure 2B) was observed for each RH level, thus at 100% RH, yields 19.85 and 22.72% higher than the rain fed management (0% RH) were found at 90 and 120 DAP, respectively. From 150 DAP, a period that includes the beginning of the maximal plant growth, the AP development was influenced more significantly by RH. The AP in the treatment with 100% RH at 150 DAP was 33.77% higher when compared with the rain fed management (0% RH), with an increase of 5.13 cm for each RH level (Figure 2C).

At 180, 210 and 240 DAP, the AP development was similar within the RH levels, with differences of 21.89, 18.92, and 22.05% for the 100% RH when compared with the treatments with 0% RH and the AP growth rate remained uniformly among treatments.

In these stages, the increase in AP for each RH level was 4.83, 5.04, and 7.18 cm at 180, 210, and 240 DAP, respectively (Figure 2D, 2E, and 2F), which shows that when irrigated the sugarcane crop exhibits a growth rate much higher than those with water deficit.

In contrast, at the end of the maximal growth stage, the effect of RH on the AP was less expressive. The increments provided by the increased RH level were 6.19, 9.01, and 6.95 cm at 270, 300 and 330 DAP, respectively. The estimated AP at 100% RH was 13.46, 13.69, and 9.40% higher than the rain fed management (0% RH) (Figure 2G, 2H and 2I).

It is noteworthy that some recovery in the AP development was observed in the treatments with water restriction, probably due to the soil water availability provided by rainfall, which began at the end of September.

The mean DC for the different RH levels showed a linear tendency in all development stages with significant differences by the F test (Figure 3). The difference in the DC development as a function of different RH levels was similar in the different periods. At 120 DAP, the DC suffered an increase of 3.92% for each RH level, with an estimated average of 27.28 mm with full irrigation (100% RH) (Figure 3A).

In the early stage of maximal sugarcane growth, the increases in DC with increasing the RH levels were 3.62, 3.07, and 3.39% at 150, 180 and 210 DAP, thus achieving an increase of 3.77, 3.49, and 4.1 mm at 100% RH as compared to the rain fed management (0% RH) (Figure 3B, 3C and 3D).

At 240 DAP, certain recovery in the DC development was observed in the treatments with water restriction, with differences of only 2.66% for each RH level studied (Figure 3D). In addition, at 270 and 300 DAP, differences of 2.56 and 2.94% were observed for the RH levels. However, it is noteworthy that it was a high rainfall period (Figure 1), which generally favors the maintenance of soil moisture (Figure 3F and 3G).

However, soon after, a gap in the mean DC is observed in analogy of the different RH levels, showing that, at 330 DAP, an increase of 4.15 mm was achieved at 100% RH when compared with rain fed management (0% RH), which represents an increase of 3.13% for each level RH, obtaining a maximum value of 37.26 mm (Figure 3H).

The AF responses of sugarcane subjected to different RH levels showed linear behavior at all development stages (Figure 4). The different AF values found for the RH levels were higher according to the crop development stages. At 90 DAP, the AF suffered an increase of 1.85 $m^2$ for each RH level, with a maximum response of 22.69 $m^2$ at 100% RH (Figure 4A).

From the early stage of maximal growth, the AF development of the plants subjected to conditions of higher soil moisture was more significant, which was 58.20% higher at 100% RH when compared with the fed rain management (0% RH) (Figure 4B). At 150, 180, and 210 DAP, the AF development was quite similar, with increases of 11.22, 10.72, and 10.57% for each RH level.

Throughout the sugarcane development stages, it is observed that the difference in AF as a function of different RH levels gradually increased. At 240, 270, 300, and 330 DAP, increments of 11.37, 15.36, 17.77, and 19.79 $m^2$ were observed for each RH level, respectively. The full irrigation (100% RH) provided increments of 45.59, 61.46, 71.08, and 79.16% as compared to rain fed management (0% RH).

The significant difference between the RH levels is mainly related to the low development of AF in the

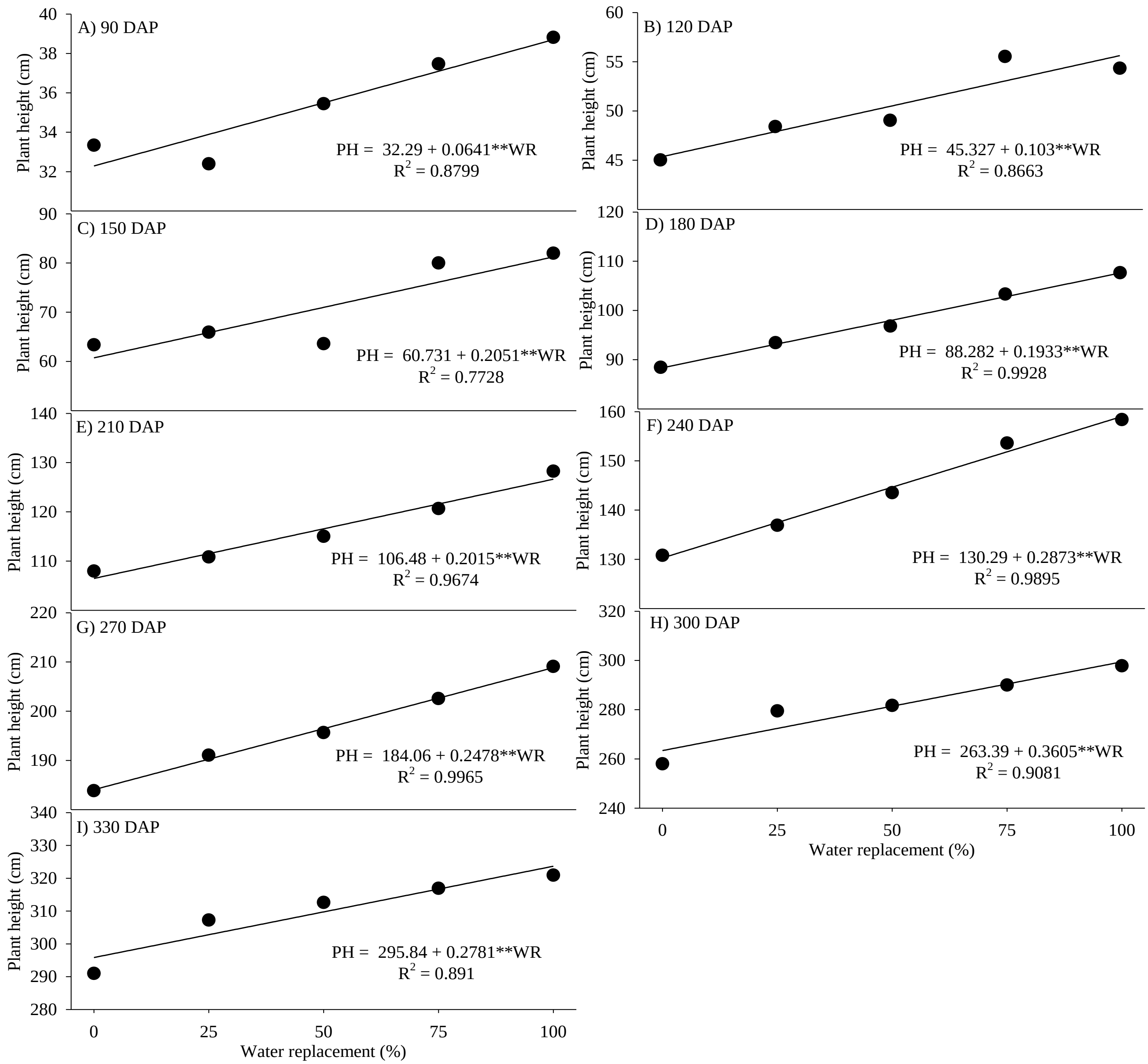


**Figure 2.** Plant cane height as a function of the water replacement levels, at different stages of crop development: A, 90 DAP; B, 120 DAP; C, 150 DAP; D, 180 DAP; E, 210 DAP; F, 240 DAP; G, 270 DAP; H, 300 DAP; and I, 330 DAP.

treatments with water restriction, evidenced by the growth rate at 0% RH of only 4.81 and 5.70 $m^2$ at 240 to 270 and 270 to 300 DAP, respectively, and a reduction of 2.84 $m^2$ at 300 to 330 DAP. It is noteworthy that, for all RH levels, the maximum AF values were found at 300 DAP, reaching maximal response of 98.05 $m^2$ at 100% RH.

Table 3 shows the summary of the analysis of variance for the variables: stalk yield (PC), water use efficiency (EUA), and number of industrially tillers (NPI). Both the factor N and the interaction RH × N caused no significance in the variables analyzed. The variable PC showed significance at 1% probability for the factor RH. The factor RH caused a 5% significant effect on NPI.

The factors evaluated did not significantly affect the variable EUA, probably due to the high rainfall throughout the experimental period (PE = 1019.2 mm). However, it is noteworthy that the rainfall in the region is concentrated in a period of the year, resulting in sharp decline in the PC yield in the rain fed management (0% RH) (Figure 5). The average PC of sugarcane followed a linear model

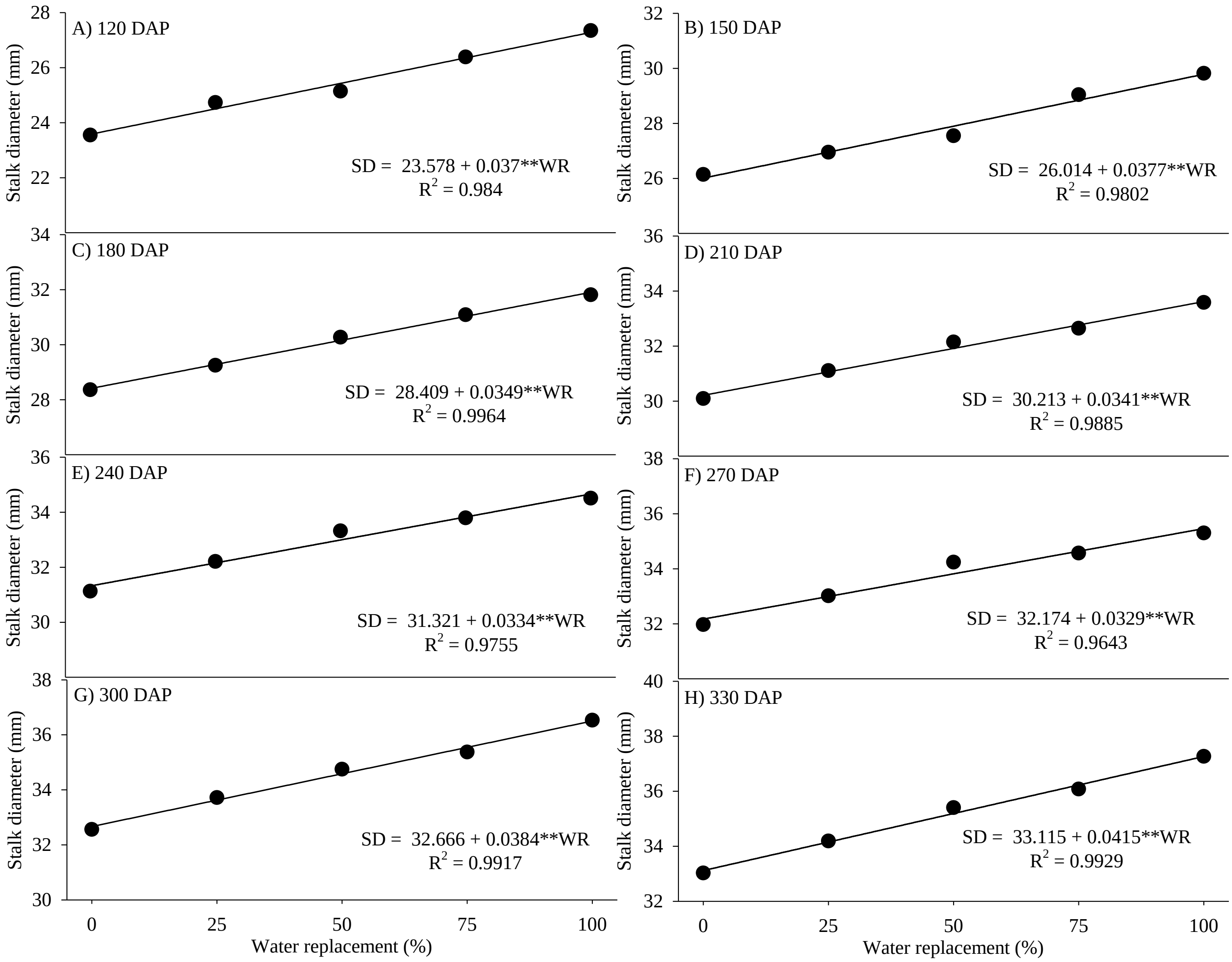


**Figure 3.** Sugarcane stalk diameter as a function of the water replacement levels, at different stages of crop development: A, 90 DAP; B, 120 DAP; C, 150 DAP; D, 180 DAP; E, 210 DAP; F, 240 DAP; G, 270 DAP; H, 300 DAP; and I, 330 DAP.

($R^2$ = 0.7471) as a function of the RH levels (Figure 5). The RH allowed an increase of 17.74 Mg $ha^{-1}$ for each level evaluated, corresponding to an increase of 9.96%. With respect to the rain fed management (0% RH), the PC was 28.5% lower than that observed at 100% RH, with an estimate yield of 249.02 Mg $ha^{-1}$.

The NPI of sugarcane showed a linear growth as a function of RH ($R^2$ = 0.9282) (Figure 6). The maximum NPI response at 100% RH was 24 tillers $m^{-2}$. The full irrigation system (100% RH) allowed increments of 5.22, 10.44, 15.66, and 20.88% at 0, 25, 50 and 75% RH levels, respectively. The plant height as a function of water replacement was much more expressive than the stalk diameter. In respect to leaf area, although higher sensitivity to the different volumes of irrigation have been observed, the water replacement had less effect on the number of tillers.

The varietal characteristics of the variety RB 85-5453, when defined under irrigated management increased with the increasing volume of water supplied, affecting the photosynthetic efficiency and consequently the stalk yield.

## DISCUSSION

Dantas Neto et al. (2006) found similar results for the variables of sugarcane growth, with no interactions between fertilization levels and irrigation regimes despite the top-dressing, which was significant. Opposite results were reported by Silva et al. (2008), who found that the stalk diameter may not reflect the differences between sugarcane grown on different water regimes. These results are in agreement with Franco et al. (2011), who reported that studies on nitrogen fertilization have very variable results regarding the effect of N application,

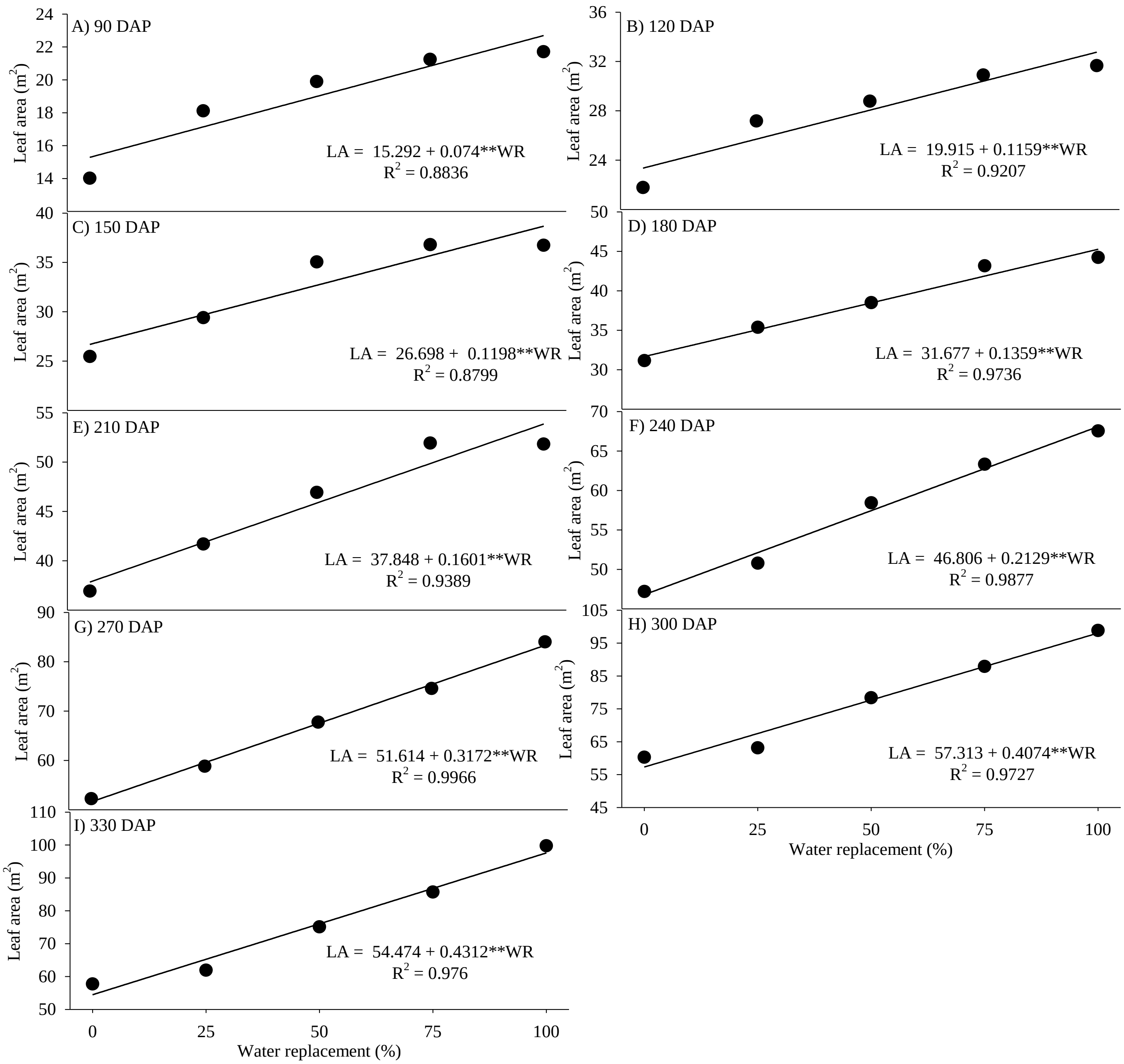


**Figure 4.** Sugarcane leaf area as a function of the water replacement levels, at different stages of crop development: A, 90 DAP; B, 120 DAP; C, 150 DAP; D, 180 DAP; E, 210 DAP; F, 240 DAP; G, 270 DAP; H, 300 DAP; and I, 330 DAP.

mainly on stalk yield, with heterogeneous responses for the plant cane and relatively homogeneous responses for the ratoon. Nevertheless, it is known that among the main factors that limit the productivity of Brazilian sugarcane include water and nutrients availability, mainly nitrogen (Wiedenfeld and Enciso, 2008; Oliveira et al., 2013).

According to Roberts (2008), the use of fertigation generally improves nutrient use efficiency, since they are applied in a fractioned way, according to the nutrient absorption of the crop. Dias et al. (2012) reported that the shoot height and biomass production were sensitive to water restriction, reaching the highest values under the full irrigation regime. Freitas et al. (2012) reported that the significant difference between sugar cane height as a function of irrigation occurred at 116 DAP, evidencing that, from then on, the water availability has become a limiting factor to the crop vegetative development. These results corroborate the findings of Oliveira et al. (2010), who demonstrated that RB varieties grown under full irrigation reached plant height values higher than 300 cm.

**Table 3.** Analysis of variance for stalk yield (PC), water use efficiency (EUA), and number of industrially tillers (NPI) of sugarcane grown under different levels of water replacement, with and without nitrogen fertilization.

| Variation | GL | Average square | | |
|---|---|---|---|---|
| | | PC | EUA | NPI |
| Water replacement (RH) | 4 | 8424.45** | 2.09$^{ns}$ | 23.33 |
| Nitrogen (N) | 1 | 6038.38$^{ns}$ | 4.36$^{ns}$ | 3.02 |
| interaction RH x N | 4 | 441.10$^{ns}$ | 0.54$^{ns}$ | 1.08 |
| Plots | 3 | 1712.05$^{ns}$ | 1.86$^{ns}$ | 10.49 |
| Residue | 27 | 1866.16 | 1.73 | 6.39 |
| CV (%) | | 20.23 | 21.29 | 11.51 |
| **Nitrogen (N)** | | **Average** | | |
| With | | 225.82$^{a}$ | 5.86$^{a}$ | 22.25$^{a}$ |
| Without | | 201.25$^{a}$ | 6.52$^{a}$ | 21.70$^{a}$ |

** and *Significant at 5 and 1% probability, $^{ns}$Insignificant at 5% probability by F test. Means followed by the same letter in the column do not differ at 5% probability by Tukey's test.

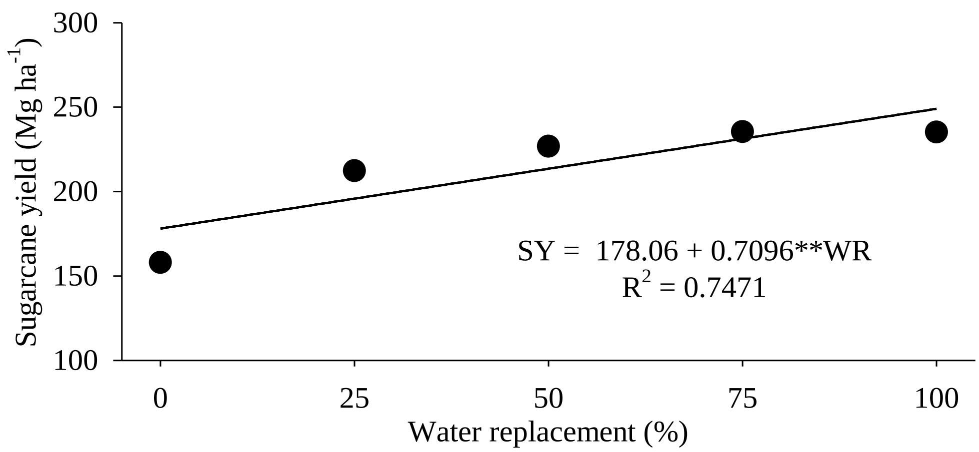


**Figure 5.** Sugarcane productivity as a function of levels of water replacement.

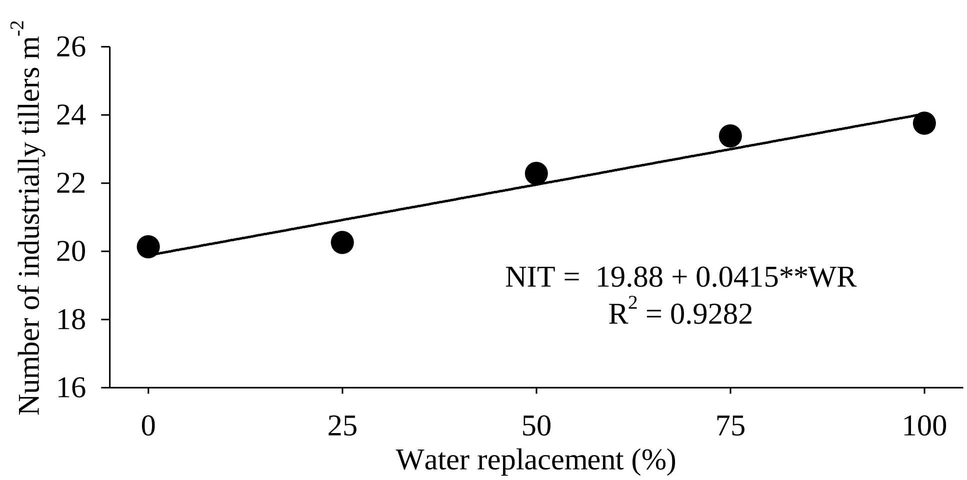


**Figure 6.** Number of industrially sugarcane tillers as a function of the water replacement levels.

This response is similar to results obtained by Dantas et al. (2006) in irrigated sugarcane crop in the Brazilian Northeast, once these authors found an increase of 30.0% with a total water of 1343 mm in relation to the rain fed management, reaching an average of 24.67 mm in stalk diameter. Farias et al. (2007) observed an increase of approximately 46.0% in leaf area index of sugarcane subjected to full irrigation.

The crop canopy is an important factor in crop yield, since it intercepts the solar radiation which promotes photosynthesis and evaporation, and causes shading on weeds (Smit and Singels, 2006). Studies have shown that the photosynthetic capacity of sugarcane decreases drastically due to the reduced leaf area (Inman-Bamber et al., 2009).

Oliveira et al. (2011) found a mean increase of 145.0% in sugarcane yield varieties subjected to full irrigation in the Pernambuco State, reaching yields of up to 255.6 Mg $ha^{-1}$. Gava et al. (2011) observed a mean increase in sugarcane yield of 24% using the irrigation system in São Paulo state. The sugarcane production can be affected significantly by the reduced tiller emission and survival, especially when water deficit occurs during crop establishment, the final number of stalks is significantly affected (Mauri, 2012).

According to Inman-Bamber and Smith (2005) and Ghannoum (2009), the morphological and physiological characteristics modified by water stress are of great importance to achieve high plant productivity. However, the productivity of the irrigated sugarcane depends on the ratio between the amount of water applied and the amount of available fertilizer, in addition to the variety, cutting cycle, soil, and climate (Gava et al., 2011).

When subjected to water stress conditions, plants exhibit several morphological and physiological changes, such as leaf rolling, changing in leaf angle, and reduction in leaf area (Chaves et al., 2008). Farias et al. (2007) observed an increase of approximately 46.0% in leaf area index of sugarcane subjected to full irrigation.

## Conclusions

The subsurface drip irrigation provided suitable conditions for the establishment and faster growth of sugarcane. The water replacement levels contributed linearly to both the growing parameters at most stages of crop development and stalk yield.

Despite the nitrogen fertilization by fractioned way through the irrigation system has contributed significantly to the development of biometric variables in the latter stages of crop development, no significant changes were observed in stalk yield.

## Conflicts of interest

The authors have not declared any conflict of interest.

## ACKNOWLEDGEMENTS

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*Full Length Research Paper*

# Macronutrients requirement of a snap bean genotype with determinate growth habit in Brazil

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**Obtaining nutrient accumulation curves is very important in knowing the plant nutritional requirement dynamics and to direct the strategies for its supply. The aim of this work was to study the uptake, compartmentalization and exportation of macronutrients of a snap bean genotype with determinate growth habit. An experiment at field conditions at Londrina State University – UEL, Londrina-PR, Br azil, was performed in a randomized block design with five replications, using UEL-1 genotype. It was observed that dry matter production, as nutrients accumulation, were slow until 20 days after emergence (DAE), V4 stage, and became more pronounced after that period. The macronutrients were more accumulated in the pods, except for Ca, which had the leaves as preferred organ. The maximum amounts of N, P, K, Ca, Mg and S uptaken were 91.0; 35.2; 131.1; 35.2; 9.1 and 4.7 kg ha$^{-1}$, respectively, while exportation to produce each ton of pod's fresh matter were 7.01 kg of N; 3.30 kg of P; 7.91 kg of K; 0.6 kg of Ca; 0.48 kg of Mg and 0.31 kg of S. One must pay attention to the proper management of quantity and epoch of N and K supply, because of the high demand and exportation of these nutrients.**

**Key words:** *Phaseolus vulgaris* L., plant nutrition, accumulation, demand.

## INTRODUCTION

The snap bean (*Phaseolus vulgaris* L.) is a Fabaceae plant, the same species of the common bean, but differing from it by the consumption of the immature fruits (pods), which are succulent with reduced fiber (Myers and Baggett, 1999). The world production of green beans in 2013 was estimated to be 21.37 million tons, more than 90% is being produced in Asia (FAOSTAT, 2013). The snap bean may have two distinct growth habits,

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determinate or indeterminate. In the first case, the plant presents the inflorescence and in the second case, the vegetative bud is present at the apexes of the stems.

In Brazil, the commercial production of snap bean occurs with the usage, mainly, of indeterminate growth habit genotypes. However, the reduced cycle, exemption from staking, concentrated harvest and the possibility of total mechanization are some of the advantages that make the determinate genotypes attractive to producers (Vidal et al., 2007; Moreira et al., 2009).

The study on nutrient uptake together with development is very relevant to crop fertilizer management. With it, the quantities and the stages of higher accumulation can be accessed (Zobiole et al., 2010), enabling to match nutrient supply to crop requirements as advocated by Zhang et al. (2011) and, thus, avoiding both limitation at plant growth and wastage by excess application.

Despite this great importance, such studies are scarce in snap bean with determinate growth habit. Therefore, the aim of this work was to describe the dynamics of nutrient uptake and compartmentalization of this vegetable crop grown in the fall/winter season in Brazil.

## MATERIALS AND METHODS

The experiment was carried in field conditions at the Agrarian Sciences Center of the State University of Londrina – UEL, Londrina-PR, Brazil (23° 23' S; 51° 11' W; 560 m altitude), between April and June 2014. The climate, according to Köppen classification, is Cfa type. The data for meteorological variables obtained during the months the experiment was performed were, respectively, 21.6; 18.3 and 16.9°C air mean temperature; 71, 74 and 75% air relative humidity and 162.5; 96.7 and 65.7 mm of rainfall, IAPAR (2015).

The soil was classified as a Red Oxisol (Santos, 2013) and presented the following characteristics of the arable layer (0.0 - 0.2 m): pH $_{CaCl2}$ = 4.82; P $_{Mehlich-1}$ = 1.84 mg dm$^{-3}$; $Ca^{2+}$ = 5.1 cmol$_c$ dm$^{-3}$; $Mg^{2+}$ = 1.7 cmol$_c$ dm$^{-3}$; $K^+$ = 0.82 cmol$_c$ dm$^{-3}$; H + $Al^{3+}$ = 6.22 cmol$_c$ dm$^{-3}$; $Al^{3+}$ = 0.1 cmol$_c$ dm$^{-3}$; CEC $_{pH\ 7.0}$ = 13.84 cmol$_c$ dm$^{-3}$; Organic Matter = 28.14 g dm$^{-3}$ (Pavan et al., 1992).

The sowing was done manually in April 3, after the soil tillage with rotary hoe, in a randomized block design with five replications. The treatments consisted of different epochs of plant evaluation. The snap bean genotype was UEL-1 (Castiglioni et al., 1993). The plots were constituted by six sowing rows with six meters long, spaced 0.5 m apart, and with 12 seeds m$^{-1}$ (240 000 plants ha$^{-1}$). The borders were the two external rows, as well as 0.5 m at the end of each row.

Representative plants were sampled weekly, the plant cycle, accounted by the days after emergence (DAE), which occurred April 10. Ten plants were collected per evaluation epoch (two per plot), by cutting them close to soil surface. The plant shoots were separated in leaves, stems and pods (when present), and washed in deionized water. The stage of development was noted according to scale proposed by Fernandez et al. (1982).

After being separated and washed, plant parts were dried in forced air system (60°C) till constant matter. Then, the plant tissues were weighed in analytical balance for obtaining dry matter (DM), ground in Wiley mill and submitted to acid digestion (sulfuric for N determination and nitricperchloric for P, K, Ca, Mg and S). The extracts were analyzed for macronutrients contents using the methods described by Silva (2009).

The accumulation of macronutrients (mg plant$^{-1}$) at different plant parts, in each sampling time, was obtained by multiplying its content (g kg$^{-1}$) by the DM (g plant$^{-1}$). Then, accumulation curves was adjusted as a function of time using the regression model "Gaussian" with three parameters, described by Equation 1, in which: $\hat{y}$ = accumulation (mg plant$^{-1}$); $a$ = value of maximum accumulation (mg plant$^{-1}$); $x0$ = value of $x$, in DAE, that proportionate the maximum accumulation; $b$ = amplitude of the interval of $x$ between the point of inflection (when the rate of daily accumulation, still positive, begins to decay) and the value of $x0$. (Zobiole et al., 2010)

$$\hat{y} = a\, e^{\left[-0,5\left(\frac{x-x0}{b}\right)^2\right]} \quad (1)$$

With the data of nutrients accumulation in each sampling time and each plant part, it was possible to obtain their percentage distribution between plant organs, calculating the relative accumulation (%). The curves and percentage distribution of DM were obtained similarly for those of the nutrients. The fresh pod yield (kg plant$^{-1}$) in ten plants harvested randomly at the blocks was evaluated, converting data to kg ha$^{-1}$ multiplied by population density (plants ha$^{-1}$). With the accumulation in the pods at the end of the cycle and the estimated yield, the exportation rates of macronutrients required to produce each ton of pod fresh matter was obtained.

## RESULTS AND DISCUSSION

The DM accumulation was low until the beginning of V4 stage, near 20 DAE, when the plant had only 13% in comparison with the maximum value. By this time, the DM was allocated mainly at the leaves (above 80%). From this point, the increment of the accumulation was accentuated and the stems participation in the relative DM was increased (Figure 1).

Higher accumulation for pods, followed by leaves and stems was observed in the respective points of maximum (Table 1). The fresh pod yield was 6 921.6 $\pm$ 2 219.6[1] kg ha$^{-1}$, similar to that obtained by Moreira et al. (2009) for different snap bean genotypes with determinate growth habit at the same location in summer season.

The leaves and stems presented a resembling dynamics of DM accumulation, reaching the point of inflection between the stages R6 and R7, while the point of maximum was observed between R7 and R8. In the reproductive phase, there was a gradual decrease in the DM accumulation of vegetative organs, especially the leaves, and a simultaneous increase in the proportion accumulated in the pods (Figure 1). This shows a redistribution of photosynthates from the sources to the reproductive sinks (Taiz and Zeiger, 2010) and also the loss of biomass, represented by the falling senescent leaves (Larcher, 2003).

The mean contents of the macronutrientes were generally higher in the leaves, with a large reduction of N, P and K, the development in this organ. The content of Ca, on the other hand, was raised, while Mg

[1] Standard deviation

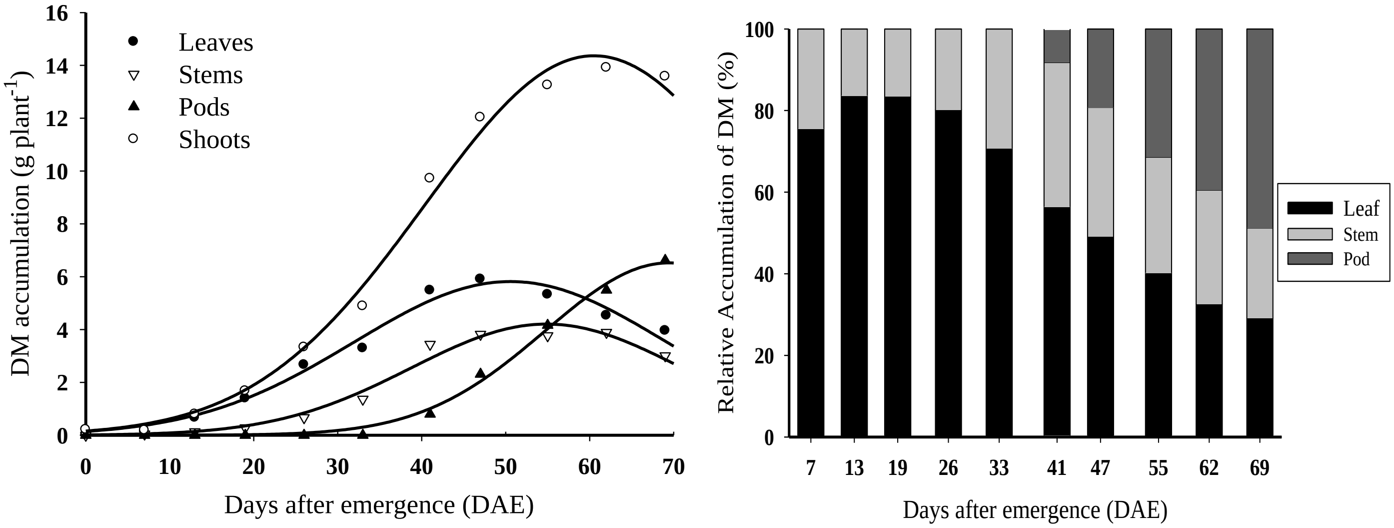


**Figure 1.** Dry matter (DM) accumulation curves and its percentage distribution between organs of the snap bean plant during its development.

**Table 1.** Estimated parameters of Gaussian model for dry matter (DM) accumulation in different parts of determinate growth habit of snap bean.

| Plant part | Estimated parameters of adjusted model (1) | | | PI (2) | $R^2$ |
|---|---|---|---|---|---|
| | $a$ | $b$ | $x0$ | ($x0$ - $b$) | |
| | g plant$^{-1}$ | Days after emergence | | | |
| Leaves | 5.82** | 18.60** | 50.57** | 31.97 | 0.98 |
| Stems | 4.21** | 16.11** | 54.88** | 38.77 | 0.98 |
| Pods | 6.52** | 14.73** | 69.46** | 54.73 | 0.99 |
| Shoots | 14.36** | 20.14** | 60.51** | 40.37 | 0.99 |

[1] $a$ = maximum accumulation (g plant$^{-1}$); $x0$ = value of $x$, in days after emergence (DAE), which is proportional to the maximum accumulation; $b$ = amplitude of the interval of $x$ between the point of inflection and the value of $x0$. [2]PI = point of inflection. **Significant at 1% probability by *t* test.

and S changed a little (Table 2). These behaviors are similar to those found by Haag et al. (1967) studying the common bean. The dynamics of contents of N, P, K, Mg and S in the stems were like that in the leaves. The content of Ca, however, was the opposite. In the pods, the contents of N, P, K, Ca and S decreased progressively, while Mg, again, remained almost constant (Table 2).

The content reduction through time can be explained, especially in the vegetative phase, by the dilution effect resulting from the DM increment. This also must be the main reason why there is a decrease in pod's content. In the reproductive phase, on the other hand, the decline of nutrients contents, especially N, P and K in the source organs (leaves and stems) is due to translocation towards the developing sinks (pods) (Marschner, 2011). The low redistribution of calcium occurs because of the little mobility in the phloem (Biddulph et al., 1958) and the input to the pods depends on the xylem stream (Grusak and Pomper, 1999).

The accumulation of nutrients was slow until close to 20 DAE (beginning of V4 stage), when related to the maximum extraction, only 18% of N, 17% of P, 21% of K, 17% of Ca, 16% of Mg and 15% of S were already accumulated in the shoots (Figures 2 and 3). The incline of uptake curves was accentuated after that period, similar to what happened for DM, reaching the log phase (Epstein and Bloom, 2005). The dynamics of accumulation of N, K, Mg and S in different parts of the plant was similar, with redistribution initially from the leaves to the stems, in V4 stage, and, from both to the pods in the reproductive phase.

