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Full Length Research Paper

Current knowledge and performance of existing charcoal coolers in improving the overall quality and shelf-life of French beans

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This study investigates current knowledge and performance of existing technology, 'charcoal coolers' in improving the overall quality and shelf-life of French beans. Data was collected primarily from a household survey using a semi-structured questionnaire administered to 45 purposively selected farmers who were selected at fixed interval. Data was analysed using the Statistical Product and Service Solutions (SPSS) Version 20 software and Pearson correlation is used to show the relationship between demographic characteristics and farmers post-harvest handling practices. The study revealed that majority of the farmers (80%) harvested late between 9 am to 12 noon and targeted temperature of 16 to 20°C. Only 32% of the farmers interviewed disinfected their produce after harvesting. Majority of farmers (57%) did not belong to any farmers' group or co-operatives. On the other hand, majority of the famers (80%) had adopted use of charcoal cooler technology and 69% stored their produce for between 1 and 6 h before delivering them to the packing shed, a distance that would take another 6 h. Farmers' age and education level had positive influence on use of cold storage facility. To sustain the use of cold storage facilities and technologies, there is need to support establishment of a community-based cold storage facilities that can be accessed by the small scale farmers to ensure their produce remain fresh before transfer to packing stations. Such a move would considerably reduce food losses and wastes along the value chain, which is an instrumental agenda of the United Nations Sustainable Development Goal.

Key words: French beans, charcoal cooler, cold storage, post-harvest practices, quality, shelf life.

INTRODUCTION

The horticultural sub-sector faces many challenges, the major one being high post-harvest losses. The major contributor to these losses is temperature (Vorster et al., 1990). The rate of deterioration of perishable goods

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increases two to three folds with every 10°C increase in temperature (Kader, 2005). Besides physiological deterioration, products may host micro-organisms like bacteria and fungi which can cause rotting and decays(Kitinoja, 2013). The higher the temperature the faster the losses through colour, flavour, nutrients and textural degradation (Vorster et al., 1990). It has been reported that when temperature is maintained at 10°C (Q10 quotient) colder than the temperature commonly experienced during ambient conditions, the shelf life of the commodity can double (Kader, 2005; Kitinoja, 2013). The higher the perishability of the produce the more it demands an effective and uninterrupted cold chain system. A cold chain system for fresh commodities can be defined as the continuous handling of the product at low temperatures during the postharvest process such as harvesting, collection, packing, processing, storage, transport and marketing until it reaches the final consumer (Kitinoja, 2013). Cold chain management should start at the time of harvesting and must take into account the time of harvesting (Kathryn and James, 2004). Temperature abuse right from the farm predisposes the produce to faster deterioration. In smallholder farms, the temperature misuse is attributed to factors such as lack of knowledge on good harvesting practice and postharvest management.

There are various options and technologies for providing cold conditions for food commodity handling. Some of the technologies that have been employed include dipping of the fruits or produce in cold water, also called hydro-cooling mixed with disinfectants such as thiabendazole and sodium hypochlorite with clean water available (Arah et al., 2016). This method is very effective in removing field heat and in the same reducing microbial loads on the harvested French beans. Some farmers, especially in developing countries, have had to assemble their produce and put them under the tree to reduce field heat (Olayemi et al., 2011). With refrigeration, possible decay of the produce is reduced and therefore increased shelf life. Vapour compression cycle is the most commonly used preservation technology. The technology depends on electricity driven and mechanical compressor (Lange et al., 2016). Another technology is the evaporative cooling system where water is applied to a porous surface like charcoal and as the temperature begins to rise, it begins to evaporate. As water changes from liquid to gas, it absorbs energy in the form of heat from the surrounding air, thus cooling it. This method releases water as a coolant, running freely over the charcoal or sand surface (Lange et al., 2016). Evaporative cooler is designed to offer an environment with both a lower temperature and a higher level of relative humidity (Nanguwo, 2000). It employs the principle of a porous structure to which water is added and as air flows within the wet wall, the air temperature is decreased due to the loss of heat to the evaporating water. The temperature is normally lowered by about 5 to 10°C, depending on the relative humidity of the ambient air. Farmers have also applied the use of ice making technology as a way of cooling the produce. This system works in two ways, first is the introduction of ice directly on the produce or by adding ice into water in which the produce is then plunged. Secondly, ice can be kept in an ice bank to cool down the surrounding stored produce through heat convection.

To address the challenge of fresh produce storage, a lot of research and innovation in Kenya over the last two years have resulted in small scale farmers increasingly adopting the use of low cost cooling chambers that use renewable energy (Toivonen, 2014). One of the most practical inventions is the use of locally made cold storage systems called charcoal cooler for use by smallholder farmers to keep their produce and increase shelf life while awaiting collection by the buyers. The objective of this study was to assess the current knowledge and extent of use of cold chain interventions in French bean production in Kenya. Kenya is a key producer of French beans for export market into Europe and UK, and most recently into Middle and Far East. Majority of the French bean production is by small scale farmers, owning less than one acre of land. French beans production in Kenya is a key income earner and generates a lot of employment in Kenya, either directly or indirectly.

Apart from technical compliance to European market standards, post-harvest losses remain a huge challenge to French bean exports in Kenya. Majority of the small scale farmers are located quite a distance from the packing facilities, and as a result, their produce takes on more hours to get to the exporters cooling facilities. Food waste reduction has become a key topic in the last two years, following realization that over eight hundred million people in the world are at the risk of starvation, while on the other hand, over 30% of edible food goes into waste along the supply chain. With renewed commitment of United Nations through the revise Sustainable Development Goals, in particular goal number 12.3, there is global focus on waste reduction along the supply and production chain. Majority of the major food traders have put in place measures to reduce losses along the supply chain, for example, Tesco, the leading UK Retailer has gone public to commit to reduce food waste along the supply chain by 50% by 2030. Tesco is a leading importer of French beans from Kenya. Findings from this study will be very useful in contributing to food waste reduction and global hunger right at the start of the French bean supply chain.

Value chain for perishable goods requires an integrated approach to cope with post-harvest challenges that are contributed majorly by temperature fluctuation. Efficient operations are essential throughout production, transportation, and storage processes. The absence of a sufficient and working infrastructure in the post-harvest value chain results in the harvested produce being lost

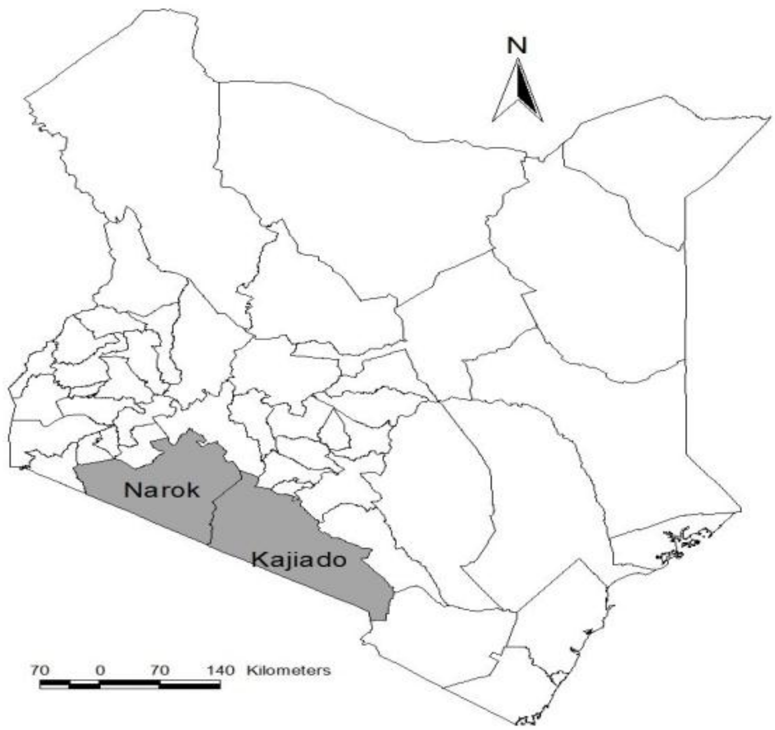

Figure 1. Map showing the study area.

before reaching the consumer market.

MATERIALS AND METHODS

Selection and description of the study site

The study was carried out in two counties of Kajiado and Narok. Kajiado County is located in the southern part of Kenya. It is situated between Longitudes 36°5'and 37°5' East and between Latitudes 1°' and 30°' South. Narok County is situated in Kenya along the Great Rift Valley. The temperature range is 12 to 30°C and average rainfall range is 500 to 1,800 mm per annum. Its geographical coordinates are 1° 5' 0" South, 35° 52' 0". This area was purposively sampled because of the large numbers of small-scale producers who mostly supply exporters. In addition, Narok and Kajiado counties have in the last decade become important French bean production regions supplementing reducing production from Central Kenya due to reducing fertility and competitive real estates in Central Kenya, thereby leaving less arable land. The prevailing weather and climatic conditions in these regions are also suitable for installation of coolers that use renewable energy (Figure 1).