Particular behaviors were observed for P and Ca, in which the first had great accumulation in the pods, with almost linearly increase, and the second had major allocation in the leaves, reinforcing the issue of low Ca

**Table 2.** Mean contents of macronutrients nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S) at different parts of the snap bean plant in each cycle epoch.

| Macronutrient | N | P | K | Ca | Mg | S |
|---|---|---|---|---|---|---|
| **Epoch of cycle (days after emergence)** | | | **g kg$^{-1}$ (1)** | | | |
| | | | **Leaves** | | | |
| 7 DAE (V2) | 59.74 | 20.02 | 72.64 | 11.52 | 3.11 | 2.25 |
| 13 DAE (V3) | 55.85 | 22.70 | 74.65 | 12.16 | 3.11 | 1.84 |
| 19 DAE (V4) | 46.66 | 17.48 | 59.60 | 13.28 | 3.47 | 2.04 |
| 26 DAE (V4) | 34.29 | 11.27 | 55.59 | 13.13 | 3.62 | 2.35 |
| 33 DAE (R6) | 34.29 | 9.40 | 52.58 | 17.18 | 3.39 | 2.12 |
| 41 DAE (R7) | 34.29 | 7.64 | 45.56 | 18.73 | 3.26 | 1.37 |
| 47 DAE (R7) | 33.23 | 7.49 | 39.54 | 21.29 | 3.72 | 1.64 |
| 55 DAE (R8) | 27.22 | 7.27 | 41.55 | 17.51 | 3.20 | 1.53 |
| 62 DAE (R8) | 29.69 | 8.48 | 53.58 | 18.42 | 2.82 | 1.57 |
| 69 DAE (R8) | 28.99 | 8.89 | 46.56 | 21.64 | 3.28 | 1.36 |
| | | | **Stems** | | | |
| 7 DAE (V2) | 43.13 | 21.22 | 105.31 | 6.23 | 2.31 | 1.20 |
| 13 DAE (V3) | 33.23 | 22.00 | 81.67 | 6.18 | 2.10 | 1.05 |
| 19 DAE (V4) | 25.45 | 17.15 | 77.65 | 5.69 | 2.21 | 1.27 |
| 26 DAE (V4) | 18.03 | 11.02 | 60.60 | 6.44 | 1.99 | 2.29 |
| 33 DAE (R6) | 14.14 | 9.88 | 56.59 | 6.72 | 2.18 | 1.53 |
| 41 DAE (R7) | 15.91 | 6.50 | 37.54 | 4.60 | 2.26 | 1.54 |
| 47 DAE (R7) | 24.04 | 6.83 | 28.51 | 4.09 | 2.28 | 1.18 |
| 55 DAE (R8) | 18.03 | 5.98 | 23.50 | 2.82 | 2.27 | 1.02 |
| 62 DAE (R8) | 21.92 | 6.17 | 33.53 | 3.06 | 2.31 | 1.45 |
| 69 DAE (R8) | 14.49 | 4.81 | 34.53 | 3.78 | 3.08 | 0.98 |
| | | | **Pods** | | | |
| 41 DAE (R7) | 33.23 | 19.28 | 57.60 | 7.70 | 2.37 | 1.70 |
| 47 DAE (R7) | 27.57 | 13.66 | 44.56 | 6.75 | 2.34 | 1.63 |
| 55 DAE (R8) | 24.75 | 13.11 | 40.55 | 5.73 | 2.41 | 1.29 |
| 62 DAE (R8) | 31.11 | 13.15 | 35.53 | 3.80 | 2.21 | 1.47 |
| 69 DAE (R8) | 30.40 | 14.62 | 35.53 | 2.85 | 2.14 | 1.35 |

[1]Mean of the plots.

redistribution. Still, except for calcium, all the nutrients evaluated had the pods as the main residence organ at the end of the cycle, with approximately 56% of N, 66% of P, 45% of K, 39% of Mg and 52% of S, which shows high exportation rates of these elements (Figures 2 and 3). The inflection points at the curves of nutrients shoot accumulation occurred in the interval of 35 to 40 DAE (R6 to R7 stage), except for phosphorus, obtained at 44 DAE (Table 3). According to Zobiole et al. (2010), the inflection point is important for the determination of index leaf sampling period. The macronutrient uptaken in higher amount by the snap bean with determinate growth habit was K, with maximum value corresponding, in the population density used, to 131.1 kg ha$^{-1}$, followed by N with 91.0 kg ha$^{-1}$, Ca and P with both 35.2 kg ha$^{-1}$, Mg with 9.1 kg ha$^{-1}$ and S with 4.7 kg ha$^{-1}$ (Table 3). The amounts of macronutrients required for uptake and exportation by plants for the production of each ton of pod's fresh matter are shown in Table 4. Lower demand of P and higher demand of N, Ca, and S for the common bean than that observed for snap bean has been reported (Soratto et al., 2013; Pegoraro et al., 2014).

Faquin and Andrade (2004) highlighted the vegetable crops larger requirement of K in relation to N, a fact observed by authors such as Grangeiro et al. (2004) for watermelon, Silva Júnior et al. (2006) for melon, Vidigal et al. (2007) for pumpkin and Tetsukabuto and Fernandes et al. (2011) for potato. However, the epoch of cultivation in this study was made and has also contributed to this result, because of the lower water supply in the region during this period, which may interfere with N mineralization from soil organic matter (Sierra et al., 2001).

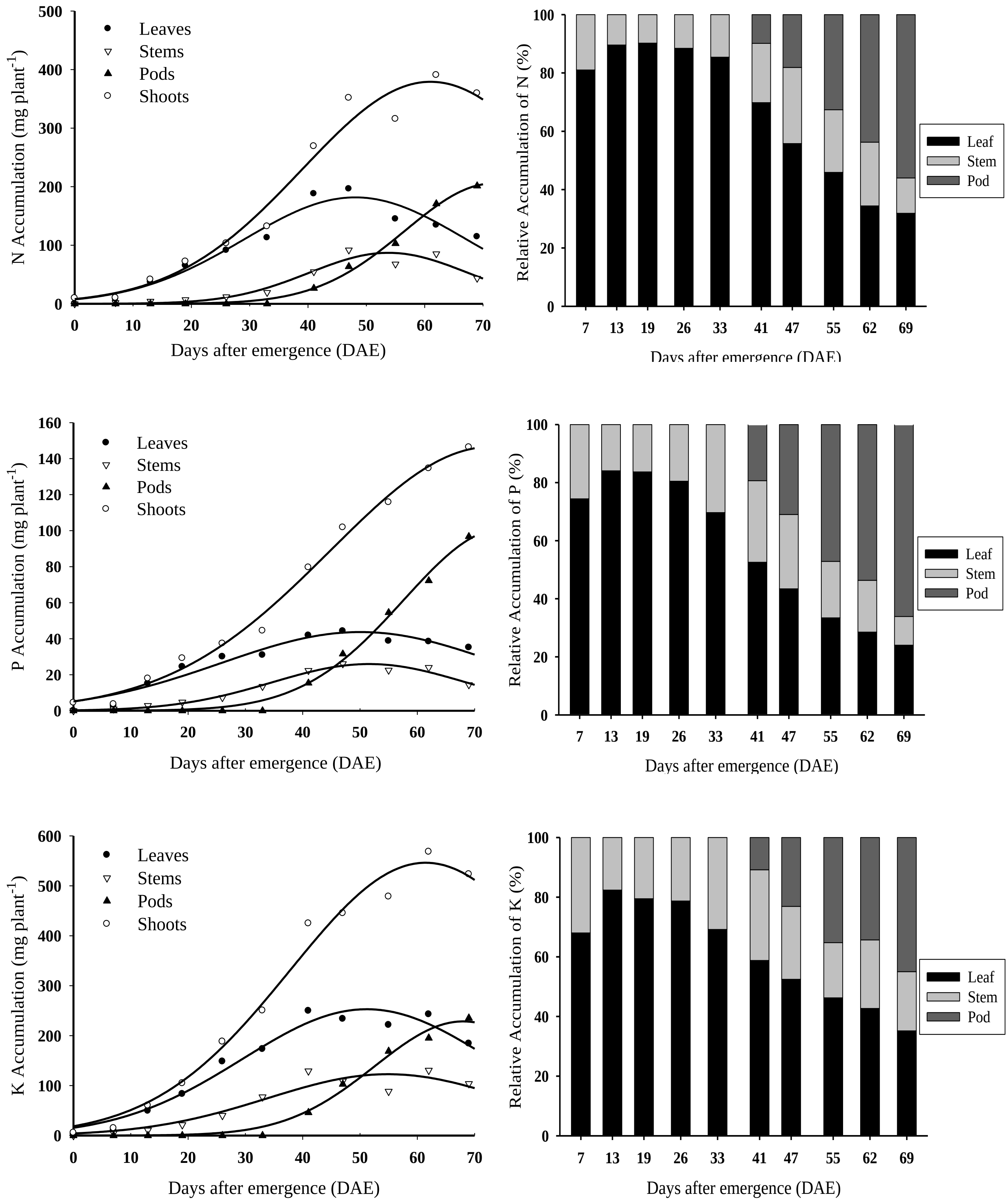


**Figure 2.** Accumulation curves of the primary macronutrients nitrogen (N), phosphorus (P) and potassium (K) and its percentage distribution between organs according to the development of determinate growth habit snap bean plant.

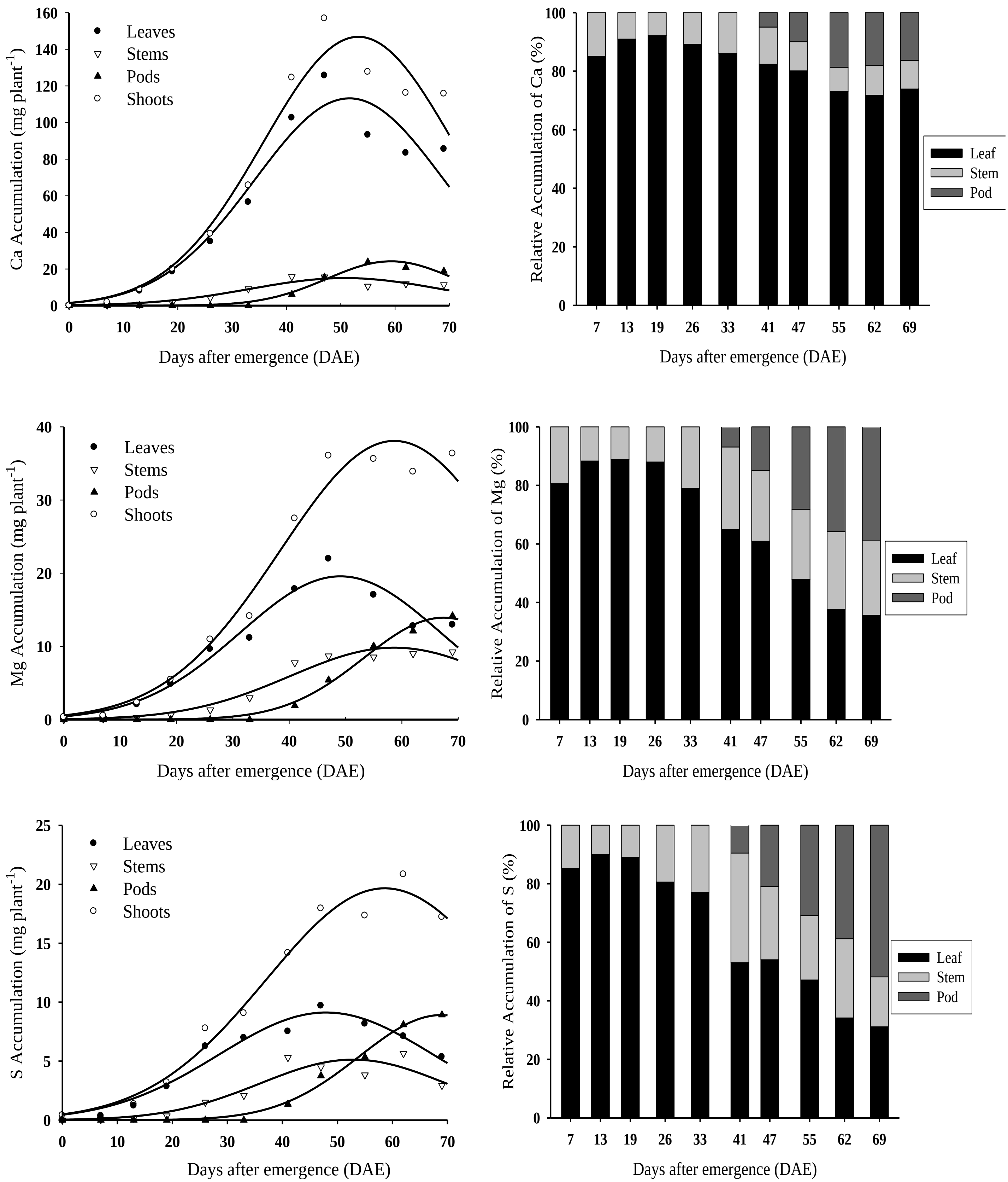


**Figure 3.** Accumulation curves of the secondary macronutrients, calcium (Ca), magnesium (Mg) and sulfur (S) and its percentage distribution between organs according to the development of determinate growth habit snap bean plant.

**Table 3.** Estimated parameters from the Gaussian model for the accumulation of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S) of different parts of the determinate growth habit snap bean plant during the development.

| Plant part | Estimated parameters of adjusted model [(1)] | | | PI [(2)] | $R^2$ |
|---|---|---|---|---|---|
| | $a$ | $b$ | $x0$ | $(x0 - b)$ | |
| | mg plant$^{-1}$ | Days after emergence | | | |
| | | | **Nitrogen** | | |
| Leaves | 181.72** | 19.03** | 48.12** | 29.09 | 0.95 |
| Stems | 87.07** | 13.65** | 53.81** | 40.16 | 0.93 |
| Pods | 205.59** | 15.23** | 71.85** | 56.62 | 0.99 |
| Shoots | 379.15** | 21.98** | 61.02** | 39.04 | 0.97 |
| | | | **Phosphorus** | | |
| Leaves | 43.71** | 24.25** | 50.05** | 25.80 | 0.94 |
| Stems | 25.94** | 16.97** | 51.59** | 34.62 | 0.98 |
| Pods | 101.36** | 17.66** | 75.27** | 57.61 | 0.99 |
| Shoots | 146.63** | 28.21** | 73.03** | 44.82 | 0.99 |
| | | | **Potassium** | | |
| Leaves | 252.87** | 21.68** | 51.19** | 29.51 | 0.97 |
| Stems | 122.79** | 21.06** | 54.88** | 33.82 | 0.90 |
| Pods | 228.48** | 15.47** | 68.02** | 52.55 | 0.99 |
| Shoots | 546.18** | 23.64** | 61.47** | 37.83 | 0.99 |
| | | | **Calcium** | | |
| Leaves | 113.20** | 17.43** | 51.59** | 34.16 | 0.94 |
| Stems | 15.04** | 17.53** | 50.83** | 33.30 | 0.90 |
| Pods | 24.19** | 11.79** | 59.27** | 47.48 | 0.98 |
| Shoots | 146.82** | 17.54** | 53.26** | 35.72 | 0.96 |
| | | | **Magnesium** | | |
| Leaves | 19.57** | 17.82** | 49.10** | 31.28 | 0.95 |
| Stems | 9.83** | 18.44** | 58.67** | 40.23 | 0.96 |
| Pods | 13.92** | 14.13** | 67.44** | 53.31 | 0.99 |
| Shoots | 38.08** | 20.26** | 58.67** | 39.41 | 0.98 |
| | | | **Sulfur** | | |
| Leaves | 9.13** | 19.55** | 47.94** | 28.39 | 0.96 |
| Stems | 5.13** | 16.92** | 52.87** | 35.95 | 0.89 |
| Pods | 8.91** | 14.96** | 68.95** | 53.99 | 0.99 |
| Shoots | 19.67** | 21.54** | 58.61** | 37.07 | 0.98 |

[1] $a$ = maximum accumulation (g plant$^{-1}$); $x0$ = value of $x$, in days after emergence (DAE), which proportionate the maximum accumulation; $b$ = amplitude of the interval of $x$ between the point of inflection and the value of $x0$. [2] PI = point of inflection. **Significant at 1% probability by *t* test.

**Table 4.** Amounts of macronutrients nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S) uptake in the shoots and exported by the pods of determinate growth habit snap bean plant, required for production of one ton of pod's fresh matter.

| Plant part | Macronutrient | | | | | |
|---|---|---|---|---|---|---|
| | N | P | K | Ca | Mg | S |
| | kg t$^{-1}$ of fresh pods | | | | | |
| Shoots | 13.15 | 5.08 [(1)] | 18.94 [(1)] | 5.09 [(1)] | 1.32 [(1)] | 0.68 |
| Pods | 7.01 | 3.30 [(2)] | 7.91 [(2)] | 0.60 [(2)] | 0.48 [(2)] | 0.31 |
| Exportation (%) [(3)] | 53.31 | 64.96 | 41.76 | 11.79 | 36.36 | 45.59 |

[1]Values equivalent to 11.66 kg $P_2O_5$; 22.82 kg $K_2O$; 7.12 kg CaO and 2.19 kg MgO; [2]Values equivalent to 7.57 kg $P_2O_5$; 9.53 kg $K_2O$; 0.84 kg CaO and 0.79 kg MgO; [3]Calculated dividing pods by shoots uptake and multiplied by 100.

## Conclusion

The uptake of macronutrients in growth habit snap bean follows the order K > N > Ca = P > Mg > S, while for the exportation rates, the order is K > N > P >> Ca > Mg > S. Except for Ca, the macronutrients are mainly in the pods at the end of the cycle.

The accumulation of all macronutrients in the shoots increased after the beginning of V4 stage, almost 20 days after emergence, and side dress fertilization, especially for N and K, should be done before that period.

## Conflict of interest

The authors have not declared any conflict of interest

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*Full Length Research Paper*

# Status of maize lethal necrosis in eastern Uganda

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**Maize lethal necrosis (MLN) is the latest emerging disease of maize in Sub-saharan Africa. It was first reported in Uganda in 2013. Consequently, information regarding its prevalence, yield loss, applicable mitigation measures and socio-economic effects is still scanty, hence this study. A survey involving 300 respondents on the status of the disease was conducted in seven districts of eastern Uganda. Results revealed that MLN became prevalent in eastern Uganda in the first season of 2012 (2012A), which stretches from March to June. The MLN disease symptoms, especially chlorosis, were prevalent in most of the farmers' fields in all the districts surveyed but particularly in Tororo, Mbale, Greater Sironko and Busia. Almost all farmers from the lowland areas reported MLN to be more destructive in the second season (B), which stretches from August to November. The disease can attack at any stage of crop growth from two weeks after planting. Very low yields (0.25 to 1.0 t ha$^{-1}$) attributed to the MLN epidemic, were obtained, causing an average yield loss of 50.5% valued at US$ 332 per hectare. Use of home saved seed especially in Busia, Tororo, Iganga and Mbale was common and partly explains the prevalence of MLN in these districts. Roguing emerged the most popular means farmers in eastern Uganda were using to control MLN. By intensifying MLN sensitization programmes, farmers will learn other management practices such as crop rotation, good sanitation practices and use of chemical sprays to control MLN. Finally expediting the breeding process for the development of MLN resistant or tolerant varieties will bring a lasting solution to the disease.**

**Key words:** Eastern Uganda, DAS-ELISA, maize chlorotic mottle virus (MCMV), maize lethal necrosis (MLN).

## INTRODUCTION

Maize (*Zea mays* L.) is the most important cereal crop in sub-Saharan Africa (FAO, 2006). In Uganda it is a key food security crop and a source of income for smallholder farmers with the eastern region accounting for over 50% of annual national production (USAID, 2010). The crop provides over 40% of the population's calorie requirements with an annual consumption of about 23 kg per capita per year (Magnay, 2004). The high, mid and low altitude areas of eastern Uganda grow different varieties of maize. This has greatly impacted on the economic growth and food security of most households. Production in the region is 1,108,554 MT as compared to the country's total production of 2,361,956 MT (MAAIF, 2011).

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However, maize on-farm yields no longer exceed 2.5 t ha$^{-1}$, against a potential of 5.0 to 8.0 t ha-1 (World Bank, 2006). The drop in yields has mainly been a result of the unpredictable weather patterns, scarcity of adapted varieties and emerging stresses such as the maize lethal necrosis (MLN). Therefore, to achieve adapted varieties for the zone, breeding initiatives need to target specific environments, and particularly address MLN which so far has been associated with 50 to 80% reduction in yield (ASARECA, 2013). The MLN results from combined infection by two viruses: maize chlorotic mottle virus (MCMV) and either maize dwarf mosaic virus (MDMV) or sugarcane mosaic virus (SCMV) or wheat streak mosaic virus (WSMV) (CIMMYT, 2004). Recent findings indicate that MCMV can be a threat on its own even in the absence of other viruses (Miano, 2014). Cabanas et al. (2013) reported that both MCMV and SCMV are seed transmitted though MCMV can also be transmitted by thrips, and SCMV by aphids and mechanical means. Other insect vectors associated with MCMV transmission and spread are maize rootworms, leaf beetles and leafhoppers. The virus may also be spread through soil and through infected plant debris since the virus can survive in plant residues (Nyvall, 1999). Continuous maize production in a field greatly increases the incidence of the viruses and vectors (Miano, 2014).

The disease was first reported in Kenya, Bomet county in 2011 at about 1900 m asl but had spread up to 2100 m asl in a period of about six months (Wangai et al., 2012). In Uganda, it was earlier reported in the eastern districts of Busia, Tororo, Iganga and Mbale (ASARECA, 2013; Plantwise, 2013) but with no concrete evidence especially regarding the prevalence, awareness, associated yield loss and socio-economic effects. The current study was therefore designed with the major objective of determining MLN current status in eastern Uganda. The specific objectives were to i) determine MLN prevalence in the different parts of eastern Uganda, ii) ascertain the yield losses associated with MLN and applicable mitigation measures.

## MATERIALS AND METHODS

### Study area, sampling size and design

The study targeted both small and large-scale maize farmers in seven districts of eastern Uganda, namely: Iganga, Busia, Tororo, Kapchorwa, Bukwo, Greater Sironko and Mbale. Notably, Greater Sironko, hereafter referred to as Sironko, is inclusive of the now independent Bulambuli district. A descriptive cross-sectional survey design using largely quantitative methods of data collection and analysis was adopted. However, it was supported by qualitative information obtained from key informants and document reviews. The study was conducted in eastern Uganda because the MLN epidemic is concentrated in this part of the country. A three-stage cluster sampling technique was used to select the 300 households based on existing reports on the presence of MLN in such districts. In the first stage of clustering, the districts of Iganga, Busia, Tororo, Kapchorwa, Bukwo, Sironko and Mbale were purposively selected. In the second stage of sampling, two subcounties were purposively selected from each district where cases of the disease were evident. The third stage involved random selection of 300 households from within the group villages based on the criteria agreed upon that such households were involved in maize cultivation at that time and at least for the previous year. A list of maize farmers within the villages was obtained from the agricultural extension officers and used for the random selection of respondents. On average, 43 farmers were interviewed from each of the seven districts. Interviews were conducted in a sub-county with highest reports of MLN incidences. Respondents were those with maize as one of the major crops on their farm, and particularly those who had planted maize on at least 250 m$^2$ in the second season (August to November) of 2014 and in the previous year (2013).

### Methods and procedure of data collection

The interview method supported by documentary review and analysis was used. The interview approach involved administering a structured questionnaire of both closed and open-ended questions to the respondents. The questionnaire was preferred because it is a quick way of data collection and it is easy to categorize, quantify and generalize the information. The questionnaire was pre-tested on ten households outside the randomly selected villages to ensure reliability and validity. Relevant documents with related information to the study at district and sub-county production offices were reviewed. These mainly comprised copies of reports submitted to the Ministry of Animal Industries and Fisheries (MAAIF). The major elements during interviews were: (i) maize production constraints with emphasis on the MLN epidemic, (ii) maize varieties grown and the source of seed, (iii) estimated maize grain yield and yield losses attributed to MLN (iv) MLN origin, symptoms and mitigation measures. Identification of MLN symptoms was aided by displaying photographs showing MLN infected plants.

Leaf isolates of about 15 cm beginning from the apex of the leaf were collected from maize fields suspected to be having the MLN disease. In total, about 42 leaf samples were collected from the seven districts, comprising on average six samples per district. The samples were packed in an 11.4 × 24 cm kraft brown envelopes and stored under cool conditions before conducting ELISA tests at the National Agricultural Research Laboratories (NARL) in Kawanda. The double antibody sandwich (DAS) ELISA technique (Hill, 1984) was conducted using a Model 680 microplate reader on the 17th June 2014. The instrument was set at a standard measurement filter of 405 nm. The samples were divided into two parts therefore making two replications. Using the DSMZ culture technology, an MCMV infected maize leaf and a clean leaf were used as positive controls and negative controls, respectively; also a buffer, making a total of 45 samples. Optical density (OD) values equal or above positive control were regarded as positive for the virus, whereas those with equal or less than that of negative control were taken as negative (no virus). In addition, OD values two times higher than the negative control were regarded as positive for the virus.

### Data analysis

Qualitative data were coded and analyzed using SPSS version 16.0 (SPSS 1989-2007). Chi-squared tests were used to compare performance between locations. The SPSS was also used to provide summaries of the farmer household's maize production characteristics such as frequencies and means. Mean results from ELISA tests were obtained per sample and compared with the positive and negative controls to deduce presence or absence of

**Table 1.** Farmers' sources of maize seed.

| District | Respondents (%) | | | | | |
|---|---|---|---|---|---|---|
| | Home saved (n=70) | Agro-input shops (n=178) | Neighbours/ friends (n=3) | Kenya (n=32) | Extension Agent (n=10) | Other sources (n=7) |
| Bukwo | 0.0 | 19.7 | 0.0 | 53.1 | 0.0 | 0.0 |
| Busia | 22.9 | 7.9 | 33.3 | 6.2 | 10.0 | 14.3 |
| Sironko | 1.4 | 20.8 | 0.0 | 3.1 | 40.0 | 0.0 |
| Iganga | 37.1 | 5.6 | 0.0 | 0.0 | 0.0 | 28.6 |
| Kapchorwa | 2.9 | 15.7 | 0.0 | 18.8 | 20.0 | 28.6 |
| Mbale | 18.6 | 23.0 | 0.0 | 0.0 | 10.0 | 28.6 |
| Tororo | 17.1 | 7.3 | 66.7 | 18.8 | 20.0 | 0.0 |
| Chisquare | 575.48 | | | | | |
| Prob. (df=42) | < 0.001 | | | | | |

**Table 2.** Most popular maize varieties planted in 2013.

| District | Respondents (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| | DK8031 (n=5) | Duma (n=4) | H614D (n=39) | Landrace (n=30) | Longe 5 (n=85) | Longe10 (n=49) | Others (n=42) |
| Bukwo | 0.0 | 0.0 | 61.5 | 0.0 | 0.0 | 0.0 | 4.8 |
| Busia | 80.0 | 25.0 | 0.0 | 16.7 | 10.6 | 6.1 | 14.3 |
| Sironko | 0.0 | 0.0 | 0.0 | 0.0 | 18.8 | 49.0 | 7.1 |
| Iganga | 20.0 | 0.0 | 2.6 | 40.0 | 16.5 | 6.1 | 16.7 |
| Kapchorwa | 0.0 | 50.0 | 35.9 | 0.0 | 8.2 | 0.0 | 11.9 |
| Mbale | 0.0 | 0.0 | 0.0 | 3.3 | 34.1 | 38.8 | 19.0 |
| Tororo | 0.0 | 25.0 | 0.0 | 40.0 | 11.8 | 0.0 | 26.2 |
| Chisquare | 1080 | | | | | | |
| Prob. (df=84) | P < 0.001 | | | | | | |

the MLN disease.

## RESULTS

### Social demographic trend of farmers and farming type

Of the 300 household heads visited, 60 were females. Majority of the respondents comprised farmers growing maize on a purely subsistence system (73%), followed by commercial producers (17.5%) and lastly the semi-commercial (9.5%). Most commercial farmers were found in Bukwo and Sironko (32%), while the subsistence farmers were mainly in Mbale (22%). Other than maize, the other popular crops in the districts visited were cassava, beans, sweet potatoes and groundnuts. Beans were by and large popular in Mbale (76.2%), cassava in Busia (39.3%), sweet potatoes in Iganga (100%), groundnuts in Busia and Tororo (50%).

### Source of maize seed and the maize varieties popular in the zone

Majority of farmers obtain maize planting seed from agro-input shops (59.3%) followed by use of home saved seed (23.3%). Notably, 10.7% of farmers especially in Bukwo, Kapchorwa and Tororo obtain planting seed from Kenya (Table 1). Other sources where farmers obtained seed include traders, middlemen and retail shops.

The most popular maize varieties planted in the year 2013 varied based on the region but with Longe 5, an open pollinated variety from Uganda, being the most popular (28.3%) across districts followed by Longe 10 (16.3%), a three-way cross hybrid from Uganda (Table 2). Other varieties were popular in specific districts such as H614D, a hybrid from Kenya, was popular in Bukwo and Kapchorwa (35.9 and 61.5%, respectively). The landraces were popular in Tororo and Iganga (40%) followed by Busia (16.7%) and Mbale (3.3%). None of the respondents admitted growing landraces in Bukwo, Sironko and Kapchorwa. Varieties such as Hybrid

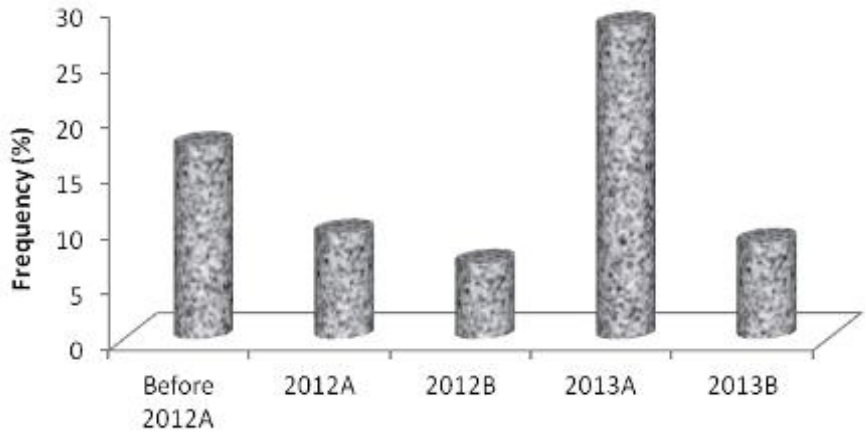


**Figure 1.** Season of the year MLN first observed.

405, 513H, 6213 & 624 and PAN 67 (not included in the table) were found in Tororo, Kapchorwa, Bukwo and Busia, respectively. Notably, all of these hybrids have their origin in Kenya except PAN 67, which is a South African variety though packed Kenya.

### Year MLN first observed and the current status

Majority of the farmers (28.2%) first encountered MLN in 2013A, which runs from March to June of each year (Figure 1). However, 17.4% of the farmers confessed observing the disease as early as the first season of 2012. This implies that the disease became prevalent in Uganda in 2012A. Currently, the disease is on farmers' fields but more destructive in the second season (August to November) than the first season. Notably, 10.6% of farmers either had no clue on when the disease began or had never observed the disease at all.

A section of farmers (51.8%) especially from Mbale and Bukwo reported that the disease is still on the increase. However, some farmers (11.8%) from Sironko and Tororo districts, earlier affected by the disease, reported a tremendous decline in MLN. Majority of the farmers (33.2%) reported having experienced the highest MLN yield losses during periods of drought. However, as regards the origin of the disease, a reasonable number of farmers (43.2%) expressed ignorance (data not shown). Other popular opinions about origin of MLN included seed from Kenya (15.8%), soil (14.3%) and use of home saved seed (12.1%).

### MLN symptoms and stage of maize at first observation of symptoms

Chlorosis was reported by 34.5% of the farmers as the most common symptom associated with MLN. Other recognizable symptoms were stuntedness (5%), dead heart and necrosis (2.9%), mottling of leaves (2.6%), and sterility or poor grain fill (1.1%). About 30.3% of the farmers reported MLN to start manifesting when the maize is at four weeks after planting (WAP), popularly referred to as the 'knee height' stage or the 7-8 leaves stage (Data not shown). Other prominent stages were booting (24.2%) and the tasseling stage (6.6%). Farmers averaging 3% reported observing MLN symptoms at germination (up to 2 WAP), grain filling and grain hardening stages.

### Area under maize in 2013, corresponding grain yields and yield losses

The study indicated that most farmers (43.3%) had only 0.41 to 0.5 ha of land under maize production (Table 3). Only 19% of the respondents, mainly from Bukwo and Sironko, had more than 1 ha of land under maize. Highest number of farmers with the least area under maize (< 0.4 ha) was found in Mbale (26.2%), Busia (23.8%) and Iganga (23.8%). Other than Bukwo and Sironko, Iganga was the only other district with farmers having more than 2 ha of land under maize.

Majority of farmers (43.4%) obtained an average yield of 0.63 t $ha^{-1}$ in the year 2013 (Table 4). Only 8.7% of farmers had yields exceeding 3.5 t $ha^{-1}$. These were mainly from the districts of Bukwo (28.9%), Sironko (11.9%) and Kapchorwa (5.0%). Iganga was the district where most farmers (42.1%) reported the least maize production per hectare as opposed to Bukwo where only 1.9% of farmers had yields below 0.25 t $ha^{-1}$. A greater number of farmers (54.5%) reported selling their maize between U. Shs. 500 to 650 per kilogramme, which gives an average price of U.Shs. 575.

The MLN disease symptoms were recorded in all the seven districts surveyed each registering minimal

**Table 3.** Area under maize in the year 2013 (average for one season)

| District | Respondents (%) | | | | | |
|---|---|---|---|---|---|---|
| | < 0.4 ha (n=42) | 0.41-0.5 ha (n=130) | 0.51-1.0 ha (n=71) | 1.1-1.5 ha (n=32) | 1.51-2.0 ha (n=19) | > 2.0 ha (n=6) |
| Bukwo | 4.8 | 13.1 | 14.1 | 53.1 | 26.3 | 16.7 |
| Busia | 23.8 | 10.0 | 11.3 | 12.5 | 0.0 | 0.0 |
| Sironko | 4.8 | 13.8 | 15.5 | 3.1 | 36.8 | 66.7 |
| Iganga | 23.8 | 16.9 | 7.0 | 0.0 | 0.0 | 16.7 |
| Kapchorwa | 2.4 | 13.1 | 19.7 | 12.5 | 21.1 | 0.0 |
| Mbale | 26.2 | 22.3 | 18.3 | 9.4 | 5.3 | 0.0 |
| Tororo | 14.3 | 10.8 | 14.1 | 9.4 | 10.5 | 0.0 |
| Chisquare | 497.089 | | | | | |
| Prob. (df=42) | < 0.0001 | | | | | |

**Table 4.** Summary of maize yields, yield losses and financial losses incurred due to the MLN epidemic.

| Grain yield ($t\ ha^{-1}$) in 2013 | Mean grain yield ($t\ ha^{-1}$) | [1]Grain yield loss due to MLN ($t\ ha^{-1}$) | [2]Monetary loss (Ug. Shs.) | Monetary loss (US dollars) | Farmers (%) |
|---|---|---|---|---|---|
| <0.25 | 0.25 | 0.13 | 72,594 | 29.0 | 15.8 |
| 0.25-1.0 | 0.63 | 0.32 | 182,936 | 73.2 | 43.4 |
| 1.1-2.0 | 1.6 | 0.81 | 464,600 | 185.8 | 18.9 |
| 2.1-3.5 | 2.8 | 1.41 | 813,050 | 325.2 | 13.1 |
| 3.6-4.5 | 4.1 | 2.07 | 1,190,538 | 476.2 | 4.7 |
| 4.6-5.5 | 5.1 | 2.58 | 1,480,913 | 592.4 | 1.0 |
| > 5.5 | 5.5 | 2.78 | 1,597,063 | 638.8 | 3.0 |
| Average per ha | 2.9 | 1.4 | 828,813 | 331.5 | 14.3 |

Calculations based on data from respondents. Note 1US$ = 2500 U. Shs. at time of the study.[1] Calculated using mean of 50.5% yield loss per hectare attributed to MLN. [2]Calculated using mean of U. Shs.575 per kg (US $ 0.23) as the average farm-gate price of dry maize grain in eastern Uganda at the time of the study.

variations. Key to note is that most farmers (38.6%) reported having experienced yield losses ranging from 31 to 70% as a result of the MLN disease (data not shown), giving a mean yield loss of 50.5%. There were also incidences of over 90% yield loss reported by 5.5% of the farmers, mainly from Nabongo sub-county in Sironko district. Precisely, the most MLN affected districts were Sironko and Tororo both with over 60% of farmers reporting above 50% yield losses due to MLN. Therefore, average grain yield loss as a result of the MLN disease in the year 2013 was 1.4 t/ha, which translates into a monetary loss of US$ 332 for each hectare of maize planted.

## MLN mitigation measures

Most respondents (51.4%) mentioned roguing as the commonest mitigation measure being used against MLN (Table 5). The practice was found commonest in Tororo (65.7%) and Mbale (64.9%). However, 20.6% of respondents, especially in Kapchorwa and Bukwo, had not started taking any precautions against MLN. Other methods being used to curb MLN but on a small scale include chemical sprays, fertilizer boosts, crop rotation and the use of certified seed. Farmers in Tororo particularly mentioned that they no longer grow a lot of maize in the second season (August - November), having experienced too much MLN in the second season of 2013. They hence associated the disease to the short rain season and long dry spell which occurs during this period of the year.

## Source of information on MLN and sensitization measures so far undertaken

Most farmers (75.8%) reported having no source of information on MLN (data not shown). Some farmers acknowledged receiving information on MLN from agriculture extension agents (11.3%), and the print and electronic media (10%). Other sources of information

**Table 5.** MLN mitigation measures.

| Variables | Respondents (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Bukwo | Busia | Sironko | Iganga | Kapchorwa | Mbale | Tororo | Mean |
| Chemical sprays | 17.3 | 2.9 | 9.3 | 0.0 | 0.0 | 5.3 | 2.9 | 5.4 |
| Roguing | 26.9 | 45.7 | 46.5 | 57.9 | 52.5 | 64.9 | 65.7 | 51.4 |
| Fertiliser boosts | 0.0 | 2.9 | 2.3 | 2.6 | 2.5 | 3.5 | 0.0 | 2.0 |
| Crop rotation | 0.0 | 8.6 | 2.3 | 0.0 | 0.0 | 3.5 | 8.6 | 3.3 |
| Use of certified seed | 0.0 | 2.9 | 0.0 | 7.9 | 0.0 | 0.0 | 0.0 | 1.5 |
| None | 32.7 | 17.1 | 16.3 | 5.3 | 37.5 | 21.1 | 14.3 | 20.6 |
| Others | 0.0 | 0.0 | 11.6 | 0.0 | 2.5 | 0.0 | 5.7 | 2.8 |
| Chisquare | 349.2 | | | | | | | |
| Prob. (df=49) | P < 0.0001 | | | | | | | |

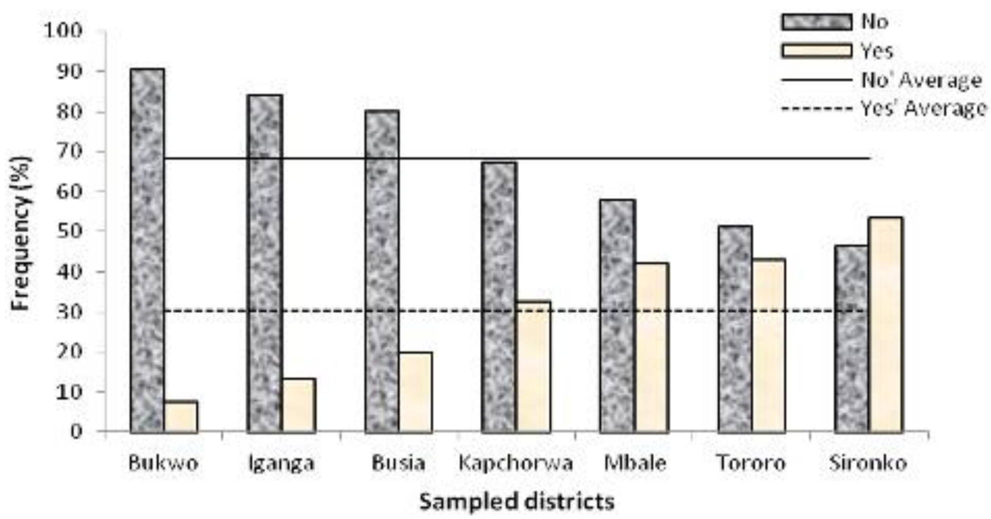


**Figure 2.** Farmers' response on whether they have got any sensitization on the MLN disease.

were the neighbours, friends, traders and politicians. Most farmers (68.3%) reported having received no sensitization on the MLN disease (Figure 2). Majority of these were from Bukwo, Iganga and Kapchorwa, respectively. Only 30.3% of farmers confirmed having received some sensitization on the MLN disease, most of whom (53.5%) were from Sironko, Tororo and Mbale. Interestingly, it is Sironko where the disease is most rampant.

### Results from the leaf ELISA test

Of the seven sets of samples tested, MCMV was confirmed in four sets from Mbale (Busiu subcounty), Busia (Busitema subcounty), Tororo (Mella subcounty) and Sironko (Nabongo subcounty) (Table 6; Figure 3). Though no MCMV was confirmed from Bukwo, results obtained had an OD value above that of the negative control, which necessitates a confirmatory test during an MLN pick period in these locations.

## DISCUSSION

In the traditional African setting, it is normally the man who talks to visitors, and by default is the head of the household. It is for this reason that majority of the respondents were men though women (80%) are the most involved in farming in sub-Saharan Africa (AfricaRenewal, 2014). Eastern Uganda contributes 50% of the total maize production in Uganda (UBOS, 2011). It is therefore not surprising that it was ranked as the most important crop of all households visited, and almost 99% of the households had planted maize in the last two years. According to Gibson et al. (2005), though farmers may buy good seed from agro-input shops, they grow them for many cropping cycles leading to a breakdown in

**Table 6.** MLN sample results following DAS-ELISA test based on presence of MCMV.

| Sample Location | S/county | Altitude (m) | Latitude | Longitude | Mean reading (405 nm) | Deduction |
|---|---|---|---|---|---|---|
| Positive control | | | | | 0.390 | + |
| Negative control | | | | | 0.125 | - |
| Buffer | | | | | 0.111 | - |
| Mbale | Busiu | 1152 | 0°55’33.3’’. | 34°10’24.6’’. | 0.474 | + |
| Sironko | Town Council | 1260 | 1°13’31.5’’ | 34°14’31.1’’ | 0.427 | + |
| Busia | Busitema | 1125 | 0°33’41.5’’ | 33°59’13.7’’ | 0.333 | + |
| Iganga | Buyanga | 1107 | 0°40.027. | 33°38’11.5” | 0.130 | - |
| Tororo | Mella | 1155 | 0°39’47.3’’ | 34°15’7.5’’ | 0.462 | + |
| Bukwo | Riwo | 1762 | 1°19’27.3’’ | 34°47’33.5’’ | 0.145 | - |
| Kapchorwa | Kawowo | 1110 | 1°21’54.3’’ | 34°19’53.9’. | 0.092 | - |

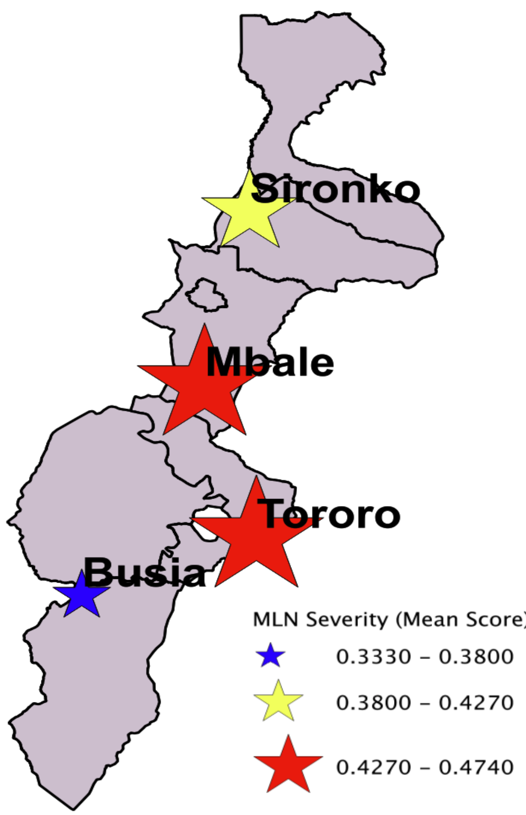


**Figure 3.** Map of eastern Uganda showing districts with highest MLN severity scores.

resistance to various stresses as a result of out-crossing in the field. Similarly, Abalo (2007) reported that peasant farmers in eastern Uganda rely on the landraces as planting seed. The popularity of Longe 5, an open pollinated variety, in the sampled households is because it is cheaper compared to hybrid maize but not necessarily the highest yielding.

Notably, the popularity of the Kenyan hybrids, especially H513 was truncated by the MLN epidemic. Cultivar H614D is still the most popular hybrid in the highland areas of eastern Uganda, namely Bukwo and Kapchorwa districts. These regions also had the least incidence of MLN at the time of the survey. However, H614D is not resistant to MLN but probably it is a bit more tolerant compared to the landraces and other varieties like H513, H520, Duma43 and DK8031, which are equally popular in the region (Das et al., 2015). Almost all respondents had abandoned the growing of hybrid H513 after being informed by MAAIF agents in 2013 that the variety is very susceptible to MLN. Farmers testified that they actually got very low yields of H513 in 2013. However, according to the Joint Assessment Report (2012), all commercial varieties are affected by MLN, of course not excluding plants from farmers' own saved seed.

The MLN symptoms observed by farmers in the study districts are similar to those earlier reported by Makumbi and Wangai (2012). However, mild mosaic or mottling symptoms and a moderate reduction of growth might be due to single infections of MCMV or SCMV. According to DSMZ (2014), in the case of mixed infections, early infected plants appear stunted and show a general chlorosis, leaf bleaching and necrosis. In addition, from the results, MLN can attack at any stage of crop growth since at every stage, right from two weeks after planting, farmers observed the MLN symptoms. This corroborates with findings from Joint Assessment Report (2012) that the disease attacks crops at all stages of growth. They further add that drought conditions enhance disease expression.

The area under maize of 0.41-0.5 ha per household was quite high compared to a national average of 0.31 ha (Okoboi, 2010). This indicates that farmers are interested in maize production despite the numerous production constraints. The corresponding yields of 0.25 to 1.0 t $ha^{-1}$ were too low compared to average yields of 2.2 t $ha^{-1}$ in eastern Uganda (Okoboi, 2010), and a potential of 4.5 to 7.0 t $ha^{-1}$ in Uganda (UBOS, 2011). The low yields were attributed to the MLN epidemic in the period of the study, which caused an average yield loss of 50.5% valued at US$ 332 per hectare. Other constraints such as use of inferior seed, inadequate rains, poor soil fertility and a plethora of pests and diseases could have exacerbated the MLN epidemic in some of the districts, especially Iganga, Busia, Tororo and Mbale. ASARECA (2013) reported MLN to cause yield losses of 50 to 80% in Uganda, which is within the range obtained in the current study.

Though roguing emerged the most popular means farmers in eastern Uganda are using to control MLN, it cannot be used in isolation. According to Nelson et al. (2011), most effective control involves an integration of cultural practices with insecticides and host resistance. Crop rotation for at least two seasons with alternative non-cereal crops has been reported to effectively control MCMV (Uyemoto, 1983). A great section of farmers (23.3%) reported using home saved seed for planting, which presents a big risk of MLN infection. According to Wangai et al. (2012), farmers should plant certified seed only instead of recycling seed. Sensitization programmes, however, need to be intensified since over 70% of farmers reported having no information on MLN.

The presence of MCMV in Mbale, Busia, Tororo and Sironko following the DAS-ELISA tests is a confirmation of presence of MLN in these regions, as earlier reported by ASARECA (2013) and Plantwise (2013), and the farmers interviewed in the current study. Other affected districts though not covered in this study but through personal observation include Kween, Bugiri, Budaka, Butaleja and Mayuge. Efforts to curb the epidemic have concentrated on sensitization of farmers on the good agronomic practices, and breeding for resistant varieties. The ministry of Agriculture should however, not relent on testing for MCMV in all seed coming into the country. There is also need to collect more isolates and test for MLN during the pick periods of the disease.

## Conflict of interests

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# Agro-physiologic effects of compost and biochar produced at different temperatures on growth, photosynthetic pigment and micronutrients uptake of maize crop

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**The production and use of biochart and compost present many opportunities for soil improvement and agricultural productivity. However, the yield and performance of biochar depend on the feedstocks, pyrolysing temperatures and rate of application. Experiments were conducted to find out the effect of compost and biochar produced from two different feed stocks (Rice husk and Mexican sunflower) and pyrolysed at different temperatures (300, 350 and 400°C) on the growth, yield, nutrient uptake and chlorophyll contents of maize (*Zea mays* L.,). These were applied at three levels (5, 10 and 15 ton/ha) and the pots were laid out in a Completely Randomized Design (CRD) with four replicates. Data were collected on growth and yield attributes of maize, photosynthetic pigments and nutrient uptake by maize crop.The results showed that the feedstock pyrolyzed at temperature between 300 to 350°C and compost applied at higher rate between 10 to 15 ton/ha performed better. On the growth and yield parameters, compost and biochar at relatively low temperature and applied at 15 t/ha performed better than other treatments including control both at the main and residual experiments On the residual effect, the two types of biochar performed better than compost most especially sunflower biochar pyrolysed at 300 and 350°C and applied at 15 t/ha. The chlorophyll formation was enhanced more in maize treated with higher rates of biochar than lower rates. The result indicates that depending on feedstock, biochar and compost have potentials to serve as nutrient sources.**

**Key words:** Biochar, bioenergy, chlorophyll, pyrolysis, plant nutrition, photosynthesis.

## INTRODUCTION

Conversion of agricultural wastes to soil amendments is now gaining attention worldwide as a sustainable method of waste management and is accepted as a good soil management practice for sustainable crop production. It

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helps in improving soil fertility through the modification of soil physical, chemical and biological properties (Haering and Evanylo, 2005). This in turn leads to the production of high quality food crops (Asuegbu and Uzo, 1984) through improved crop nutrient use efficiency (Tiessen et al., 1994; Bol et al., 2000; Diels et al., 2004). Several research findings have shown that improving plant nutrition through the use of organic amendments for sustainable crop production is a promising approach (Quansah, 2000; Basha et al., 2005).

Recently, the use of carbon rich organic amendments called black carbon or biochar for boosting soil fertility, sequestering atmospheric carbon and reducing the impact of agriculture on the environment is being promoted. Biochar is a kind of charcoal made from the pyrolysis of a range of biomass or feed stocks (Novak et al., 2009). It is the porous carbonaceous solid produced by thermo-chemical conversion of organic materials in oxygen depleted atmosphere. This has physiochemical properties suitable for safe and long-term storage of carbon in the environment and for soil improvement (Steinbeiss et al., 2009). Biochar in contrast to other organic manure or fertilizers is rich in carbon and is therefore not being easily susceptible to biological degradation. This makes it more stable and gives it the ability to stay longer in the soil (Skjemstad et al; 1996; Goldberg1985; Schmidt and Noack, 2000; Pessenda et al. 2001; Krull et al. 2006; Lehmann and Rondon, 2006).

Though, not like other fertilizers in term of soil fertility improvement, but the ash from biochar could have strong effect on yields after application (Chan et al., 2008). It can supply nutrients such as calcium, potassium and magnesium to the plants and also retain nutrients for plant uptake. Its application helps farmers in several ways; it reduces the amount of fertilizer needed, slowly releases nutrients to plants and improves soil moisture retention thereby securing the crops against drought. Biochar can also be used in all types of agricultural systems and unlike manure that emits methane into the atmosphere, biochar, reduces the greenhouse gases in the environment by increasing soil carbon sequestration thereby reducing atmospheric $CO_2$ concentrations (Laird, 2008; Liang et al., 2008; Woolf et al., 2010). Compared to other soil amendments, the high surface area and porosity of biochar enable it to adsorb or retain nutrients, water and provide a habitat for beneficial micro-organisms to flourish (Glaser et al., 2002; Lehmann and Rondon, 2006; Warnock et al., 2007).

Biochar production, yield and quality however pose a lot of challenges to its use and applicability. Biochars generally, are produced from a range of organic materials and under different conditions resulting in products of varying properties (Baldock and Smernik, 2002; Nguyen et al., 2004; Guerrero et al., 2005) but the ability of biochar to store C and improve soil fertility depends on its physical and chemical properties. This in turn varies based on the pyrolysis process and the choice of feedstock. The conditions under which a biochar is produced greatly affect its relative quality as a soil amendment (McClellan et al., 2007, McLaughlin et al., 2009). For instance, at high temperatures (400 to 700°C) according to Lehmann and Baldock (2002), biochar has fewer ion exchange functional groups due to dehydration and decarboxylation and this limits its usefulness in retaining soil nutrients. On the other hand, biochars produced at lower temperatures (250 to 40°C) have higher yield recoveries and contain more C=O and C-H functional groups that can serve as nutrient exchange sites after oxidation (Glaser and Lehmann, 2002). Moreover, biochars produced at these lower pyrolysis temperatures have more diversified organic character, including aliphatic and cellulose type structures which may be good substrates for mineralization by bacteria and fungi which have an integral role in nutrient turnover processes and aggregate formation (Thompson, 1978).

Feedstock selection also has a significant influence on biochar surface properties (Downie and Crosky, 2009) and its elemental composition (Amonette and Joseph, 2009). Meanwhile, the extent to which source of feedstock and pyrolysing temperature could increase the efficiency of applied biochar in sustaining soil and crop productivity has not received much research attention. Since, both feedstock and pyrolysis conditions affect physical (Downie and Crosky, 2009) and chemical properties likewise quantity and quality of biochar, there is need therefore to study the effect of pyrolysis temperature and processing time on biochar yield from different feedstocks as well as their consequent effects on crop production.

Similarly, compost is also considered as a valuable soil amendment for centuries. There is greater awareness that using composts is an effective way of increasing healthy crop production, reduce overdependency on chemical fertilizers and conserve natural resources (Storey, 1995; Epstein, 1997). The decomposition process converts potentially toxic or putricible organic matter into a stabile non-toxic product for soil improvement and plant growth. Compost fertilizer has been used as mulch for weed control as well as soil fertility improvement (Roe et al., 1997). This study was therefore conducted to determine the optimum temperature for pyrolysing different feedstocks (Rice husk and dry Mexican sunflower) on biochar yield and efficiency as well as assessing the variations in gaseous emission during pyrolysis in relation to different feed stocks and different temperatures. Effects of biochar made from different feed stocks and compost made from Mexican sunflower and poultry manure were also compared on soil fertility improvement and crop production. Maize which is one of the most important cereal crops world-wide was used as test crop for this study. In Nigeria, the cultivation of maize is very popular for its high productivity and diversity of use. The average yield of maize is considerably reduced due to decline in soil fertility and unfavourable climatic conditions. Soil fertility improvement through the use of biochar and compost would help in boosing maize production.

## METHODOLOGY

The experiments which consisted of first and residual trials were carried out at the roof top of the Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, Oyo state from March to November, 2014. The geographical location is 7° 24'N, 3° 54'E, and Elevation 234 m above sea level. The soil for the experiment was first homogenized and sieved before distributing them into 5 L capacity experimental pots with each containing 5 kg soil. Representative samples were taken for laboratory analysis. In the laboratory, the soil sample were air-dried, crushed using ceramic mortar and pestle and then sieved through a 2 mm mesh and the pre-cropping physicochemical properties of the soil were determined. Soil pH was measured in a 1:1 soil-water ratio using a glass electrode (H19017 Microprocessor) pH meter. Soil organic carbon was determined by the modified Walkley-Black method as described by Nelson and Sommers (1982). Total nitrogen was determined by macro-kjedahl method. Available P by Bray one method as described by Bray and Kurtz (1945). Exchangeable bases (calcium, magnesium, potassium and sodium) in the soil were determined in 1.0*N* ammonium acetate ($NH_4OAc$) extract. The results showed that the soil was slightly acidic (6.8) but it is within the pH range for agricultural production. The organic matter, total nitrogen and available phosphorus were 21.5, 1.01% and 60.3 g/kg respectively. Ca, Mg, K, and Na were 3.29, 0.54, 0.27 and 0.14 cmol/kg respectively. Mn, Fe, Cu and Zn were 73.9, 73.8, 0.30 and 1.8 mg/kg respectively.

### Biochar and compost production

Biochar was produced from two types of feedstocks (Rice husk and dried Mexican sunflower). Rice husk was obtained from AfricaRice at International Institute of Tropical Agriculture (IITA) Ibadan Oyo state. The pyrolysis was carried out using the biochar reactor fabricated by the Department of Mechanical Engineering, University of Ibadan Oyo state. The reactor was allowed to warm for about 30minutes so as to gain heat energy. Then 10 kg of the rice husk was put in a piece of pyrolysis apparatus which consisted of a stainless reactor of 500 mm length with a 150 mm inside diameter. The rice husk was then heated at different temperature; which are 300, 350 and 400°C. This temperature was regulated by the thermocouple connected with the reactor. The gas analyzer was also used for analyzing and recording all the gases emitted during the pyrolysis process. Since the rice husk pyrolysis was being carried out at different temperature, at the end of each pyrolysis of a particular temperature, the thermostat stopped and it was allowed to cool for 30 min before opening so as to reduce the rate of burning when exposed to atmosphere. The biochar produced was then collected through an open channel in a tray and spread for further cooling. This process was used for all the biochar produced at different temperatures. The pyrolysis temperatures tested in this study can be classified as "slow pyrolysis" which is meant for soil improvement for planting.

### Production of Mexican sunflower (*Tithonia diversifolia*) biochar

The Mexican sunflower plant was obtained from the crop garden of Crop Protection and Environmental Biology, University of Ibadan Oyo state. The plant was manually cut into 2 mm before pyrolyzing. The pyrolysis was carried out as described for rice husk. The Biochar moisture content was measured by oven drying a sub sample of 2 g at a temperature of 80°C for 24 h.

### Composting

Compost was made from Mexican sunflower (*Tithonia diversifolia*) and poultry manure. The materials were laid out in ratio 3:1 of plant materials to poultry manure (on dry weight basis) after sorting and chopping using Partially Aerated Composting Technique (PACT-2) proposed by Adeniran et al. (2001). The heap was left to decompose for 3 months to allow for the proper decomposition of the organic materials for plant growth. Continuous turning and watering was done to quicken the decomposition rate, after which the matured composts were evacuated from the heap, air-dried, shredded and samples taken to the laboratory for physico-chemical analysis.

### Chemical analysis of compost and biochar

The organic materials used in this experiment were characterized by determining their pH, organic carbon, exchangeable bases, and macro and micronutrients according to the method described by Ahmedna et al. (1997). pH was measured with a pH-meter (Jenway 3305). Total C was done using the method described in ASTM D 3176 (ASTM, 2006) and the total P was read with a spectrometer (Vitatron). Calcium, magnesium, potassium, sodium, manganese, iron, copper and zinc in the compost and biochar were determined using the Atomic Absorption Spectrometer (AAS). Sodium (Na) and Potassium (K) were read on a flame photometer. The percentage of oxygen that was generated during the pyolysis was recorded and stored in gas analyzer, the efficiency of the reactor during the pyrolysis was also determined with the use of gas analyzer. The percentage of the carbondioxide and other gases emitted during the pyrolysis was also monitored and saved in the gas analyzer.

### Experimental procedure and treatments

The pots were arranged in Complete Randomized Design (CRD) replicated four times. The treatments consisted of three levels of compost (5, 10 and 15t/ha), three levels of rice husk and sunflower biochar (5, 10 and 15 t/ha) at 300, 350 and 400°C. The treatments were denoted as, Control (No compost or biochar), Compost at 5, 10 and 15t/ha as CR1, CR2 and CR3 respectively, while rice husk biochar as RR1, RR2 and RR3, Sunflower biochar as SR1, SR2, SR3 for temperatures T1, T2 and T3 respectively. The organic materials were mixed thoroughly with soil according to treatment rate for one week before planting of maize.

### Data collection

The data collection commenced two weeks after planting and data were collected on vegetative and yield parameters of maize, nutrient uptake as well as chlorophyll contents. The plant nutrient uptake was determined using standard procedures while chlorophyll contents were determined using SPAD meter as well as the method described by Sarropoulou et al. (2012) with slight modifications. 1 g of fresh leaves was placed in 25 ml glass tubes and 15ml of 96% (v/v) ethanol was added to each tube. The tubes with the plant material were incubated in a water bath at a temperature of 79.8°C until complete discoloration of sample, after

**Table 1.** Biochar yield under different temperature.

| Biomass | Temperature (°C) | Input (kg) | Duration (min) | Output (kg) | Efficiency (%) |
|---|---|---|---|---|---|
| Rice husk | 300 | 10 | 30 | 2.80 | 28 |
| Rice husk | 350 | 10 | 30 | 2.20 | 22 |
| Rice husk | 400 | 10 | 30 | 3.5 | 35 |
| Sunflower | 300 | 2.5 | 30 | 1.55 | 62 |
| Sunflower | 350 | 2.5 | 30 | 0.85 | 34 |
| Sunflower | 400 | 2.5 | 30 | 1.50 | 60 |
| Sunflower | 300 | 2.5 | 30 | 0.92 | 37 |
| Sunflower | 350 | 2.5 | 30 | 0.82 | 33 |
| Sunflower | 400 | 2.5 | 30 | 0.92 | 37 |

about two to three hours. The absorbance of chlorophylls a and b was measured at 665 and 649nm respectively using visible spectrophotometer. Total chlorophyll was determined from the equations shown below:

Chl (*a*+*b*) = 6.10* A665 + 20.04*A649) * 15/1000/F.W (mg/g F.W)

The residual experiment was carried out after the first planting in order to determine the residual effect of the treatments on maize growth.

## RESULTS

### Biochar yield under different temperatures

The yields recorded after pyrolysing different feedstocks under different temperatures showed that the system was able to pyrolyze between 2.5 and 10 kg of biomass (Mexican sunflower and rice husk) with appropriate and moderate yield output of about 1:4 ratio per run. The relative yield of products from biochar varies with temperature, feedstocks and other factors.Temperature of 400°C produces more char from rice husk while 300 and 400°C were more effective in the case of sunflower biochar (Table 1).

### Biochar and compost nutrients analysis

The physical and chemical properties of biochar produced from rice husk and Mexican sunflower under different temperatures of 300, 350 and 400°C showed that different pyrolysing temperatures and feedstocks influenced the nutrient compositions of biochar. Variations were observed in the carbon contents and concentrations of primary macro and micro nutrients which are essential for soil fertility improvement. The carbon content of Rice husk biochar was the highest (53 g/kg) under the pyrolysing temperature of 350°C followed by that of 400°C and the carbon content at 300°C was the lowest for Rice husk. Similarly, Fe and Mn were more in Rice husk under this temperature than other materials. The carbon content, potassium, calcium iron and manganese concentrations of Sunflower biochar however increased with increasing temperature and the highest concentrations were found at 400°C. However, it was observed that the dried compost contain high amount of primary nutrients and carbon content when compared to biochar. Organic Carbon content of the *Tithonia diversifolia* dry compost was 63g/kg. Nitrogen, Phosphorus and Calcium were 1.46%, 1.20 and 2.87 cmol/kg respectively. These analytical results revealed that both biochar and compost are good soil amendments to improve crop yield (Table 2).

### Growth, yield, Chlorophyll content and micronutrient uptake by maize crop

There was a general increase in growth parameters of maize crop grown on soil amended with either compost or biochar throughout the growing period compared to control. However, there were variations among the treatments and application rates. For instance, among the compost rates CR3 recorded the highest mean values for plant height and leaf area. The trend was similar in other amendments with higher rate performing better than the lower rate. In the case of temperature, rice husk pyrolysed at 300°C and applied at 15 t/ha (RT3R3) and ST3R2 (sunflower biochar pyrolysed at 400°C and applied at the rate of 10 t/ha) increased the plant height and leaf area more than other treatments. It was observed that soil amended with RT1R3 (rice husk biochar 300°C at 15 t/ha) had the highest mean value (86.13 cm) for the plant height which was significantly different ($P \leq 0.05$) from every other treatments while sunflower biochar pyrolysed at 400°C and applied at the rate of 15t/ha gave the highest mean value for leaf area. The least mean value was recorded in maize crop treated with rice husk biochar (400°C) at the rate of 15 t/ha. The number of leaves produced by the maize plant in the soil treated with sunflower biochar pyrolysed at 350°C and

**Table 2.** Chemical properties of compost and Biochar produced from Rice husk and Mexican Sunflower under different temperature.

| Properties | Compost | RT1 | RT2 | RT3 | ST1 | ST2 | ST3 |
|---|---|---|---|---|---|---|---|
| Carbon (%) | 63 | 14 | 52 | 36 | 11 | 11 | 16 |
| Total Nitrogen (%) | 1.46 | 1.63 | 1.99 | 1.32 | 0.79 | 0.74 | 0.53 |
| **Exchangeable base (cmol/kg)** | | | | | | | |
| Potassium | 0.62 | 0.53 | 1.34 | 0.86 | 2.11 | 2.24 | 2.66 |
| Calcium | 2.87 | 0.02 | 0.84 | 0.09 | 1.44 | 0.81 | 1.64 |
| Magnesium | 0.03 | 0.02 | 0.02 | 0.02 | 0.04 | 0.02 | 0.02 |
| Sodium | 0.10 | 0.02 | 0.07 | 0.03 | 0.08 | 0.06 | 0.07 |
| Phosphorus | 1.20 | 0.42 | 0.71 | 0.80 | 1.09 | 0.91 | 0.98 |
| **Extractable micronutrient (mg/kg)** | | | | | | | |
| Iron | 1285.00 | 276.00 | 2230.00 | 366.50 | 139.50 | 106.00 | 187.50 |
| Zinc | 6.00 | 4.50 | 6.90 | 5.20 | 8.00 | 10.20 | 5.50 |
| Copper | 19.45 | 43.15 | 4.15 | 1.85 | 10.55 | 5.60 | 8.40 |
| Manganese | 276.50 | 81.50 | 310.00 | 175.50 | 93.00 | 59.50 | 123.00 |

RT1= Rice husk biochar at 300°C, RT2= Rice husk biochar at 350°C, RT3= Rice husk biochar at 400°C, ST1 = Sunflower, biochar at 300°C, ST2= Sunflower biochar at 350°C, ST3= Sunflower biochar at 400°C

applied at the rate of 15 t/ha recorded the highest mean values (Table 3)

On biomass production, soil amendment with RT2R2, ST3R3, ST1R3 and CR3 gave the highest shoot fresh weights which were significantly different (P≤ 0.05) from others including control. The root fresh weight was enhanced by the application of RT1R3, ST1R3 and ST3R3. These treatments also gave the highest shoot and root dry weight compared to all other treatments. Rice husk biochar 300°C at the rate of 15t/ha (RT1R3) recorded the highest root dry weight which was not significantly different (P≤ 0.05) from sunflower biochar 400°C at 15 t/ha (Figure 1a and b). On the chlorophyll content, rice husk generally performed better than sunflower biochar and compost. The chlorophyll content of maize grown on the soil amended with rice husk which was pyrolysed at 400°C and applied at the rate of 5 t/ha (RT3R1) was the highest compared to other treatments. This was followed by RT3R2, RT1R2 and RT1R3 though, with variations based on different temperatures and application rate. The addition of RT1R1, RT1R3, RT3R2, ST1R3 and ST2R2 increased the chlorophyll contents of maize crop compared with control. When comparing the chlorophyll content of maize crop from soil treated with compost, sunflower biochar and rice husk biochar at different rate and temperature, sunflower biochar at 300°C at 10 t/ha (ST1R2) gave the lowest chlorophyll concentration (Figure 2).

Micronutrient uptake by maize was also enhanced by biochar and compost. Rice husk biochar pyrolysed at 400°C and applied at 15 t/ha increased Zn accumulation in maize more than other treatments. This was followed by those of RT2R1, ST1R3, ST2R3, ST3R1 and ST3R2. They were all significantly different from those treated with compost and control. No significant difference was observed in the zinc concentration of the maize treated with RT2R1, RT2R2 RT3R2, ST1R2, ST3R1 and ST3R3. Similarly, pyrolysing rice husk at 400°C also increased Fe uptake by maize and performed better than control and those treated with compost. There were also no significant differences (P≤ 0.05) among compost treatments at 5, 10 and 15 t/ha (Figures 3a and b). The distribution of nutrient uptake among the maize plant varies.

### Residual effect on growth and yield of maize

The residual effect of compost and biochar on plant height indicated that there was general increase over the growth period (Data not shown). As observed in the main planting, there were variations in the performance of different amendments based on feedstock, period of data collection, rate of application and pyrolysing temperatures. Rice husk biochar pyrolysed at 400°C and applied at 15 t/ha gave the highest mean value while rice husk biochar 300°C at 10 t/ha recorded the lowest mean value. On the leaf area, RT1R3 (Rice husk biochar at 300°C and at 15 t/ha) gave the highest mean value followed by compost at 15 t/ha while the lowest was recorded from rice husk biochar pyrolysed at 300°C and applied at 10 t/ha (Table 4). The number of leaf was also enhanced in all amended soils compared with control except that there were no significant differences among the organic treatments. Application of the rice husk biochar pyrolysed at 400°C and applied at 15 t/ha and

**Table 3.** Effect of compost and biochar produced at different temperature on vegetative parameters of maize.

| Treatment | Plant height (cm) | Leaf Area ($cm^2$) | Number of leaf |
|---|---|---|---|
| CR1 | 57.13 | 118.60 | 6.43 |
| CR2 | 54.54 | 131.45 | 6.75 |
| CR3 | 61.75 | 134.79 | 6.68 |
| RT1R1 | 57.93 | 119.52 | 6.48 |
| RT1R2 | 56.63 | 118.48 | 6.83 |
| RT1R3 | 58.76 | 126.89 | 6.70 |
| RT2R1 | 62.63 | 132.76 | 6.48 |
| RT2R2 | 61.91 | 139.47 | 6.78 |
| RT2R3 | 58.33 | 145.10 | 6.63 |
| RT3R1 | 59.18 | 116.54 | 6.63 |
| RT3R2 | 57.72 | 131.55 | 6.60 |
| RT3R3 | 55.04 | 111.18 | 6.50 |
| ST1R1 | 59.87 | 127.72 | 6.43 |
| ST1R2 | 61.20 | 130.96 | 6.65 |
| ST1R3 | 56.83 | 119.58 | 6.75 |
| ST2R1 | 55.62 | 119.23 | 6.45 |
| ST2R2 | 68.24 | 126.40 | 6.83 |
| ST2R3 | 65.16 | 134.00 | 7.00 |
| ST3R1 | 60.52 | 106.37 | 6.45 |
| ST3R2 | 59.38 | 116.14 | 6.10 |
| ST3R3 | 66.82 | 136.96 | 6.63 |
| Control | 63.70 | 121.36 | 6.39 |
| LSD ($P \leq 0.05$) | 9.89 | 30.66 | 0.78 |

Means followed by the same letter in a column are not significantly different from each other at $P \leq 0.05$ by DMRT. R1, R2 and R3, = Application rate at 5, 10 and 15t/ha. R= Rice husk biochar, S=sunflower biochar, T1, T2 and T3 = 300, 400 and 500°C.

sunflower biochar 400°C at the rate of 10 and 15 t/ha gave the highest shoot fresh weight of 17.83, 17.37 and 17.06 g respectively in residual trial. It was clearly observed that most of the treatments with higher rate performed better than their lower rates. Also application of ST3R2 (sunflower biochar 400°C at 10 t/ha) and ST2R2 (sunflower biochar 350°C at 10 t/ha) and compost gave the shoot fresh weight values of 13.16, 12.37 and 12.32 g respectively. As observed with the shoot fresh weight, rice husk biochar pyrolysed at 400°C and applied at 15 t/ha also gave the highest dry matter yield and it was significantly different from other treatments. There was no significant difference between the sunflower biochar (at 300°C at 10 t/ha) and control. Among all the treatments, rice husk biochar pyrolysed at 300°C and at the rate of 5 t/ha gave the lowest shoot dry weight of 8.55 g (Figure 4). Sunflower biochar produced at 400°C and at the rate of 10 t/ha recorded the highest mean value of 17.50 g for the root fresh weight followed by compost at 15 t/ha while the lowest mean value was observed in rice husk biochar pyrolysed at 300°C and applied at 5 and 10 t/ha. Application of ST3R2 also gave the highest root dry weight of 6.23 g when compared to other treatments. Higher rate of all the treatments also performed better than their lower rates (Figure 5).

## DISCUSSION

The result of this study showed clearly the potential of compost and biochar amendments under different conditions for improving maize yield. All the plants that received biochar treatments at low temperature and at higher rate between 10 to 15 t/ha performed better and this was supported by the report of Antal and Grønli (2003). The feedstock pyrolyzed at a low temperature possessed most of the essential nutrients required for plant growth.This is because as pyrolysis increases, volatile compounds in the biochar matrix are lost, surface area and ash increases but surface functional groups that can provide exchangeable capacity decreases (Giardina et al., 2000). Research has also shown that biochars are known to contain some condensed volatile compounds which can be easily converted to gaseous substances at high temperature (Antal and Gronli, 2003) and as a result of this, biochar yield decreases with increase in pyrolysis

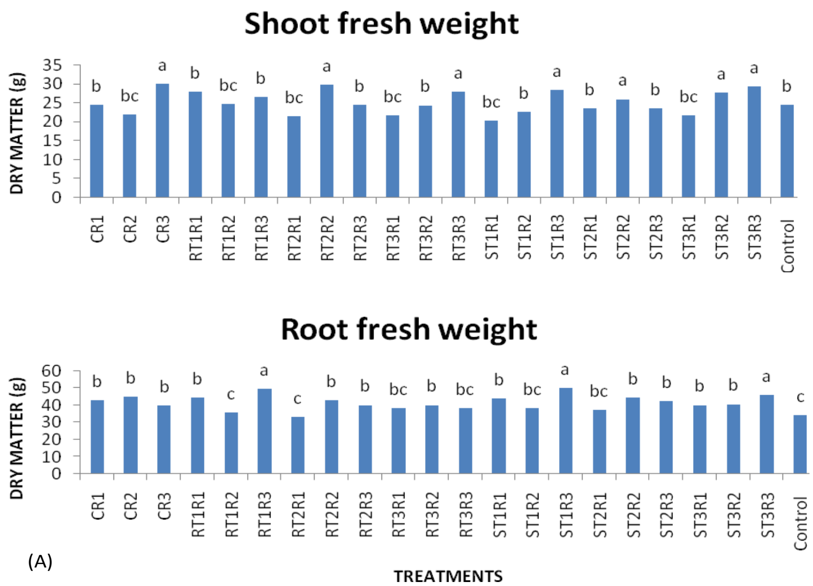


**Figure 1a.** Maize fresh weight in response to compost and biochar produced at different temperature. Footnotes: R1, R2 and R3, = Application rate at 5, 10 and 15t/ha. R= Rice husk biochar, S=sunflower biochar, T1, T2 and T3 = 300, 400 and 500°C.

**Table 4.** Residual effect of compost and biochar produced at different temperature on the growth parameters of maize.

| Treatment | Plant height (cm) | Leaf Area ($cm^2$) | Number of leaf |
|---|---|---|---|
| CR1 | 50.76 | 81.28 | 6.00 |
| CR2 | 44.46 | 88.92 | 5.43 |
| CR3 | 49.88 | 107.10 | 6.30 |
| RT1R1 | 42.21 | 82.70 | 5.75 |
| RT1R2 | 37.78 | 71.99 | 5.18 |
| RT1R3 | 52.74 | 109.67 | 6.20 |
| RT2R1 | 42.04 | 83.80 | 5.60 |
| RT2R2 | 51.87 | 101.59 | 6.35 |
| RT2R3 | 45.83 | 87.21 | 5.90 |
| RT3R1 | 45.85 | 80.17 | 5.68 |
| RT3R2 | 46.25 | 95.73 | 6.08 |
| RT3R3 | 52.89 | 99.00 | 6.10 |
| ST1R1 | 45.32 | 89.09 | 6.05 |
| ST1R2 | 42.88 | 81.51 | 5.60 |
| ST1R3 | 45.54 | 88.24 | 6.20 |
| ST2R1 | 45.46 | 98.51 | 6.10 |
| ST2R2 | 42.74 | 99.04 | 6.10 |
| ST2R3 | 43.60 | 89.84 | 5.78 |
| ST3R1 | 44.98 | 67.51 | 5.98 |
| ST3R2 | 41.00 | 88.10 | 6.08 |
| ST3R3 | 41.88 | 90.48 | 5.88 |
| Control | 45.03 | 83.81 | 5.80 |
| LSD (P≤0.05) | 9.22 | 25.13 | 1.06 |

Footnotes: R1, R2 and R3, = Application rate at 5, 10 and 15t/ha. R= Rice husk biochar, S=sunflower biochar, T1, T2 and T3 = 300, 400 and 500°C.

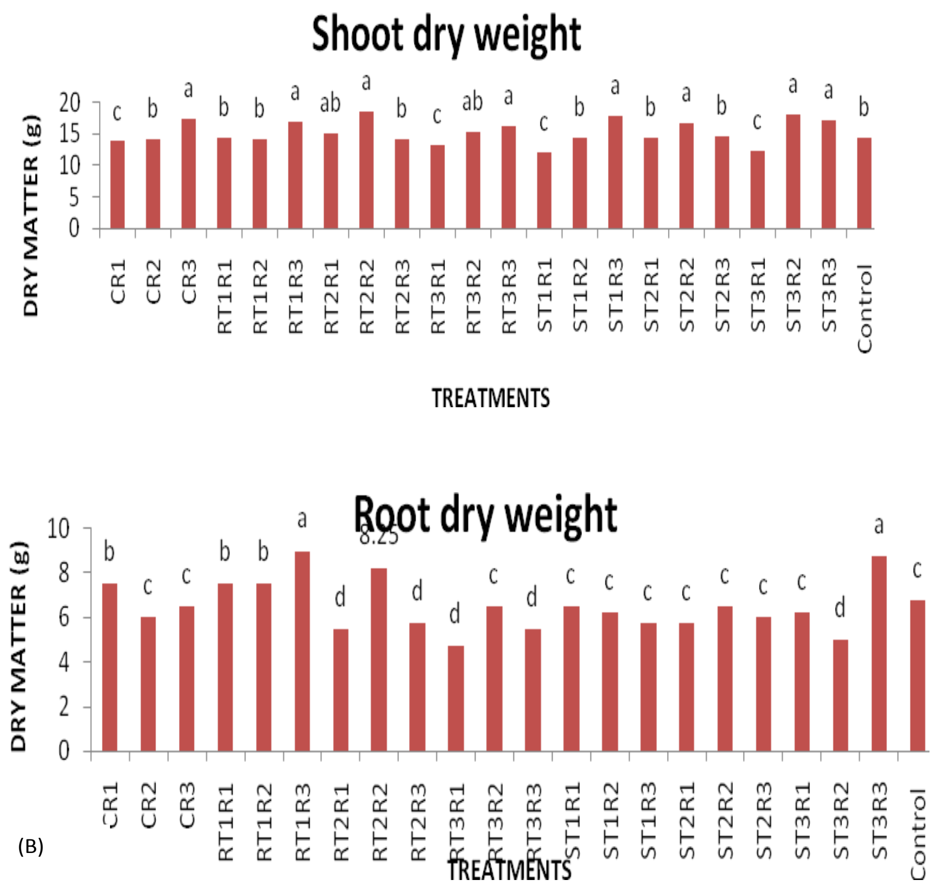


**Figure 1b.** Maize dry weight in response to compost and biochar produced at different temperatures. Footnotes: R1, R2 and R3, = Application rate at 5, 10 and 15t/ha. R= Rice husk biochar, S=sunflower biochar, T1, T2 and T3 = 300, 400 and 500°C.

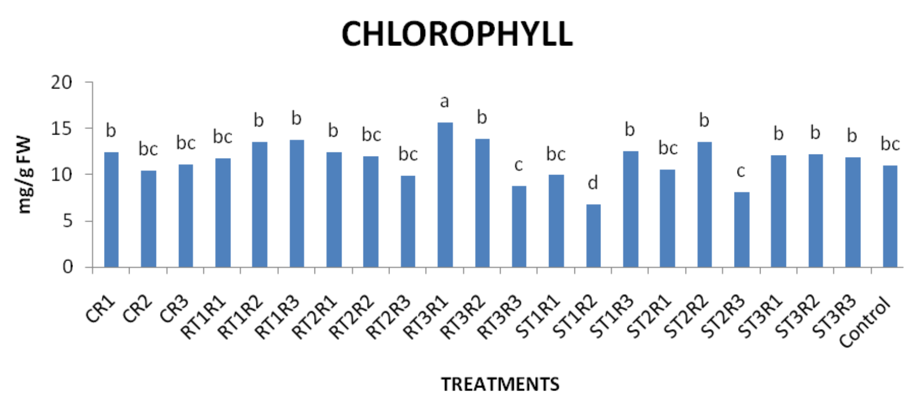


**Figure 2.** Effect of treatments on photosynthetic pigment (chlorophyll contents). Footnotes: R1, R2 and R3, = Application rate at 5, 10 and 15t/ha. R= Rice husk biochar, S=sunflower biochar, T1, T2 and T3 = 300, 400 and 500°C.

temperature. Most of the nitrogen contents are also lost to the atmosphere due to high pyrolysis but the proportion of the feedstock was conserved at low pyrolysis temperature (Knicker et al., 2000).