Sampling technique and data collection

Data used in the study was obtained primarily from a household survey that targeted French bean producing farmers in both Kajiado and Narok Counties. Farmers were selected through a systematic random sampling procedure. Farmer register from the two counties was used to serve as the sampling frame and each farmer in this list was numbered sequentially. The targeted farmers were selected at fixed interval from the list to come up with 45 farmers from the two counties. A semi-structured questionnaire was used to collect both quantitative and qualitative data. Data was collected on attitude and extent of use of charcoal coolers, temperature at harvesting and at storage, the type of storage facility, produce storage time, changes in French bean and washing of the produce.

Data analysis

The data was checked and open-ended questions were coded before data entry. The date was analysed using the Statistical Product and Service Solutions (SPSS) Version 20, an IBM product acquired by IBM in 2009 (Hejase and Hejase, 2013) by computing descriptive statistics including frequencies, percentages, means and averages. The Pearson correlation was used to show the relationship between demographic characteristics and farmers post-harvest handling of French beans and knowledge on cold storage usage.

RESULTS

Household socio-economic characteristics of the farmers

Results show that 75% of the respondents are male farmers across the counties and the proportion of male farmers is also higher in both Kajiado (71%) and Narok (86%) (Table 1). 33.3% of the interviewed farmers are between the ages of 41 and 50 years, however, the age ranged from 19 years to over fifty years. 97.8% of all the farmers were married except one who was a widow. The

Table 1. Socio-economic characteristics of French bean farmers in Narok and Kajiado counties.

Variable	Description	Frequency	Percentage
Gender	Female	11	24.4
	Male	34	75.6
Age	Over 51 years	11	24.4
	41 to 50 years	15	33.3
	31 to 40 years	12	26.7
	19 to 30 years	7	15.6
Marital status	Widowed	1	2.2
	Married	44	97.8
Literacy level	Post-secondary	12	26.7
	Secondary	18	40.0
	Primary	15	33.3

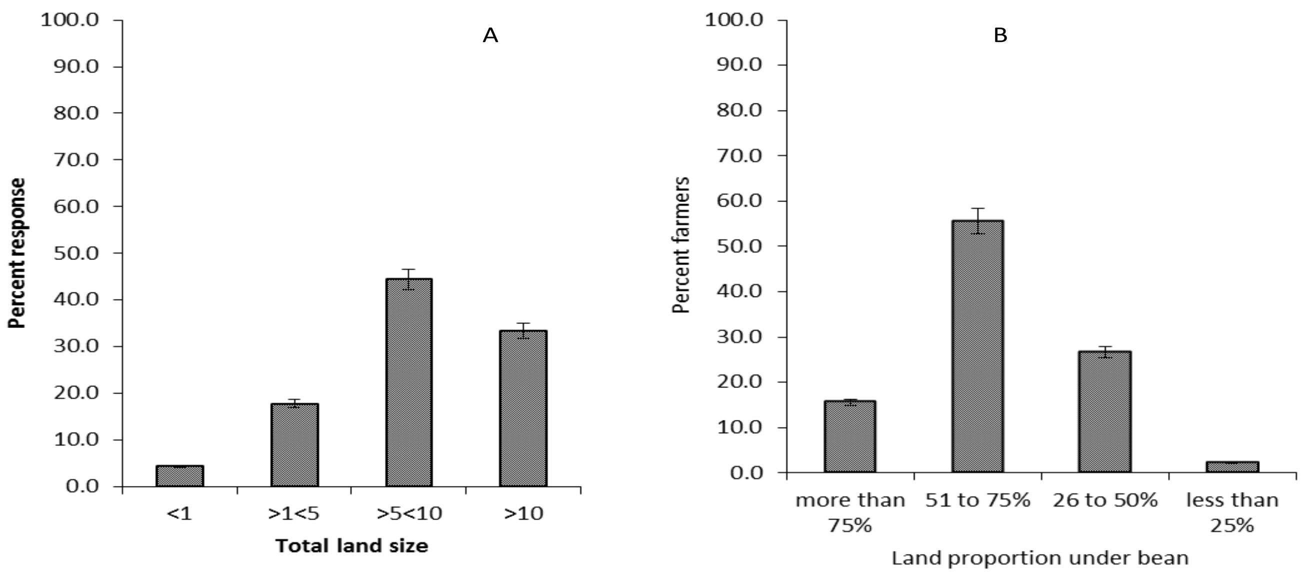

Figure 2. Proportion (%) of (A) land owned by the farmers and (B) land allocated to French bean production.

distribution of sampled farmers in terms of education showed that 40% of the respondents have basic education followed by 33.3 and 26.7% who have primary then post-secondary education, respectively.

Land size and land allocated to French bean production

French bean farmers in the two counties own land in various categories out of which 44.4% own land between 5 and 10 acres. Few farmers own land less than 5 acres while others own land above 10 acres (Figure 2). 55.5% of the interviewed farmers allocate 51 to 75% of their land to French bean production. Farmers in Kajiado County had significantly more land allocated to French bean production than farmers in Narok County.

Daily farm operations

Farmers (91.1%) report that they were solely responsible for their daily farm operations. However, 8.9% employ people either on permanent basis or as casual labourers to handle the daily farm operations (Table 2). Among the few employees, around 50% had post-secondary education, while the rest had either secondary or primary education levels. 50% of the employees are either salary employees, 25% are casual labourers or 25% offer free helping hands to the farmers.

Table 2. Characteristics of farmers responsible for daily farm operations.

Description	Category	Frequency	Percentage	Std. Dev
Daily management operation	Farmer	41	91.1	0.31
	Employee	4	8.9	
Education level of employee responsible	Pre-primary	1	25.0	0.95
	Secondary	1	25.0	
	Post-Secondary	2	50.0	
Occupation of employee responsible	Salaried employee	2	50.0	1.50
	Casual labour	1	25.0	
	Not employed	1	25.0	

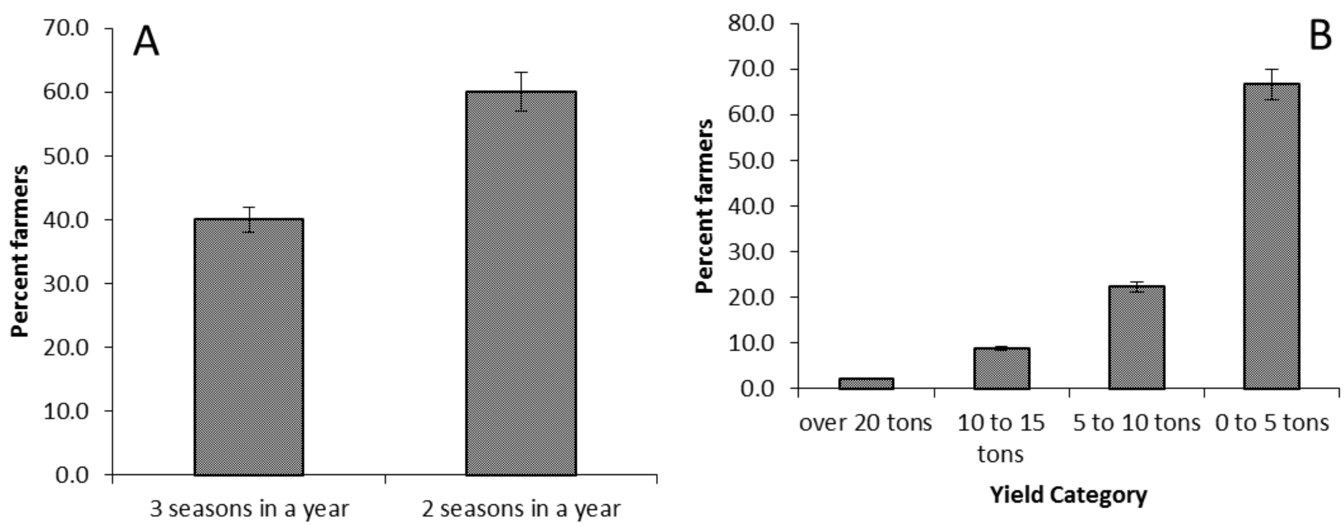

Figure 3. Main seasons of French bean production and yield achieved by the farmers.

Yields and main season of French been production

Figure 3 shows that 60% of the farmers interviewed produce French bean in two seasons in a year while the rest or 40% produce French beans in three seasons per year. The study reveals that 67% of the interviewed farmers harvest 0 to 5 tonnes of French beans from their farms, while only a few or 2.2% obtain yield above the 20 ton mark (Figure 3).

Current status post-harvest practices

Table 3 shows some of the post-harvest handling activities that were practiced by the farmers in the two counties. 68% of the farmers do not wash the French beans, 80% harvest late when the sun is up, 76% target the highest temperature of 16 to 20^{o}C and 69% store their produce for 1 to 6 h. 20% of the farmers, however, store their produce for less than an hour then deliver them to the buyer.

Farmer practices recommended by the buyers

Buyers always specify the qualities of the produce they require from farmers. To achieve the required qualities the farmers performed certain practices (Table 4). 40% of the farmers report management of pest and diseases, 24% report good fertilizer application as a way of achieving the demanded quality. Other practices performed by the farmers included planting certified seeds, timely irrigation, timely harvesting and grading and proper land preparation.

French bean qualities required by the buyer

Many farmers understood the qualities demanded by the buyers (Table 5). Some of the qualities mentioned are pests and disease-free products, products with no

Table 3. Post-harvest practices by French bean farmers.