However, it was clearly observed from this study that the performance of biochar was lower than that of

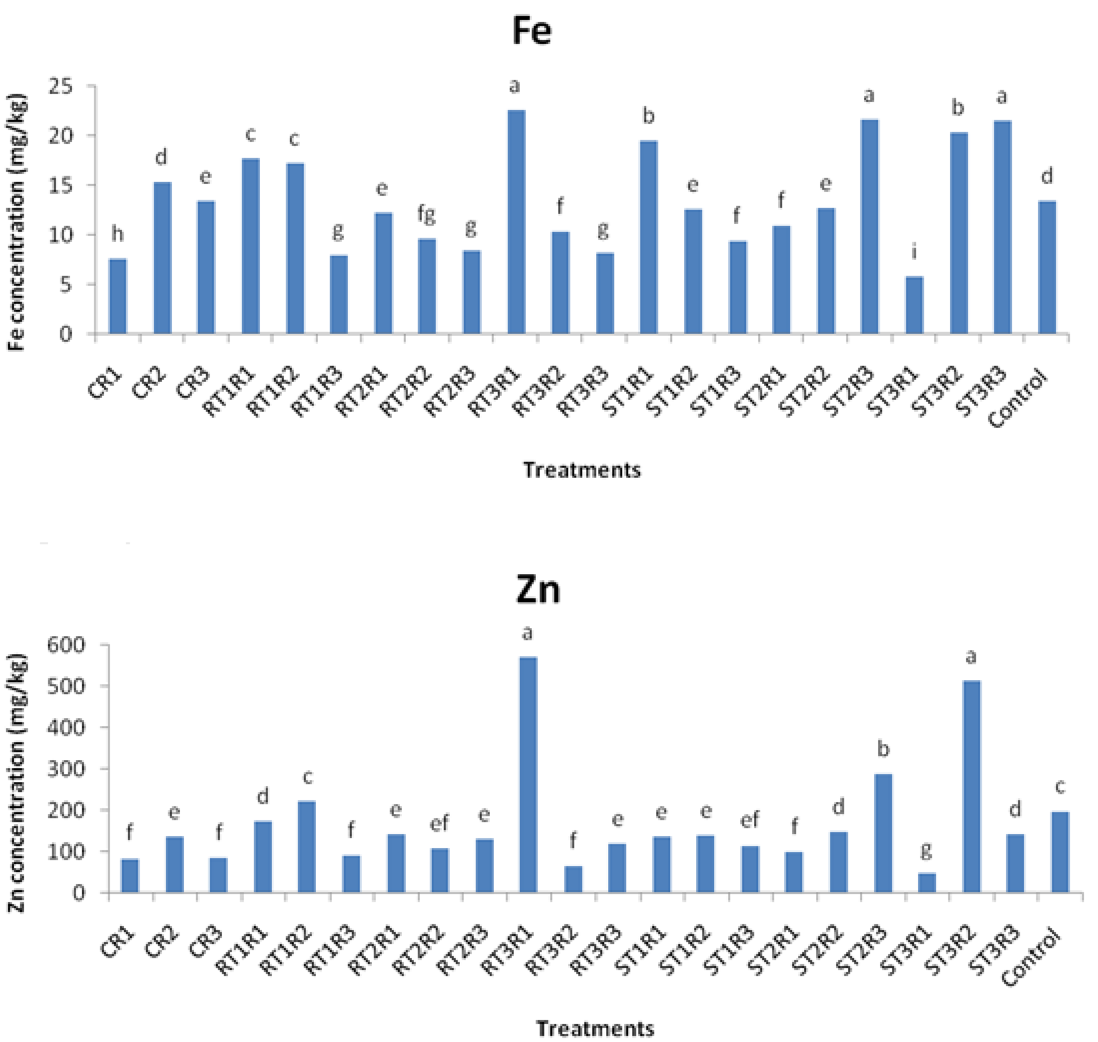


**Figure 3.** Effect of compost and biochar produced at different temperature on micronutrient uptake by maize. Footnotes: R1, R2 and R3, = Application rate at 5, 10 and 15t/ha. R= Rice husk biochar, S=sunflower biochar, T1, T2 and T3 = 300, 400 and 500°C.

compost in terms of vegetative growth. Comparatively, it was clearly observed that compost treatment performed much better than biochar alone probably because of reported properties of compost in terms of nutrient compositions (Adediran et al., 2006). This performance could therefore be attributed to availability of nutrients in the compost. The improvement observed in plant growth parameters and in dry matter yield was similar to the observation of Garllardo and Nogales (1987) that compost made available sufficient nutrients required for plant growth. It has also been asserted that nutrients availability in sufficient amount improves plant leaf area development (Akande et al., 2000). All the plants treated with compost treatment in the first experiment gave higher number of leaves, plant height, stem girth, leaf area and dry matter accumulation more than biochar and control treatments. This also confirmed the finding of Dale et al. *(*2006) that the application of compost contributed greatly to the plant growth when compared to control. This was in line with the reports of Asai et al. (2009) and Gaskin et al. (2010) that, though biochar possesses some essential elements required for plant growth but biochar can only be effective and improve plant growth when combined with other fertilizers (Blackwell et al., 2009). Many reports have also shown that there was regular decrease in plant growth with the application of biochar alone when not used in combination with other fertilizers (Gaskin et al., 2010).

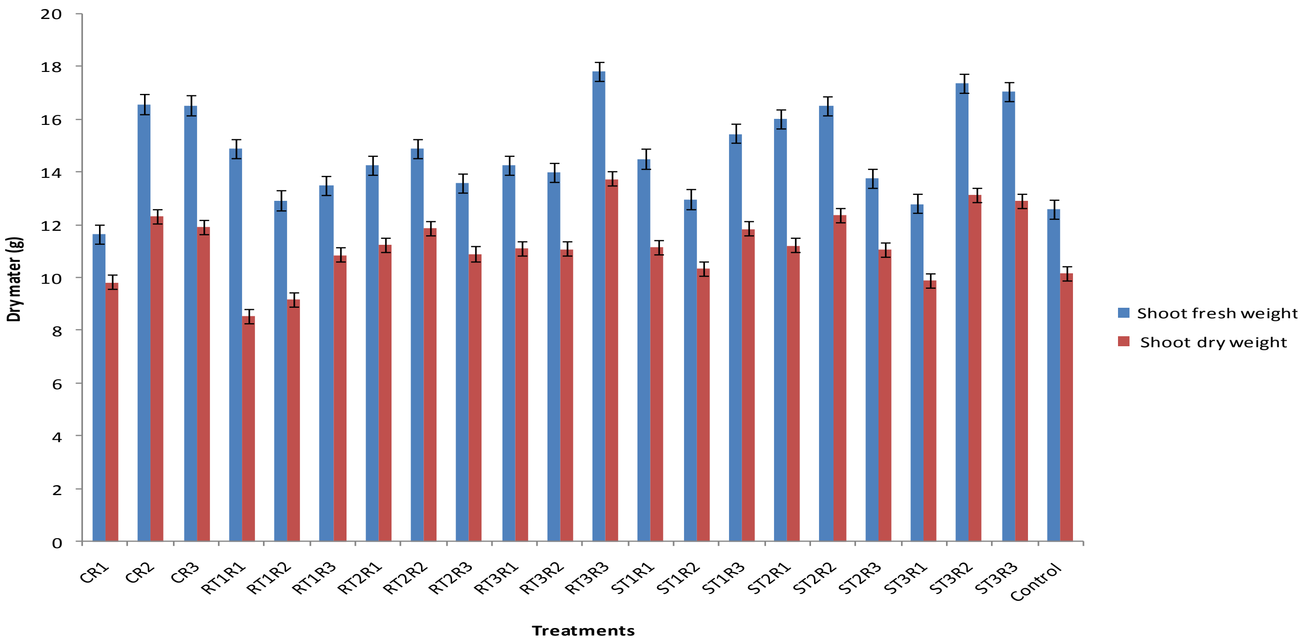


**Figure 4.** Residual effect of compost and biochar on shoot fresh and dry weight.

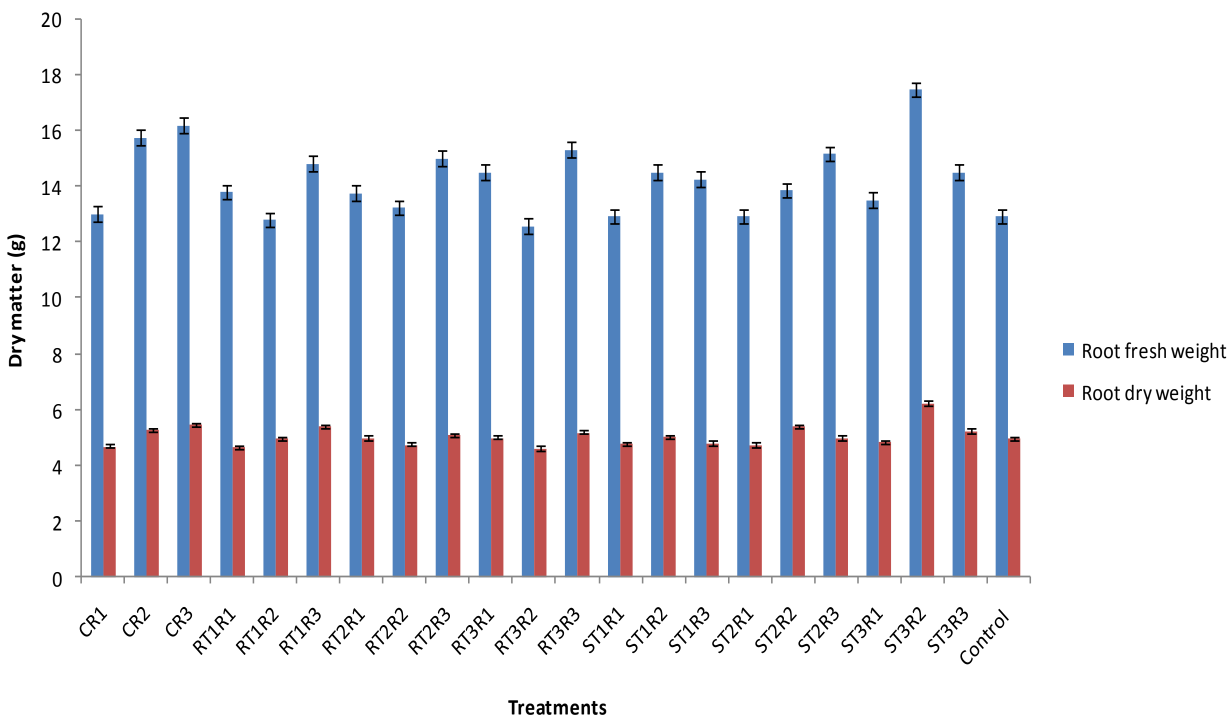


**Figure 5.** Residual effect of compost and biochar on root fresh weight and dry weight.

Chan et al. (2008) therefore gave a conclusion that biochar is not an actual fertilizer based on these observations. Better yield observed from 15t/ha treatment with compost could be attributed to the higher nitrogen availability as beneficial effect of tithonia compost on crop yield have been previously reported (Qureshi, 1990;

Adejumo et al., 2011).

Although biochar was not as effective as compost but nutrients such as calcium, potassium and magnesium which are usually limiting in poor soils have been reportedly supplied by the ash from biochar. This could in turn explain the strong effect of biochar on dry matter yield. Variation observed on the growth parameters with regards to different feedstocks (rice husk and Mexican sunflower) used for the biochar production could be due to variations in their nutrient compositions. For instance, rice husk has been reported to contain high content of silicon and potassium which have great potential for amending soil and also improve water holding capacity in soil (Oshio et al., 1981). This probably contributed to the improvement in the growth parameters of the plants treated with higher rates of this biochar. Similarly, the Mexican sunflower used as biochar feedstock is a good source of plant nutrients and according to previous research, it was reported to have high level of nutrients (Sacred Africa, 2007).

Application of biochar however, increased the photosynthetic pigment of maize when compared to control and compost. The plants amended with sunflower and rice husk biochar at 15t/ha showed maximum content of chlorophyll as compared to other treatments. This study indicated that maize plants responded better to the application of biochar pyrolysed under 300 to 350°C temperature and applied at 15 t/ha with respect to chlorophyll content. The effect of organic fertilizer on amount of chlorophyll pigments and rate of photosynthesis was studied by Fernendez-Luqueno et al. (2010) and concluded that application of organic fertilizers like biochar did not only enhance the synthesis and amount of chlorophyll but also increased the rate of photosynthesis. Photosynthesis is a complex process that is sensitive to availability of micronutrients (Marschner, 1995). The increase in the photosynthetic pigment with the addition of organic amendments might be attributed to high contents of Mg and Fe. Iron and Mg are the two important nutrients involved in chlorophyll synthesis (Nelson and Cox, 2004). The green pigment (chlorophyll) with magnesium at the core of heterocyclic protoporphyrin ring is the principal pigment responsible for light absorption and photosynthesis. This was also confirmed by the result of micronutrients (Fe and Zn) analysis where higher concentrations of the elements were found in maize treated with biochar compared to those of compost and control.

Residual effect of organic amendments is important for sustainability of nutrients and this was reflected in the residual trial. Maize growth and yield were found to be higher in the second trial than the first trial. The finding agreed with the report of Ramanurthy and Shawasshankar (1996) that residual effect of organic matter improves plant height, leaf area and dry matter production of maize crop at different growth stages. This was also confirmed by Tejada and Gonazalaz (2006) on rice. Biochar most especially can remain in soil for a longer period of time. It has therefore, been hypothesized that the long term effect of biochar on nutrient availability in the soil is due to an increase in surface oxidation and cation exchange capacity (CEC) of the soil (Liang et al., 2006) which can in turn lead to greater nutrient retention in soil. Therefore, high growth rate and dry matter accumulation observed in the residual experiment of this study indicated that biochar has a carrying over benefit on the succeeding crop. Besides, organic manures are generally known for their slow release of nutrients which probably explains the reasons for better residual effect.

Zinc and Iron uptake also increased in maize most especially in higher rates of biochar under residual trial. The same was observed with compost application and this is because the residual effect of compost application also maintained crop growth for a longer period of time. All these were attributed to the ability of organic amendments in maintaining crop yield for several years after application has ceased the explaination was that since only a fraction of the Nitrogen and other nutrients in the amendments become available in the first year of application (Motavilli et al., 1989; Eghball et al., 2002) others remain in the soil for soil improvement over a long time. The finding of Van Zwiten et al. (2010) also suggested that while biochar may not provide a significant source of plant nutrients, they can improve the nutrient assimilation capability of crops thereby positively influencing the soil environment.

## Conclusion

This study showed that pyrolytic biochar has the potential to be used in agricultural production. Although pyrolysis conditions are known to affect the chemical and physical characteristics of biochar, at the relatively low pyrolysis temperature used in this study, feedstock characteristics had the greatest influence on key agricultural characteristics. Also, compost made from Tithonia diversifolia is an excellent source of plant nutrients which was confirmed by the increase in yield from 15 t/ha compost treatment which gave better crop yield and performed excellently. In addition, both sunflower and rice husk biochar at the low pyrolysis temperatures between 300 to 350°C at higher rate of 15 t/ha gave better performance. Therefore, the nutrient contents in biochar and performance depend on the source of the feedstock, pyrolysis conditions and rate of application (Kookana et al., 2011; Bagreev et al., 2001). Overall, compost performed better than biochar in terms of growth parameters but biochar increased the micronutrient contents in maize maore than compost. For increasing the efficiency of biochar, combining biochar with other organic amendments at the right propotions could be an efficient approach.

## Conflict of Interest

The authors have not declared any conflict of interest.

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# Risk factors associated with the post-harvest loss of milk along camel milk value chain in Isiolo County, Kenya

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**Camel milk is an important commodity in the arid and semi-arid lands where it forms their basic diet, a major source of income and serves significant cultural function to the pastoralists. However, camel milk production is faced with challenges that contributes to the camel milk post-harvest losses due to poor quality and safety. This study aimed at determining the risk factors that may contribute to camel milk quality losses along the Isiolo camel milk value chain. The survey data was collected through structured questionnaire and key informant interview while the microbiological counts data were determined using ISO methods. There was poor hygiene at the herd level where high *Staphylococcus aureus* count was found on the camel udder swab, milkers' hand swab, and milking container which recorded counts of $1.4\times10^4$ cfu/cm$^2$, $1.5\times10^4$ cfu/cm$^2$, and $5.9\times10^3$ cfu/ml, respectively. In the other chain nodes, the hygiene was significantly ($p<0.05$) different with milk hands of retailers around Isiolo town, at the cooling hub/bulking milk and milk retailers in Nairobi Eastleigh area recording *S. aureus* counts of $4.9\times10^3$, $1.3\times10^4$, and $3.7\times10^3$ cfu/cm$^2$, respectively. There was problem accessing adequate potable water at the herd level than at the other chain nodes. The plastic milk containers were not disinfected with any chemical sanitizes after washing, however the smoke fumigated them. Camel disease management was poor. Both sick and health camels were milked and the milk bulked together. This therefore indicates that hygiene could be one of the most important contributor to milk deterioration along the chain. Improvement of hygiene along the Isiolo camel milk value chain can help reduce milk post-harvest losses.**

**Key words**: Camel milk, handling practices, safety, risk factors, post-harvest losses, Isiolo.

## INTRODUCTION

Camel (*Camelus dromedarius*) husbandry is mainly practiced in the arid and semi-arid land (ASAL) by the pastoral communities who keep the one humped camel (Farah et al., 2007). One humped camel (*C.dromedarius)*

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plays an important role in the livelihood and culture of pastoralists in the ASALs of Northern Kenya; which are characterized with persistent draught and limit production browse and water limit production (Guliye et al., 2007; MoLD, 2007; Mehari et al., 2007a, b; Mahmoud, 2010).

The world's camel population is estimated to be about 27 million (FAOSTAT, 2015) of which about 82.5% are found in Africa; with about 60% of these are found in Eastern Africa region countries of Somalia, Sudan, Ethiopia, and Kenya (FAOSTAT, 2015). Kenya camel population is estimated to be 3 million (KNBS, 2010; FAOSTAT, 2015); the third largest population in Africa after Somalia and Sudan. In pastoral Northern Kenya, camels are mainly kept for milk production. During the dry season when milk from other livestock is scarce, camel produce more milk and for a longer period of time than other livestock (Kaufmann, 1998). In 2008, the annual camel milk production in Kenya was estimated at 338 million tonnes, valued at USD 107 million, representing 12% of the national milk production and 20% of commercially marketable milk (Musinga et al., 2008), however this increased to 937 million tonnes in 2013 (FAOSTAT, 2015), indicating that camel dairy sub-sector can offer a huge potential for improving the livelihoods of ASAL communities.

Camel milk is a major source of food security and income and also serves as a significant cultural function to the pastoral communities (Guliye et al., 2007; MoLD, 2007; Mehari et al., 2007a, 2007b; Mahmoud, 2010). It forms basic diet for the pastoral communities, where it contributes up to 50% of the total nutrient intake and 30% of their annual caloric intake (Farah and Fischer, 2004). Surplus camel milk is usually sold in urban centres and the raised money contributes to the household cash income.

Despite the major contribution of camel milk to pastoral communities livelihoods, camel milk production is still faced high post-harvest quality deterioration and quantity losses. The risk factors contributing to these high post-harvest losses have not been determined quantitatively and qualitatively at different stages along the camel milk value chain. The objective of this study was therefore to determine the risk factors along the camel milk value chain that lead to the postharvest losses of camel milk at different stages along the camel milk value chain.

## MATERIALS AND METHODS

### Study area

The study was conducted in Isiolo County, a typical ASAL area in North-Eastern Kenya with both peri-urban and pastoral camel production systems and a thriving camel milk trade and Nairobi (Eastleigh estate) the terminal market for the Isiolo camel milk value chain. Isiolo County is a semi-arid area that experiences recurring droughts with devastating losses of livestock. Most parts of the county have mean annual temperature of between 24 and 30°C (Herlocker et al., 1993).

The camel milk production in Isiolo County is done by Somali tribesmen who form the majority of the camel owners in both peri-urban (80%) and pastoral (90%) systems. The Borana tribesmen who traditionally keep cattle until recurrent prolonged drought threat spells in ASAL areas, awakened their interest in camel keeping. The Borana tribesmen form 18.3% of peri-urban camel and 10% of pastoral system (Noor et al., 2013). About 87.5% of the produced camel milk is consumed either at the local trading centres or for subsistence at the household level (Musinga et al., 2008). Isiolo County has about 39,084 camels and a human population of about 143,294 (KNBS, 2010).

### Data collection

### Cross-sectional survey

Survey data was collected using structured questionnaires, focused group discussions (FGDs), key informant interviews, and personal observations. Purposive sampling was used to select the respondents. The respondents were 75 herdsmen (who take care of the camels and milk the camel), 75 women at the collection/bulking centre in Isiolo town and 85 women retailing camel milk within Isiolo town and its environs. The structured questionnaires aimed at determining the risk factors associated with post-harvest quality and quantity losses along the camel milk value chain. The questionnaire comprised three sections which included sanitation and hygiene, camel milk handling, and disease management in camels. Three focused group discussions were held separately with the herdsmen, women at retailing site and women at the collection/bulking centres met.

### Sampling framework

The sampling framework used was as described by Bonfoh et al., (2003). The sampling points/stages along the Isiolo camel milk value chain were herd milking level, collection level within the herd area (primary collection points), collection/bulking centre at Isiolo town (secondary collection point) where milk is cooled waiting transportation the next day to Nairobi, retailing point at Isiolo town and its environs, and finally terminal market point (tertiary point) at Eastleigh estate, Nairobi. At camel herd level, the samples collected from 10 purposively sampled camel herds were: 22 udder swabs from camels before milking and 20 hand swabs from herders doing milking, 8 swabs from milking containers.

Personnel hand swabs were 16, 10, and 12 at collection/bulking centre at Isiolo town (secondary collection point), retailing point at Isiolo town, and terminal market point (tertiary point) at Eastleigh estate, Nairobi, respectively. The time of delivering the milk taken from one level of the market chain to another was also determined.

Swabs samples were preserved in cool boxes containing ice packs and transported on a daily basis to nearby Isiolo County Referral and Teaching hospital laboratory within 2 to 3 h after sampling for microbiological analysis.

### Microbiological analysis of swab samples

The swab samples were analysed for total viable counts, total coliform count and *Staphylococcus aureus* counts.

Total viable counts (TVC) were determined using plate count agar according to ISO 4833:2003 method (ISO, 2003a). The plates were incubated at 30±1oC for 48 ±2 hours. Total coliform counts were determined according to ISO 4832:2006 method (ISO, 2006) using MacConkey agar and incubating the plate at 300°C for 48±2

h. *S. aureus* counts were determined according to ISO 6888-1:1999/Amd 1:2003 method (ISO, 2003b). The swab samples were surface plated onto the surface of Baird Parker agar. The plates were incubated at 37oC for 24 ±2 hours then re-incubated for a further 24 ±2 hours. Typical *S. aureus* colonies black or grey, shining and convex and surrounded by a clear zone which were partially opaque were counted. The TVC, coliform counts and *S. aureus* counts were expressed as colony forming units (cfu) per square centimeter of swabbed area.

#### Data analysis

Data from questionnaires were analyzed using SPSS Statistics Version 20.0 for descriptive statistics (frequencies, means, and percentages). Qualitative data from the focused group discussion and the key informant interview were transformed into thematic components and written into descriptive prose.

The microbiological data was entered into Microsoft Excel 2013 to generate graphs and table presentation of the results. GENSTAT statistical packages 15th Edition was used to determine if there was significant differences in the counts along the Isiolo camel milk value chain.

## RESULTS AND DISCUSSION

### Containers used for milking and milk transportation

The containers used both for milking and transportation were exclusively plastic. The herdsmen milking reported that they use plastic containers for milking, because they are light (47%) and cheap to buy (36.3%). Camel milk traders reported that they use plastic containers for transportation because they are cheap to buy (41.7%) and easier to transport (23.7%). Some herdsmen (9.7%) and traders (17.3%) reported that they use plastic container for milking to prevent spoilage. This indicates poor knowledge among the pastoralists on the causes of spoilage. The convenience of the containers in terms of use and transportation accounted for 2.4 and 3.2% by traders and herdsmen, respectively (Figure 1).

The milking was done while the milkers were standing due to the high level of the udder that demands that the milking container has to be lifted up during milking to prevent spillage. Wayua et al. (2012) reported similar result that milking is done with one leg raised to support the milking container.

### Hygiene and sanitation along the camel milk value chain

Table 1 shows the microbial counts, as indicated by total viable counts (TVC), coliforms counts, and *S. aureus* counts of swabs from milkers' hands, camel udder, and milking containers at the herd level. There was no significant difference ($p<0.05$) in TVC and *S. aureus* counts of swabs from milkers' hands and the camel udder. However, milking container showed significantly ($p<0.05$) lower count for TVC ($1.1\times10^5$ cfu/cm$^2$) and *S. aureus* counts ($5.9\times10^3$ cfu/cm$^2$). There were significant differences $p<0.05$) between coliforms counts of swabs from milkers' hands, camel udder, and milking containers. The udder swabs showed significantly ($p<0.05$) lower coliform count ($4.6\times10^2$ cfu/cm$^2$), while the milkers' hands showed significantly higher coliform count ($7.2\times10^2$ cfu/cm$^2$). The high *S. aureus* counts in milking personnel hand swabs was not significantly ($p<0.05$) different from camel udder swabs. The counts in swabs from milkers' hands for all the three indicator organisms were higher, indicating that milkers' demonstrate poor hygiene during milking, hence could be the main source of microbial contamination of camel milk. Although udders are also not washed before milking and the camel udder swab showed significantly lower swab counts than the milkers' hand swabs. This could be due to the calf suckling before milking. The suckling cleans the udder by removing dirt and microorganisms on the teat. The camel morphology is such that the udder is in direct contact with the soil during resting periods which could results in contamination of the udder with microbes on the ground. The calf may compromise the udder hygiene since after suckling no cleaning of the udder is done before milking. The calf may therefore introduce some microbes to the udder before milking (Noor et al., 2013).

The camel's udder was not washed because the pastoralist believed that the camel udder is always clean. However, our study found that camel's udder was highly contaminated (Table 1). About 37% of the milkers' wet their hands directly with milk in the milking container to lubricate the teat during milking. This act could result into introduction of microbial and dirt contaminants into the milk from the unhygienic milkers' hand, as shown by the microbial load of their hand swabs (Table 1). This results in the milk having higher microbial load, hence facilitating microbial deterioration of the milk leading to post-harvest losses of the milk. This poor hygiene could be due to the fact that the herdsmen do not wash their hands regularly due to lack of water.

Milking container had significantly ($p<0.05$) low TVC and *S. aureus* counts compared to milkers' hand and camel udder, indicating that washing of the milking containers was not effective as the containers still had high counts. The most important risk factor for possible milk contamination is therefore the herdsmen who milk the camel and handle the camel at the same time.

As indicated by the wide count range in all indicator organisms count, different herds showed significance differences ($p<0.05$) in hygiene status of the milking personnel, udder, and milking container. The differences in hygiene status in different herds shows that mixing/pooling or bulking of milk from different herds is a risk factor as herds higher microbial counts could compromise the camel milk quality; thus, supporting findings of study by Younan and Abdurahman (2004) among South Somali milk producers/traders. Milk from different herds are usually brought to a central point along

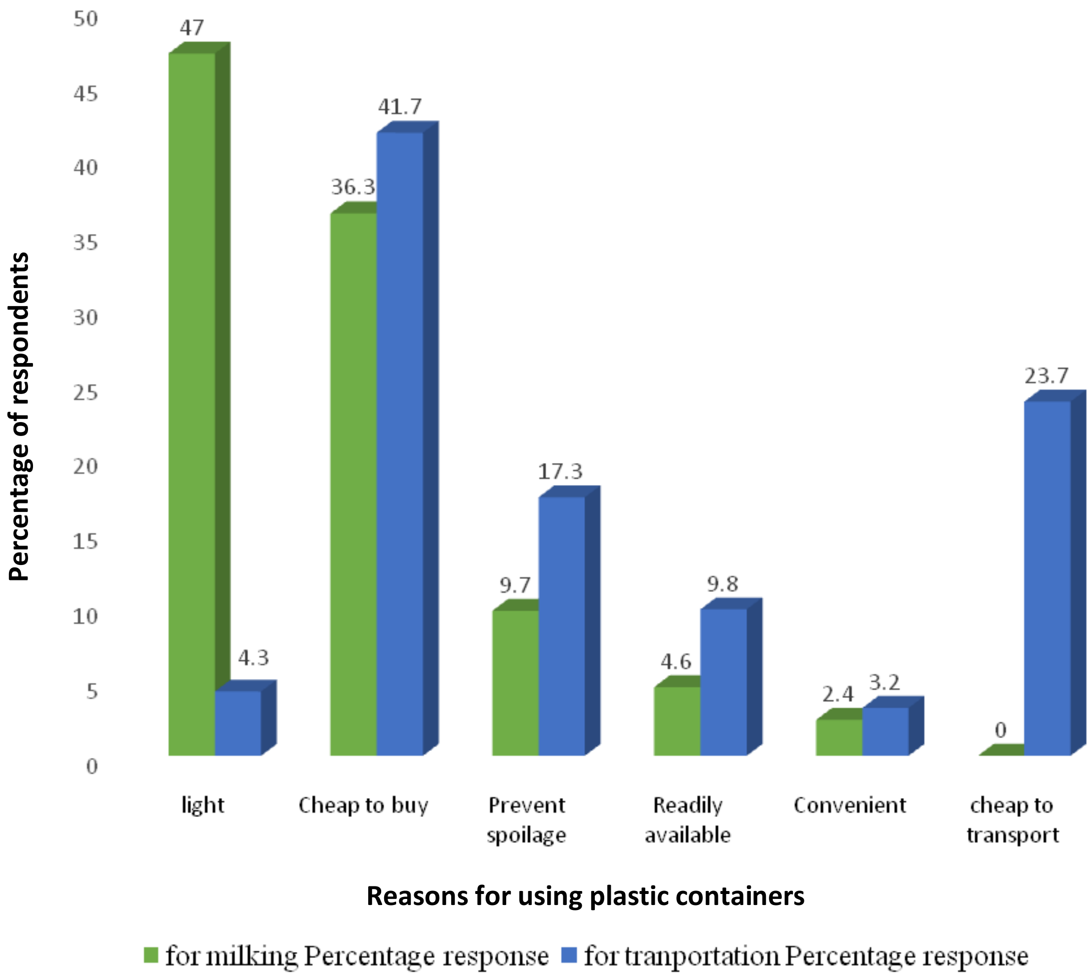


**Figure 1.** Reasons for use of plastic containers at the herd level and during transportation of camel milk.

the road or designated place in area where transporters on either motorbikes or pickup trucks or donkey pick the milk and transport it to bulking centre at Isiolo town.

Table 2 shows microbial counts, as indicated by total viable counts, coliforms counts, and *S. aureus* counts of hands swabs of women at the camel milk cooling hub and those retailing camel milk at Isiolo town and Eastleigh, Nairobi. The results showed that there was no significant difference ($p>0.05$) in TVC of hand swabs of women retailing camel milk around Isiolo town ($1.5\times10^5$ cfu/cm$^2$), women at the bulking/cooling hub at Isiolo town ($1.3\times10^5$) and women retailers at Eastleigh, Nairobi ($9.6\times10^4$ cfu/cm$^2$). However, there was significant difference ($p>0.05$) in *S. aureus* counts of hand swabs of women retailing camel milk around Isiolo town ($4.9\times10^3$ cfu/cm$^2$), women at the bulking/cooling hub at Isiolo town ($1.3\times10^4$), and women retailers at Eastleigh, Nairobi ($3.7\times10^3$). As demonstrated by *S. aureus* counts, an indicator organism of personnel hygiene, the women at the bulking/cooling hub in Isiolo town showed poor personnel hygiene, while the women in Eastleigh, Nairobi showed the best personnel hygiene. There was also significant difference ($p<0.05$) in coliform counts of hand swabs of women at the bulking/cooling hub at Isiolo town ($1.5\times10^2$), women retailing camel milk around Isiolo town ($1.1\times10^2$ cfu/cm$^2$) and women retailers at Eastleigh, Nairobi ($1.4\times10^2$ cfu/cm$^2$). The women at the bulking/cooling hub at Isiolo town showed significantly poor personnel hygiene ($1.5\times10^2$ coliform cfu/cm$^2$), compared to women retailing camel milk around Isiolo town who demonstrated significantly better personnel hygiene ($1.1\times10^2$ coliforms cfu/cm$^2$). As shown in the wide range of count (Table 2), there was significant differences ($p<0.05$) among the women at the bulking/cooling hub at Isiolo town, with some showing better personnel hygiene, while other showing poor personnel hygiene.

The cooling hubs, where different herds milk are bulked into group bulking and cooling tank or into private individual freezers, could therefore be one of the major risk points along the camel milk value chain for camel

**Table 1.** Microbial load of milkers', camel udder and milking containers at milk harvesting at herd level.

| Type of organism | Hand swabs for milkers (n*= 20) | | Camel udder swab (n=22) | | Milking container swab (n=8) | |
|---|---|---|---|---|---|---|
| | Counts (cfu/ cm$^2$) | | | | | |
| | Geometric mean | Range | Geometric mean | Range | Geometric mean | Range |
| Total viable counts (TVC) | $6.5\times10^{5}$ **b** | $1.5\times10^{4} – 3.5\times10^{6}$ | $5.8\times10^{5}$ **b** | $1.4\times10^{4} – 2.4\times10^{6}$ | $1.1\times10^{5}$ **a** | $1.9\times10^{3} – 2.0\times10^{5}$ |
| Coliforms | $7.2\times10^{2}$ **c** | $1.8\times10^{1} – 3.3\times10^{3}$ | $4.6\times10^{1}$ **a** | $0 – 2.0\times10^{2}$ | $4.5\times10^{2}$ **b** | $3.6\times10^{1} – 1.2\times10^{3}$ |
| *Staphylococcus aureus* | $1.5\times10^{4}$ **b** | $2.0\times10^{3} – 4.2\times10^{4}$ | $1.4\times10^{4}$ **b** | $2.0\times10^{1} – 5.1\times10^{4}$ | $5.9\times10^{3}$ **a** | $3.8\times10^{2} – 2.1\times10^{4}$ |

*n= Number of samples. **The generic mean values with similar letters in the same row are not significantly different at 5%.

**Table 2.** Microbial Counts of hand swabs of women at camel milk retailing and cooling hub at Isiolo town, and women retailing milk in Eastleigh, Nairobi.

| Type of microorganism | Women retailers around Isiolo (n=10) | | Women at the cooling hubs (n=16) | | Women retailer in Nairobi-Eastleigh (n=12) | |
|---|---|---|---|---|---|---|
| | Counts (cfu/cm$^2$) | | | | | |
| | Geometric mean | Range of counts | Geometric mean | Range of counts | Geometric mean | Range of counts |
| Total viable counts (TVC) | $1.5\times10^{5}$ **a** | $1.0\times10^{4} – 6.4\times10^{5}$ | $1.3\times10^{5}$ **a** | $1.8\times10^{4}$- $4.6\times10^{5}$ | $9.6\times10^{4}$ **a** | $8.0\times10^{3} – 6.4\times10^{5}$ |
| Coliforms | $1.1\times10^{2}$ **a** | $10 – 7.9\times10^{2}$ | $1.5\times10^{2}$ **c** | $10 – 1\times10^{3}$ | $1.4\times10^{2}$ **b** | $12 – 9.7\times10^{2}$ |
| *Staphylococcus aureus* | $4.9\times10^{3}$ **b** | $2.4\times10^{2}$- $2.3\times10^{4}$ | $1.3\times10^{4}$ **c** | $6.8\times10^{2}$- $8.1\times10^{4}$ | $3.7\times10^{3}$ **a** | $2.6\times10^{2} – 1.2\times10^{4}$ |

The generic mean values with similar letters in the same row are not significantly different at 5%. n= number of samples

milk contamination. Ninety three (93) percent of women at bulking/cooling centre and 73% of women retailing milk at Isiolo town were accessible to potable water provided by county government. However, at herd level water sources were reported to be either river or borehole, or dam or in plastic containers delivered to them when empty milk containers are returned to the herds. This shows that most important risk factor at the camel milk production level water hygiene and sanitation which is more difficult and expensive to control. Therefore, herders have no choice, but to use the water that is available for cleaning milking container. The source of water for use at the herd level therefore presented one of the most important risk factor of camel milk contamination. Other studies also reported that water in the ASALs is grossly contaminated and its availability in the camel milk production areas is either scarce or unavailable (Kaindi et al., 2011). The water source for cleaning milk containers should be potable (Musinga et al., 2008; Lore et al., 2006). To prevent milk contamination, hygiene and sanitation of personnel, and milking and milk handling containers and water quality are extremely important (Gran et al., 2002). These are hardly exercised at the herd level making camel milk contamination highly likely.

Table 3 shows the duration of time milk takes from milking to various stages along the camel milk value chain.

The results show that camel milk is held for 25 to 30 h before it reaches the terminal market at Eastleigh, Nairobi County.

Milk takes about 11 h at high ambient temperatures (28 to 30°C) before it is cooled at the bulking/cooling centre at Isiolo town. This coupled with poor milk handling hygiene practices contributes a lot to the deterioration of milk quality.

Therefore, holding milk at high ambient temperature for long time is other risk factor associated with final milk quality. Holding of camel milk at low temperatures for 18 to 30 h can allow for growth of psychrophilic spoilage and pathogenic bacteria, which are mostly associated poor hygiene practices. Therefore, holding of poor quality raw milk at low temperature is also a risk

**Table 3.** Time milk takes from milking to reach various stages along the camel milk value chain.

| Parameter | Stage along the chain | | | | |
|---|---|---|---|---|---|
| | Individual camel milk | Primary collection within the herd areas | Delivered to bulking/cooling hubs in Isiolo | Milk at retailing point in Isiolo | Bulked in in Nairobi Eastleigh |
| Cumulative time (h) elapsed after milking | < 1 | 4 - 7 | 4-11 | 5-13 | 25-30 |

factor to quality and safety of camel milk.

## Cleaning of plastic milk handling containers

The milking containers at the herd level were cleaned exclusively by the herdsmen (100%) the following morning, about 20 h after use, by rinsing the container only with water, which may not be effective to remove the accumulated dirt and microorganisms. The rinse water may not be potable thereby increasing the chances of contamination of container and hence by extension contamination of milk. The water used at the herd level also was inadequate for proper cleaning. Containers used to transport milk from the herds to bulking/cooling centre at Isiolo town were cleaned exclusively by the women (94.8%) who receive the milk at bulking/cooling centre or by hired women who clean the containers for a pay (5.2%). Hot water, detergents, and sanitizers are not used during cleaning. This leads to ineffective cleaning of the milk containers with likelihood of residual microorganisms remaining in the containers which contaminates added milk hence leading to poor quality milk, and post-harvest loss of the milk. The containers used to transport the milk to Eastleigh, Nairobi are brought back to Isiolo town uncleaned where they are cleaned more than 12 h after the milk had been drained from the containers.

Delay in cleaning milk handling containers is a risk factor as it presents adequate time for microorganisms to multiply and increase to microbial load microbial load to levels that are difficult to reduce to acceptable level during cleaning. This could result into high microbial counts in milk handled in these containers and hence accelerated microbial spoilage leading to post-harvest losses of the milk.

## Smoke fumigation of plastic milk handling containers

After cleaning, the milk handling plastic containers are normally smoke fumigated using special trees/shrubs. Other studies have also reported on the use of smoke fumigation in the pastoral system (Wayua et al., 2009; Kipsang, 2011).

Our study found that there are eight tree/shrub varieties commonly used for smoke fumigation of milk handling containers along the chain (Table 4). The most commonly used tree/shrub variety was *Acacia nilotica* known as "*Bil-ill"* by the Somali community and "*Sabans"* by Borana community. While the second most used tree/shrub variety was *Cardia quercifolia (C. sinensis)* known as "Marer" by the Somali community and "Madeer" by Borana community. Seifu et al., (2007) in Ethiopia reported three main tree varieties which included *Olea Africana, Balanites galabra*, and *Acacia ethaica*. Wayua et al. (2012) also reported four tree varieties used in Isiolo which included *Olea africana*, *Acacia nilotica, Balanities aegyptica*, and *Combretum* species. The usage is dependent on the effectiveness of the tree/shrub variety and its availability in the area of use.

According to the respondents, the herders use the best tree/shrub varieties for smoke fumigation, because they are accessible to them during grazing of the camels. Some of the women at bulking/cooling and retailing points sent to their cleaned containers to the herders for smoking (8.8%). This was because the herders are more accessible to the tree/shrubs species as they graze camels. The smoking is done by lighting the tree/shrub until it flames for sometimes, then the fire is extinguished and smoke is directed into the container. This is repeated several times depending on the container size until the container gets hot.The container is then closed to enclose the smoke inside it for about 15 min. Some respondents rinse the excess smoke out after some time (32.1%), while others (67.9) do not rinse. During smoking, some particles of the burning tree/shrub drop into the container, some pastoralists' remove them, while others do not.

This usually changes the colour of the milk. This could lead to post-harvest loss of the milk if the targeted consumers or customers do not prefer smoke flavour and taste in the milk, a case typical to most non-pastoral communities. The foreign taste and flavour further makes the milk unacceptable by processors leading to to rejection of milk by processors, hence leading to post-harvest losses.