Description	Category	Frequency	% of responses
Produce washing	Yes	15	33.3
	No	30	66.7
Time of harvesting	6 am - 9 am	8	17.8
	9 am - 12 pm	36	80.0
	12 pm - 3 pm	1	2.2
Measure Temperature	Yes	9	20.0
	No	36	80.0
Targeted temperature (°C)	6 - 10	3	6.7
	11 - 15	7	15.6
	16 - 20	34	75.6
	More than 20	1	2.2
Storage duration (h)	less 1	9	20.0
	1 to 6	31	68.8
	6 to 12	5	11.1

Table 4. Distribution of respondents based on the farming practices recommended by the buyer.

Factors considered to meet buyers demand	Frequency	% of responses
Fertilizer application	11.0	24.4
Control of pests and diseases	18.0	40.0
Planting certified seeds	9.0	20.0
Proper land preparation	1.0	2.2
irrigation in time	8.0	17.8
harvesting and grading	12.0	26.7
Total	59.0*	-

Table 5. Distribution of the farmers based on qualities of French beans demanded by the buyer.

Quality demanded by buyers	Frequency	% of responses
Pest and disease-free products	33.0	73.3
products with no blemishes	9.0	20.0
Non-dehydrated products	7.0	15.6
seed diameter 8 mm (11 - 15 cm)	5.0	11.1
well graded seed	4.0	8.9
Total	58*	-

blemishes, non-dehydrated products, pods diameter 8 mm and length of (11 to 13 cm), and well graded pods. 73% of the interviewed farmers report pest and disease free produce as the most demanded quality by the buyers. 20% mention products with no blemishes and 15.6% mention non-dehydrated produce. A few other farmers mention pods with 8 mm diameter and well graded pods.

Farmers' group membership

The results show that there is low participation in farming groups amongst the interviewed farmers (Figure 4A). The

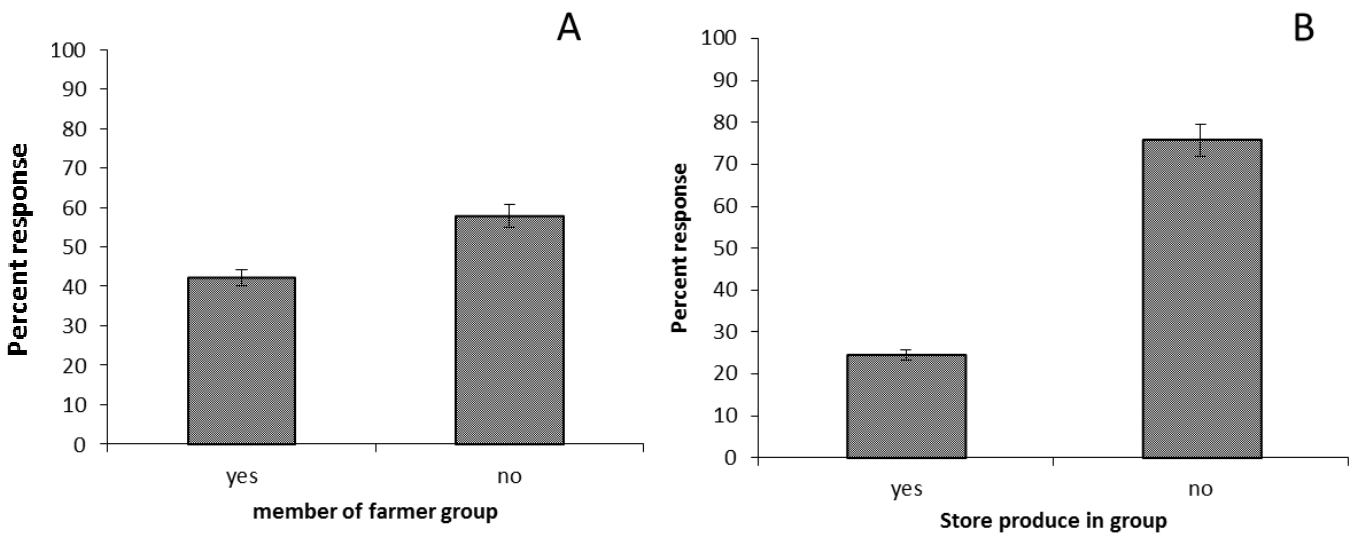

Figure 4. Group membership by the farmers and storage of produce in group storage facilities.

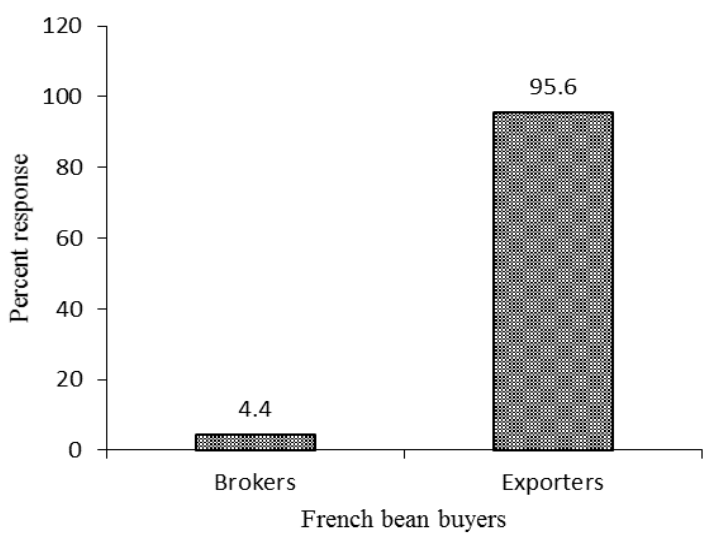

Figure 5. Market outlets for French beans from farmers.

interviews revealed that 57.7% of the farmers are not members of farmer groups and therefore were not involved in farmers-group activities. 42% are members of a known group; however, only 24% of these farmers collectively store their produce in farmer groups (Figure 4B).

French bean buyers

When the farmers were asked about the buyers, 95.6% supply their produce directly to exporting companies while only a few (4.4%) sell their produce through the brokers (Figure 5).

Charcoal cooler usage and construction materials

The usage of charcoal cooler among the farmers was common knowledge as majority (80%) of the respondents report having either used the charcoal cooler or borrow from another farmer, while only a few of the farmers did not use the cooler (Table 6). The farmers were also asked about the materials they used in constructing the charcoal cooler in terms of the floor, the walls and roofs. 73% cement their floor while others either put concrete floor or wooden planks. All the farmers use iron sheets as the roofing materials while they use the charcoal to construct the wall. However, in terms of humidifier installation, only 31% install humidifiers while the rest did

Table 6. Distribution of farmers based on the use and the material for construction of charcoal coolers.

Variable	Description	Frequency	Percentage
Charcoal cooler usage	Yes	36	80.0
	No	9	20.0
Floor	Cemented floor	33	73.0
	Concrete floor	2	4.4
	Wood planks	1	2.2
Roof	Iron sheets	36	80.0
Walls	Charcoal wall	36	80.0
Presence of humidifiers	No running water	22	48.9
-	water	14	31.1

Table 7. Factors employed by the farmers to improve the efficiency of the charcoal cooler.

Description	Category	Frequency	% of responses
Efficiency of the cooler	Yes	32.0	71.1
	No	5.0	11.1
What could be done to improve the cooler	Cementing floor	1.0	2.7
	More charcoal	2.0	5.4
	Size enlargement	2.0	5.4
	Wall mesh	3.0	8.1
	Roof cooling	5.0	13.5
	Air circulation	5.0	13.5
	Installing humidifiers	19.0	51.4

not.

Effectivity of the charcoal cooler

Results in Table 7 show that 71% of the respondents confirm that the charcoal cooler was effective as a storage facility while only 11% of the respondents suggest otherwise. In addition, when the farmers were asked how to improve the storage cooler, over 50% of the respondents suggest installation of humidifiers. 5% suggest improvement in air circulation within the coolers while a further 5% suggest installation of cooling system in the roof. Other suggestions included enlargement of the cooler plant, addition of more charcoal, and reinforcing of the wall by using wire mesh.

Correlation coefficient among demographic characteristics of farmers and various post-harvest practices

Demographic characteristics were correlated with various post-harvest practices (Table 8). Harvesting time was both negatively correlated and statistically significant ($P \leq 0.05$, r= - 0.332) with age. Temperature storage was also both positively correlated and statistically significant ($P \leq 0.05$, r = 0.367) with gender. However, education level ($p \leq 0.01$, r = -0.383) and harvesting ($p \leq 0.01$, r = -0.444) were both negatively correlated and statistically significant with charcoal cooler effectivity.

DISCUSSION

Demographic and socio-economic characteristics play an important role in understanding the differences between farmers and hence explaining their behaviour regarding adoption of certain technologies. The major characteristics of farmers covered in the survey were distribution of farmers by gender, educational level, marital status and farmers' age. Results show that 75% of the interviewed farmers were males. This implies that French bean production in the two regions was dominated by males. This result could be due to the fact that French bean production is labour intensive (Usman et al., 2016). However, this contradicts many findings which have reported women as the leaders in agricultural

Table 8. Correlation coefficient among demographic characteristics of farmers and various post-harvest practices.