Several studies have recommended that

**Table 4.** Tree/shrub varieties commonly used for smoke fumigation of milk handling containers at various points along the value chain.

| Vernacular name Somali/Borana | Scientific name of tree/shrub varieties | Herdsmen N=74 (%) | Retailing women around Isiolo N=82 (%) | Women at collection point N = 72 (%) |
|---|---|---|---|---|
| Bil-ill/Sabans | *Acacia nilotica* | 81.2 | 74.8 | 76.4 |
| Marer/Madeer | *Cardia quercifolia* (*C. sinensis*) | 71.8 | 41.8 | 57.0 |
| Qualangal/Qualqulch | *Maerua denhardtiorum* | 55.7 | 61.5 | 35.8 |
| Degayera/Ilqabachi | *Cadaba ruspolii* | 42.6 | 12.4 | 26.4 |
| Kulan/Bedan | *Balanites pedicillaris* (*B. rotundifolia*) | 33.8 | 23.4 | 19.4 |
| Fulay /Wachu | *Acacia zanzibarica* | - | 17.2 | 15.3 |
| Qote | *Cardia ovalis* | - | 5.8 | 12.5 |
| Da'ar/Bires | *Terminalia kilimandscharicum* | - | 13.6 | 11.4 |

N= number of samples.

containers for milking, transporting, and storing milk should be adequately cleaned using good quality water and disinfected to avoid microbial contamination and improve the microbiological quality of milk (Bonfoh et al., 2006; Lore et al., 2006; Musinga et al., 2008).

## Camel health

About 78% of herdsmen reported that camels had painful and swollen teats an indication of early stage of mastitis and 9% reported milk discolouration an indication of advanced mastitis. Just like using uncleaned milking machine for several cows, the herdsmen usually milks both infected and uninfected camels at same time, a milking practices that has been reported to increase spread of mastitis. Our other study, which screened the same camels for mastitis using California Mastitic Test (CMT), also showed high prevalence of mastitis in the herds examined (Lamuka et al – manuscript submitted).

Prevalence of mastitis in camel milk production system has been reported in Kenya (Younan and Abdurahman, 2004; Kaindi et al., 2011) and Ethiopia (Abera et al., 2010). Milk from camels showing sign of mastitis were mixed with the rest of the milk from healthy camels. This poses health risk as pathogenic microorganisms are likely to be present in the mastitic milk (Tesfaye et al., 2011); hence, mastitic milk should not be consumed resulting to post-harvest losses.

About 97% of the herders indicated that they self-medicate camels. Therefore, the milk is likely to have high levels of veterinary drug residues than levels acceptable for consumption leading to its being rejection.

The herdsmen do not segregate milk from sick camels instead they mix it with milk from healthy camels, a practice that is likely to pose a food safety public health risk due to the likely presence of zoonotic organisms. This increases food safety risk to the population who consume the raw milk without even boiling, a practice common among pastoralists (Wayua et al., 2012).

About 95% of the herdsmen responded that there is no health risk in drinking milk from sick camels and that there is no need to observe the withdrawal period as this will be milk wastage. Several studies have shown that foodborne pathogens in the camel milk can be associated with unhealthy camels (Meile, 2010; Wanjohi et al., 2010; Megersa et al., 2011; Tesfaye et al., 2011).

Unlike a study by Younan and Abdurahman (2004) who listed several factors as contributors to the poor hygiene; this study has used quantitative and qualitative data to extensively demonstrate that poor sanitation and hygiene practices along the camel milk value chain are as a result of interaction of many risk factors which may lead to the post-harvest losses of milk and/or make milk unacceptable for consumption and processing.

## Conclusion

The Isiolo camel milk value chain is associated with a number of risk factors which results to the spoilage of camel milk or the camel milk being unfit for processing and unsafe for consumption. Among the risk factors include poor personnel hygiene, inadequate cleaning and sanitation of milk handling containers, lack of potable water for cleaning of milking containers, poor camel health management, unfavorable high ambient temperatures, longer time taken for milk to be cooled at cooling facilities. Sanitation and hygienic practices at various stages along the Isiolo camel milk value chain were found to be significantly different. Strategies to improve pastoralists' income and livelihoods from camel milk should therefore focus on innovative ways of reducing camel milk contamination, spoilage and post-harvest losses along the camel milk value chain. This can be achieved through well-tailored hygiene and food safety education aimed at improving pastoralists' knowledge on food hygiene and sanitation and hence improved quality and safety of marketed milk.

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## Conflict of interests

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# A mathematical model for the selection of an economical pipe size in pressurized irrigation systems

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**The selection of an economical pipe size for pumping plant and pipelines (mains and submains) in pressurized irrigation system should be based on careful economic analysis. A small diameter pipe may require a lower initial investment, but the head loss due to friction is greater and this increases the power cost. Similarly, a larger pipe involves a higher initial investment with less power cost. In this study, various mathematical or empirical models were formulated to select an economical pipe based on pipe diameter. These models were formulated for six different pipe materials such as reinforced cement concrete non pressure (RCC-NP2), RCC-NP3, galvanized iron, poly vinyl chloride (PVC) grey plain, PVC grey socket, and PVC grey rubber riveted (RR) joint. Each pipe material with diameters 40, 50, 75, 90,110, and 160 mm were selected to derive mathematical formulae. The prices for the different pipe materials of varying sizes were collected from various retailer shops. Further, mathematical formulation was done for calculating fixed and operating costs of these six pipe materials based on diameter of pipe, flow rate, electricity cost, and length of pipe.**

**Key word:** Pressurized irrigation system, pipes, fixed cost, variable cost, diameter of pipe.

## INTRODUCTION

The pressurized irrigation system implies an application of different pipelines in the irrigation fields. These pipelines have a long life and low maintenance costs when properly installed. They are essentially leak proof and water supply to the field plots are controlled precisely through the water distribution system. Since, this system operate under pressure, they can be applied in uphill or downhill sections, permitting delivery of irrigation water to areas not accessible to open channels or other distribution systems. Their initial cost is high as compared to lined channels, but is more economical under many field conditions and for long term use.

There are different types of pressurized irrigation methods, such as sprinkler, drip, and micro-sprinkler. These methods become increasingly popular due to their higher water application and distribution efficiencies. The

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sprinkler irrigation technology was considered to be the most efficient one prior to the development of trickle/drip irrigation technology. The drip irrigation is now one of the fastest growing technologies in modern agriculture and has proved to be the most efficient one. It has gained worldwide popularity, particularly in water scarce areas, undulating hill areas, and saline areas.

The modernization of irrigation distribution systems is mainly based on replacing the open channels by pressurized systems, which include the application of pipeline systems. The soluble fertilizers, herbicides, and fungicides can be applied through irrigation water economically and with little extra equipment. The pressurized systems guarantee higher and better distribution efficiency by eliminating the seepage and evaporation losses that occur in open channel systems and allow better control on the volume withdrawn by the farmers. The design of any water conveyance system for this system involves the selection of different sizes of pipes, materials, and equipment for utmost economy. The Indian farmers depend mainly on the earthen channels, from which much amount of water gets lost by evaporation and seepage, that is, of the order of 10 to 40% of the total water supply (Kumar, 1976).

There are different types of pipe materials which are used in water distribution system. The pipelines are normally design to deliver fluid at the required head and flow rate in a cost effective manner. The optimal selection, design, and managing of irrigation systems at farm level is an important factor for rational use of water, economic development of the agriculture, and its environmental sustainability. Some of the acceptable materials for this research work are commercial pipes, such as poly vinyl chloride (PVC), galvanized iron, and reinforced cement concrete (RCC) pipe. The increase in conduit diameter leads to increase in annual capital costs and increase in operating costs. The selection of an optimum conduit diameter for a particular fluid flow will therefore be a vital economic decision. When water is to be pumped at a known rate through a long pipeline, the designer may select a small diameter pipe to save the cost but this normally results in high pumping costs due to high friction loss in small size pipes.

In this system of water distribution, there are many factors which affect the flow of water in the pipes, such as, Reynolds number which is inversely proportional to friction factor (governed by roughness coefficient) for the pipe material. Koo and Blasius correlation factors are governed by laminar flow ($Re < 2000$) and turbulent flow ($Re > 4000$). The average velocity profile and average pressure drop is also governed by the types of flows and are being assumed for different sections. The pumps and other machineries play an important role in the flow of water through the pipes, controlled by valves/recycle valves. There are various studies on selection of an optimal economical pipe sizes (diameter) for pressurized irrigation system. Some of them are presented and reviewed herein.

Featherstone and El-Jumaily (1983) developed a computer programming approach for the selection of an optimal diameter pipe networks by incorporating the various capital and operating costs. Valiantzas et al. (2007) used very simple empirical pipe selection methods based on arbitrary concepts, which do not lead to an optimal solution instead. They presented a simple method, which allows the user to determine directly the optimum pipe size to use in simple irrigation delivery systems with pumps. Two simulation models were developed by Calejo et al. (2007) for the analysis of pressurized irrigation systems (sprinkling or micro-irrigation) operating on-demand to deliver water with the flow rate and pressure required with respect to the time, duration, and frequency decided by the farmers. Pedras et al. (2008) developed a decision support system (DSS) to design micro-irrigation systems and to advise farmers based on field evaluations. It was written in Visual Basic 6.0 language, runs in a Windows environment, and uses a database with information on emitters and pipes available in the market. Akintola and Giwa (2009a) developed a computer-aided optimization technique for the determination of optimum pipe diameter for a number of idealized turbulent flows. Relationships were formulated by connecting theories of turbulent fluid flow with pipeline costing. Akintola and Giwa (2009b) worked on an iterative optimization procedure for laminar flow in pipelines for the selection of optimum pipe diameter. Ohirhian and Ofoh (2010) developed various equations for direct calculation of the volumetric flow rate during laminar and turbulent flow of an incompressible fluid in pipes. The various equations were derived from simultaneous solution of the Bernoulli equations of Hagen Poiseulle and Darcy-Weisbach formula for the head loss and the Reynold’s number.

The aforementioned literature review indicates that most of the works regarding the optimization of pipe sizes selection was done by computer simulation models, which was not feasible for most of the Indian farmers as well as the farmers from Odisha. The average per capita water availability (both surface and ground) here is 3359 $m^3$ per year (www.dowrorissa.gov.in). With the projected future application of Odisha, the per capita water availability will reduce to 2218 $m^3$ in 2051, which is much above the water stressed condition of 1700 $m^3$, and 1000 $m^3$ was considered as water scarce condition (Water Resources Department, Government of Odisha, August, 2007). Odisha is primarily an agrarian state, where irrigation sector holds a key position in the economic development of the people and state as a whole. The application of pressurized irrigation systems for the irrigation and other purposes can neutralize the overload of water distribution in various sectors like, industrial sectors, domestic purposes, etc. One of the major criteria in the pressurized irrigation, selection of optimum pipe sizes considering the economic indicator is very important.

## MATERIALS AND METHODS

### Variable costs of irrigation projects

Variable costs of irrigation projects include the cost on regulation of the conveyance system as well as maintenance and repairs. In case of wells and pumps, they include the cost of power/fuel (electricity/diesel), costs of lubricants, labour charges for operating the pumping units, and the expenditure on repairs and maintenance of the equipment and accessories. The cost of power is often the most important component of variable costs in the case of pumping systems. The usual practice is to calculate the requirement of energy per hour of operation from the known discharge rate of the pumping plant, total operating head and its overall efficiency. The requirement of power is expressed in kilowatt-hours for electricity and liters of diesel per hour of operation of engines. The energy consumption of an electric motor is computed as follows:

$$\text{Energy consumption} = \frac{\text{Break horse power}}{\text{Motor efficiency}} \times 0.746 \quad (1)$$

Efficiencies of electric motors may be obtained from the performance data supplied by the manufacturers. Motor efficiencies usually vary from 75 to 90%. The demand of electrical power for hourly operation is multiplied by the annual hours of operation to arrive at the total annual energy consumption. The annual power cost is determined by multiplying the annual energy demand by the prevailing cost per unit of electrical energy. Variability in expected life can occur for many of these components. Due to different physical conditions like the quality of ground water, the level of repair, operation and maintenance practiced, and the length of time, the system is used each year. In case of poor quality ground water and abnormal working conditions like excessive silt-load in water, the expected service life will be less. Sound engineering judgment should be exercised in estimating the economic life during the analysis.

### Discounting rate, present worth, and capital recovery factor

The present worth is determined by multiplying the future amount by the expression:

$$\frac{1}{(1+i)^n} \quad (2)$$

The present worth of a future value at the end of $n^{th}$ period at an interest rate of i is computed using the following formula,

$$PV = F\left[\frac{1}{(1+i)^n}\right] \quad (3)$$

$$P' = A\,\frac{(1+i)^n - 1}{i(1+i)^n} \quad (4)$$

It may be seen that the capital recovery factor (CRF) is the reciprocal of the present worth of an annuity factor. The compounding factor is used to calculate the future worth of a present amount at the end of a particular period, using the following relationship:

$$F_V = P(1+i)^n \quad (5)$$

Where i = the discount rate indicating the opportunity cost of the capital. Discount rate is also called discount factor or present worth factor. (It is the reciprocal of the compounding factor for 1). The mathematical expression for compounding factor is:

$(1 + i)^n$,

Where i = rate of interest, n = period, PV = present value of the future income stream, F = future value of the income stream, A = amount of each payment, $F_V$ = future worth, P' = present amount, and P = present worth of a sequence of level payments.

### Capital recovery factor

Capital recovery factor (CRF) is used to calculate the amount of each level payment to be made at the end of each 'n' period, to recover the present amount at the end of the period, at a predetermined interest rate of 'i'. The factor is computed as follows:

$$CRF = \frac{i(1+i)^n}{(1+i)^n - 1} \quad (6)$$

The amount of the level (even) payments to be made is computed by the formula:

$$A = P\,\frac{i(1+i)^n}{(1+i)^n - 1} \quad (7)$$

Where A = amount of each level payment to be made at the end of each of 'n' periods, P = present amount. The annual cost of capital investment for an irrigation system can be determined from the present worth value of the investments, plus the interest during the period of analysis. The annual cost is usually determined by calculating a uniform series of annual values for depreciation and interest over the analysis period which is equivalent to the single present worth value. The value of this uniform series of annual cost is determined by the application of capital recovery factor (CRF).

### Operating costs

***Energy/fuel consumption***

**Electricity motor:** Efficiencies of electric motors usually vary from 80 to 90%.

**Energy consumption, kilowatt-hours =** $\frac{\text{brake horse power}}{\text{Motor efficiency}} \times 0.746$

**Engine:** An estimate of the rate of fuel consumption for a given engine can most accurately be made if the manufacturer's fuel consumption curve for that engine is available. Cost per hour of operation:

$$= BHP \times \{\text{Fuel consumed in liters per hour}\} \times \{\text{Cost of fuel per liter}\}$$

### Fixed and operating costs

The fixed cost ($C_f$) for a pipeline, L is expressed as:

$C_f$ = P×L× $\frac{i(1+i)^n}{(1+i)^n - 1}$ (8)

The operating cost ($C_o$) also depends upon on the following factors:

$C_o = f(d, Q, h_f, t, c_e, \eta)$ (9)

Annual operating cost to overcome friction is:

$C_o = \frac{0.746 \times WQ(h_f) \times t \times C_e}{75\eta}$ in Rupees (10)

On substituting the value of $h_f = \frac{8flQ^2}{\pi^2 g d^5}$ in equation (9),

$$C_o = \frac{8.103 \times 10^{-4} \times wflQ^3 t(C_e)}{\eta d^5} \quad (11)$$

Power loss due to friction (PS) in horse power is calculated as:

$$PS = \frac{WQh_f}{75\eta} \quad (12)$$

Annual power loss due to friction (PS) in kilowatt-hour is calculated as:

$$PS = \frac{0.746 \times W \times Q \times h_f \times t}{75\eta} \quad (13)$$

Thus, $C_T = C_f + C_o$ (14)

Substituting the value of $C_f$ and $C_o$ respectively,

$$C_T = Pl \times \frac{i(1+i)^n}{(1+i)^n - 1} + \frac{8.103 \times 10^{-4} \times wflQ^3 t(C_e)}{\eta d^5} \quad (15)$$

The total cost per unit length of pipeline is,

$$C_T = P \times \frac{i(1+i)^n}{(1+i)^n - 1} + \frac{8.103 \times 10^{-4} \times wfQ^3 tc_e}{\eta d^5} \quad (16)$$

Where d- diameter of pipe (m), Q = discharge (cumec), $h_f$ = head loss due to friction (m), t = pump use hr/year , $c_e$ = cost of electricity (Rs/KWH), $\eta$ = efficiency of power unit (fraction), W = Unit weight of water (kg/m$^3$), P = price per unit length of pipe (Rs/m), i = interest rate, (fraction), n = life of pipe (years), $C_T$ = total cost per annum (Rs/year), $C_f$ = fixed cost per annum (Rs/year), $C_o$ = Operating cost per annum (Rs/year), and L = length of pipe (m).

### Economical pipe size selection

#### *Optimization analysis*

The optimum diameter of pipe will be that diameter at which the present value of capital cost and operation and maintenance charges is minimum. Capital cost includes cost of pipes, cost of laying the pipe and the cost of pump set. The operation and maintenance (O & M) charges comprise of energy charges, and establishment costs pertaining to rising mains. Since the horse power of the pump sets depends upon frictional loss, energy charges are also a frictional loss, energy charges are also a function of the pipe diameter. The formula for determining the present value for the purpose of optimization analysis is given as follows:

$$\text{Present Value} = \text{Capital cost} + (\text{Operation \& Maintenance charges}) \times \frac{(1+i)^n 1}{i(1+i)^n}$$

(17)

Where i = rate of interest as applicable, and n = life of the project.

By minimizing the present value, optimum diameter is estimated and results for different discharges and types of pipes are computed and compared for their relative costs. The diameter of the pipe selected should be nearest to the optimum size thus arrived at.

### Cost of pipe diameter relationship

In this, different pipes of varying sizes and materials, their price per unit length are considered. The prices for the different pipe materials have been obtained by collecting from various retailers and shops or standard farms. An empirical equation relating price with diameter which is used here is:

$P = a (d)^b$ (18)

where P = price per unit length (Rupees), d = diameter (mm), a and b = constants. The criteria for deciding the most economical pipe diameter is that the diameter at which the sum of fixed and variable costs per annum is minimum. The total cost consists of fixed cost and operating cost. The fixed cost depends upon the initial value and life expectancy of the equipment. The variable cost is a function of head loss ($h_f$); flow rate (Q), the number of hours the system used in a year. The value of ($h_f$) was obtained from Darcy Weisbach formula, getting the value of Moody's diagram and the empirical relationship between price per unit length and diameter was substituted to give an equation which can be differentiated with respect to diameter and equated to zero to yield the most economical diameter.

## RESULTS AND DISCUSSION

### Empirical formulation of cost diameter relationships of pipes

To derive an exponential based mathematical equation between cost and diameter, various pipe diameters of different materials were selected. The different pipe materials include: reinforced cement concrete non pressure (RCC-NP2), RCC-NP3, galvanized iron, poly vinyl chloride (PVC) grey plain, PVC grey socket, and PVC grey rubber riveted (RR) joint. Each pipe material with diameters 40, 50, 75, 90,110, and 160 mm were selected to derive mathematical formulae. The prices for the different pipe materials of varying sizes were collected from various retailer shops and standard farms. The present costs of different pipes such as RCC, GI, and PVC are shown in Tables 1 to 3, respectively. The cost and diameter relationship of pipes is shown in Table 4. From this table, it is evident that there exists an

**Table 1.** Present retail cost of RCC pipes.

| Diameter of pipe (mm) | Present rate per unit length (Rs.) | |
|---|---|---|
| | RCC NP2 | RCC NP3 |
| 100 | 92.0 | 113.0 |
| 150 | 117.0 | 146.0 |
| 200 | 145.0 | 205.0 |
| 250 | 173.0 | 252.0 |
| 300 | 256.0 | 406.0 |
| 350 | 299.0 | 640.0 |
| 400 | 336.6 | 710.6 |
| 450 | 404.0 | 785.0 |
| 500 | 455.4 | 897.6 |
| 600 | 640.0 | 1220.0 |
| 700 | 836.0 | 1500.4 |
| 800 | 1034.0 | 1957.0 |
| 900 | 1314.0 | 2455.0 |
| 1000 | 1566.0 | 2972.0 |
| 1100 | 1794.1 | 3440.8 |
| 1200 | 2090.0 | 3988.0 |

Note: RCC NP = Reinforced Cement Concrete Non Pressure

**Table 2.** Present costs of GI pipes.

| Pipe diameter (mm) | Present cost per unit length (Rs.) |
|---|---|
| 12.7 | 136.55 |
| 19.1 | 193.30 |
| 25.4 | 278.95 |
| 31.8 | 420.35 |
| 38.1 | 497.95 |
| 50.8 | 631.50 |
| 63.5 | 690.79 |
| 76.2 | 797.72 |
| 127 | 1103.00 |

**Table 3.** Present costs of PVC pipes.

| Pipe | Diameter (mm) | Present cost per unit length (Rs.) |
|---|---|---|
| PVC Grey Plain | 40 | 38.90 |
| | 50 | 41.60 |
| | 75 | 46.40 |
| | 90 | 65.65 |
| | 110 | 61.00 |
| | 160 | 128.08 |
| PVC Grey Socket | 40 | 48.50 |
| | 50 | 58.65 |
| | 75 | 62.01 |
| | 90 | 60.58 |
| | 110 | 87.61 |
| | 160 | 123.52 |
| PVC Grey RR joint | 40 | 77.98 |
| | 50 | 88.90 |
| | 75 | 95.90 |
| | 90 | 135.12 |
| | 110 | 195.05 |
| | 160 | 292.01 |

exponential relationship between the cost and diameter of different pipes. Further, a value of coefficient of regression ($R^2$) was derived for different pipe materials. The RCC- NP3 pipe material exhibited a highest value of $R^2$ = 0.986 as compared to other materials. The PVC Grey Plain pipe material exhibited a lowest value of $R^2$ = 0.941 as compared to other materials. However, there was not much difference in $R^2$ values were observed among different materials. Therefore, from these results one can say that the RCC- NP3 is the best material to consider for pipe construction during installation of pressurized irrigation system in the study region.

The cost and diameter of various pipes of different materials were plotted in the form of exponential plots and are shown in Figures 1 to 6. From the representation of the exponential plots, power curve equations were developed between the present cost (P) and diameter of the pipe (d) manufactured with different materials along with the coefficient of regression ($R^2$) values. Trend lines were also plotted in Figures 1 to 6. From these plots, it is evident that the RCC- NP3 plot (that is, Figure 2) developed a well as established power curve relationship with highest $R^2$ value. The similar type of results was also drawn from the Table 4.

## Economic analysis

The selection of pipe size of pumping plants and pipelines should be based on careful economic analysis. A small pipe may require a lower initial investment but the head loss due to friction is greater and this increases the power cost. A larger pipe in many cases will save more in power cost than the additional investment. Further, the larger pipe may so reduce the total pump head that a lighter and lower priced pump and power unit may be used. The actual cost of the installation based on the cost of pump and electric motor, taking into account the friction head under the two cases, should be estimated and the choice of the pipe size finalized. There are two types of costs are involved in deriving cost analysis i.e. fixed costs and operating costs. The fixed cost of the selected pipes was formulated considering the cost

**Table 4.** Derived empirical relationship between cost and diameter of pipe.

| Name of pipe | Empirical equation | Coefficient of regression ($R^2$) |
|---|---|---|
| RCC- NP2 | $P = 0.126(d)^{1.345}$ | 0.974 |
| RCC- NP3 | $P = 0.071(d)^{1.530}$ | 0.986 |
| Galvanised Iron | $P = 288.02(d)^{1.007}$ | 0.979 |
| PVC Grey Plain | $P = 1.842(d)^{0.790}$ | 0.941 |
| PVC Grey Socket | $P = 4.862(d)^{0.610}$ | 0.943 |
| PVC Grey RR Joint | $P = 1.983(d)^{0.960}$ | 0.979 |

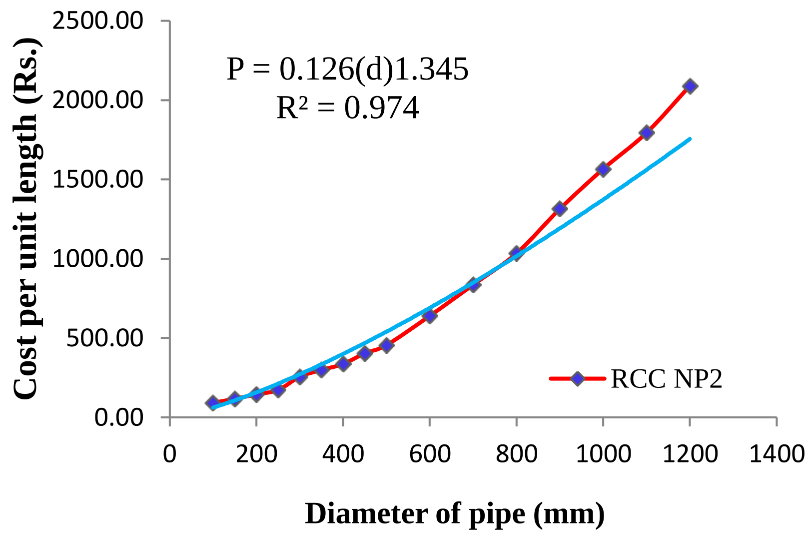


**Figure 1.** Relationship between cost and diameter of RCC NP2 pipe.

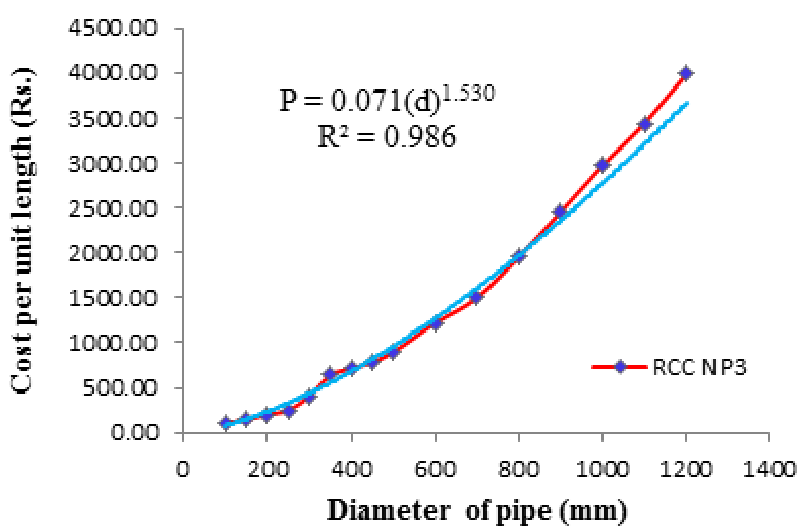


**Figure 2.** Relationship between cost and diameter of RCC NP3 pipe.

diameter relationship through equation (8) the fixed costs for the selected pipes are given in Table 5. The operating cost indicates the amount of power consumed while operating the power system for overcoming the frictional head losses in various pipe sizes. It has been evaluated on yearly basis, which decreases with increase in pipe

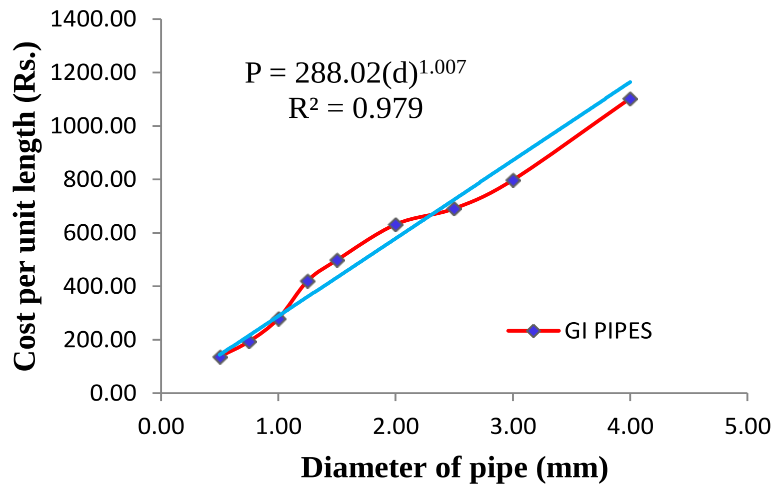


**Figure 3.** Relationship between cost and diameter of GI pipe.

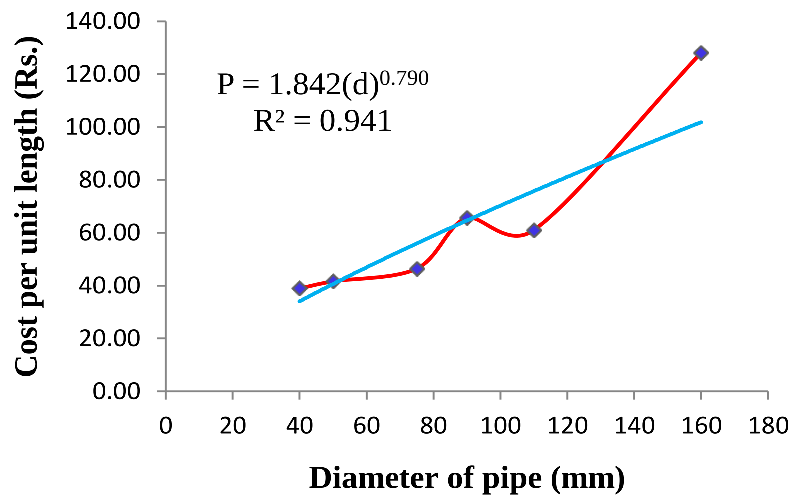


**Figure 4.** Relationship between cost and diameter of PVC Grey Plain pipe.

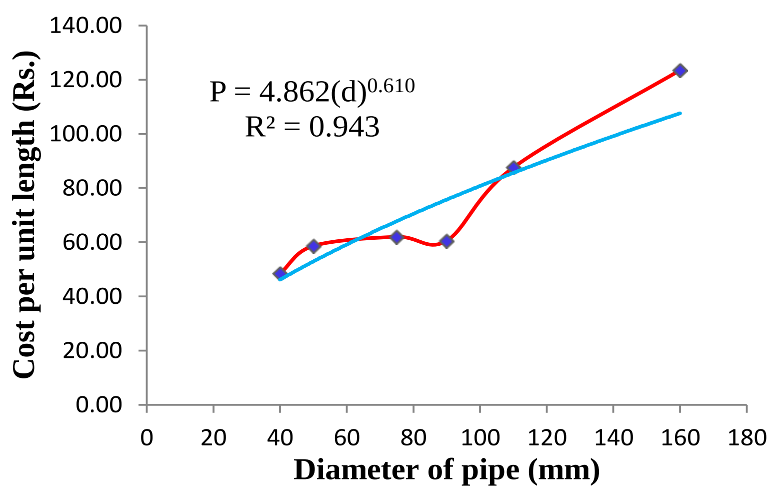


**Figure 5.** Relationship between cost and diameter of PVC Grey Socket pipe.

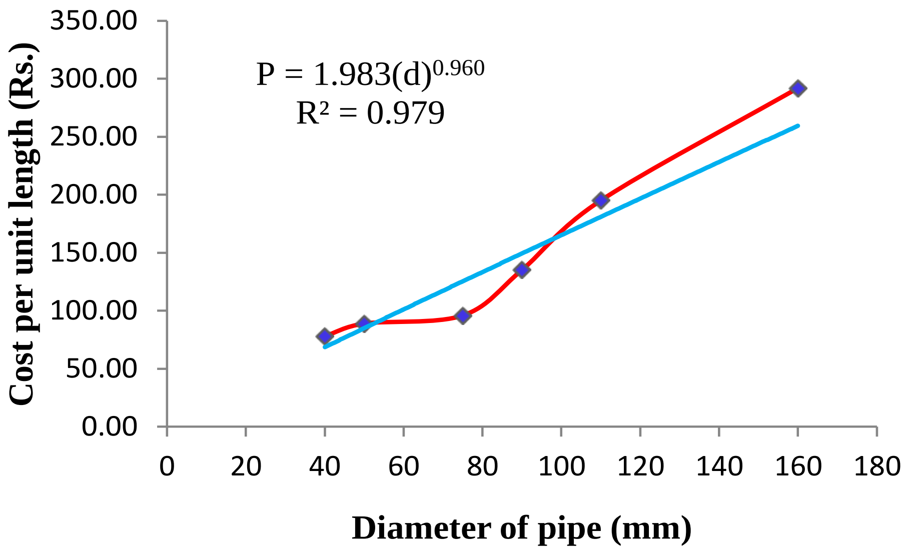


**Figure 6.** Relationship between cost and diameter of PVC Grey Rubber Riveted (RR) Joint.

**Table 5.** Values of fixed cost for the pipes of different materials.

| Name of pipe | Fixed cost ($C_f$) |
|---|---|
| RCC- NP2 | $0.126(d)^{1.345} \times L\left[\frac{i(1+i)^n}{(1+i)^n - 1}\right]$ |
| RCC- NP3 | $0.071(d)^{1.53} \times L\left[\frac{i(1+i)^n}{(1+i)^n - 1}\right]$ |
| Galvanised Iron | $288.02(d)^{1.0074} \times L\left[\frac{i(1+i)^n}{(1+i)^n - 1}\right]$ |
| PVC - Grey plain | $1.842(d)^{0.79} \times L\left[\frac{i(1+i)^n}{(1+i)^n - 1}\right]$ |
| PVC - Grey Socket | $4.862(d)^{0.6103} \times L\left[\frac{i(1+i)^n}{(1+i)^n - 1}\right]$ |
| PVC - Grey RR Joint | $1.983(d)^{0.96} \times L\left[\frac{i(1+i)^n}{(1+i)^n - 1}\right]$ |

Note: d = diameter of the pipes (m), i = interest rate, n = life of the pipes (years), and L = length of the pipe (m).

**Table 6.** Values of operating cost for the pipes of different materials.

| Name of pipe | Operating cost ($C_o$) |
|---|---|
| RCC- NP2 | $C_o = \frac{8.103\times10^{-4}\times wflQ^3t(C_e)}{\eta d^5}$ |
| RCC- NP3 | $C_o = \frac{8.103\times10^{-4}\times wflQ^3t(C_e)}{\eta d^5}$ |
| Galvanised Iron | $C_o = \frac{8.103\times10^{-4}\times wflQ^3t(C_e)}{\eta d^5}$ |
| PVC Grey Plain | $C_o = \frac{8.103\times10^{-4}\times wflQ^3t(C_e)}{\eta d^5}$ |
| PVC Grey Socket | $C_o = \frac{8.103\times10^{-4}\times wflQ^3t(C_e)}{\eta d^5}$ |
| PVC Grey RR Joint | $C_o = \frac{8.103\times10^{-4}\times wflQ^3t(C_e)}{\eta d^5}$ |

Note: d = diameter of pipe (m), Q = discharge (cumec), t = pump use (hr/year), $C_e$ = cost of electricity (Rs./KWH), $\eta$ = efficiency of power unit (fraction), W = Unit weight of water (kg/m$^3$), $C_o$ = Operating cost per annum (Rs/year), and l = length of pipe (m).

diameter in all types of pipes. The operating cost depends upon the various factors, such as diameter of the pipe, discharge rate, head loss due to friction, pump operation hours in a year, cost of electricity per kilowatt hour, and efficiency of the power unit. The annual operating costs for various pipes of different variable sizes are calculated following equation (10), and is given in Table 6.

## Conclusion

Irrigation system design substantially affects application efficiency and involves numerous variables, whose principal objective is to maximize benefits and minimize costs. The pressurized irrigation system can attain a reasonable level of efficiency, when they are well designed, appropriately selected, and adequately operated. The work was initiated with the collection of present rates of the selected pipes from the nearby market of Bhubaneswar, Odisha. The cost and diameter relationship of different pipe materials was established by developing an empirical equation in the power form. The fixed cost is calculated by using the present market value of the pipes the capital recovery factor per unit length of the pipe. The operating cost is calculated considering the variable diameters, flow rate, head loss due to friction, time of operation per annum, cost of electricity per annum, and the efficiency of the power unit. The cost is considered for the mathematical model ($C_T = C_o + C_f$). Thus, the mathematical model for the economical diameter was obtained. The minimum pipe sizes selection involves low fixed cost but result in high frictional head loss which results is more power consumption resulting is the high cost of operation. Again the selection of high pipes sizes results is high initial cost but less operating cost.

## Conflict of interest

The authors have not declared any conflict of interest

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Full Length Research Paper

# Evaluating the logistics performance of Brazil's corn exports: A proposal of indicators

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**Despite significant advances in the Brazilian agriculture, the logistics costs, particularly transportation and storage costs continue to act as the main barriers that limit the potential of the Brazilian agribusiness. This study analyzes the logistics efficiency of the main flow routes for Brazil's agricultural production and exports - in particular, corn production and exports, among the major producing states and the main ports of Brazil. For this purpose, we develop a performance measurement system based on the Balanced Scorecard (BSC) using four models for efficiency analysis: financial, customer, internal business processes, and learning and growth. Tthe efficiency of the main routes was calculated using the data envelopment analysis (DEA). Our results suggest that the routes from Mato Grosso state to Santarem port using a road-waterway intermodal transport system were the most efficient on three of the four criteria. Thereafter, the relative efficiency of all main Brazilian routes was analyzed based on the four criteria, establishing the references and developing a standard model to evaluate other logistics systems.**

**Key words:** Data envelopment analysis, corn, logistics index, route analysis.

## INTRODUCTION

The spatial arrangement change of the Brazilian agricultural production is a recurring phenomenon and agricultural businesses are exploring new frontiers through activities that incorporate modern production technologies (Oliveira, 2014). However, modern agriculture must foster sustainable development, including production systems that promote efficient water management (Valipour, 2015).

Brazil's market share in global corn exports is approximately 17% (22 million tons) which represents 28% of the national produce and supports the Brazilian commercial trade results (USDA, 2014). The productivity of Brazilian corn is approximately 5,051 kg/ha, which is lower than the global average of 5,495 kg/ha (USDA, 2014). Besides the economic importance, corn is an essential component of animal feed for livestock such as poultry, pork, and cattle. On a regional basis, the main Brazilian producer states that export corn are Mato

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Grosso (59% of the total exports), followed by Paraná (13%) and Goiás (12%). Together, they account for over 84% of the national export volume. The most important Brazilian ports used for corn exports are the Port of Santos (SP) and the Port of Paranaguá (PR) with 43% and 15% of the total volume of corn exports, respectively (MDIC, 2014).

The spatial reconfiguration of agriculture in Brazil increases production; nevertheless some weaknesses are becoming apparent, in particular, those related to transportation and storage. Bartolacci et al. (2012) stated that Brazil's agricultural competitiveness can only be increased by directing investments and solving logistical barriers. In the Brazilian case, product competitiveness can be increased by overcoming problems such as the shortage of storage capacity, reducing bureaucracy and increasing port organization, redistributing the cargo transportation matrix, as well as increasing the capacity and efficiency of rail and waterway transport.

Difficulties in the Brazilian infrastructure are not just limited to transportation, but also to the storage network. In addition to the deficit capacity, the storage location is inadequate and largely concentrated in urban centers. The ideal solution would be that the storage units focus on farms and the countryside. As stated by Junqueira and Morabito (2012), storage is a strategic factor in the agricultural sector to correct the seasonality issue in production, and therefore, to meet demand during the off-season period, in addition to maintaining stability in product prices and cargo rates.

According to Oliveira (2014), constant developments in agriculture, increase in productivity, and expansion of domestic corn in areas previously considered unproductive can further increase production. However, aspects such as increasing the efficiency of ports, obtaining international quality certifications for the produced corn, improving logistics network development, and reducing the bureaucracy process and costs are still key elements for the efficiency of this production chain (Amirteimoori, 2011).

Therefore, the overall objective of the study is to develop a performance measurement system that makes it possible to evaluate the relative logistics efficiency of the Brazilian corn export process through the data envelopment analysis (DEA) method in the main logistics routes.

## MATERIALS AND METHODS

This study used MDIC (2014) and USDA (2014) data and bibliographical research to build the indicators and data envelopment analysis (DEA) to calculate the logistics efficiency of the main flow routes for Brazil's corn production and exports. Four strategic indices based on the balanced scorecard (BSC) were used to evaluate logistics efficiency. The BSC was developed by Kaplan and Norton (1997) to translate corporate strategy into performance indicators, unfolding it into four perspectives – financial; customer; internal business processes; and learning and growth - not restricting the analysis to financial criteria.

The indices for all routes were organized and analyzed using the DEA methodology, thus providing information on which route is the most efficient in which index. The DEA, introduced by Farrel (1957) and generalized by Charnes et al. (1978), is a non-parametric technique that allows the handling of multiple outputs and inputs to facilitate comparative performance measurement of independent units, that is, the efficiency of each unit. DEA allows several inputs and outputs to be used when analyzing the performance of various similar organizational units (DMUs) through a standard linear programming, which seeks to establish the maximum efficiency of a DMU, expressed in the rate of ratio of inputs to outputs, comparing the performance of a unit in relation to the group of similar units. This technique allows us to identify units with relatively more efficient performance (Talluri et al., 2013).