Correlation	Age	Gender	Marital status	Study level	Harvesting time	Measuring temperature	Targeted temperature	Storage	Storage time	Charcoal cooler	Effectivity
Age	1										
gender	-0.017	1									
Marital status	0.050	0.265	1								
Study level	-0.114	0.116	-0.182	1							
Harvesting time	-0.332*	-0.159	0.056	0.037	1						
Measuring temperature	0.028	0.071	-0.247	0.142	-0.215	1					
Targeted temperature	-0.072	0.079	0.066	-0.132	0.532**	-0.273	1				
Storage	-0.051	0.367*	-0.047	0.128	-0.257	-0.057	-0.247	1			
Storage time	0.065	-0.204	-0.249	-0.171	0.147	0.186	0.336*	-0.442**	1		
Charcoal cooler	-0.169	0.323*	0.351*	-0.111	-0.130	-0.129	-0.099	0.295	-0.410**	1	0^b
Effectivity	0.413*	0.011	0b	-0.383*	-0.444**	-0.071	-0.093	0.255	-0.304	0^b	1

*, **: Correlation is significant at the 0.05 level (2-tailed) and at the 0.01 level (2-tailed), respectively.

activities, mainly in large commercial set-ups like for the case of the exporters. Gender of the farmers captures the differences in post-harvest handling between female and male farmers with either expected to have higher tendency to carefully handle the produce (Garikai, 2014). Age is an important demographic feature because it determines the quality of labour (Babalola et al., 2010). 33.3% of the farmers were aged 41 to 50. This implicates that majority of the farmers are still in their active age of agricultural production and therefore there is possibility of high productivity. However, there was evidence that the youth (ages between 19 and 30 years) were the least involved, a true reflection of the diminishing interest of the young generation towards Agriculture. The study further revealed that 97.8% of the farmers were married and therefore had family responsibilities. These results concur with the findings by Adamu (2005) who found that the majority of arable farmers were married and therefore they were enthusiastically engaged in agricultural production activities in order to improve the living standard of their households (Siri et al., 2012). Older farmers with huge farming experience are expected to easily adopt new technologies and that includes appropriate post-harvest handling technology to reduce losses associated with increased temperature (Martey et al., 2012).

The interviewed farmers had different levels of education with 40% attaining the basic level of the Kenyan education system. Education enables farmers to make choices based on their own understanding as well as work together easily with other farmers (Martey et al., 2012). It is often assumed that farmers with better education standards have better managerial skills and are able to search for appropriate technologies (Siri et al., 2011) and would find it easy to invest in post-harvesting technologies to avoid losses (Garikai, 2014). Farmers with post primary education can appreciate and use post-harvest technologies (Babalola et al., 2010). This implies that farmers in the present study are open to new ideas and may not necessarily stick to old methods of agriculture. With introduction of a new technology, adoption rate is expected to be high amongst educated farmers.

Land ownership and land size are factors often considered important in technology adoption. Farmers with larger pieces of land are more likely to adopt an improved technology compared with farmers owning small pieces of farms. Of the sampled respondents, majority were small scale farmers owning small pieces of land. Land ownership, size and quality positively influence adoption of technology (Abera, 2009). Majority of the farmers were not members of any farmer group. Farmer group membership helps in accessing information important in production, post-harvest practices and marketing (Garikai, 2014). Ortmann and King (2007) stated the reasons for the formation of farming cooperatives and farmer group organisations including promotion of self-help, improving negotiating strength with input suppliers and buyers of farm products, assurance of input supplies and/or product markets mainly for perishable crops like fruits and vegetables, and reduction of opportunistic behaviour brokers. The lack of participation in these farmer groups meant lack of access to information on production, post-harvest handling of French bean and marketing and this may have negative effect on the overall yield achieved by the farmers.

Farmers (80%) interviewed harvested French beans from 9.00 am to 12 noon while others also harvested in the afternoon. The time a farmer decides to harvest may impact positively or negatively on losses associated with post-harvest handling. Harvesting in the morning when the temperatures are not high results in low post harvest losses. Morning hours are characterized by high humidity and low temperatures with the produce still turgid while in the afternoon the weather is characterized by high temperatures and high evaporation resulting in less turgid and shrivelled produce (Kereth et al., 2013). Therefore, biological reactions such as respiration are expected to increase in the afternoon (Mashau et al., 2012). Field heat is usually high and detrimental at harvesting and should be removed quickly as possible (Arah et al., 2016).

When handling perishable produce like French beans, high standards of hygiene are paramount. Unfortunately, cleaning or disinfecting was not a common practice in the two counties as majority of the interviewed farmers admitted not to cleaning or disinfecting their produce. This can be attributed to very stringent rules on post-harvest wash as outlined in the Food Safety Standards that most exporters subscribe to, mainly Global GAP that has strict control on the quality of water that can be used to wash fresh produce, such water must be potable/drinkable quality, a requirement that may not be achieved by most of the smallholder farmers or sheer ignorance of the practice by the farmers (Arah, 2016). Few of them use water guard to wash their produce. Kader (2005) reiterated that good hygiene while handling the produce minimises contamination hence reduction in post-harvest losses.

Precooling minimizes the effect of microbial activity and immediate cooling is very important. Low temperature for French bean handling can be attained either in the early hours of the morning or late in the evening (Kader, 1984). Results show that many farmers stored their produce for around 1 to 6 h. French bean in its harvested form contains high percentage of water and therefore carries out physiological respiration (Idah et al., 2007). As the produce respires and loses water, their quality depreciates as they shrivel. This leads to deterioration during transit and storage, hence the need for minimal storage times. It has been reported that the longer the produce is stored the higher the change in texture, aroma and even spoilage (Yahia, 2006). Post-harvest handling and processing of fresh produce may impact on the biochemical changes and microbial contamination (Rico et al., 2007). To avoid contamination and degradation of the fresh produce, Kader (2005) suggests reducing potential contamination of the produce during growth, harvesting and up to storage. Conformity to sanitation standards is necessary to limit losses.

Considering 80% of farmers have cold storage rooms, storage period of up to 6 h was reported. Storage period is an important aspect when handling French beans, majority of the interviewed farmers' stored French beans for between 2 and 3 h. It has been reported that longer storage periods result in quality deterioration. Fresh produce like French beans continues to break down and utilize their nutrients from harvesting through packing, distribution, marketing and even sale. This results in rapid loss of the produce when the temperatures are high (Gikaria, 2013). Carbohydrates, proteins and other nutrients are broken down into simpler compounds often resulting in reduced quality or quantity of the foods (Kitinoja, 2013). Minimizing the storage time at the farm will help in reducing postharvest losses as suggested by Kader (2005) who argued that as the time from the time of purchase, the produce in the market increases, and its deterioration increases.

The present study revealed that many of the farmers used a cooling storage facility before transporting to the respective customers; this ensured reduced post-harvest losses associated with high temperatures. Kader (2005) reported that overheating during storage and transportation results in increased water loss. Provision of optimum temperature and relative humidity is the most important tool for maintaining quality of vegetables (Kader, 2003). 73% of the farmers in the present study used mainly cement for floor construction, charcoal for wall and iron sheets for roofing. As suggested by the farmers, increasing the size of the cooler is important. Enough floor space provides ready access to all the produce stored in the room. It is recommended that the floor should be equipped with a suitable inside drain to dispose of waste water from cleaning and condensation. A charcoal cooler employs the principal of evaporative cooling to maintain cool interior temperature for produce preservation. The facility is constructed from an open timer frame with charcoal filled wall which should be continuously moist. Many of the farmers with cold storage facility did not install humidifiers. As the warm dry air passes through the moist charcoal, water is evaporated into the air resulting into the cooling. This facility has benefits of increasing the air moisture content, preventing produce from drying out, then extending the shelf life. Evaporative cooling is based on the principle that water requires heat energy to evaporate. In hot environment, the evaporation of water in hot dry air creates a cooling effect.

In most cases, small scale farmers depend on middlemen or brokers for selling their produce. In this study, 95.6% farmers preferred to sell their produce to well-established exporters. According to Lange et al. (2016), large number of middlemen involved in value chains can significantly affect the viability of introducing and maintaining cold chains.

This study is very important, considering that majority of the small holder farmers rely on the use of charcoal coolers as a means of cooling their produce, and majority of them are convinced that the charcoal coolers are effective in terms of cool chain improvement, the findings from this study provide an excellent opportunity for policy makers to include provision of cold storage facilities in the local areas, especially targeting export small scale farmers.

For the exporters who have contracted the small scale farmers to grow French beans for export, findings on time of harvesting is very important, considering that majority of the farmers harvest between 9 am and 12 noon, at this time normally the sun is always up and temperatures high, it is indeed a good opportunity for the exporters to review their operating procedures to ensure that the farmers harvest early in the morning between 6 and 9 am before temperatures rise.

With regards to the efficacy of the existing charcoal coolers, majority of the farmers were not bothered to measure the produce temperature, as such the effectiveness of the charcoal coolers was not a priority. There is definitely a gap in terms of working to improve the effectiveness of the charcoal coolers, which can only be validated by measuring the produce temperature.

From the results, majority of the small scale farmers did not belong to any farmers group, a factor that would make it impossible to benefit from economies of scale with regards to National Government Infrastructural Development. In addition, this provides an opportunity for the Kenyan Government to implement the Food Crops Act, which requires that farmers get organized into groups and sell their produce through an organized group through a collective consolidation centre.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Abera G (2009). Commercialization of smallholder farming: Determinants and welfare outcomes. A cross-sectional study in Enderta District, Tigrai, Ethiopia (M.Sc. Thesis). An MSc Thesis Presented to the University of Agder, Kristiansand, Norway.

Adamu AA (2005). Efficiency of resources use in irrigated wheat production among farmers in Hadeja Jamaare River Basin, Nigeria (Unpublished M.Sc thesis), Department of Agricultural Economics and Extension, ATBU Bauchi.

Arah KI, Ahorbo GK, Anku EK, Kumah EK, Amaglo H (2016). Post-harvest handling practice in developing countries: A mini review. Adv. Agric. 8p.

Babalola DA, Makinde YO, Omonona BT, Oyekanmi MO (2010). Determinants of post- harvest losses in tomato production: A case study of Imeko – Afon Local Government Area of Ogun State. J. Life Phys. Sci. 3(2):14-18.

Garikai M (2014). Assessment of vegetable postharvest losses among smallholder farmers in Umbumbulu area of Kwazulu-Natal province, South Africa. (MSC Thesis). University of KwaZulu-Natal, South Africa.

Hejase AJ, Hejase HJ (2013). Research Methods A Practical Approach for Business Students. (2nd edn.), Philadelphia, PA, USA: Masadir Inc.

Idah PA, Ajisegir ESA, Yisa MG (2007). Fruits and vegetables handling and transportation in Nigeria. Aust. Technol. 10(3):175-183.

Kader AA (1984). Effects of postharvest handling procedures on tomato quality. In Symposium on Tomato Production on Arid Land 190(21):209-222.

Kader AA (2003). Perspective on postharvest horticulture. Hortic. Sci. 38:1004-1008.

Kader AA (2005). Increasing food availability by reducing postharvest losses of fresh produce. Acta Hortic. 682:2169-2175.

Kathryn CT, James WR (2004). Harvesting and handling peaches. Accessed online at http://www.pre-coolers.net/documents/HarvestingandHandlingPeaches.pdf

Kereth GA, Lyimo M, Mbwana HA, Mongi RJ, Ruhembe CC (2013). Assessment of post-harvest handling practices: knowledge and losses of fruits in Bagamoyo district of Tanzania. J. Food Sci. Qual. Manage. 11:8-16.

Kitinoja L (2013). Use of cold chains for reducing food losses in developing countries. PEF White Paper No. 13-03.

Lange B, Priesemann C, Geiss M, Lambrecht A (2016). Promoting food security and safety via cold chains: Technology options, cooling needs and energy requirements. Internationale Zusammenarbeit (GIZ) GmbH. https://www.giz.de/expertise/downloads/giz2017-en-food-security-cold-chains.pdf Accessed on 1 December 2017

Martey E, Al-Hassan RM, Kuwornu JKM (2012). Commercialization of smallholder agriculture in Ghana: A Tobit regression analysis. Afr. J. Agric. Res. 7(14):2131-2141.

Mashau ME, Moyane JN, Jideani IA (2012). Assessment of postharvest losses of fruits at Tshakhuma fruit market in Limpopo Province, South Africa. Afr. J. Agric. Res. 7(29): 4145-4150.

Olayemi FF, Adegbola JA, Bamishaiye EI, Daura AM (2011). Assessment of post-harvest challenges of small scale farm holders of tomotoes, bell and hot pepper in some local government areas of Kano State, Nigeria. Bayero J. Pure Appl. Sci. 3:39-42.

Ortmann GF, King RP (2007). Agricultural cooperatives II: Can they facilitate access of small-scale farmers in South Africa to input and product markets? Agrekon 46(2):219-244.

Rico D, Martín-diana AB, Barat JM, Barry-Ryan C (2007). Extending and measuring the quality of fresh-cut fruit and vegetables: A review. J. Food Sci. Technol. 18:373-386.

Toivonen PMA (2014). Relationship of typical core temperature after hydrocooling on retention of different quality components in sweet cherry. Hortic. Technol. 24:457-462.

Usman SI, Abdulahi A, Qasimu AI, Adamu T (2016). Farmers' perception on organic manure usage among arable crop farmers in Jalingo Local Government Area of Taraba State, Nigeria. Econ. Eng. Agric. Rural Dev. 16:3.

Vorster LL, Toerien JC, Bezuidenhout JJ (1990). Temperature management of avocados an integrated approach. South Afr. Avocado Growers' Assoc. Yearbook 13:43-46.

Yahia EM (2006). Controlled atmospheres for tropical fruits. In: Yahia, E.M. (ed). The current status and future application of modified and controlled atmospheres for Horticultural Commodities. Stewart Postharv. Rev. 5(6):1-10.

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Full Length Research Paper

Coffee straw used as mulch for germination and strength of crops and spontaneous species seedlings

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The objective of this study was to evaluate the allelopathic effect of using conilon and arabica coffee straw as mulch on the emergence and development of three crops (*Lactuca sativa, Cucumis sativu, Solanum lycopersicum*) and three natural plants (*Eupatorium maximilianii*, *Amaranthus hybridus* and *Bidens pilosa*). The experiment was carried out in a greenhouse in a completely randomized design, with six repetitions. The treatments were arranged in a 3×6 factorial scheme, with three treatments of soil cover: Control (uncovered), conilon coffee straw and arabica coffee straw and six plant species. Conilon coffee straw harmed the emergence of *C. sativus*, *L. sativa* and *E. maximilianii*; it reduced the emergence speed of the studied species, except for *S. lycopersicum* and *A. hybridus*; it reduced both fresh and dried *C. sativus* masses. Arabica coffee straw only compromised the emergence of *E. maximilianii*; it reduced the emergence speed of *L. sativa*, *E. maximilianii*, and *A. hybridus*; and it increased both fresh and dry masses of *C. sativus* seedlings. It is possible to conclude that conilon coffee straw can be used as mulch in *S. lycopersicum* crops, in the management of the weed *E. maximilianii*, and also to delay the emergence of *B. pilosa*. Arabica coffee can be used in *C. sativus*, *S. lycopersicum* and *L. sativa* crops in the control of *E. maximilianii* and to slow down the emergence of *A. hybridus*.

Key words: *Cucumis sativus*, *Lactuca sativa*, *Solanum lycopersicum* L., *Eupatorium maximilianii* Schrad, *Amaranthus hybridus* L, *Bidens pilosa* L.

INTRODUCTION

Brazil is the largest producer and exporter and the second largest consumer of coffee in the world, with a production of about 55 million benefited bags in 2016 (ICO, 2017). During the fruit processing, a large volume of residues is produced, especially coffee straw (outer skin + parchment), which is often incorrectly discarded, resulting in damage to the environment (Magalhães et al., 2008).

In agriculture, the importance of the application of straw mulch has been tested as useful tool to improve soil quality and to reduce soil erosion. Especially, coffee straw, that is widely used as a composition of organic substrates for seedling production (Godoy et al., 2008; Coelho et al., 2016; Cerdà et al., 2017). It can also be used as mulch, both for crop nutrition, since this residue is an excellent organic matter, potassium and nitrogen source (Costa et al., 2007), as well as for managing natural plants, because of its negative effect on some natural plants due to its allelopathic potential.

Allelopathy is a common phenomenon in plant communities, in which certain plants or plant residues release allelochemicals substances that interfere with the development of nearby plants (Zhang et al., 2011). The allelochemicals can act in pre and post-emergence stages and in the soil seedbank (Han et al., 2008), which is an important factor in the control of certain undesired species, thus obtaining more productive crop systems (Goldfarb et al., 2009).

Caffeine and phenols found in coffee are secondary metabolites that act as allelochemicals in several plant species (Lima et al., 2007). It is known that conilon coffee has higher caffeine and polyphenols content than arabica coffee does (Ribeiro et al., 2014). This fact indicates that the straw of each one of these species of coffee may present different allelopathic responses that might interfere positively or negatively in the plants present therein, including the main crop (Rizzardi et al., 2008; Tesio et al., 2011; Campiglia et al., 2012).

In spite of presenting a great allelopathic potential over invasive plants and of being a source of nutrition for several crops, there are still few studies in literature that correlate the use of coffee straw as mulch in cultivation and weeds management. May et al. (2011) reported that coffee skin can also be tested for the creation of natural herbicides less aggressive to the environment, and show the great importance of studies that seek new options for the usage of coffee straw.

In this research the crop species tested are among the most cultivated in Brazil. Besides being highly sensitive to allelopathic substances, they are widely used as bioindicator plants for bioassays. The natural plants tested are the most present in cultivated areas, and they compete for the production factors with the cultivated species, causing reduction of productivity and product quality (Lorenzi, 2008).

The objective of this study was to evaluate the effect of mulches (conilon and arabica coffee straws) on the germination and development of three crops (*Lactuca sativa*, *Cucumis sativus* and *Solanum lycopersicum*) and three weeds (*Eupatorium maximilianii*, *Amaranthus hybridus* and *Bidens pilosa*).