According to Azambuja et al. (2015), DEA models can work under conditions of constant returns to scale (Charles, Cooper and Rhodes (CCR) model, also known as CRS) or variable returns to scale (Banker, Charnes and Cooper (BCC) model, also known as VRS). In this study, we used the BCC model oriented to output, which has its mathematical formulation defined as:

$$Max\ h_o \quad \text{s.t} \tag{1}$$

$$x_{jo} - \sum_{k=1}^{n} x_{ik} \cdot \lambda_k \geq 0, \forall\, i \tag{2}$$

$$-h_o \cdot y_{jo} + \sum_{k=1}^{n} y_{jk} \cdot \lambda_k \geq 0, \forall\, j \tag{3}$$

$$\sum_{k=1}^{n} \lambda_k = 1 \tag{4}$$

$$\lambda_k \geq 0, \forall\, k \tag{5}$$

The proposed model maximizes outputs, keeping the inputs unchanged. In Equation 1 $h_o$ is the efficiency. The Equation 2 guarantees that the reduction in inputs does not get through the frontier defined by the reference DMUs. The Equation 3 guarantees that the reduction in inputs does not affect the output values. The Equation 4 guarantees that the contribution of the DMU does not exceed 1 and Equation 5 guarantees that the contribution of the DMU is not negative. Note that $\lambda_k$ is the DMU contribution $k$ in the formation of the DMU target $o$.

### Defining routes to analyze the most favorable option

When developing the study, we conducted logistics process mapping for corn and identified the main routes of flow for export, which were defined as the objects of study, the DMUs. Seventeen 17 such routes were defined, of which eight were unimodal, using only the road modal and nine were intermodal, using a combination of road/rail and road/waterway transport systems. Therefore, we have the origins of the routes in the states of Mato Grosso, Paraná, and Goiás and destinations to the ports of Santos (SP), Paranaguá

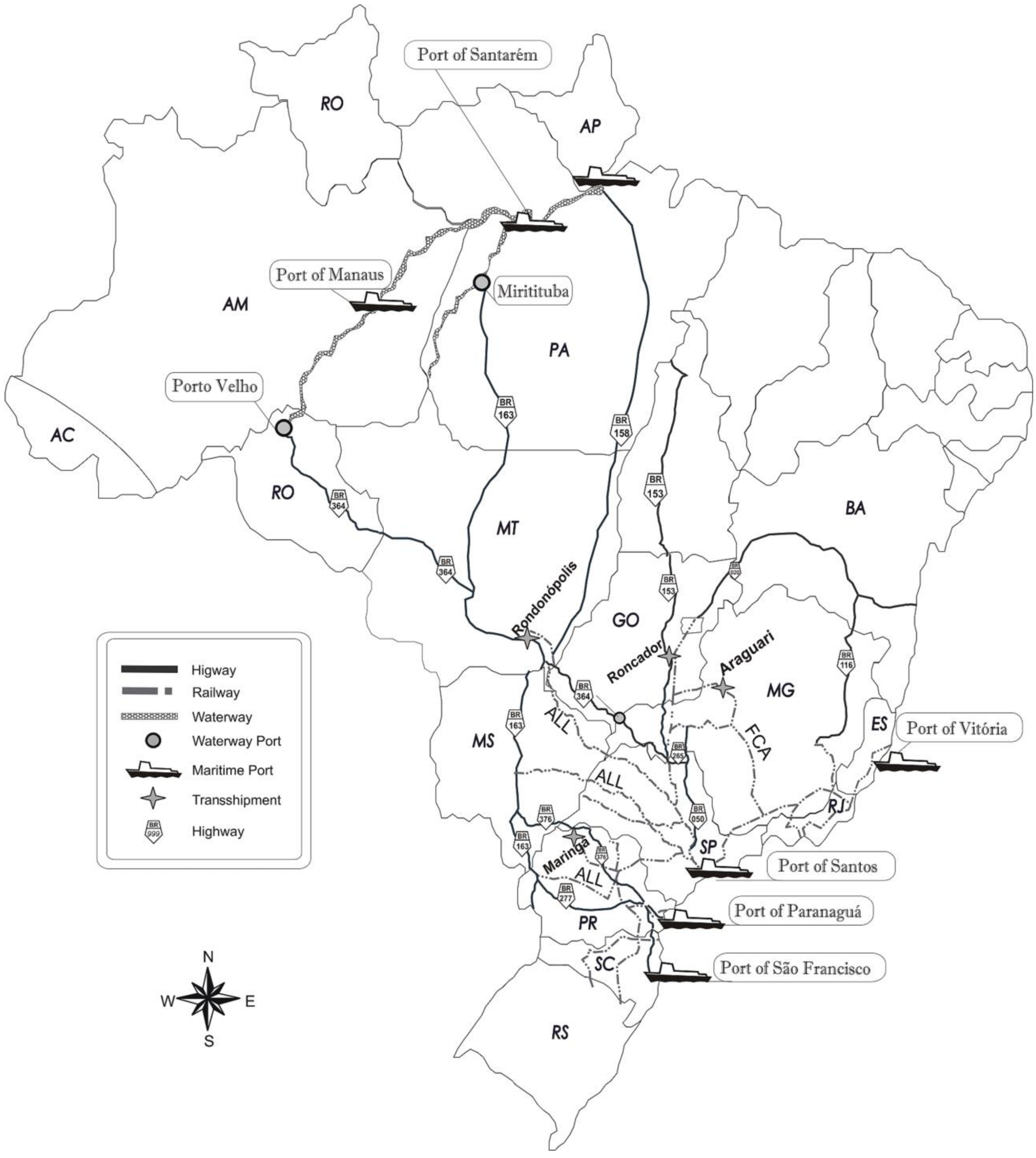


**Figure 1.** Primary logistics routes for corn in Brazil.

(PR), São Francisco do Sul (SC), Vitória (ES), Santarém (PA) and Manaus (AM) (Figure 1). For example, the MT-SP-ROAD route (DMU01) is characterized by the corn flow between the producing state of Mato Grosso to the Port of Santos (SP) using the exclusive road transport option. The MT-SP-ROAD-RAIL (DMU02) considers the route between the producing state of Mato Grosso to the Port of Santos (SP) using the road-waterway intermodal transport. The produced corn travels a distance of approximately 600 km using the BR-163 and BR-364 highways to the intermodal terminal of Rondonópolis-MT, covering the remainder of the route by rail for 1551 km. The rail transshipment terminals Rondonópolis-MT, Araguari-MG, Maringá-PR, and Roncador-GO were considered. As

**Table 1.** Measuring efficiency models.

| Output | Input |
|---|---|
| Logistics costs (Financial Dimension) | Composition of the transportation matrix<br>Availability of warehouses<br>Corn production cost<br>Productivity of corn in field |
| DMU Participation (Customer Dimension) | Transportation cost<br>Queue in the ports/ships<br>Route extension |
| Lead time delivery<br>(Internal Business Processes Dimension) | Average speed<br>Queue in the ports/ships<br>Route extension |
| Sustainable development<br>(Learning and Growth Dimension) | Fuel consumption<br>Fleet age<br>Composition of the transportation matrix |

for waterway terminals, the Miritituba-PA Terminal (DMU06) and the Porto Velho-RO Terminal (DMU07) were considered (Figure 1).

#### Definition of variables

To define the variables, the Balanced Scorecard (BSC) based on four dimensions was used as reference:

#### Financial dimension

This dimension represents the costs and analyzes the contribution of all indicators in minimizing the final cost of the Brazilian corn in the international market. The strategic objective representing this dimension is the Logistics costs, calculated as the total cost of the Brazilian corn delivery in the Port of Shanghai, China, since it is the largest port in terms of activity in the world and the consumer market for Brazilian corn is focused on Asia.

#### Customer dimension

From the customer's perspective, the DMU Participation indicator was established, which represents the total volume of export by a particular route compared to the total national volume of export.

#### Internal business processes dimension

In this dimension, the activities that should be developed were evaluated, taking into account the history of bureaucratic procedures of customs and excessive time for delivery. The strategic objective that analyzes these factors is the Lead Time Delivery, which considers the queue of ships in the port and transportation time to the Port of Shanghai, China.

#### Learning and growth dimension

From the learning and growth perspective, the factors that should be developed for the future business success was analyzed. For this, we adopted sustainable development, which represents the adoption of clean practices and which measures the flow impact of corn production in accordance with the modals and the route used. The estimate adopted in this case was the $CO_2$ emission. After analyzing the indicators proposed in the literature, indicators that reflect the supply chain strategy vision used in the study (Table 1) were selected as inputs. Aiming to verify the relationship of each input indicator with strategic indicators (output), Table 1 was structured, which shows the four models and the respective indicators, constituting the analysis. In the resolution process of the models, we used the BCC method. As a way of dealing with undesirable outputs, the data was treated in order to adjust them through the multiplicative inverse method, which transforms undesirable output indicators (Logistics Costs, Lead Time Delivery, and Sustainable Development) as inputs through the formula $F_{(u)} = -u$. In order to highlight the efficient DMUs, the inverted frontier method was used:

$$Inverted\ Frontier\ Efficiency = \frac{Standard\ Efficiency\ + (1 - Inefficiency)}{2} \quad (6)$$

The final efficiency was obtained by calculating the inverted frontier, which identifies the most efficient DMU and provides a comparative efficiency analysis of the remaining DMUs.

## RESULTS AND DISCUSSION

Initially, the results obtained by the routes in each of the four proposed models were analyzed: logistics costs/financial dimension, DMU participation/customer dimension, lead time delivery/internal business processes dimension and sustainable development/learning and growth dimension. Thereafter, the routes were analyzed as a whole.

**Table 2.** Final efficiency of DMUs (financial/logistics costs model).

| DMU/Indicator | DMU | Efficiency | Inefficiency | Inverted frontier efficiency | Final efficiency |
|---|---|---|---|---|---|
| MT-SP-ROAD | DMU01 | 0.572050 | 0.838137 | 0.366957 | 0.482665 |
| MT-SP-ROAD-RAIL | DMU02 | 1.000000 | 0.773996 | 0.613002 | 0.806293 |
| MT-PR-ROAD | DMU03 | 0.529067 | 0.906229 | 0.311419 | 0.409616 |
| MT-PR-ROAD-RAIL | DMU04 | 0.532161 | 0.944073 | 0.294044 | 0.386762 |
| MT-PA/AM-ROAD | DMU05 | 0.819424 | 0.585113 | 0.617155 | 0.811756 |
| **MT-PA/AM-ROAD-WATER** | **DMU06** | **1.000000** | **0.479456** | **0.760272** | **1.000000** |
| MT-PA/AM-ROAD-WATER 2 | DMU07 | 0.767949 | 0.763861 | 0.502044 | 0.660348 |
| MT-ES-ROAD | DMU08 | 0.479456 | 1.000000 | 0.239728 | 0.315319 |
| MT-ES-ROAD-RAIL | DMU09 | 0.637890 | 0.993624 | 0.322133 | 0.423708 |
| PR-PR-ROAD | DMU10 | 0.880385 | 0.907441 | 0.486472 | 0.639866 |
| PR-PR-ROAD-RAIL | DMU11 | 1.000000 | 0.798897 | 0.600551 | 0.789917 |
| PR-SC-ROAD | DMU12 | 0.798897 | 1.000000 | 0.399449 | 0.525402 |
| PR-SC-ROAD-RAIL | DMU13 | 0.893217 | 0.894404 | 0.499406 | 0.656878 |
| GO-SP-ROAD | DMU14 | 0.891240 | 0.747728 | 0.571756 | 0.752041 |
| GO-SP-ROAD-RAIL | DMU15 | 1.000000 | 0.619565 | 0.690217 | 0.907856 |
| GO-ES-ROAD | DMU16 | 0.666405 | 1.000000 | 0.333202 | 0.438267 |
| GO-ES-ROAD-RAIL | DMU17 | 1.000000 | 1.000000 | 0.500000 | 0.657659 |

Source: Survey data (2015).

## Financial dimension

Following the financial model resolution, the road and waterways intermodal route was analyzed through the Miritituba terminal for obtaining an overall efficiency rate of 76.02% (inverted frontier efficiency), being the most efficient route (MT-PA/AM-ROAD-WATER). From this route, the relative efficiency of others was calculated, highlighting the road and waterways intermodal route to export corn from the state of Goiás to the Port of Santos through the railway, which reached a relative efficiency rate of 90.78% (GO-SP-ROAD-RAIL). Table 2 shows the financial model results.

## Customer dimension

As main references in the customer model, the logistics process of corn flow between the producing state of Mato Grosso and the Port of Santos via road (MT-SP-ROAD) was verified, which obtained an efficiency rate of 98.07% (inverted frontier efficiency). This is an expected result, since Mato Grosso is the largest exporter state and Port of Santos is the largest Brazilian port in export volume, with the distribution of 74% of the total transport, according to the Brazilian transportation matrix of CNT (2014). This result demonstrates that the route has reached the maximum limit of its capacity, which calls for the need for distribution by other routes. The main alternative is using the Ports of Santarém and Manaus (MT-PA/AM-ROAD), with relative efficiency rate of 96.39%, followed by alternatives using the highway for transport between the State of Paraná and the Port of Paranaguá (PR-PR-ROAD) and Port of São Francisco do Sul (PR-SC-ROAD) with relative efficiency rates of 94.42 and 87.92%, respectively, as detailed in Table 3.

## Internal business processes dimension

As a result of the internal business processes efficiency model, all routes had partial efficiency rates of above 93% owing to the representativeness of the transshipment time between the Brazilian ports and the Port of Shanghai, China, which directly influences the total lead time delivery process. After application of the inverted frontier method, the analyzed routes had a very high inefficiency rate (above 90%). The highlighted routes of this model were that from the state of Mato Grosso to the Ports of Santarém/Manaus, using the road and waterway intermodal route through the Miritituba waterway terminal (MT-PA/AM-ROAD-WATER 2), with a relative efficiency rate of 96.07%. Also, from the State of Paraná to the Port of São Francisco do Sul using the railway (PR-SC-ROAD-RAIL), with a relative efficiency rate of 98.66%. The data on internal business processes model are available in Table 4.

## Learning and growth dimension

The highlighted routes were from the State of Paraná,

**Table 3.** Final efficiency of DMUs (customer/DMU participation model).

| DMU/Indicator | DMU | Efficiency | Inefficiency | Inverted frontier efficiency | Final efficiency |
|---|---|---|---|---|---|
| **MT-SP-ROAD** | **DMU01** | **1.00** | **0.03861** | **0.98070** | **1.00000** |
| MT-SP-ROAD-RAIL | DMU02 | 0.38 | 0.11232 | 0.63270 | 0.64515 |
| MT-PR-ROAD | DMU03 | 0.11 | 0.34375 | 0.38539 | 0.39297 |
| MT-PR-ROAD-RAIL | DMU04 | 0.04 | 1.00000 | 0.01968 | 0.02007 |
| MT-PA/AM-ROAD | DMU05 | 1.00 | 0.10938 | 0.94531 | 0.96392 |
| MT-PA/AM-ROAD-WATER | DMU06 | 1.00 | 1.00000 | 0.50000 | 0.50984 |
| MT-PA/AM-ROAD-WATER 2 | DMU07 | 0.11 | 1.00000 | 0.05469 | 0.05576 |
| MT-ES-ROAD | DMU08 | 0.27 | 1.00000 | 0.13569 | 0.13836 |
| MT-ES-ROAD-RAIL | DMU09 | 0.09 | 1.00000 | 0.04664 | 0.04756 |
| PR-PR-ROAD | DMU10 | 1.00 | 0.14796 | 0.92602 | 0.94425 |
| PR-PR-ROAD-RAIL | DMU11 | 1.00 | 0.43044 | 0.78478 | 0.80023 |
| PR-SC-ROAD | DMU12 | 1.00 | 0.27538 | 0.86231 | 0.87928 |
| PR-SC-ROAD-RAIL | DMU13 | 1.00 | 0.80111 | 0.59945 | 0.61125 |
| GO-SP-ROAD | DMU14 | 0.48 | 0.18122 | 0.64713 | 0.65987 |
| GO-SP-ROAD-RAIL | DMU15 | 0.19 | 0.52719 | 0.33268 | 0.33923 |
| GO-ES-ROAD | DMU16 | 0.19 | 0.33036 | 0.43078 | 0.43926 |
| GO-ES-ROAD-RAIL | DMU17 | 0.06 | 0.96105 | 0.04899 | 0.04996 |

Source: Survey data (2015).

**Table 4.** Final efficiency of DMUs (internal business processes/ lead time delivery model).

| DMU/Indicator | DMU | Efficiency | Inefficiency | Inverted frontier Efficiency | Final efficiency |
|---|---|---|---|---|---|
| MT-SP-ROAD | DMU01 | 0.94795 | 0.98711 | 0.48042 | 0.86976 |
| MT-SP-ROAD-RAIL | DMU02 | 0.94247 | 0.98342 | 0.47953 | 0.86814 |
| MT-PR-ROAD | DMU03 | 0.93573 | 1.00000 | 0.46787 | 0.84703 |
| MT-PR-ROAD-RAIL | DMU04 | 0.93260 | 1.00000 | 0.46630 | 0.84419 |
| MT-PA/AM-ROAD | DMU05 | 1.00000 | 0.93573 | 0.53214 | 0.96338 |
| **MT-PA/AM-ROAD-WATER** | **DMU06** | **1.00000** | **0.89527** | **0.55236** | **1.00000** |
| MT-PA/AM-ROAD-WATER 2 | DMU07 | 1.00000 | 0.93869 | 0.53065 | 0.96070 |
| MT-ES-ROAD | DMU08 | 0.95657 | 1.00000 | 0.47829 | 0.86589 |
| MT-ES-ROAD-RAIL | DMU09 | 0.91415 | 1.00000 | 0.45707 | 0.82749 |
| PR-PR-ROAD | DMU10 | 1.00000 | 0.96480 | 0.51760 | 0.93706 |
| PR-PR-ROAD-RAIL | DMU11 | 1.00000 | 0.96458 | 0.51771 | 0.93726 |
| PR-SC-ROAD | DMU12 | 1.00000 | 0.95489 | 0.52256 | 0.94604 |
| PR-SC-ROAD-RAIL | DMU13 | 1.00000 | 0.91000 | 0.54500 | 0.98667 |
| GO-SP-ROAD | DMU14 | 0.97808 | 0.96604 | 0.50602 | 0.91610 |
| GO-SP-ROAD-RAIL | DMU15 | 1.00000 | 100000 | 0.50000 | 0.90520 |
| GO-ES-ROAD | DMU16 | 0.97956 | 0.95525 | 0.51216 | 0.92721 |
| GO-ES-ROAD-RAIL | DMU17 | 1.00000 | 0.97987 | 0.51006 | 0.92342 |

Source: Survey data (2015).

which is closest producing state for all export routes. The reference is the railway route from state of Paraná to the Port of Paranaguá with a final efficiency rate of 97.55% (PR-PR-ROAD-RAIL), followed by the road route from state of Paraná to the Port of Paranaguá (PR-PR-ROAD) (91.76% relative efficiency), and intermodal route

**Table 5.** Final efficiency of DMUs (learning and growth/sustainable development model).

| DMU/Indicator | DMU | Efficiency | Inefficiency | Inverted frontier efficiency | Final efficiency |
|---|---|---|---|---|---|
| MT-SP-ROAD | DMU01 | 0.06084 | 0.80321 | 0.12882 | 0.13204 |
| MT-SP-ROAD-RAIL | DMU02 | 0.13159 | 0.49400 | 0.31880 | 0.32678 |
| MT-PR-ROAD | DMU03 | 0.05544 | 0.88153 | 0.08696 | 0.08913 |
| MT-PR-ROAD-RAIL | DMU04 | 0.06696 | 0.80806 | 0.12945 | 0.13269 |
| MT-PA/AM-ROAD | DMU05 | 0.11830 | 0.55221 | 0.28304 | 0.29013 |
| MT-PA/AM-ROAD-WATER | DMU06 | 0.13664 | 047907 | 0.32878 | 0.33702 |
| MT-PA/AM-ROAD-WATER 2 | DMU07 | 006317 | 0.78861 | 0.13728 | 0.14072 |
| MT-ES-ROAD | DMU08 | 0.04887 | 1.00000 | 0.02444 | 0.02505 |
| MT-ES-ROAD-RAIL | DMU09 | 0.06944 | 1.00000 | 0.03472 | 0.03559 |
| PR-PR-ROAD | DMU10 | 1.00000 | 0.20964 | 0.89518 | 0.91760 |
| **PR-PR-ROAD-RAIL** | **DMU11** | **1.00000** | **0.04887** | **0.97557** | **1.00000** |
| PR-SC-ROAD | DMU12 | 0.73216 | 0.23976 | 0.74620 | 0.76489 |
| PR-SC-ROAD-RAIL | DMU13 | 0.80549 | 0.06067 | 0.87241 | 0.89426 |
| GO-SP-ROAD | DMU14 | 0.23325 | 0,40161 | 0.41582 | 0.42624 |
| GO-SP-ROAD-RAIL | DMU15 | 0.24990 | 019556 | 0.52717 | 0.54037 |
| GO-ES-ROAD | DMU16 | 0.11389 | 0.56225 | 0.27582 | 0.28273 |
| GO-ES-ROAD-RAIL | DMU17 | 0.20136 | 0.28717 | 0.45709 | 0.46854 |

Source: Survey data (2015).

(roadway and railway) from state of Paraná to the Port of São Francisco do Sul (PR-SC-ROAD-RAIL) (89.42% relative efficiency). Detailed results of all routes are shown in Table 5.

## Conclusions

This study analyzed the logistics efficiency of the main flow routes of the Brazilian corn production for export among the major producing states and the main Brazilian ports. In the evaluation process, the input and output indicators that influenced the final process outcome were defined.

By analyzing the four proposed models - financial, customer, internal business processes, and learning and growth, it was found that the export route DMU06, which represents the flow from the state of Mato Grosso to the ports of Santarém/Manaus using the road and waterway intermodal route through the waterway terminal Miritituba in the Tapajós-Amazonas Waterway (MT-PA/AM-Road-Waterway-DMU06) was the most efficient route, both in the financial and the internal business processes models. Moreover, in the sustainable development model, it was the most suitable alternative when the origin of corn was from the state of Mato Grosso - the largest producing state. When we use the state of Paraná as the reference, it was possible to highlight the road and rail route to the port of Paranaguá, and when corn comes from the state of Goiás, the road and waterway intermodal route through the port of Santos was used as the reference.

Through the financial efficiency evaluation model, the influence of the participation of rail and waterway modes in the process of defining efficient routes was observed; four of the five most favorable results were part of this intermodality. It is also important to note that the flow to the ports of Santarém and Manaus were the most suitable alternatives to the already overloaded port of Santos. In the efficiency analysis model in sustainable development, the routes with lower extension between the producer state and the export port were privileged, such as the state of Paraná route to the port of Paranaguá through intermodal road-rail. This study allows for a route-level logistics efficiency analysis. Moreover, the results imply targeting of investments on routes that had unsatisfactory results, that is, low levels of efficiency. The proposed models become relevant owing to their replication potential in other logistics process efficiency analyses for other agricultural commodities, as well as their abilities in facilitating comparison with competing countries in the corn chain.

## Conflict of Interests

The authors have not declared any conflict of interests.

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# Soil moisture and water use efficiency in cotton plants grown in different spacings in the Brazilian Cerrado Region

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**The aim of this study was to assess soil moisture and water use efficiency in cotton plants (*Gossypium hirsutum* L.) grown in different row spacing in the *Cerrado*, Brazil. The crop was irrigated by drip irrigation, and the amount of supplied water was equal to 90% of crop evapotranspiration (ETc). Spacing of 0.4, 0.7 and 1.0 m between rows were assessed. Soil moisture was assessed up to 1.0 m deep, and water use efficiency for seed cotton and shoot dry matter yield was assessed based on the total water supplied to the crop (rain + irrigation). Soil moisture variations were minimal from 0.6 m deep, regardless of the spacing used. From this same depth, the soil cultivated under the denser system (0.4 m) was drier than the other spacing. In traditional planting (1.0 m), the cotton plant has lower water use efficiency for both seed and shoot dry matter yield. Water use efficiency is optimized in 0.6 m spacing, although the nitrogen dose applied should be observed.**

**Key words:** *Gossypium hirsutum* L., drip irrigation (SDI), moisture profile.

## INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is the most important textile fiber in the world. It is produced in more than 30 countries, with emphasis to China, in the first position. Brazil is the fifth largest producer and third largest exporter (FAO, 2015). The culture has great economic value and is especially used in regions with irregular rainfall or limited water amounts for irrigation, where it is an important income source for small and large-scale farmers (Papastylianou et al., 2014; Valipour, 2015a).

In Brazil, cotton growth has become common in denser spacing. Traditionally, the culture is seeded at 0.8 to 0.9 m spacing between rows, with final stand ranging from 100 to 120 thousand plants ha$^{-1}$. In dense cultivation, spacing is typically reduced to 0.45 m between rows, or even smaller. Some preliminary results show that this new system may reduce total yield costs, while increasing yield per area (Belot and Vilela, 2010).

However, if there is economic motivation stimulating the adoption of this planting system on the one hand, there is lack of scientific information on the consequences of this crop management. It is not known, for example, if such management change would affect crop water

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consumption, consequently reducing soil moisture faster. In addition, the effect of this management on water use efficiency is also unknown.

Moisture profile analysis has provided important information on crop growth. In some cases, moisture along the profile shows little variation (Zhou et al., 2010). However, variations are accentuated in other cases (Hu et al., 2009). Such variations are probably due to uneven vertical root density distribution (Zhao et al., 2010). With respect to water use efficiency, it seems that there is negative correlation with spacing between rows, as shown in different crops (Zhou et al., 2010, 2011).

In this context, this experiment aimed to assess soil moisture and water use efficiency in cotton plants under drip irrigation and different planting spacing in an Oxisol from the Brazilian *Cerrado*.

## MATERIALS AND METHODS

The experiment was conducted in field, in the experimental area of the Federal University of Mato Grosso (UFMT), Rondonopolis Campus (-16° 27'45"latitude, -54° 34' 49"longitude, 290 m altitude), between May to September 2015.

The experimental design was of randomized blocks in Box-Behnken design (Box and Draper, 1987). 15 treatments and 4 repetitions were used, totaling 60 experimental units of 30 m². Treatments were, as follows: three cultivation spacing (dense - 0.4 m, intermediate - 0.7 m - traditional - 1.0 m), three nitrogen fertilizer levels, applied throughfertigation as a percentage of the recommended dose (low dose - 20%, intermediate dose - 110% and High dose - 200%) and irrigation three levels, through subsurface drip irrigation (deficit - 30% of ETc; intermediate - 90% of ETc, and water surplus - 150% of ETc).

The irrigation practice has become increasingly common in agricultural systems to achieve high productivity (Valipour, 2014a, b, c). The method adopted was drip irrigation due to greater efficiency of water supply to the plants because, in that system, in addition to located application near the root system, only a portion of soil surface is wet which minimizes losses related to evaporation (Valipour, 2012 a, b, c).

The experimental area soil was classified as Oxiisol (EMBRAPA, 2013). For fertilization and liming recommendation, chemical and particle size characterization was conducted (Table 1) according to EMBRAPA (1997).

Soil acidity was corrected rising base saturation to 50%, and fertilization was carried out with 155 kg ha$^{-1}$of nitrogen in urea form, 120 kg ha$^{-1}$of phosphorus ($P_2O_5$), as Single superphosphate, 100 kg ha$^{-1}$ of potassium ($K_2O$), in potassium chloride form, and 30 kg ha$^{-1}$of micronutrients (FTE). Both were conducted according to Sousa and Lobato (2004). Nitrogen was applied by fertigation, with the aid of Dosmatic® MiniDos 1% (Hydro systems, USA) fertilizer injector.

Irrigation management was carried out from reference evapotranspiration (ETo) (Valipour and Eslamian, 2014; Valipour, 2014d,e,f,g,h; Khoshravesh et al., 2015; Valipour, 2015b,c), which was estimated by Penman-Monteith/FAO-56 (Equation 1), using the following meteorological variables: net radiation (MJ m$^{-2}$ d$^{-1}$), wind speed (m s$^{-1}$), air temperature (°C) and relative humidity (%). Data were obtained from an automatic weather station installed about 270 m from the experimental area, in which data were stored every 10 min.

Irrigations were carried out on alternate days based on crop evapotranspiration (ETc), according to Equation 2, and the water volume to be applied was corrected by the drip irrigation system efficiency (90%). Three irrigation depths were used: LI30 - water deficit (30% of ETcdemand), LI90 - intermediate (90% of ETc) and LI150 - high water surplus (150% of ETc). Crop coefficients (Kc) for the four plant development stages were chosen as proposed in the FAO 56 (Allen et al., 1998) for cotton crop.

$$ETo = \frac{0.408\Delta\left(Rn - G\right) + \gamma \frac{900}{T+273} u_2\left(e_s - e_a\right)}{\Delta + \gamma\left(1 + 0.34u_2\right)} \quad (1)$$

Where; ETo - reference evapotranspiration (mm d$^{-1}$),Δ - tangent to the vapor pressure curve (kPa °C$^{-1}$), Rn - available netsolar radiation (MJ m$^{-2}$ d$^{-1}$), G - heat flow in the soil (MJ m$^{-2}$ d$^{-1}$),γ - psychrometric constant (kPa °C$^{-1}$), u2 - Wind speed at 2 m (m s$^{-1}$), s - atmospheric water vapor saturation pressure (kPa),$e_a$ - current atmospheric water vapor pressure (kPa) and T - average daily air temperature (°C).

$$ETc = ETo \times K_c \quad (2)$$

Where; ETc - crop evapotranspiration (mm d$^{-1}$), ETo - reference evapotranspiration (mm d$^{-1}$) and Kc - crop coefficients.

Treatments related to planting spacing were defined according to the current recommendations of cotton crop productive system in Mato Grosso, Brazil. Sowing was held on May 1, 2015, with IMA 5675B2RF cotton seeds and 8 plants per linear meter. Plant densities, according to crop spacing, were, as follows: E0.4 - 225,000 plants ha$^{-1}$; E70 - 128,571 plants ha$^{-1}$and E100 - 90,000 plants ha$^{-1}$.

Soil profile moisture was continuously observed from 06/06/2015 using Diviner 2000®model (Sentek Pty Ltd, Australia) capacitance probe, with readings at every 0.1 m. For moisture readings, nine access tubes were installed, three for each planting spacing, at 1 m deep in default random plots with intermediate irrigation (90% ETc) in each spacing. Of the three access tubes for each spacing, one was installed in the row-crop, and the other two between the lines (on opposite sides). Soil moisture was a result of the average of the three measuring points.

At 142 days after sowing, experiment harvesting was conducted, and fruits and stems were separated. The collected material was weighed and dried in an oven with forced air at 65°C for 72 h. Subsequently, it was weighed to determine seed cotton and shoot dry matter yield (stem + fruit). Thus, seed cotton ($WUE_Y$) and shoot dry matter ($WUE_{DM}$) water use efficiency for yield were calculated. Efficiency was calculated through the ratio between the interest variable and the total water amount supplied to the crop (rain + precipitation).

Water use efficiency for yield and shoot dry matter variables were statistically assessed through response surface analysis, using the SigmaXL® 7.0 software tool to the maximum significance level of 5% for all statistical tests.

## RESULTS AND DISCUSSION

Soil moisture distinctly varied for the various soil profile layers in different spacing (Figure 1). It was observed that upper layers (up to 0.4 m) had higher moisture oscillations compared to the deeper layers (0.4 to 1.0 m) in all spacing. This is probably due to higher root density in the upper layers compared to deeper layers. Thus, Zhao et al. (2010), for example, studied the drip irrigation influence on cotton plant root length density, and found

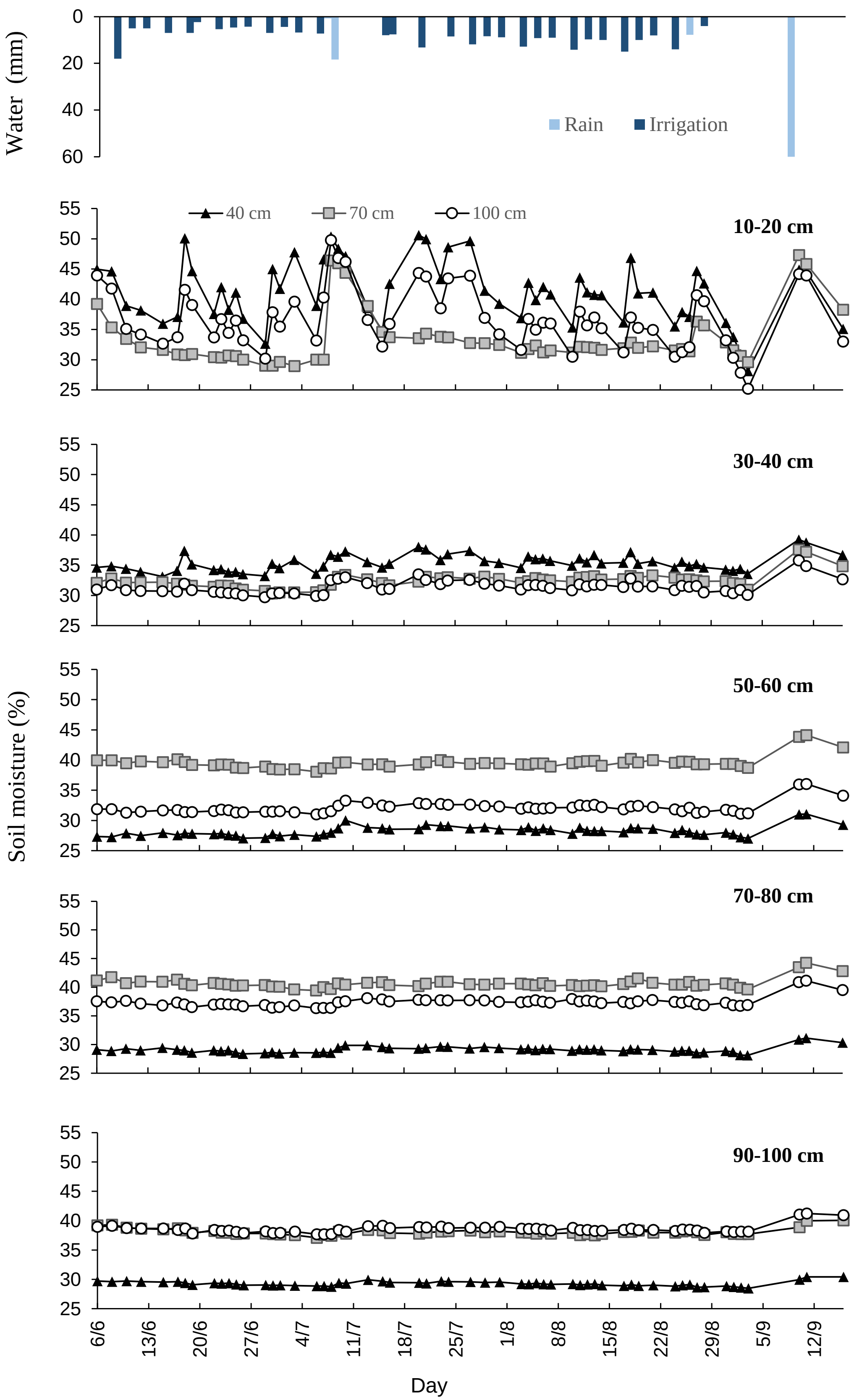


**Figure 1.** Soil moisture by layers under different spacing between rows and irrigation depths (irrigation + rainfall) for cotton plant grown in a*Cerrado* Oxisol in 2015.

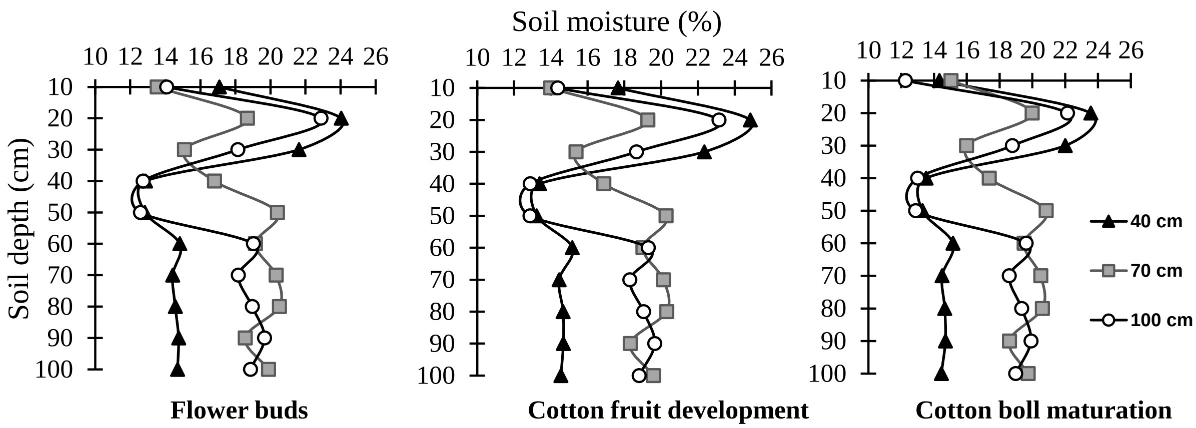


**Figure 2.** Soil moisture profile (%) until 1.0 m deep in three phenological stages for cotton plant grown under different row spacings in a *Cerrado* Oxisol in 2015.

that most of roots were concentrated until the depth of 0.5 m.

Among spacing assessed, the highest soil moisture values to a depth of 0.4 m were found in the denser crop (Figure 1). From this depth, the situation is reversed, that is, the soil was more dried in the 0.4 m spacing. For other spacing, it was observed that soil moisture in intermediate spacing was initially lower in the first layer (Figure 1). Subsequently, moisture values on the same spacing were higher, up to the depth of 0.8 m, and were identical in the last layer, when compared to the largest crop spacing (Figure 1).

From the 0.5 to 0.6 m layer, soil moisture variation was minimal in all spacing assessed (Figure 1), which could be an indication of maximum plant water extraction depth, according to analysis by Farahani et al. (2008). At the time, Farahani et al. (2008) assessed the moisture profile of a soil cropped with cotton plants, and concluded that most of water extraction occurred up to the depth of 1.20 m. However, it does not explain lower soil moisture values from the depth of 0.5 m to the 0.4 m spacing.

In a similar analysis, Hu et al. (2009) concluded that soil moisture increase in higher depths is the result of higher water supply than what is consumed by the plant. Although the soil had a plastic cover in the study by Hu et al. (2009), which reduces evaporative water loss and favors moisture increase, tendencies found by the authors were, in part, similar to those of this study. It was noted that between the 0.5 and 1.0 m layer, soil moisture was always lower than 30% for 0.4 m planting (Figure 1). For other spacing, soil moisture was kept almost constant at 40% for 0.7 m planting, increasing from approximately 33 to 40% in the largest planting. The reason why the soil was drier at higher depths in denser crops should be further investigated. Probably, vertical root distribution was different between spacing, as shown by Zhao et al. (2010)

The soil moisture profile in the three spacing was similar during flower bud, apple development and boll maturing stages (Figure 2). Moisture initially increased with depth, with subsequent decrease and, finally, little variation was observed in higher depths. Once again, little soil moisture variation was observed in depths higher than 0.6 m, regardless of spacing used. In addition, the lowest soil moisture amounts were observed under 0.4 m spacing.

The total water amount stored throughout the profile was lower under denser row spacing, followed by the large stand later by the intermediate row spacing (Figure 3). Difference between spacing were statistically different among them, with values of 163, 184 and 176 mm for 0.4, 1.0 and 0.7 m spacing (Figure 4). Thus, smaller differences may indicate that the total water consumption of cotton plant grown under denser water system is higher compared to the traditional system. Water use efficiency for seed yield (kg ha$^{-1}$ mm$^{-1}$) was statistically significant (Figure 5), whose mathematical model was, as follows:

$$WUE_Y = 4.292 - 0.468ET_L + 0.443N_L - 0.677RS - 1.262RS^2 \qquad R^2 = 0.56 \quad (3)$$

Where: $WUE_Y$: Water use efficiency for seed cotton yield (kg ha$^{-1}$ mm$^{-1}$), $ET_L$:crop evapotranspiration level(mm), RS: Row spacing (cm).