MATERIALS AND METHODS

The experiment was carried out at the weed and medicinal plants sector of the Center of Agricultural Sciences and Technology of the Universidade Estadual do Norte Fluminense Darcy Ribeiro, located in the city of Campos de Goytacazes-RJ, at latitude of 21°19'23" S and longitude of 41°19'41" W. According to the classification of Köeppen, the climate is tropical, hot and humid with rainy summers, dry winters, and average altitude of 11 m.

A completely randomized design of six repetitions was used. The treatments were arranged in a 3×6 factorial scheme, with three treatments of soil cover: Control (uncovered), conilon coffee straw (*Coffea canephora*) and arabica coffee straw (*Coffea arabica*); and six plant species (*Lactuca sativa*, *Cucumis sativus*, *Solanum lycopersicum*, *Eupatorium maximilianii*, *Amaranthus hybridus* and *Bidens pilosa*).

Conilon coffee straw was obtained from the production and processing of grains of plants from the variety G35 cultivated in the municipality of Muqui, Espírito Santo, at latitude: 20º57'06" S, and longitude: 41º20'45" W and altitude, 250 m. The arabica coffee straw was obtained from the production, pulping and processing of grains of *Catuaí Vermelho* IAC 81 cultivar, cultivated in the city of Venda Nova do Imigrante, Espírito Santo, Brazil at Latitude: 20º20'23" S, longitude: 41º08'05" W and altitude 63 m.

The seeds of *L. sativa*, *C. sativus* and *S. lycopersicum* were acquired in the local commerce. They present fast germination and high sensitivity to allelopathy. The seeds of *E. maximilianii*, *A. hybridus* and *B. pilosa* were purchased from *Cosmos Agrícola Produção e Serviços Rurais Ltda* (Agrocosmos), a company specialized in the control of prepared seeds, located in the city of Engenheiro Coelho, São Paulo.

The seeds were sown in polyethylene trays (25 cm × 40 cm) in an 18 cm long line and 5 cm between the rows. Different number of seeds was used for each specie, in order to allow adequate densities of germination and plant growth. 18 seeds of *L. sativa*, 8 of *C. sativus*, 12 of *S. lycopersicum* and *black pickle* and 20 of *E. maximilianii* and *A. hybridus* to the depth of 1 cm was used.

The substrate used was 150 mL of sand that had received irrigation for five days before planting, in order to leach possible compounds present in the material. Subsequently, the substrate was covered, between rows, with 150 mL of dry coffee skin per 90 cm^2 of area, except the control, which remained uncovered. The treatments were irrigated daily to maintain the moisture content of the soil close to the field capacity.

The allelopathic activity was evaluated by the following tests:

1. Percentage of emergence (PE): Determined by the count of the number of seedlings emerged in the last emergence assessment, according to the species, on the seventh day after installation of the test for *L. sativa*; on the eighth day for *C. sativus*; on the fourteenth day for *S. lycopersicum*, *A. hybridus* and *B. pilosa*; and on the twenty-fifth day for *E. maximilianii*. The results were expressed as percentage of emerged seeds (Brazil, 2009).
2. Emergence speed index (ESI): The count of emerged seedlings was performed daily, from first seedling emergence to the last one according to each species, and subsequent conversion to the index proposed by Maguire (1962). Fresh matter (FM) and dry matter (DM) of the seedlings: At the end of the total emergence test, the

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Table 1. Variance analysis of the percentage of emergence (PE), of the emergence speed index (ESI), fresh matter weight (FMW) and dry matter weight (DMW) of cultivated and invasive species under the effect of the tested mulches.

VF	DF	PE (%)		ESI		FMW (mg)		DMW (mg)	
		MS	F	MS	F	MS	F	MS	F
Mulch (M)	2	0.560	16.304*	57.458	30.291*	0.157	32.535*	0.00055	26.305*
Species (S)	5	1.234	35.99*	89.987	47.441*	1.024	211.955*	0.00460	220.11*
Interaction	10	0.131	3.815*	15.694	8.274*	0.797	16.472*	0.00020	9.656*
Residue	90	0.034		1.897		0.484		0.00002	

*Significant effect according to F test (P<0.05).

Table 2. Percentage of emergence (PE) and emergence speed index (ESI) of seedlings of cultivated and invasive species, under the effect of mulches of conilon and arabica coffee straws on sand substrate in a greenhouse, in Campos dos Goytacazes, RJ.

Specie	Control	Conilon coffee straw	Arabica coffee straw
Percentage of emergence (%)			
Lactuca sativa	88.89 ± 7.85^{Aab}	28.70 ± 26.62^{Bbc}	87.03 ± 8.36^{Aa}
Cucumis sativus	93.75 ± 10.45^{Aa}	79.17 ± 18.81^{Ba}	95.83 ± 6.45^{Aa}
Solanum lycopersicum	27.78 ± 15.51^{Ad}	25.00 ± 27.88^{Abc}	36.11 ± 18.00^{Ab}
Eupatorium maximilianii	62.50 ± 26.78^{Abc}	9.17 ± 9.16^{Bc}	33.33 ± 14.02^{Bb}
Amaranthus hybridus	24.17 ± 58.45^{Ad}	17.50 ± 11.72^{Abc}	18.33 ± 23.59^{Ab}
Bidens pilosa	51.39 ± 32.23^{Acd}	45.83 ± 18.81^{Ab}	44.44 ± 19.48^{Ab}
Emergence speed index (ESI)			
Lactuca sativa	10.24 ± 3.720^{Aa}	1.63 ± 1.683^{Cab}	6.66 ± 0.614^{Ba}
Cucumis sativus	4.75 ± 1.246^{ABb}	3.29 ± 1.194^{Ba}	5.50 ± 0.948^{Aa}
Solanum lycopersicum	0.64 ± 0.384^{Ad}	0.51 ± 0.563^{Ab}	0.76 ± 0.440^{Ab}
Eupatorium maximilianii	2.44 ± 1.249^{Abcd}	0.45 ± 0.547^{Bb}	1.03 ± 0.491^{ABb}
Amaranthus hybridus	1.30 ± 0.468^{Acd}	0.79 ± 0.663^{Ab}	0.65 ± 0.723^{Ab}
Bidens pilosa	3.54 ± 2.751^{Abc}	1.14 ± 0.669^{Bab}	1.91 ± 1.115^{ABb}

Means (± Standard Deviation) followed by the same capital letters in the line (LSD=0.255) and lowercase letters in the column (LSD=0.3119) do not differ according to the Tukey test (P≤0.05, n=6, N=108).

seedlings were removed from the trays, washed in running water, taken to the laboratory, where they were weighed to establish the amount of fresh matter. Then the seedlings were placed in a forced air convection oven at 40°C for 72 h. The weighing were realized on a precision scale, with the results expressed in milligrams per seedling (mg plant^{-1}).

The data were submitted to Liliefors test for normality of variance and Cochran and Barttlet test for homogeneity. In these tests, the need to transform the PE and ESI variables by the arcsine of the square root (x / 100) and the FM and DM by the square root (x + 1) was identified. The variance analysis and mean separation were done by the Tukey test at 5% probability level. The software SAEG 9.0 was always used.

RESULTS AND DISCUSSION

The variance analysis indicated a significant interaction effect (P<0.05) between the type of mulch and the species studied for all variables analyzed, as shown in Table 1.

Among the evaluated species, *L. sativa* and *C. sativus* were the ones that presented the highest percentage of emergence both in the control (without mulch) and in the covered with arabica coffee straw treatments. However, the straw from conilon coffee reduced the emergence of *L. sativa* to 28.70% (a reduction of 60.19% in relation to the control), as shown in Table 2.

It was verified that conilon coffee straw affected the ESI of the studied species, except for *S. lycopersicum* and *A. hybridus*. However, this mulch increased the ESI of *C. sativus* and *B. pilosa*. Arabica coffee straw reduced *L. sativa* ESI (Table 2), but there was no effect on the ESI of the other studied species.

In relation to spontaneous species, *E. maximilianii* was the only one that had the emergence of the seedlings influenced by the mulches, in which the conilon coffee straw was reduced by 85.33% and the arabica coffee straw was reduced by 46.67%. This fact indicates that *E. maximilianii* is highly sensitive to this type of soil cover, and that the use of coffee straw (especially that of conilon coffee) may be a viable alternative in the control of this invader plant.

Table 3. Fresh and dry mass of the cultivated species and natural plants, under the effect of mulches of conilon and arabica coffee straws on sand substrate in a greenhouse, Campos dos Goytacazes, RJ, 2013.

Specie	Control	Conilon coffee straw	Arabica coffee straw
Fresh mass of the seedlings (mg)			
Lactuca sativa	0.048±0.014^{Ab}	0.018±0.020^{Ab}	0.063±0.012^{Abc}
Cucumis sativus	0.669±0.213^{Ba}	0.310±0.157^{Ca}	0.899±0.102^{Aa}
Solanum lycopersicum	0.109±0.016^{Ab}	0.029±0.028^{Bb}	0.144±0.037^{Ab}
Eupatorium maximilianii	0.026±0.012^{Ab}	0.031±.039^{Ab}	0.029±0.006^{Abc}
Amaranthus hybridus	0.015±0.004^{Ab}	0.009±0.088^{Ab}	0.012±0.008^{Abc}
Bidens pilosa	0.061±0.021^{Ab}	0.027±0.010^{Ab}	0.059±0.022^{Abc}
Dry mass of the seedlings (mg)			
Lactuca sativa	0.0021±0.0004^{Ab}	0.0014±0.0012^{Ab}	0.0058±0.0077^{Ab}
Cucumis sativus	0.0432±0.0109^{Ba}	0.0260±0.0113^{Ca}	0.0569±0.0061^{Aa}
Solanum lycopersicum	0.0054±0.0007^{Ab}	0.0016±0.0014^{Ab}	0.0081±0.0021^{Ab}
Eupatorium maximilianii	0.0023±0.0014^{Ab}	0.0025±0.0030^{Ab}	0.0038±0.0010^{Ab}
Amaranthus hybridus	0.0006±0.0003^{Ab}	0.0005±0.0005^{Ab}	0.0010±0.0010^{Ab}
Bidens pilosa	0.0042±0.0017^{Ab}	0.0015±0.0005^{Ab}	0.0046±0.0019^{Ab}

Means (± Standard Deviation) followed by the same capital letters in the line (LSD=0.0958) and lowercase letters in the column (LSD=0.1170) do not differ according to the Tukey test (P≤0.05, n=6, N=108).

Arabica coffee straw can be used as mulch for the cultivation of *C. sativus* and *S. lycopersicum* without causing damage to the emergence of the seedlings. However, conilon coffee straw harmed the emergence of *C. sativus* (14.58% reduction) and *L. sativa* (60.19% reduction), indicating that it should not be used in the cultivation of these crops.

The ESI for the *C. sativus* was higher in the presence of arabica coffee mulch (5.50) in comparison to the conilon coffee (3.29). This result indicates that conilon coffee straw interferes negatively with *C. sativus*, and reveals that arabic coffee straw can benefit the culture of this crop, since it does not harm its emergence and increases its ESI.

Although the Arabic coffee straw positively influenced the percentage of emergence of *L. sativa* seedlings, there was a negative effect on ESI, indicating that this coffee straw may present a negative allelopathic effect on this specie. Moreira et al. (2016) observed that *L. sativa* plants have their growth considerably inhibited when cultivated in soil obtained close to the coffee root, due to the release of allelochemicals, which corroborates the data of this study.

E. maximilianii and *B. pilosa* have shown an ESI reduction when conilon coffee straw was used as mulch. These results suggest that this cover can be used to reduce the emergence speed of these species.

In addition to the reduction of the emergence and ESI caused by conilon coffee straw in *C. sativus* seedlings, radicular anomalies were observed, such as size reduction, thickening and necrosis, evident symptoms of the allelopathic effect of the mulch (Ferreira and Áquila, 2000). It is known that the conilon coffee has a higher content of caffeine, soluble solids and polyphenols than the arabica coffee does (Ribeiro et al., 2014). This fact may explain the great negative influence of conilon straw on the emergence and ESI for most species studied, since caffeine and phenols are the main secondary metabolites of coffee that act as allelochemicals (Lima et al., 2007) .

However, similar symptoms of the negative allelopathic effect were also observed by May et al. (2011) when studying the effect of arabica coffee straw extract on the development of *C. sativus*. It is believed that this effect occurred due to the mode of use, in which the extract may have presented higher concentration of allelochemicals than the straw used as mulch, which usually presents a slower release of its allelochemicals.

Regarding the fresh mass, an increase in the fresh matter of the *C. sativus* seedlings was observed when arabica coffee straw was used as mulch, in comparison to the control. However, the conilon coffee straw reduced the fresh mass of the *C. sativus* and of the *S. lycopersicum* and there was no effect on the *S. lycopersicum*, in relation to the control (Table 3).

It can be observed that the seedlings of *S. lycopersicum* and *C. sativus* suffered a negative influence of the conilon coffee straw mulch, with 53.66 and 73.39% fresh mass reduction, respectively. The arabica coffee straw influenced only the *C. sativus*, increasing its fresh mass by 34.37% in relation to the control.

It was found that the conilon coffee straw provided a reduction of 39.81% in the dry mass of *C. sativus* seedlings, whereas the arabica coffee straw provided a 31.71% increase in the dry mass of its seedlings in relation to the control. The other evaluated species behaved indifferently in relation to this variable, which indicates that, although the mulches affect the germination and emergence of these species, they do not influence the development of the seedlings.

Caffeic acid (3,4-dihydroxycinnamic acid) present in coffee straw can alter root activity by inhibiting the absorption of phosphate and potassium by depolarization of root membranes (Barkosky et al., 2000). This fact may explain the lower amount of fresh and dry mass of the *S. lycopersicum* and *C. sativus* seedlings, when conilon coffee straw was used as mulch.

Barkosky et al. (2000), when evaluating the allelopathic effects of caffeic acid in *Euphorbia escola*, verified that the exposure of the plant to caffeic acid for 30 days reduced the leaf area, the leaf mass, root mass, root length, above-ground length and total plant weight, when compared to the control. This result corroborates with those found in this work, since conilon coffee straw probably has a higher concentration of caffeic acid than arabica coffee straw does, thus reducing the dry mass of the *C. sativus* seedlings.

Conclusion

Conilon coffee straw, as mulch, can be used in *S. lycopersicum* crops cultivation, in the control of the weed *E. maximilianii*, and also to delay the emergence of *B. pilosa*. Arabica coffee straw can be used as mulch in *C. sativus* and *S. lycopersicum* crops cultivation, in the control of the weed *E. maximilianii* and in the reduction of the emergence speed of *A. hybridus*.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Barkosky RR, Einhellig FA, Butler JL (2000). Caffeic acid-induced changes in plant–water relationships and photosynthesis in leaf y spurge *Euphorbiae sula*. J. Chem. Ecol. 26(9):2095-2110.

Brazil (2009). Ministério da Agricultura, Pecuária e Abastecimento. Regras para análise de sementes. Brasilia, Secretaria Nacional de Defesa Ambiental 399p.

Campiglia E, Radicetti E, Mancinelli R (2012). Weed negative control strategies and yield response in a pepper crop (*Capsicum annuum* L.) mulched with hairy vetch (*Vicia villosa* Roth.) and oat (*Avena sativa* L.) residues. Crop Prot. 33(1):65-73.

Cerdà A, Rodrigo-Comino J, Giménez Morera A, Keesstra SD (2017). An economic, perception and biophysical approach to the use of oat straw as mulch in Mediterranean rainfed agriculture land. Ecol. Eng. 108:162-171.

Coelho GF, GonÇalves Jr AC, Nóvoa-Muñoz JC, Fernández-Calviño D, Arias-Estévez M, Fernández-Sanjurjo MJ, Álvarez-Rodríguez E, Núñez-Delgado A (2016). Competitive and non-competitive cadmium, copper and lead sorption/desorption on wheat straw affecting sustainability in vineyards. J. Clean. Prod. 139:1496-1503.

Costa RSC, Leônidas FC, Rodrigues VGS, Santos JCF (2007). In: EMBRAPA RONDÔNIA: Consórcio Brasileiro de Pesquisa e Desenvolvimento do café. Available at: https://www.infoteca.cnptia.embrapa.br/infoteca/bitstream/doc/1027715/1/Doc159cafe.pdf

Ferreira AG, Aquila ME (2000). Alelopatia: uma área emergente da ecofisiologia. Rev. Bras. de Fisiologia Veg. 12(1):175-204.

Godoy WI, Farinacio D, Funguetto RF, Borsatti F, Scarioti A, Soligo E, Sbardelotto G (2008). Production of broccolis seedlings in different alternatives substrates. Hortic. Bras. 26(2):3878-3882.

Goldfarb M, Pimentel LW, Pimentel NW (2009). Alelopatia: relações nos agroecossistemas. Tecnologia e Ciências Agropecuárias, João Pessoa 3(1):23-28.

Han CM, Pan KW, Wu N, Wang JC, Li W (2008). Allelopathic effect of ginger on seed germination and seedling growth of soybean and chive. Sci. Hortic. 116(1):330-336.

International Coffee Organization (ICO) (2017). Trade Statistics Tables. London, United Kingdom - 2017. Available at: http://www.ico.org/trade_statistics.asp?section=Statistics.

Lima JD, Moraes WS, Mendonça JC, Nomura ES (2007). Resíduos da agroindústria de chá preto como substrato para produção de mudas de hortaliças. Ciênc. Rural 37(1):1609-1613.

Lorenzi H (2008). Plantas Daninhas do Brasil: terrestres, aquáticas, parasitas e tóxicas. In: Lorenzi, H. editor geral Harri Lorenzi. 4ª ed. Nova Odessa. Instituto Platarum. pp. 21-32

Magalhães EA, Silva JS, Silva JN, Filho DO, Dorneles SML, Martin S, Dutra L (2008). Casca de café associada à lenha como combustível para aquecimento indireto do ar de secagem. Rev. Bras. de Armazenagem 10(1):66-72.

May D, Rocha L, Oliveira CMR, Maranho LT (2011). Efeito de extratos de casca de café (*Coffea arabica* L.) na germinação e crescimento de *Cucumis sativus* (*Cucumis sativus* L.). Rev. Bras. de Biociências 9:180- 186.