Considering the nitrogen dose as fixed (maximum dose) and the water amount applied (≈ETc), in which only the spacing varied (40, 70 and 100 cm), it was noted that the highest water use efficiency was obtained in the intermediate spacing (Figure 6), with a value of 4.7 kg ha$^{-1}$ mm$^{-1}$. Ranging the spacing between 40 and 100 cm, increasing 10 cm and fitting and deriving a model to the data, as to obtain the maximum point, it was observed that the highest efficiency was achieved for the 60 cm spacing, with a value of 4.83 kg ha$^{-1}$ mm$^{-1}$. From the $WUE_Y$ point of view, this value is the most suitable

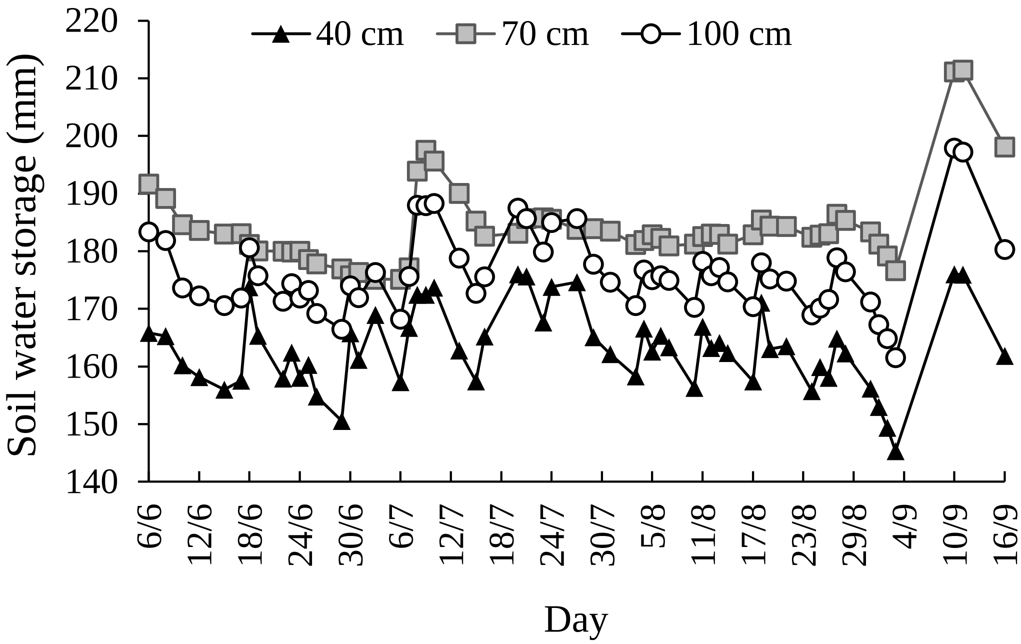


**Figure 3.** Total soil water (mm) in the 0-1.0m layer for cotton plant grown under different spacing between rows in a *Cerrado* Oxisol in 2015.

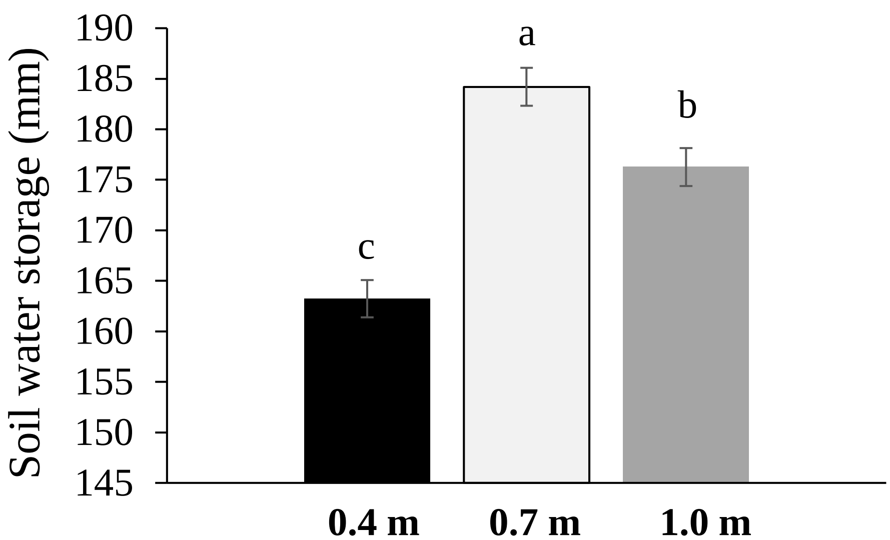


**Figure 4.** Soil total water mean (mm) in the 0-1.0m layer of cotton plant grown under different row spacings in a *Cerrado* Oxisol in 2015. Bars indicate mean confidence interval (α = 0.05). Means with different letters indicate statistical difference (α = 0.05).

spacing for cotton planting in a condition in which the water amount applied is equal to 90% ETc, considering the fixed nitrogen dose.

The maximum $WHE_Y$ value found was within the limits described by FAO to several cultures (Sadras et al., 2007). For cotton plant, values are of kg ha$^{-1}$ mm $^{-1}$. Zonta et al. (2015) found values of 6.9 kg ha$^{-1}$ mm $^{-1}$, considering 100% ETc irrigation. Regarding shoot dry matter (Figure 7), with the same conditions described above, the following model was obtained:

$$WUE_{DM} = 7.967 - 1.351ET_L + 1.050N_L - 1.461RS - 2.136RS^2 \qquad R^2 = 0.60 \qquad (4)$$

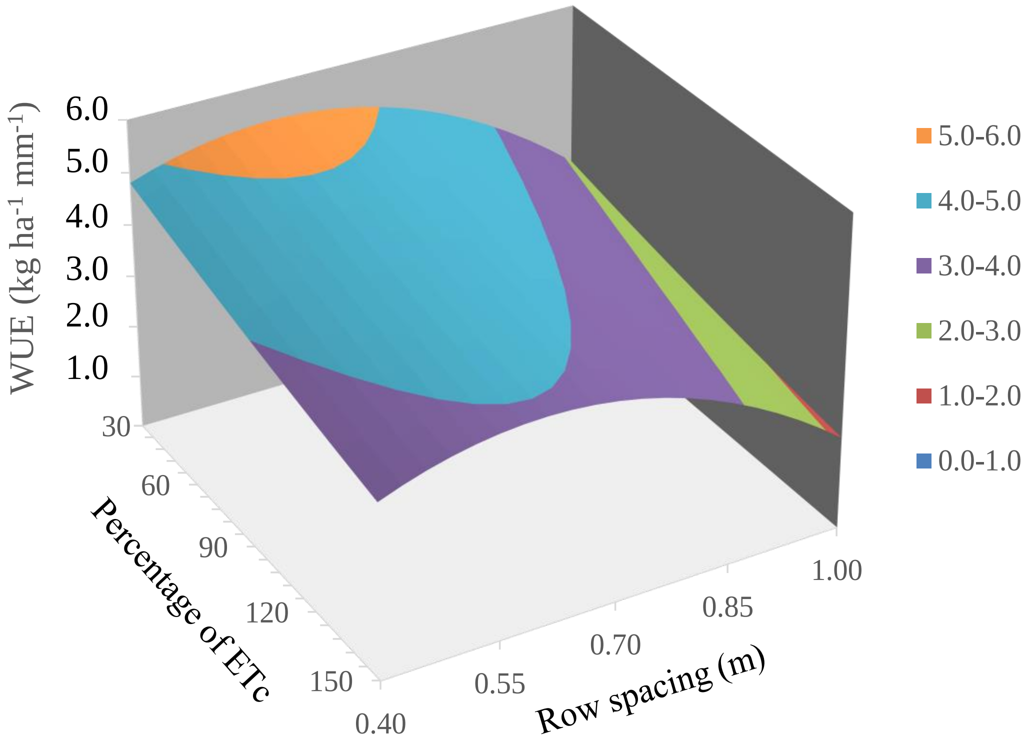


**Figure 5.** Water use efficiency for seed cotton yield (kg ha$^{-1}$ mm$^{-1}$) according to crop evapotranspiration levels (mm) and crop spacing, with fixed nitrogen dose a *Cerrado* Oxisol in 2015.

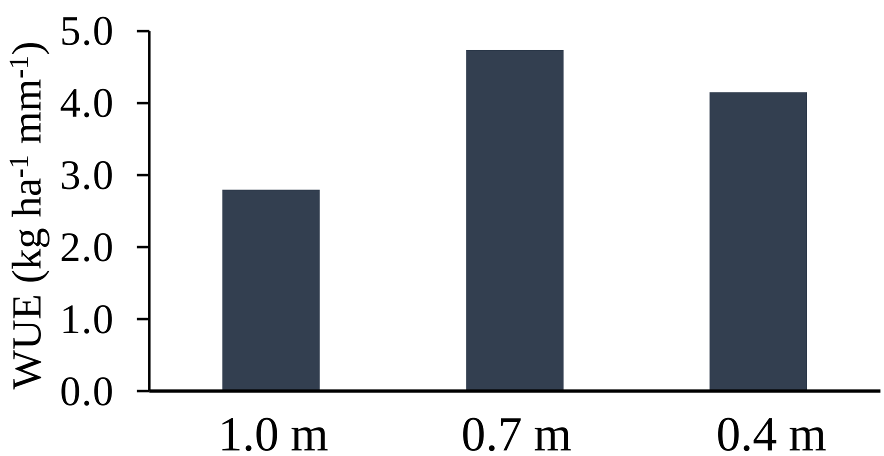


**Figure 6.** Seed cotton yield water use efficiency (kg ha$^{-1}$ mm$^{-1}$) according to crop spacing, grown in a *Cerrado* Oxisol in 2015.

Where: $WUE_{DM}$: Shoot dry matter water use efficiency (kg ha$^{-1}$ mm$^{-1}$), $ET_L$: Crop evapotranspiration level (mm), RS: Row spacing (cm).

Considering the same analysis aforementioned, $WUE_{DM}$ values were 8.3, 9.0 and 5.4 ha$^{-1}$ mm$^{-1}$ for spacing of 40, 70 and 150 cm (Figure 8). Adequate spacing was 60 cm again, with 9.6 kg ha$^{-1}$ mm$^{-1}$$WUE_{DM}$.

## Conclusions

(1) In denser spacing (0.4 m), soil moisture is lower in the soil deeper layers (> 0.6 cm) compared to larger spacing.
(2) Cotton plant grown in conventional spacing (1.0 m) has lower water use efficiency for seed cotton yield compared to the denser system (0.7 and 0.4 m).

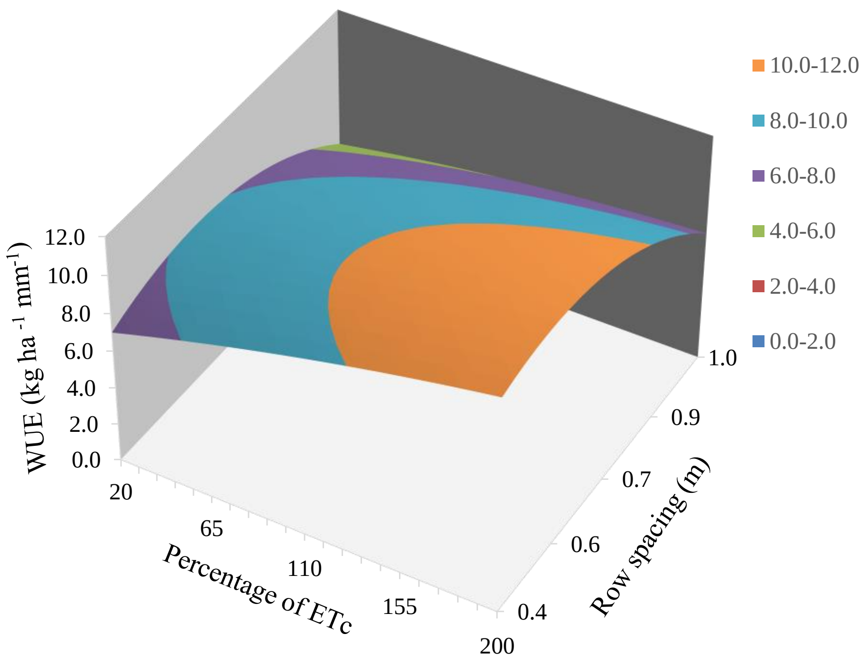


**Figure 7.** Shoot dry matter water use efficiency (kg $ha^{-1}$ $mm^{-1}$) according to crop evapotranspiration levels (mm) and crop spacing, with fixed nitrogen dose, in a *Cerrado* Oxisol in 2015.

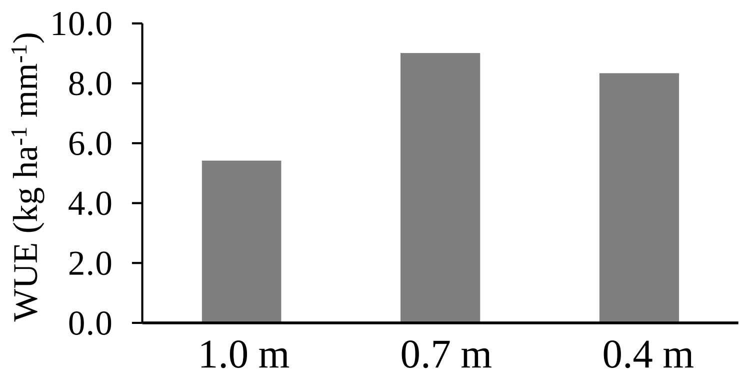


**Figure 8.** Cotton plant shoot dry matter water useefficiency (kg $ha^{-1}$ $mm^{-1}$) according to crop spacing grown in a *Cerrado* Oxisol in 2015.

(3) Higher cotton plant water use efficiency can be obtained in intermediate spacing, 0.6 m.

## Conflict of Interests

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# The relationship between organic acids, sucrose and the quality of specialty coffees

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**There is an increasing demand for specialty coffees in the worldwide coffee market, which justifies the need for research focused on understanding the effect of genetic factors and environmental conditions on coffee quality. The aim of this study was to assess the occurrence of arabica coffee genotypes with a high potential for specialty coffee production under three different environmental conditions. Green coffee beans of arabica genotypes largely cultivated in Brazil were chemically evaluated: one Mundo Novo line and three Bourbon lines. The experimental sites were established in fields in three different municipalities (Lavras, Santo Antônio do Amparo and São Sebastião da Grama) located in Brazil. The level of sucrose and oxalic acid was a good discriminant marker for the beverage quality of the genotypes assessed, in which coffees with higher scores also showed higher levels of sucrose and lower levels of oxalic acid. Yellow Bourbon IACJ9 and the Yellow Bourbon/Origin SSP were indicated as the most suitable genotypes for specialty coffee production.**

**Key words:** Specialty coffees, sugar, acids, sensory profile, principal component analysis.

## INTRODUCTION

The worldwide demand for specialty coffees has been increasing in larger proportions than for regular coffees. The quality of specialty coffees is related to their intrinsic characteristics, which are represented by the chemical composition of coffee beans. The chemical compounds found in coffee beans provide flavor, aroma, acidity, sweetness and sourness to the beverage (Figueiredo et al., 2015; Giomo and Borém, 2011).

Coffee breeding programs have developed cultivars with the objective of increasing yield and resistance to pests and diseases and have developed short-stature plants that are adapted to several environmental conditions (Petek et al., 2006; Sera, 2001). However, beverage quality is rarely considered as part of this development.

The Bourbon coffee cultivar has received more attention from the coffee market than existing arabica coffee

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**Table 1.** Studied genotypes, environments and their codes[a].

| Environments | Genotypes |
|---|---|
| A1 = Lavras | G1 = Mundo Novo IAC 502/9 |
| A2 = São Sebastião da Grama | G2 = Yellow Bourbon IAC J9 |
| A3 = Santo Antônio do Amparo | G3 = Yellow Bourbon/Origin SSP[b] |
| | G4 = Yellow Bourbon/Origin CM[b] |

a A1, A2, A3, G1, G2, G3 and G4 = codification of the genotypes and environments used in the discussion of the results. bSSP = São Sebastião do Paraíso, MG; CM = Carmo de Minas, MG.

**Table 2.** Geographic region, climatic variables and characterization of the three studied environments.

| Municipality | Lavras | São Sebastião da Grama | Santo Antônio do Amparo |
|---|---|---|---|
| Region | Southern Minas Gerais | Mogiana Paulista | Southern Minas Gerais |
| Altitude | 919 m | 1300 m | 1050 m |
| Mean temperature | 20.4°C | 20°C | 19.9°C |
| Mean annual precipitation | 1460 mm | 1560 mm | 1700 mm |
| Latitude | 21°14'43"S | 21°44'50"S | 20°56'47"S |
| Longitude | 44°59'59"W | 46°55'33"W | 44°55'08"W |
| Soil type | Clayey oxisol | Clayey oxisol | Clayey oxisol |

cultivars. That is because it has a high potential to produce differentiated coffees regarding tastes and aromas. However, differences in the production of high-quality beans have been reported among Bourbon genotype lines (Ferreira et al., 2012; Figueiredo et al., 2013, 2015; Taveira et al., 2014).

Coffee expresses its quality differently depending on the place where it is grown. It is essentially a *terroir* product that is directly affected by environmental aspects including nature and human actions (Alves et al., 2011). The production of arabica coffees in Brazil is large, and it is located in places with several different environmental conditions. This diversity of the environments associated with the wide genetic variability allows Brazil to produce coffees with very distinct sensory profiles.

The acceptance of coffee by consumers is related to its quality, which in turn is related to the chemical composition of the coffee beans. Among several classes of chemical compounds, organic acids and sucrose are known to contribute to coffee flavor. Nevertheless, the levels of organic acid and sucrose in coffee beans have already been quantified (Alcázar et al., 2003; Campa et al., 2004; Jham et al., 2002; Ky et al., 2001; Rogers et al., 1999), and there are no reports relating these chemical compounds to the sensory quality of Bourbon genotypes.

The increasing demand for specialty coffees justifies the need for producing countries to invest in research focused on understanding the impact of genetic and environmental factors on final coffee quality. Therefore, the objective of this study was to identify the occurrence of coffee genotypes that have an increased potential for producing specialty coffees in three different Brazilian municipalities as well as to understand the influence of the interaction of genetic and environmental factors on the levels of organic acids and sucrose. In addition, the authors focused on determining the relationship between these chemical compounds and the sensory characteristics of the Bourbon genotypes.

## MATERIALS AND METHODS

### Experimental conditions

Four genotypes of *Coffea arabica* L. (Table 1) were grown in experimental field plots since 2005 in the southern region of the state of Minas Gerais and in the region of Mogiana in the state of São Paulo, including the municipalities of Lavras, MG; Santo Antônio do Amparo, MG and São Sebastião da Grama, SP. Both regions are highlighted for their production of Arabica coffee on a large scale. The distinct edaphoclimatic conditions of these important Brazilian coffee producing regions were represented in this study, and their main characteristics are shown in Table 2.

### Coffee harvest and processing

Coffee fruits were handpicked and selectively harvested when the fruits were completely mature to guarantee the complete uniformity of the material from the different parcels. Although, the selective harvest of mature fruits was performed, a small portion of immature fruits was still found in the cherry portion. Those unripe fruits were manually removed from the samples, resulting in approximately 20 L of coffee fruit, thus guaranteeing the retention of only mature fruits. Then, the coffee fruits were peeled to obtain the pulped coffee. Drying was carried out immediately after processing according to the method of Borém et al. (2008), until coffee beans were at the level of 11% (w.b) moisture content.

### Sample preparation

After drying, the samples were packed in paper bags and covered with plastics bags, identified and stored in chambers at a controlled temperature of 18°C for 60 days. Then, the samples were benefited and the defects were removed in order to standardize the samples and minimize interferences unrelated to the genetic material or the environment. Chemical analysis and roasting were performed in beans retained on sieves 16 and higher (16, 17 and 18/64 inches). For the chemical analyses, raw beans were milled for one minute in an 11A basic mill (IKA, Brazil) by adding liquid nitrogen to facilitate the milling and avoid sample oxidation. After milling, the samples were kept in a freezer at -80°C until analysis.

### Roasting and sensory evaluation

All procedures were performed according to the protocol described by the Specialty Coffee Association of America - SCAA (Lingle, 2011). The sensory attributes included the aroma, uniformity, absence of injuries, sweetness, flavor, acidity, body, balance, completion and overall impression. The final sensory grade was generated from the sum of all of the evaluated attributes. For each evaluation, five cups of coffee representing each genotype were evaluated, with one session of sensory analysis for each repetition and a total of three repetitions. Each environment was evaluated separately, and the results of the sensory analyses were scored on a scale representing the quality level in intervals of 0.25 points.

In addition to the final grade obtained from the sensory evaluation, the attributes of aroma, acidity, body and flavor were also analyzed statistically in order to complement the analysis, considering that these are the main attributes responsible for distinguishing the different sensory profiles of the coffee.

### Chemical analysis

In total, 0.5 g of ground green coffee beans were deposited into a 100 mL flask, mixed with 70 ml of deionized water (18.2 MQ) at 70°C, agitated and then placed into a water bath set at 70°C for 30 min. The flask content was filled to 100 mL with deionized water (18.2 MQ) and then filtered in paper (Schleicher and Schuell filter paper 597.5). Three milliliters of the resulting extract was filtered again in a C18 cartridge (SEP PAK) that had been previously balanced with methanol and 3 mL of water (Rogers et al., 1999). The filtrate was used for further quantitative determination of sucrose and organic acids.

### Quantitative determination of sucrose

The concentration of sucrose was measured in two replicates (Pezzopane et al., 2012) using a high performance liquid chromatography (HPLC) Bio-inert quaternary system (Shimadzu Kyoto, Japan), model FCV-10AL-VP, with a pump (Shimadzu, Kyoto, Japan), model LC-10Ai (Kyoto, Japan); automatic injector (Shimadzu, Kyoto, Japan), model SIL-10Ai; electrochemical detector (Dionex, CA, USA), model ED 50; and suppressor (Dionex, CA, USA), model ASRS 300, 4 mm. The column used was a PA1, 250 x 4 mm (Dionex®). Elution was performed in the isocratic mode with a flow rate of 1 $mL.min^{-1}$ at 30°C using 50 $mMol.L^{-1}$ NaOH as an eluent.

Concentrations were calculated from the peak area of the standard solution (Sigma, cat. no. 7903, Sigma. St. Louis, MO). The sucrose levels of the samples collected in 2010 and 2011 were quantified in percentage of dry matter (% d.m.).

### Quantitative determination of organic acids

The concentration of organic acids was measured in two replicates using a high performance liquid chromatography (HPLC) Bio-inert quaternary system (Shimadzu, Kyoto, Japan), model FCV-10AL-VP, with a pump (Shimadzu, Kyoto, Japan), model LC-10Ai (Kyoto, Japan); automatic injector (Shimadzu, Kyoto, Japan), model SIL-10Ai; electrochemical detector (Dionex, CA, USA), model ED 50; and suppressor (Dionex, CA, USA), model ASRS 300, 4 mm (Rogers et al., 1999).

Standard solutions of lactic acid, acetic acid, malic acid, oxalic acid and citric acid were used for peak identification in the chromatograms and for the calculation of the sample concentration. The organic acid levels of the samples collected in 2010 and 2011 were quantified in percentage of dry matter (% d.m.).

### Statistical analysis

Four genotypes grown in three different environments were assessed. The experiment in each environment was performed in a randomized block design with three replicates comprising 10 plants each.

The sensory profile, final sensory score and organic acid and sucrose content were subjected to ANOVA. The Scott-Knott test was applied with $P<0.05$ using the software SISVAR® (Ferreira et al., 2011) for the significant differences found by the F test.

The dataset comprising the sensory and chemical results underwent a multivariate analysis (Principal Component Analysis-PCA) to get a deeper understanding of the factors using the statistical Chemoface software (Nunes et al., 2012).

## RESULTS AND DISCUSSION

### Chemical composition and sensory profile

The organic acid levels, sucrose levels, sensory attributes and final score of the sensory analysis are shown in Table 3. Citric acid was found at higher concentrations in the green coffee extracts (~1.32% d.m.). Malic acid was found at an intermediate level in the samples (~0.5% d.m.). Lactic acid, acetic acid and oxalic acid were found to be lower than 0.09% d.m. The sucrose concentration had an average value of 9.72% d.m. The levels of the compounds analyzed are in accordance with reports found in the literature on organic acids (Alcázar et al., 2003; Jham et al., 2002; Rogers et al., 1999) and sucrose (Campa et al., 2004; Ky et al., 2001; Redgwell and Fischer, 2006).

The most abundant organic acids in green coffee beans are citric and malic acids (Ginz et al., 2000). Sucrose represents almost the total of amount of free sugars in green coffee beans. However, its content varies among species. *C. arabica* L. has sucrose levels ranging from 5.1 to 9.4% d.m., whereas *Coffea canephora* has much lower levels, ranging between 4 and 7% d.m. (Campa et al., 2004; Ky et al., 2001).

**Table 3.** Average concentration of the organic acids, sucrose, final sensory score and sensory attributes (fragrance, taste, acidity and body) of four genotypes grown in three environments. Average and significance of the F test was determined by ANOVA.

| Genotype/Environment | | Lactic acid | Acetic acid | Malic acid | Oxalic acid | Citric acid | sucrose | Fragrance | Taste | Acidity | Body | Final score |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| G1 | | 0.08 | 0.08[a] | 0.55[a] | 0.05 | 1.36 | 9.5[a] | 7.25[a] | 7.11[a] | 7.25[a] | 7.37[b] | 80.38[a] |
| G2 | | 0.08 | 0.08[a] | 0.51[a] | 0.05 | 1.36 | 10.17[b] | 7.60[b] | 7.39[b] | 7.38[b] | 7.37[b] | 81.61[b] |
| G3 | | 0.08 | 0.06[b] | 0.46[b] | 0.04 | 1.29 | 9.88c | 7.58[b] | 7.44[b] | 7.43[b] | 7.33[b] | 81.76[b] |
| G4 | | 0.09 | 0.06[b] | 0.53[a] | 0.05 | 1.42 | 9.34[a] | 7.26[a] | 7.07[a] | 7.15[a] | 7.17[a] | 79.87[a] |
| *F* | | 0.17 | 0.04 | 0.00 | 0.16 | 0.31 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 |
| A1 | | 0.08 | 0.08 | 0.49 | 0.04[a] | 1.32 | 9.03[a] | 7.36 | 7.20 | 7.22 | 7.24 | 80.59 |
| A2 | | 0.09 | 0.07 | 0.53 | 0.04[a] | 1.32 | 10.25[b] | 7.52 | 7.35 | 7.37 | 7.36 | 81.42 |
| A3 | | 0.08 | 0.06 | 0.52 | 0.06[b] | 1.43 | 9.88c | 7.38 | 7.22 | 7.32 | 7.33 | 80.70 |
| *F* | | 0.11 | 0.07 | 0.06 | 0.00 | 0.08 | 0.00 | 0.11 | 0.20 | 0.07 | 0.12 | 0.12 |
| A1 | xG1 | 0.06[a] | 0.07[a] | 0.50 | 0.05[b] | 1.26 | 8.86[a] | 7.09[a] | 6.95[a] | 7.12[a] | 7.25[b] | 79.64[a] |
| | xG2 | 0.06[a] | 0.10[b] | 0.49 | 0.04[a] | 1.37 | 9.4[b] | 7.53[b] | 7.25[b] | 7.27[b] | 7.31[b] | 80.93[b] |
| | xG3 | 0.09[a] | 0.06[a] | 0.45 | 0.04[a] | 1.25 | 9.31[b] | 7.58[b] | 7.51[b] | 7.46[b] | 7.36[b] | 81.96[b] |
| | xG4 | 0.12[b] | 0.07[a] | 0.51 | 0.05[b] | 1.39 | 8.56[a] | 7.22[a] | 7.07[a] | 7.02[a] | 7.05[a] | 79.86[a] |
| *F* | | 0.00 | 0.01 | 0.22 | 0.04 | 0.50 | 0.00 | 0.01 | 0.01 | 0.00 | 0.05 | 0.03 |
| A2 | xG1 | 0.09 | 0.08 | 0.56 | 0.04 | 1.34 | 9.98[a] | 7.62[b] | 7.40[b] | 7.54[b] | 7.40 | 81.89[b] |
| | xG2 | 0.09 | 0.07 | 0.53 | 0.05 | 1.34 | 10.6[b] | 7.62[b] | 7.45[b] | 7.35[b] | 7.37 | 81.76[b] |
| | xG3 | 0.09 | 0.06 | 0.50 | 0.04 | 1.31 | 10.49[b] | 7.68[b] | 7.51[b] | 7.45[b] | 7.37 | 82.28[b] |
| | xG4 | 0.09 | 0.06 | 0.53 | 0.04 | 1.31 | 9.95[a] | 7.19[a] | 7.03[a] | 7.15[a] | 7.30 | 79.77[a] |
| *F* | | 0.81 | 0.47 | 0.31 | 0.46 | 0.97 | 0.01 | 0.01 | 0.04 | 0.03 | 0.87 | 0.02 |
| A3 | xG1 | 0.09 | 0.08 | 0.57[a] | 0.06 | 1.48 | 9.68[a] | 7.04[a] | 6.99[a] | 7.10 | 7.47[a] | 79.63[a] |
| | xG2 | 0.08 | 0.05 | 0.50[b] | 0.05 | 1.36 | 10.51[b] | 7.64[b] | 7.48[b] | 7.52 | 7.42[a] | 82.15[b] |
| | xG3 | 0.08 | 0.05 | 0.44[b] | 0.05 | 1.32 | 9.86[b] | 7.48[b] | 7.29[b] | 7.36 | 7.26[b] | 81.06[b] |
| | xG4 | 0.07 | 0.06 | 0.55[a] | 0.06 | 1.57 | 9.50[a] | 7.36[b] | 7.12[a] | 7.28 | 7.15[b] | 79.98[a] |
| *F* | | 0.57 | 0.19 | 0.00 | 0.18 | 0.13 | 0.00 | 0.00 | 0.04 | 0.06 | 0.02 | 0.02 |

G1= Mundo Novo IAC 502/9, G2= Bourbon Amarelo IAC J9, G3= Bourbon Amarelo/Origem SSP, G4= Bourbon Amarelo/Origem CM, A1= Lavras, A2= São Sebastião da Grama, A3= Santo Antônio do Amparo.

Among the chemical compounds analyzed, sucrose was the only one that was different (P<0.05) in green coffee bean extracts according to different genotypes, environments and interactions between genotype and the environment. Most of the genotypes did not differ

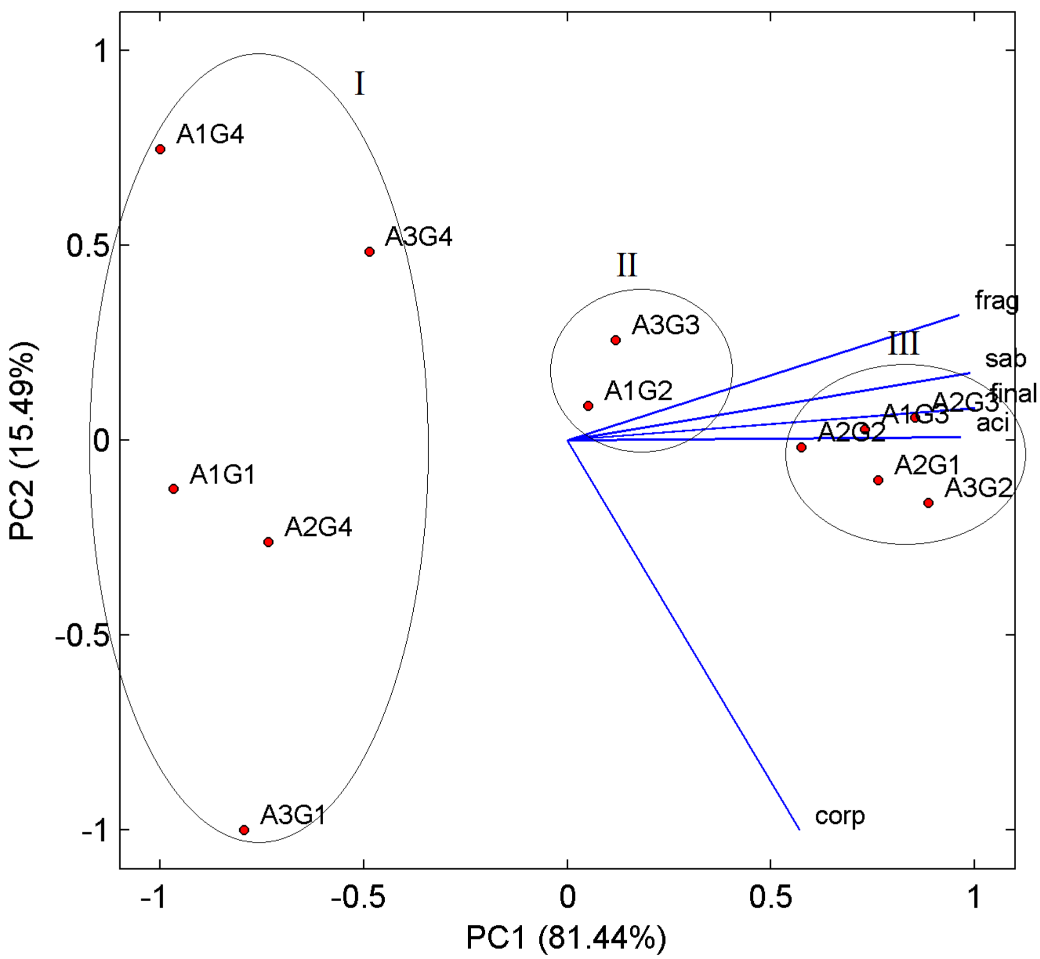


**Figure 1.** Principal component analysis biplot of PC1 and PC2 for the dataset of four genotypes (G) and three environments (A) as functions of the sensory attributes and the final sensory score. frag=fragrance; sab=taste; aci=acidity; corp=body; final= final sensory score. G1: Mundo. Novo IAC 502/9, G2: Yellow Bourbon IAC J9, G3:Yellow Bourbon/Origin SSP, G4: Yellow Bourbon/origin CM, A1: Lavras, A2: São Sebastião da Grama, A3: Santo Antônio do Amparo.

in the organic acids analyzed (Table 3). The sucrose content in commercial green coffee beans varies according to the coffee species, variety, geographical origin and roasting conditions (Knopp et al., 2006; Oosterveld et al., 2003).

The interaction between genotype and the environment was significant ($P<0.05$) for the sucrose content, final sensory score and most of the attributes analyzed. These results emphasize the effect of the genotype and environmental conditions on the final quality of the coffee beans, as previously reported (Bertrand et al., 2006; Malta and Chagas, 2009).

## Principal component analysis (PCA)

The biplots were obtained according to the score dispersion of the first principal components in the axes. The first component showed the highest variance, followed by the second component. The main characteristics that determined the formed groups were also detected from the biplot outputs.

Figure 1 is a projection of the results obtained from the PCA. It refers to the distribution of the genotype/ environment ($A_xG_y$) scores as functions of the sensory attributes and the final sensory score. PC1 and PC2 showed 81.44 and 15.49% of the total variance, respectively, totaling 96.93% of the total variance. This result is an excellent explanation of the variation occurring among the samples regarding their sensory characteristics.

In the biplots shown (Figures 1 and 2), the sensory attributes are represented by vectors and the interaction between genotypes and environments ($A_xG_y$) are represented by dots. PC1 suggested similarity among the dots (Figure 1), which formed three distinct groups of

**Table 4.** Correlation values of the assessed parameters (final sensory score and sensory attributes) of the principal component analysis.

| Parameter | PC1 (%) | PC2 (%) |
|---|---|---|
| Fragrance | 0.96 | 0.32 |
| Taste | 0.99 | 0.17 |
| Acidity | 0.97 | 0.01 |
| Body | 0.57 | -1.00 |
| Final | 1.00 | 0.08 |

genotypes x environment: group I (A1G1, A1G4, A3G1, A2G4 and A3G4); group II (A3G3 and A1G2); and group III (A2G3, A1G3, A3G2, A2G1 and A2G2).

Coffee samples belonging to group I showed little relation according to fragrance, taste and acidity and had lower final sensory scores (below 80 points) (Table 3) when compared with group III (Figure 1). All the genotypes that showed scores above 80 had potential for the production of specialty coffees, especially those with scores above 81 points (group III).

Fragrance, taste and acidity were the most important attributes for the discrimination of the coffee samples, which characterize PC1 (Table 4). Acidity in coffee is an important organoleptic parameter, which might be desirable or not, depending on the nature of the predominant acid in the beverage. The desirable acidity contributes to the vivacity of the coffee drink, increasing the sweetness perception and providing a dry fruit taste. An over-expressed acidity may be unpleasant, and it may be related to unusual tastes in coffee drinks (Illy and Viani, 2005; Lingle, 2011).

Body showed higher correlation with PC2. This attribute was important for discriminating the sensory profile of coffee samples with lower sensory scores (group I). Body is an attribute that is used to describe and characterize the physical properties related to density and texture (Avelino et al., 2005; Illy and Viani, 2005).

The genotypes expressed their sensory characteristics in different ways (Figure 1). The Yellow Bourbon genotype (G1) showed lower scores for taste, acidity and fragrance when compared with the Yellow Bourbon genotype IAC J9 (G2) and Yellow Bourbon (G3). The genotype G4 showed the lowest potential for the production of specialty coffees regardless of the environment where it was grown. The G4 samples were located on the opposite side of the vectors that indicate the scores of the attributes. Differences among Bourbon genotype lines regarding the potential for the production of coffees with higher quality has also been reported (Ferreira, et al., 2012; Figueiredo, et al., 2013).

São Sebastião da Grama (A2) was higher when compared with Lavras (A1) and Santo Antônio do Amparo (A3). The A2 environment showed higher scores for the sensory attributes acidity, taste and fragrance (Figure 1) and had the highest final sensory scores (Table 3). Its samples were located at the right side of the biplot (group III), with the exception of genotype G4.

The three studied environments (A1, A2 and A3) are known for their large production of commodity coffees and also for producing specialty coffees. However, even regions propitious for producing specialty coffees have a climatic diversity that causes variation in the beverage (Alves et al., 2011). Variation in climatic conditions may interfere with the formation and maturation of coffee fruits (Dal Molin et al., 2008), which allows differences in the final sensory profile of the beans.

The Mundo Novo cultivar (G1) is widely cultivated in Brazil, mainly because it has a high yield (Carvalho et al., 2006; Fazuoli et al., 2005). However, it has shown restrictions for the production of specialty coffee, which indicates that its beverage is highly dependent on the environment where it is grown. This cultivar only had an outstanding sensory score when grown in São Sebastião da Grama (A2), the most suitable environment for the production of specialty coffee.

The Yellow Bourbon genotype grown in Lavras (A1G3) and the Yellow Bourbon IAC J9 (A3G2) genotype grown in Santo Antônio do Amparo showed outstanding sensory scores and allowed the production of high quality coffees (Figure 1). In this case, the interaction between the genotype and environment was a determining parameter for the flavor expression of the samples. These results confirm the high potential of G2 and G3 for providing specialty coffees under different environmental conditions. Genetic diversity is one of the most important factors that contribute to the definition of the final coffee quality (Dessalegn et al., 2008; Leroy et al., 2006; Pereira, et al., 2010).

Considering studies correlating the chemical composition of green coffee beans with the final sensory quality, a new biplot was generated (Figure 2). It was generated from the PCA of the four genotypes and the three environments in the study, and its variables included sensory attributes, final sensory score, organic acids and sucrose. PC 1 explained 47.91% of the total variance, and PC 2 explained 15.82%. Together, the first two principal components explained 63.73% of the total variance.

The correlation results among organic acids, final sensory score and sensory attributes are represented in Figure 2, and the weight of each variable is shown in Table 5. Figure 2 also shows that the groups of dots previously obtained as a function of the sensory attributes (Figure 1) were similar, although the percentage of the explained variance was lower. The sensory attributes were clearly represented along PC1 in the biplot (Figure 2), with the exception of the body; the chemical variables were represented along PC2, with the exception of oxalic acid and sucrose.

The inclusion of the chemical compounds as variables in the principal component analysis allowed for a higher dispersion of the dots ($A_xG_y$). An increase in dissimilarity among the genotypes x environments ($A_xG_y$) was

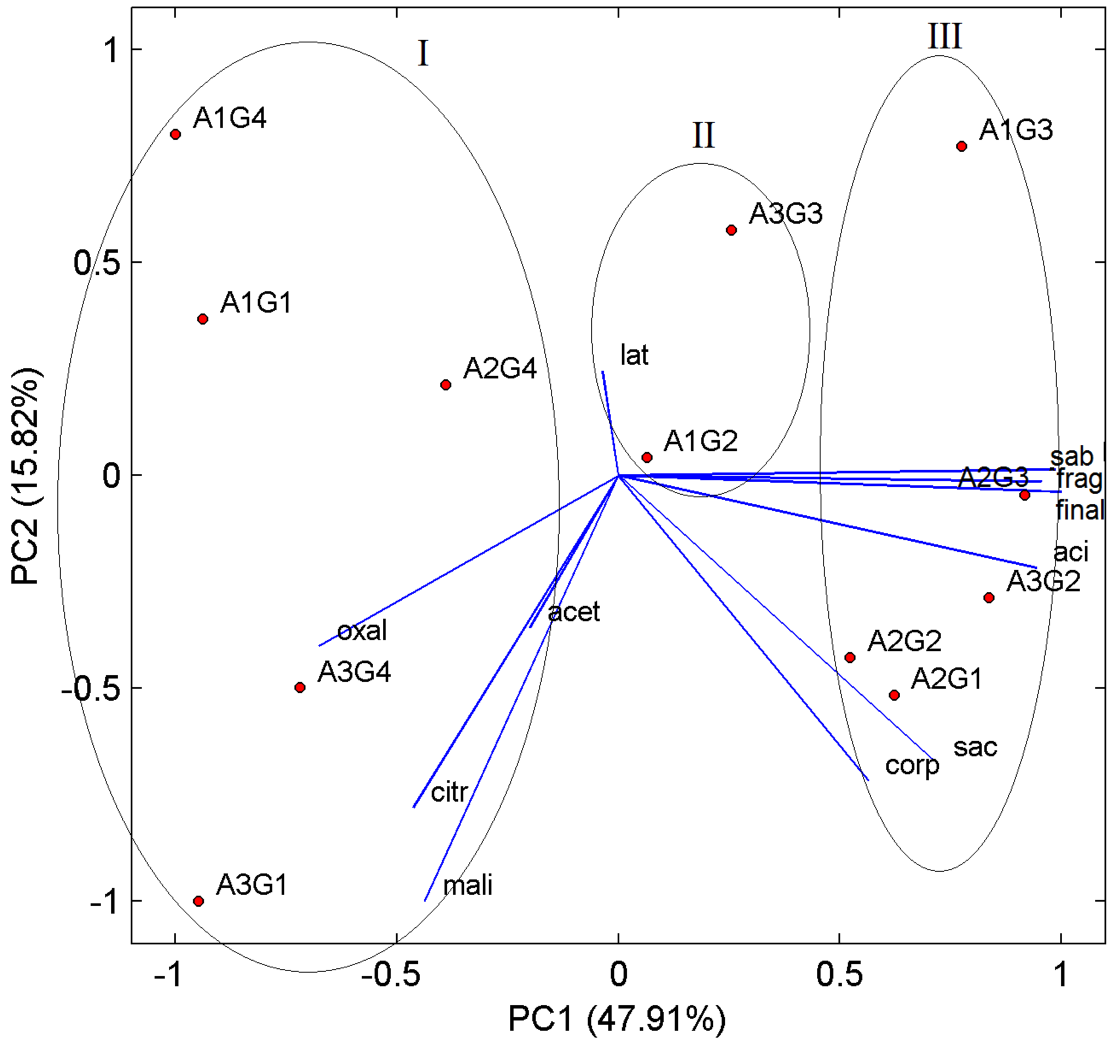


**Figure 2.** Biplot of principal component 1 (47.91%) and 2 (15.82%) of the principal component analysis of four genotypes (G) and three environments (A). The variables considered for the analysis included organic acids, sucrose, final sensory score and sensory attributes. frag=fragrance; sab=taste; aci=acidity; corp=body; final=final sensory score; lat=lactic acid; oxal=oxalic acid; citr=citric acid; acet=acetic acid; mali=malic acid; sac=sucrose. G1: Mundo Novo IAC 502/9, G2: Yellow Bourbon IAC J9, G3: Yellow Bourbon/Origin SSP, G4: Yellow Bourbon/Origin CM, A1: Lavras, A2: São Sebastião da Grama, A3: Santo Antônio do Amparo.

observed. This increase in the distance between the samples occurred mainly along PC2, which showed a larger influence of the chemical compounds (Figure 2).

Sucrose and oxalic acid had a higher correlation with PC1 (Table 5), which contributed to the discrimination of groups I and III (Figure 2). The weights of these compounds (value in module) were higher than 0.65, indicating that they were important for the scores on PC1 spreading. Coffees with the best sensory characteristics (group III) were positively correlated with the content of sucrose and negatively correlated with oxalic acid. The opposite behavior was observed for the samples belonging to group I (Figure 2 and Table 5).

Sucrose is a compound that is present in green coffee beans and has been the focus of studies as an important precursor of taste and aroma. This compound is rapidly degraded, and it can be found only in vestigial levels after the coffee is roasted. The decrease in sucrose level may reach 98% during roasting (Trugo and Macrae, 1984). Sucrose is also an important contributor to the formation of reducing sugars that are involved in the fragmentation and caramelization roasting reactions as well as the Maillard reaction. Several compounds resulting from the reactions are important contributors to coffee flavor, which may be related to beverage sweetness (Ginz et al., 2000; Rogers et al., 1999). This sensory attribute is one of the most appreciated characteristics in gourmet coffee drinks. That is why sucrose is expected to be present at higher levels in coffees with higher quality, as was observed in the present study.

**Table 5.** Correlation between the analyzed variables (organic acids, final sensory score and sensory attributes) in principal components 1 and 2.

| Parameter | PC1 (46.52%) | PC2 (26.69%) |
|---|---|---|
| Fragrance | 0.96 | -0.01 |
| Taste | 0.99 | 0.01 |
| Acidity | 0.94 | -0.22 |
| Body | 0.56 | -0.72 |
| Final | 1.00 | -0.04 |
| Lactic acid | -0.03 | 0.24 |
| Acetic acid | -0.20 | -0.36 |
| Malic acid | -0.44 | -1.00 |
| Oxalic acid | -0.68 | -0.40 |
| Citric acid | -0.46 | -0.78 |
| Sucrose | 0.72 | -0.68 |

Sucrose content has also been positively associated with coffees with higher acidity (Bertrand et al., 2003; Decazy et al., 2003), which confirms the results of our study. The sucrose vector and acidity attribute showed proximity in the biplot (Figure 2) and a positive correlation pointing to the same group of samples. Those attributes were extremely important in the discrimination of coffee samples A3G2, A2G2 and A2G1, which showed the highest scores in the sensory analysis. Therefore, the contribution of the sweetness and acidity attributes was important for the taste and aroma of the coffee samples.

Oxalic acid is a toxic dicarboxylic acid and is present in plants, such as spinach and wood sorrel. Although, the consumption of pure oxalic acid is deadly, its level in edible plants is very low to present any serious risk (Snyder, 2002). Oxalic acid was the organic acid found in the lowest amount in the samples (average values ~0.05% d. m.). No study in the literature correlates the oxalic acid content with its sensory perception in food. However, its content in this study was negatively related to beverage quality.

The other organic acids (lactic acid, acetic acid, malic acid and citric acid) showed higher weights when contributing to sample dispersion in PC2 (Table 5). Malic acid and citric acid were the organic acids with the highest correlation coefficients in PC2. PC2 allowed for the discrimination of the samples ($A_xG_y$) as a function of the malic and citric acidS in each group previously formed by PC1.

Carboxylic acids are responsible for coffee fragrance in low concentrations. In addition, each acid has its own taste, e.g., lime taste for citric acid, buttery taste for lactic acid and apple taste for malic acid. Those acids are more perceptible as an odor rather than as a taste. Acetic acid is an exception in coffee because its presence is the result of fermentation in coffee. Green coffee beans, which have high levels of this acid, usually favor a fermented taste, which is very distasteful (Lingle, 2011). It was highlighted that all of the correct procedures for the production of specialty coffees were followed during the harvest and post-harvest of the coffee fruit samples in this study. Such care guaranteed samples free of defects and fermentation.

Although, organic acids are responsible for the formation of taste and aroma in coffee, PC2 explained only 15.8% of the total variance. These results indicated that the quantified organic acids (lactic acid, acetic acid, malic acid and citric acid) may not, by themselves, explain the sensory characteristics assessed.

## Conclusions

Yellow Bourbon IAC J9 and Yellow Bourbon/origin SSP were the genotypes most indicated for the production of specialty coffees. This information brings strong insight to breeding programs that are beginning to focus on coffee quality and to farmers that want to develop a business in specialty coffees in Brazil.

Sucrose content and oxalic acid content were found to be good discriminant markers for green coffee beans regarding the final quality of the coffee samples. Conversely, lactic, acetic, malic and citric acids did not allow the discrimination of the coffee samples in relation to their quality.

## Conflict of Interests

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# Financial analysis of poultry production in Kwara State, Nigeria

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**This study examined the economics of poultry production in Kwara State, Nigeria using budgetary and profit index analysis. Primary data were collected through a set of structured questionnaire from 80 registered poultry farmers using a systematic random sampling technique. The results of the study showed that 72.5% of the respondents were youth with the mean age of 38.7 years and 63.8% choose poultry farming as main occupation. Analysis of costs and returns revealed that poultry production is profitable in the study area. The gross income and net income for eggs production were found to be ₦4,062,422 and ₦1,255,965, respectively, while the gross and net incomes for broilers production were ₦1,683,209 and ₦499,187, respectively. The regression analysis showed that stock capacity, variable, and fixed costs including labour, feed, and equipment were the significant factors affecting poultry farm business in the study area. These costs increase as the size of the business increases. To achieve optimum output and maximise profit, poultry producers in the study area would have to stem down cost of production. Provision of technical education through extension agents would greatly help in achieving this lofty goal.**

**Key word:** Poultry production, budgetary analysis, benefit cost ratio, regression analysis.

## INTRODUCTION

One of the major challenges facing Nigeria is the satisfaction of the ever-increasing demand for protein. Most Nigerian diets are deficient in animal protein. The FAO recommends that the minimum intake of protein by an average person should be 65 g per day; of this, 36 g (that is, 40%) should come from animal sources. The country is presently unable to meet this requirement. The animal protein consumption in Nigeria is 15 g per person per day (Tijjani et al., 2012) which is a far cry from the FAO recommendation. As a result, wide spread hunger, poor, and stunted growth as well as increase in spread of diseases are evident in the country.

Animal protein sources include fish, egg, poultry meat, beef, milk, beacon, pork, and mutton; but they are not affordable. The common sources accessible to most Nigerians are frozen fish, beef, and local chickens. Many

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farmers are involved in poultry production especially in Kwara State Nigeria, but the level of the productivity still remain local and small-scale. Various studies (Tijjani et al., 2012; Nurudeen, 2012; FAO, 2010; Yusuf and Malomo, 2007; Ojo, 2003) reached a consensus that intensification of production of meat and eggs derived from prolific animals like poultry birds is germane to meet animal protein requirements from domestic sources. Poultry meat and egg offer considerable potential for bridging the nutritional gap in view of the fact that high yielding exotic poultry are easily adaptable to our environment and the technology of production is relatively simple with returns on investment appreciably high.

Poultry is a sub-sector in the livestock industry constituting a major component of the agricultural economy. The sector provides animal protein to the populace as well as employment for a considerable percentage of the population. According to FAO Report (2010), poultry comes fourth among sources of animal proteins for human consumption in Nigeria and contributes about 27% of the national meat production.

Okoli et al. (2004) revealed that 85% of rural families keep small ruminants and local fowls primarily as an investment and sources of manure or meat at home or for use during festivals. In spite of this, livestock production is not keeping pace with the protein requirements of the rapidly increasing Nigeria population. Demand is more than supply. Since the responsibility of any civilized government is to provide adequate food and assure an atmosphere free from hunger and malnutrition, the Federal Government of Nigeria placed a ban on importation of frozen chicken and turkey parts to encourage massive poultry production locally (Agricultural Transformation Agenda, 2012). This policy has encouraged many investments in poultry production in Nigeria. It has therefore, becomes a full time job for many and is considered to be a commercially viable enterprise.

Considering poultry production as a commercially viable business demands the application of the knowledge of farm management (Olukosi and Ogungbile, 1989) in the area of economic measurement of it profitability, with the utmost aim of guiding the farmers in the appropriate use of resources/combination to maximize profit and encourage potential entrants to increase output and bridge the gap between national demand and supply of animal protein. This study is therefore analysing the economics of poultry production in Kwara State.

The study investigated the cost and returns of poultry production and the factors affecting its productivity in the state. The outcome of the study is to show the profitability level and the branch of the business that is more profitable and remunerative. The result of the study may also point to the fact that poultry business is the quickest and easiest way to reduce poverty level in Nigeria. Another major policy challenge inhibiting the overall development of the country's economy.

## MATERIALS AND METHODS

### Study area

This study was carried out in Kwara State, Nigeria. Kwara State was one of the seven states created on 27th of May, 1967. It extends from latitude 7° 45`N in its southern end, latitude 2° 45`E to the west and longitude 6° 40`E to south east. It covers an area of 35,705 km$^2$ and has a total population of 2,371,089 (NPC, 2007) with a population density of 66 people/km$^2$. The population in the state makes up 1.7% of Nigeria's total population. The state is basically agrarian. 80% of the population resides in the rural areas and 90% of this rural population are farmers. Livestock production, including sheep, goat, and poultry are also popular in the state. Kwara State is divided into 16 Local Government Areas, including Asa, Baruteen, Edu, Ekiti, Ifelodun, Ilorin East, Ilorin South, Ilorin West, Isin, Irepodun, Kaiama, Moro, Offa, Oke-ero, Oyun, and Pategi. Moro is the project area. The main ethnic groups are Yoruba, Hausa, Ibibio, Nupe, and Baruba.

### Sampling techniques and sample frame

Proportion sample formula (Bowley's, 1977 quoted from Yusuf, 2011) was employed to determine the sample size for the study. The formula is presented as:

$$n = \frac{N}{1 + N(e)^2} \quad (1)$$

Where n is the sample size sought; N is the research population, e is the level of confidence (taken as 95%).
The sample size (n) for this study was calculated using an assumed mean of 100 as:

$$n = \frac{100}{1 + 100(0.05)^2} = 80$$

Sample size therefore = 80

The primary data were therefore collected from 80 respondents selected from the registered poultry farmers in the state through systematic random sampling technique. Each respondent was selected after the interval of six from the register provided by the KWSADP office on the premise that the selected respondent produces layers and broilers in the same production cycle. The selected sample was served with the structured questionnaires designed in line with the objectives of the study during one of their general meeting early this year.

Information gathered included the value of eggs, layers, and broiler outputs obtained by adding cash receipts from eggs, layers, and broilers produced and the values of each of the outputs consumed by the farmers household and gifts to their friends. Inputs including size of the flocks, feed intake (₦), variable expenses (cost of feeds, labour and other operating expenses) (₦), and depreciation values (durable items). Socio-economic characteristics such as farmers' age (years), education (years), household size, and experience of farmers in poultry production which were considered for their influence on poultry outputs.

### Data analysis

Descriptive statistics, budgetary analysis, benefit cost ratio, and ordinary least squares regression (OLS) were used for the analysis.

**Descriptive statistics**

This involves the use of means, percentages, and frequency distributions, to show the various findings about the respondents in the study area. The mean and percentage will be derived from the following formula:

$$\text{Mean} = \bar{x} = \frac{\sum_{i=0}^{n} Xif}{\sum_{i=0}^{n} f}$$

$X_i$ = Observed Variable
f = Number of time the variable occurs

$$\text{Percentage} = \frac{X1x100}{\sum_{i=0}^{n} X1}$$

**Budgetary technique**

According to Olukosi and Sonaiya, (2003), farm budgeting is a detailed physical and financial plan for operating farms for certain period. It enables the estimation of total expenses (costs) as well as various receipts (returns) within a production period. This technique was employed to analyse the net farm income of the poultry business in the study area.
The model for estimating farm budgeting is outlined thus as:

NFI = GI – (TVC+TFC)

Where NFI is the net farm income, GI is the gross income (total revenue), TVC is the total variable cost, and TFC is total fixed cost.

**Total variable cost of production (TVC):** TVC comprises expenses on water, electricity, hired labour, marketing, vaccinations, and drugs.

**Total fixed cost (TFC):** Depreciation expenses on land, equipment, generator, houses, and machineries.
To obtain the worth of each of the fixed cost items, the straight line method of depreciation was used and it was assumed that the salvage value of the fixed items used in the business is zero. The formula for depreciation using straight line method is given as:

$$\text{Depreciation} = \frac{\textit{Purchase price}}{\textit{No of useful years of the asset}}$$

**Benefit-cost ratio:** The viability of the poultry enterprise was determined using the benefit-cost ratio (BCR), which is the division of total revenue by total cost. The BCR measures the ability of the business to upset its financial obligations and still remain standing.

**Regression model:** OLS regression analysis was employed to determine the factors influencing the profitability of poultry production.

The double-log function considered is presented as:

$$LnY = Ln\hat{a}_o + \hat{a}_1 LnX_1 + \hat{a}_2 LnX_2 + \hat{a}_3 LnX_3 + \hat{a}_4 LnX_4 + \hat{a}_5 LnX_5 + \hat{a}6\ LnX6 + U_i$$

Where Y = total value of output produced per year (including layers, eggs, and broilers in naira), $X_1$ = flock size (In number), $X_2$ = operating expenses (cost of labour, drugs, and transportation in naira), $X_3$ = cost of feed (in naira), $X_4$ = depreciation (cost of equipment in naira), $X_5$ = farmers experience (years), $X_6$ = level of education (in years), $U_i$ = Error term, and $\hat{a}_o$ = Constant term, $\hat{a}_i$ to $\hat{a}_5$ = regression coefficients to be estimated

## RESULTS AND DISCUSSION

Table 1 shows the summary statistics of poultry production in Kwara State. 57% of the total revenue comes from the sales of eggs meaning that this branch of the poultry business is highly lucrative. It was also revealed that the variable cost covered about 70% of the total cost of production with feed and labour contributing close to 80% of the cost. This is in line with the findings of Nurudeen (2012), Yusuf and Malomo (2007) and Ojo (2003). The farm size (stock capacity) was found to be 2036 with standard deviation of 2980. This shows that an average poultry farm in the study area belongs to a medium scale category. This classification is premised on Ojo (2003) classification of poultry farms: small scale category = 1000 birds, medium scale category = 1001 and 4999 birds, while large scale category = more than 5000 birds.

The table also shows that the commercial poultry farmers in Kwara State are highly experienced, well educated and young and showed keen interest in poultry business as revealed by the number choosing poultry production as their main occupation. Yusuf and Malomo (2007) and Ojo (2003) also discovered that majority of the poultry farmers in their areas of studies were young and highly educated. This finding is therefore, a pointer toward a brighter future poultry production in the state in particular and Nigeria as whole.

### Profitability analysis of poultry farms in Kwara State

#### *Gross margin and farm income analysis*

The study revealed (Table 1) that the mean values of eggs and broilers produced in a poultry farm within a production cycle were ₦4,062,422 and ₦ 2,182,396, respectively, while the total cost incurred in their production were ₦2,806,457 and ₦1,683,290, respectively. This implies that an average poultry farm in the state earns a net revenue of ₦1,255,965 and ₦499187 from eggs and broiler business in a production cycle. In addition, each farm had a gross revenue of ₦839,254 from the sales of old layers. The net revenue per bird stands at ₦862. The analysis showed that poultry business is very profitable and confirmed the findings of various researchers (Nurudeen, 2012; Tijjani, 2012; Ibrahim et al., 2009; Yusuf and Malomo, 2007; Ojo, 2003) in different states of the federation who concluded that poultry business was highly profitable.

### Benefit cost ratio

The viability of the business was determined through

**Table 1.** Summary statistics of the poultry variables.

| Items | Mean (₦) | Standard deviation |
|---|---|---|
| **Sale receipts** | | |
| Eggs | 4,062,422 | 10530536 |
| Layers | 839,254 | 1976305 |
| Broilers | 2,182,396 | 10944400 |
| Gross margin | 7,084,072 | 14678347 |
| Total fixed cost (dep) | 853,017 | 1679650 |
| | | |
| **Variable costs** | | |
| Labor | 231,000 | 324856 |
| Water | 1594 | 12292 |
| Electricity | 26040 | 21700 |
| Fuel | 21927 | 42185 |
| Feeds (for layers) | 1,608,090 | 1739531 |
| Feeds for broilers) | 277,002 | - |
| Vaccination | 28,789 | 24284 |
| Day old chicks (pullets) | 360,000 | 260435 |
| Day old chicks (broilers) | 243,840 | 227650 |
| Total variable cost (layers) | 1,953,440 | - |
| Total variable cost (broilers) | 830,192 | 2814729 |
| Total cost (eggs) | 2,806,457 | 3512930 |
| Total cost (broilers) | 1,683,209 | 1589000 |
| Net revenue (eggs) | 1,255,965 | 6145287 |
| Net revenue (broilers) | 499187 | 325670 |
| Net revenue/bird | 862 | 657 |
| BCR | 1.6 | - |
| | | |
| **Socio-economic characteristics** | | |
| Age | 39.4 | 9.4 |
| Years of education | 14.2 | 2.7 |
| Household size | 5.6 | 1.4 |
| Mean capacity (no) | 2,036 | 2980 |
| Years of experience | 8 | 6.0 |
| Main occupation | - | 63% |

benefit cost analysis. The ratio of benefit (mean gross revenue) to mean total cost of production was found to be 1.6. This implies that poultry business in Kwara State is viable, capable of offsetting its own cost, and still generate 60 kobo from every one naira invested.

Analysis of the result in Table 2 shows that all the parameters estimated carried positive signs, except household size which was negative. This finding implied that age ($X_1$), education ($X_3$), stock capacity ($X_4$), depreciation value of fixed items ($X_5$), and operating cost (TVC) ($X_6$) all have direct relationship with farm output in poultry production. The negative sign carried by households' coefficient showed that farm output reduces with increased number of house hold members probably, because large households consume more. The T-ratios showed that stock capacity ($X_4$), depreciation value of fixed items ($X_5$), and operating cost (TVC) ($X_6$) have significant relationship with output. The high significant level of these variables showed that they increased as the revenue increases. This finding collaborates that of Nurudeen (2012), Yusuf and Malomo (2007), and Ojo (2003), who explained the fact that stock capacity stands for the number of birds in the farm . Farm revenue increases with number of flocks. The variable cost includes cost of feeds, labour, vaccination, and other operating cost; all these increase with the size of the farm and the technical and allocative understanding of the farmer, that is, the ability to use minimum input to produce more output and ability to implore appropriate combination of inputs at least cost to maximize revenue. While, the depreciation cost includes depreciating values of poultry pens, generator, feeding, and watering troughs

**Table 2.** Regression analysis for input/output relationship in poultry production.

| Variable | Estimated parameter | Coefficient | Standard errors | t-values | Significant levels |
|---|---|---|---|---|---|
| Constant | $X_0$ | -7254826 | 5429452 | -1.336 | 0.186 |
| Age (year) | $X_1$ | 0.036 | 7914.74 | 0.718 | 0.475 |
| HHSIZE | $X_2$ | -0.011 | 536227.76 | -0.227 | 0.821 |
| EDU (YEAR) | $X_3$ | 0.36 | 277428.83 | 0.711 | 0.479 |
| STOCK CAP | $X_4$ | 0.634 | 473.28 | 8.732 | 0.000* |
| DEP VALUE | $X_5$ | 0.521 | 0.521 | 3.315 | 0.001* |
| VAR. COST | $X_6$ | 0.325 | 0.325 | 3.539 | 0.001* |

Diagnostics statistic: $R^2$=0.826; F Value = 57.68; Dependent variable = TR; N=80; *Significant at1%.

and other equipments and all these increase with use and handling.

F-value 57.7 showed that all explanatory variables taken together have a significant effect on the dependent variable (TR). $R^2$ value of 0.826 implies that 83% of the variation in the dependent variable has been explained by the independent variables, such as stock capacity ($X_4$), depreciating cost of equipment ($X_5$), and TVC ($X_6$) and that the remaining 17% was as a result of random variable.

## Conclusion

An attempt to examine the economics of poultry production in Kwara State was the principal focus of this study. The specific objectives were to estimate profitability of poultry production and determine the factors influencing total revenue obtained in poultry production in the study area. Primary data were collected from 80 poultry farmers selected from the registered poultry farmers in the state through systematic random sampling technique using structured questionnaire. Descriptive statistics, farm budgetary technique, benefit cost analysis, and ordinary least square regression analysis were used for the data analysis. Three important findings emerged; first, poultry production in Kwara State was dominated by highly educated youth with the mean age of 38.7 years with keen interest in poultry production. This is a positive sign for future poultry activities. Second, poultry production is highly profitable in Kwara State; however, egg production contributed the highest return. Net farm income from eggs production stood at ₦1,255,965, while that of broilers was ₦ 499,187 in one production cycle, respectively. Third, the costs of variable inputs including: feeds, labour, other operating cost, and depreciating cost of equipment were high and increasing with output thereby depriving the farmers the full benefit of their efforts.

## RECOMMENDATIONS

1. To reduce cost of production, farmers should form agricultural cooperative groups which will enable them to benefit from the economy of scale through bulk purchases of farm inputs.
2. Government should encourage the youths involved by providing them technical education in the area of resource management through the extension agents to reduce production cost.
3. The e-wallet program of the federal government to supply subsidized feeds, drugs, and vaccine to poultry farmers is in the right direction and should be intensified to benefit large farmers at the right time to reduce the stress and cost of production in order to improve profitability.

## Conflict of Interests

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# Ergonomic characterization of three sugar cane harvester machinery models

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**Due to the latest expansion on Brazilian agricultural area, there has been growing demand for field machinery usage aiming at increasing operational efficiency along with cost reduction on production as well as searching for improvement of working conditions. This paper aimed at ergonomic characterization comparing three models of agricultural harvesters on sugar cane plantations in order to provide information to help decision takers. Each harvester was analysed in 157 items. Results showed that ergonomic design in the three analyzed harvesters had high safety level to operators. Nevertheless, there is the necessity of improvement in some analysed items within operator's working place.**

**Key words:** Anthropometry, agricultural mechanization, ergonomics, *Saccharum* species, safety.

## INTRODUCTION

Agricultural mechanization within Brazilian agriculture has great importance for competitiveness (Peloia and Milan, 2010). Therefore, agricultural machinery usage is essential and crucial for Brazilian agribusiness for minimizing worker's physical effort, improve operational productivity, and also add quality and reliability in field work.

Considering sugar cane as one of the most important Brazilian agricultural products which has been growing and having high demand on production, is likely to occupy vast farming areas and thus mechanized harvesting has high importance for such cultivation (Pezzin Junior et al., 2013; Silva et al., 2011).

Agricultural machinery operators have driving position in the cabin as their main activity, therefore, the importance for observing ergonomic criteria which may establish the correct adaptation within man-machinery components (Fontana and Seixas, 2007).

Within the current agricultural development farming, machinery industries have been closely observing operator's workplace through technical ergonomic knowledge. However, several requirements are still neglected (Nietiedt et al., 2012).

Ergonomics is the science which studies man and working environment interaction, considering the positive and negative effects of such relationship (Almeida, 2011). Recently, the inclusion of ergonomics into the design of products has gained importance (Souza et al., 2012).

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**Table 1.** Technical specification of evaluated sugar cane harvester machineries.

| Technical characteristic | Harvester brands and models | | |
|---|---|---|---|
| | **CASE IH 8800** | **JOHN DEERE 3522** | **SANTAL S11** |
| Hour meter (h) | 6.943 | 3.257 | 2.390 |
| Motor (brand/model) | CASE IH C9 | John Deere 6090T | Scania DI 12 54 A |
| Power (HP) | 330 | 342 | 336 |
| Wheel sets | Crawler | Crawler | Crawler |

Villarouco and Andreto (2008) consider that a full ergonomic evaluation covers a wide range of variables requiring efforts from several different areas.

According to Leite and Carvalho (2011), ergonomics covers the study of previous working conditions, that is, the concept of work as well as its consequences which interact in the relation of man, machine, and environment, while relating with such production system.

Pizo and Menegon (2010) also say that the interaction which occurs among such parts of the process leads to increased knowledge or consciousness level of activity which will be key factor when implementing ergonomic actions resulting from the diagnostic as well as from proposed transformation.

For Silva et al. (2009), it is the employer's duty to conduct ergonomic analysis in workplace taking into account the working conditions according to Regulatory Convention 17 – Ergonomics, established by Ministry of Labour through Ordinance No. 3751, of November 23rd, 1990, which defines the parameters that allow the adaptation of working conditions to worker paraphysiological characteristics aiming at generating maximum comfort, safety, and efficient performance.

In this context, by means of ergonomic analysis, it is also possible to identify physical, cognitive and social risks and also to provide actions aiming at improving comfort and safety for the worker. Therefore, this paper aimed at compared ergonomic characterization of three models of harvester machinery used at sugar cane plantation in order to provide information to help decision makers.

## MATERIALS AND METHODS

Ergonomic data from three harvester machinery (Table 1), from sugar and ethanol plant, were collected from March to May, 2014. Harvester machinery is used in sugarcane (*Saccharum* species) plantations in the Midwest of São Paulo State.

According to Volpato et al. (2012) due to the lack of a specific manual for ergonomic evaluation for sugar cane harvester machinery, the methodology proposed by Eriksson et al. (2006) was used. One hundred and fifty seven items were measured in order to obtain an overview of ergonomic profile of harvesters according to the following aspects:

**Access to cab:** 19 items were evaluated, highlighting height measurement of the first step for accessing the cab as well as measurements of the other steps, stair width and depth, height and diameter of access handle, dimensions of access door, and emergency exit;

**Cabin:** for this step, 10 items were evaluated such as cabin height, distance between dashboard and seat, front and rear windshields in relation to height of operator's head, room for legs, place for personal items storage, and sharp corners or edges among other items;

**Visibility:** eight items were evaluated such as viewing distance between operator and the ground and vice-versa, viewing angle, windshield cleaning systems, view obstruction points;

**Operator seat:** 22 items were evaluated in this section emphasizing height, depth and width of the seat, backrest height, type of suspension, size and moving angles of armrest, seat position configurations;

**Commands and instruments**: 13 items were collected such as the distance from primary and secondary controls, control identification and positioning, distance among commands moved by the feet, strengths for moving commands;

**Machinery operation:** 7 items evaluated the operator's control over automatized functions, movement of grip controls, break reliability, number of platform activation, and speed;

**Machine information:** 6 evaluated items of circumstances in which the operator can hear, see or feel alert signs of danger, an overview of control dashboard, color and symbols on operation displays;

**Operator's position:** 4 items were evaluated for seat adjustments, armrest, controls and instruments, adequacy of operator station for different biotype operators;

**Noise:** 4 different situations were evaluated on working conditions according to noise level. Equivalent level (Leq) was obtained from Regulatory Convention 15, established by Ministry of Labour, presented by Atlas Team (2014);

**Cabin climate control:** among 6 evaluated items, temperature was highlighted as well as solar radiation controls;

**Gas and particles:** 6 evaluated items of air inlet, dust removal filters, pollen and soot, and regulation of gas emission which are of greatest importance in this section;

**Lighting:** 10 items were evaluated, emphasizing on lighting within operational and conduction area, lighting reduction, lighting direction, reflection on glass or on parts of the machine;

**Instruction manual:** in this section, 13 items were evaluated: scope of instructions, language, level of instructions, diagnosis of

**Table 2.** Ergonomic classification of evaluated harvesters according to used methodology.

| Classification | Characteristics |
|---|---|
| A | Operation is highly productive in all kinds and conditions of geographical relief, with high level of safety |
| B | Operation can be developed within a highly productive operational efficiency but under easier cultivation and smoother relives conditions than those elicited in classification A, with a higher safety level but not as class A pattern |
| C | This classification is considered within medium safety level. Operation should be developed under easy working conditions. Thus, it should be in a lower operational pace, within a shorter time and lower safety level than in B classification |
| D | Operation requires easy working conditions, lower operational pace and shorter time than C classification |
| E | Machinery either does not meet mandatory safety requirements or has a high level of injury for the operator being necessary the correction of such items to be used |

failures, maintenance routine, safety, ergonomics, warranty conditions;

**Maintenance:** this section had the highest number of evaluated items totalizing 29, emphasizing failure diagnosis graphic, diagnosis on engine problems, auxiliary lifting devices and lighting, hood or protective covers, floor on maintenance platform, among others. Result interpretations (Table 2) were based according to Eriksson et al. (2006) classification.

For evaluation development, the following materials were used: digital sound pressure measurer Instruterm, DEC-416 model, used for noise level evaluation; digital light intensity Lux measurer Instrutherm, LDR 208 model; tape measure light intensity Lux measurer, Instrutherm, LDR 208 model; tape measure, scale and measuring tape with millimetric records and a protractor for measuring curvature degrees.

Goodman test with multiple comparisons within and among multinomial populations (Goodman, 1964, 1965) was carried out for section proportion comparisons in each evaluation score (classification) among evaluated harvester machinery as well comparisons of section proportions within the same classification among harvesters. It was considered a 5.0% significant level and each section evaluation was independently conducted.

## RESULTS AND DISCUSSION

Due to great physical effort to get in and get off the operation platform in agricultural machinery, it is necessary for the development of technical-scientific studies which may result in improvement of access and exit at workplaces.

So access to cab, in the three evaluated harvester models, were classified as E score for showing high injury risk to operators due to height of the first step in relation to the ground as well as the distance between stair steps. This is endorsed by Mattar et al. (2010) when affirming that national agricultural machinery do not meet minimum requirement of Regulatory Norm NBR 4252 regarding access and exit from operating platform. Monteiro (2010) defines the cabin on agricultural machinery as a place to protect the operator from the sun or from the rain, cold, dust, emissions, noise as well as minimize vibrations thus providing thermal comfort.

Within such context, the main detected problem in evaluated cabs was the distance from the rear windshield with the height of operator’s head, which has an inadequate distance what may cause injury to operator (average 575 mm). It was also observed that the lack of a place to keep personal belongings or even first aid items may jeopardize operational safety for these items may shift due to machinery jerking. Thus, this section was rated C for the three evaluated harvesters.

Visibility from operational platform, that is, from the cabin is extremely important; for it can decrease or eliminate accidents besides providing an adequate positioning, which may consequently result in a lower physical effort from the operator. For Fiedler (1995), operator’s visibility must not have interference from fogged, small or even badly distributed windshield glass. Visibility must also not be obstructed by narrow screens, hydraulic hoses or by windshield wiper.

According to evaluations, it was observed that the three studied harvesters provide operator with a vertical viewing angle (on working position) ≥ 65°. The three machineries also have windshield and side window defroster systems as well as a cleaning system. However, visibility is blocked in rear view due to a structure on the machinery used for operation and it also has limitation for viewing ratoon cane, that is, distances of 2 m inferior from the side of the harvester resulting in a C rating for evaluated harvester models.

Agricultural machinery is relatively complex and is made up of several compounds. Operator’s seat is one of this important compound. If it is not correctly adjusted to the different biotypes, it may cause tiredness or even musculoskeletal disorders. For Santos et al. (2008), when any diagnosed disorder is connected to sedentary work, it is considered an ergonomic factor in seat project designs along with working environment. In this context, seat evaluation section had a B rating for the three evaluated harvesters for they showed adequate suspension systems, backrest angle, height and convexity of lumbar support. However, the reduced space between seat and dashboard does not allow operator to bend legs within 60° angle.

Performance of harvesters should be simple and continuous. Nevertheless, such situation is not always possible to be carried out. There are some actions which should be hand operated or even requiring a high level of concentration from operator. Fontana and Seixas (2007) stated that it is necessary to consider operator's characteristics and performed work trying to reach a higher productivity efficiency as well as a higher comfort and safety degrees, thus having better working conditions. So the three evaluated agricultural harvesters had a C rating for they did not show function indicators which can be easily understood by operator as well as the position and the distance of control commands.

Alonço et al. (2006) stated that graphic symbols used to indicate machitrol functions accomplish a safer operation. Symbols were standardized according to Alonço et al. (2007) after Regulatory Norm NBR 11379, aiming at standardizing graphic symbols for operational command identification and machinery maintenance. In machinery information section, it was observed that harvesters showed some deficiency and were rated B. Nevertheless, they have information monitors with texts and symbols which are clearly visible and easily read by operators.

Agricultural machinery operation is an activity which encompasses basically two factors: man (operator) and engine (tractor) (Debiasi et al., 2004). Such performance carried out for long hours can be considered hard chore. Therefore, the correct body posture directs to an adequate comfort for the operator who will consequently have a better operational efficiency. Thereafter, operator's position in the three analysed harvesters is not impaired and was rated B.

Noise is a sound wave, or a complex of waves, which can cause discomfort and gradual hearing loss (Cunha et al., 2009). Constant or recurrent noise can also be defined as any undesirable sound being that for agricultural machinery the greatest noise sound comes from the engine. For Alves et al. (2011), agricultural machinery engine noise along time may cause hearing problems for operators. It is observed that noise levels are much higher than the one established by Brazilian Legislation in the three analysed harvesters especially when air conditioning is on, thus rating them as C. Facing such situation is necessary either the use of earplugs for noise level reduction or working time reduction for operators.

One of the main causes of working stress is laid on adverse climate conditions as hot or cold in excess (Lima et al., 2005). Poorly ergonomic designed cabins may impair internal climate control causing unsteadiness and may compromise the internal climate control, making them unstable or even complex in addition to solar radiation that can reach directly the operator causing discomfort or even an accident. John Deere harvester was rated A for this evaluated item for it had the best climatic conditions in the cabin. The other analysed harvesters were rated B for they do not offer solar radiation protection.

Exposure to airborne particles and machinery exhaust emissions may cause several health damages to operators. Silva (2007) states that gas or dust inhaling oil smell may cause headaches, nausea, eye and mucous irritation, allergies and breathing pathologies. In this section, the three analysed harvesters were rated B for exhausting gases from engine may go into the cabin.

Regarding lighting, illuminance (lux) was the used standard. Its correct light dosage helps reduce accidents, keeps trained personnel within the company and decreases operational failures (Brito, 2007). Nowadays, agricultural operations are also carried out at night shifts making it a must that the adequate illumination of all operational areas specially for avoiding operator's sight tiredness. The three analysed harvesters were rated A for this section thus showing no problems.

Manuals of agricultural machinery are intended to give information in order to guide operators as well as machinery managers, having detailed descriptions on technical, ergonomic, and safety data and also information on maintenance.

For Eriksson et al. (2006), it is of high importance that the operator can identify and manage engine mechanic failures from manual information. Therefore, the analysed harvester manuals were rated B for none of them showed detailed information on ergonomic items.

Agricultural machinery maintenance may be considered a procedure set which aims at maintaining the equipment within the best working conditions which may lead to lifespan increase avoiding premature damages, solving the current ones and tending to higher work safety (Reis et al., 2005).

When maintenance on agricultural machinery occurs, it should be considered, among other items, access to stairs and maintenance platform, failure system indicators, auxiliary lighting equipment, essential tools for maintenance and especially fast and safe accessibility for power sources. Under such conditions, the three analysed harvesters showed acceptable safety conditions and were B rated.

Table 3 shows results on comparisons of multiple proportions according to section evaluation scores (classification) for different harvesters. Due to the sample size, it was decided to reduce evaluation score numbers,

**Table 3**. Number and percentage of sugar cane harvesters evaluated in each classification group.

| Harvesters | Classifications | | | |
|---|---|---|---|---|
| | A and B | C | D and E | Total |
| Case | 9 (64.3%)$^{Aa}$ | 4 (28.6%)$^{Bab}$ | 1 (7.1%)$^{Ab}$ | 14 |
| John Deere | 9 (64.3%)$^{Aa}$ | 4 (28.6%)$^{Bab}$ | 1 (7.1%)$^{Ab}$ | 14 |
| Santal | 8 (57.2%)$^{Ba}$ | 5 (35.7%)$^{Aab}$ | 1 (7.1%)$^{Ab}$ | 14 |
| Total | 26 (61.9%) | 13 (31.0%) | 3 (7.1%) | 42 (100%) |

thus using three classifications. Therefore, classification was grouped according to safety level offered by harvesters during operation as follows: A and B (optimum and good security level); C (medium security level); D and E (low security level).

Goodman test for proportions among and within multinomial populations where different capital letters indicate significant difference ($p<0.05$) among harvesters for each classification group and different lowercase indicate different group proportion classification ($p<0.05$) among harvesters.

Analyzing A and B classification groups, statistical difference ($p<0.05$) was observed being that Santal harvester showed lower evaluation percentage for this safety level (optimum and good). Regarding medium safety harvester classifications (C), Case and John Deere machinery showed lower percentages, statistically different from Santal machinery. Concerning low safety level (D and E), the three analysed harvesters were similar. Regarding classification groups, there is statistical difference ($p<0.05$) for all analysed harvesters.

## Conclusions

The three analyzed harvesters showed high safety levels for operators concerning ergonomics design. However, Case and John Deere machinery show predominance. In the three analysed harvesters, it is necessary to review accessibility conditions for the three of them showing high injury risk for operators. The distance from rear wind shield in relation to the height of operator's head, level of noise, visibility blocking in some parts of harvester as well as limiting of ground viewing are the highest deficiencies on evaluated machinery.

## Conflict of interests

The authors have not declared any conflict of interests.

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