Moreira MHO, Almeida GRR, da Cunha LT, Queiroz RL (2016). Interações alelopáticas sobre o desenvolvimento de *Lactuca sativa* (*Lactuca sativa*, L. cv. Vanda) cultivada em solo cafeeiro. Revista da UIIPS 4(4):16.

Ribeiro BB, Mendonça LMVL, Assis GA, Mendonça JMA, Malta MR, Montanari FF (2014). Avaliação química e sensorial de blends de Coffea canephora Pierre e Coffea arabica L. Coffee Sci. 9(2):178-186.

Rizzardi MA, Neves R, Johann LB (2008). Potencial alelopático da cultura da canola (*Brassica napus L.* var. *oleifera*) na supressão de *Bidens pilosa* (*Bidens sp.*) e soja. Rev. Bras. de Agrociência, Pelotas 14(2):239-248.

Tesio F, Wenston LA, Ferrero A (2011). Allelo chemicals identified from Jerusalem artichoke (*Helianthus tuberosus* L.) residues and their potential inhibitory activity in the field and laboratory. Sci. Hortic. 129:361-368.

Zhang S, Zhu W, Wang B, Tang J, Chen X (2011). Secondary metabolites from the invasive *Solidago canadensis* L. accumulation in soil and contribution to inhibition of soil pathogen *Pythium ultimum*. Appl. Soil Ecol. 48:280-286.

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Full Length Research Paper

Effects of sewage sludge and length on initial growth of guava seedlings

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Substrate and light intensity exert influence on the quality of seedlings produced by increasing plant nutritional efficiency and growth. The objective of this work was to study the emergence and early growth of guava seedlings (*Psidium guajava* L.) in substrate enriched with sewage sludge and under different light intensities. In the conduction of this work, guava seeds of the cultivar Pedro Sato were used and five substrates formed by dystrophic Red Yellow Latosol, washed sand, and sewage sludge: S1-soil + sand (Control); S2 - soil + sand + silt (40 t ha^{-1}); S3 - soil + sand + silt (80 t ha^{-1}); S4 - soil + sand + sludge (120 t ha^{-1}); S5 - soil + sand + sludge (240 t ha^{-1}); and S6 - soil + sand + sludge (480 t ha^{-1}). Each experimental plot consisted of four repetitions of 25 seeds for the analyzed emergence (%) and emergence speed index. For fresh and aerial part dry mass (mg L^{-1}), root dry mass (mg L^{-1}), root volume (cm^3 plant^{-1}), number of leaves, levels of chlorophyll *a*, *b* and total chlorophyll each experimental plot consisted of four repetitions of 16 seeds. The production of guava seedlings with sewage sludge should be conducted with the dosages of 240 and 480 t ha^{-1}. Shading with two screens is recommended for the production of guava seedlings.

Key words: Biosolids, emergency, luminous intensity, *Psidium guajava* L., residues.

INTRODUCTION

Guava (*Psidium guajava* L.) is a shrub native to South America that produces fruit with great acceptance in the market for consumption *in natura* and production of various industrialized product lines (Osório et al., 2011). In recent years, the state of Espirito Santo has excelled in fruit, mainly at the family farming level, reaching a production of 7,656 tons, distributed in 306 ha of harvested area, according to the Brazilian Institute of Geography and Statistics (Ibge, 2015).

The use of biosolids as organic fertilizer in agriculture is recognized as one of the most promising alternatives for final disposal of this waste (Pinto et al., 2014). Environmental practices with use sewage sludge, as nutrient source for plants, have been studied in the latest

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years in order to reduce the impact of their deposition and reduce production costs (Freitas et al., 2015). Research shows that sewage sludge has a high potential for agricultural use in some plant species because of the chemical composition and high organic concentration. For the implementation of a productive orchard, the formation of good quality seedlings is necessary, which highlights the substrate, which should promote nutritional efficiency, be of low cost and provide suitable environmental conditions for the early growth of plants. Thus, the use of this residue in agriculture and even in seedling production can contribute to reducing environmental contamination by potentially toxic elements, reducing the impact to the environment (Kelessidis and Stasinakis, 2012; Trigueiro and Guerrini, 2014).

The seed germination depends on the your origin, highlighting soil nutrient availability, since it directly influences embryo formation, the genetics and chemical composition of the species as it was verified in several studies on nutritional management of the "mother-plant", mainly in the beginning of the reproductive stage when nutrients are translocated to the seeds, and the seed storage after its physiological maturation, which may compromise the physiological quality, which are factors considered as mandatory steps in a seed production program (Carvalho and Nakagawa, 2012; Silva et al., 2015).

Despite the benefits of the application of sewage sludge in the physical and chemical characteristics of the soil and on plant growth, the accumulation of high concentrations of sludge elements in the soil is potentially toxic to plants (Singh and Agrawal, 2008; Nascimento et al., 2014). When in contacts with plants, heavy metals may limit their growth and development by inhibiting physiological processes such as transpiration, respiration and photosynthesis (Nagajyoti et al., 2010; Taiz et al., 2015), making necessary the knowledge of the chemical characteristics of the sludge and the observation of the effect of different concentrations on soil and plants. Thus, sewage sludge must meet the necessary requirements for the concentration of heavy metals and pathogens, according to the legal norms which determine the limit of metal concentrations, the maximum cumulative loads permitted for use in agricultural soils, presence of pathogens and the species for which its use is recommended (Brasil, 2006). Salvador et al. (2013), working with the waste use substrate to yield guava seedlings, observed that the treatment containing sewage sludge presented a negative effect on the growth of the plants.

In addition to plant nutrition, light intensity exerts influence on plant growth, acting as a direct source of energy for vegetative growth (Taiz and Zeiger, 2013). According to Baliza et al. (2012), the light intensity promotes the formation of micro-climates, alters temperature and influences the soil water availability to the plants.

The objective of this study was to analyze the emergence and early growth of guava plants (*P. guajava* L.) in substrate enriched with sewage sludge and under different light intensities.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse and Seed Analysis Laboratory of the Center for Agricultural Sciences and Engineer at the Federal University of Espírito Santo, in Alegre-ES, geographical coordinates 20°45'49"S and 41°31'59" W with altitude 112 m and rainfall of 1,200 mm year^{-1}. Guava seeds (*P. guajava* L.), obtained from fruits orchards in the municipality of Guaçuí-ES, geographical coordinates 38°46'32"S and 41°40'46"W, altitude 590 m, which were transported to the seed analysis laboratory were used.

The seeds were manually extracted from the fruit, using tap water flowing over a sieve with a one mm mesh diameter. The substrates used composed of Dystrophic Red Yellow Latosol/Oxisol, washed sand and sewage sludge, which were screened in 4 mm meshes, being: S1-soil + sand (control); S2- soil + sand + sludge (40 t ha^{-1}); S3- soil + sand + sludge (80 t ha^{-1}); S4- soil + sand + sludge (120 t ha-1); S5- soil + sand + sludge (240 t ha^{-1}); and S6- soil + sand + sludge (480 t ha^{-1}). The substrates were placed in plastic containers with a 45 cm^3 capacity and the seeds were sown at a depth of 20 mm.

The sewage sludge used was collected in the Waste Water Treatment Plant (WWTP) of the Espírito Santo Sanitation Company (CESAN), in Joana D'arc, Vitória-ES, which analyses were performed to determine the physical and chemical characteristics and heavy metals, which revealed: As = 11.20; Ba = 518.00; Cd = 1.41; Ca = 8221.00; Pb = 45.40; Cu = 315.00; Cr = 41.10; S = 18306.00; Mg = 3761.00; Mn = 220.00; Hg = 2.33; Mo = 1.41; N = 5505.00; Ni = 20.3; K = 6.51; Se = 25.3; Na = 2764.00; Zi = 738.00 mg kg^{-1}; Total P = 0.115%; Organic C = 6.55%; OM = 11.30%.

The experiment was conducted in a greenhouse under different light intensities obtained with black polyolefin screens (2 mm): full sun (0.85 μmol m^{-2} s^{-1}); covered with one screen (0.74 μol m^{-2} s^{-1}); two screens (0.70 μmol m^{-2} s^{-1}); three screens (0.40 μmol m^{-2} s^{-1}).

The variables analyzed were emergence (%), emergence speed index, according to Maguire (1962), shoot fresh and dry weight (mg L^{-1}), root dry weight (mg L^{-1}), root volume (cm^3 plant^{-1}), number of leaves, content of chlorophyll a, b and total, with the help of ClorofiLOG (CFL 1030 model), after 110 days.

For variable emergence (%) and emergence speed index, each plot consisted of four replicates of 25 seedlings. After emergence of seeds, each plot consisted of four replicates of 16 seedlings for seedling formation in greenhouse seedlings in the period of 110 days. The seeds were sown at a depth of 20 mm in plastic containers with a 45 cm^3 capacity.

The experimental design was completely randomized in a factorial 6 × 4, consisting of six substrates and four shade levels. The data relating to the variables analyzed were transformed: emergence ŷ = [to arcsine (x/100)1/2] and the others, by [(x + 0.5) 1/2], subject to the assumptions of normality test and homogeneity of variance and regression analysis. For data analysis, R software was used (R Development Core Team, 2017).

RESULTS

All heavy metals in the sewage sludge were found at limits below the maximum established by Conama (2006); this biosolid was considered suitable for use in

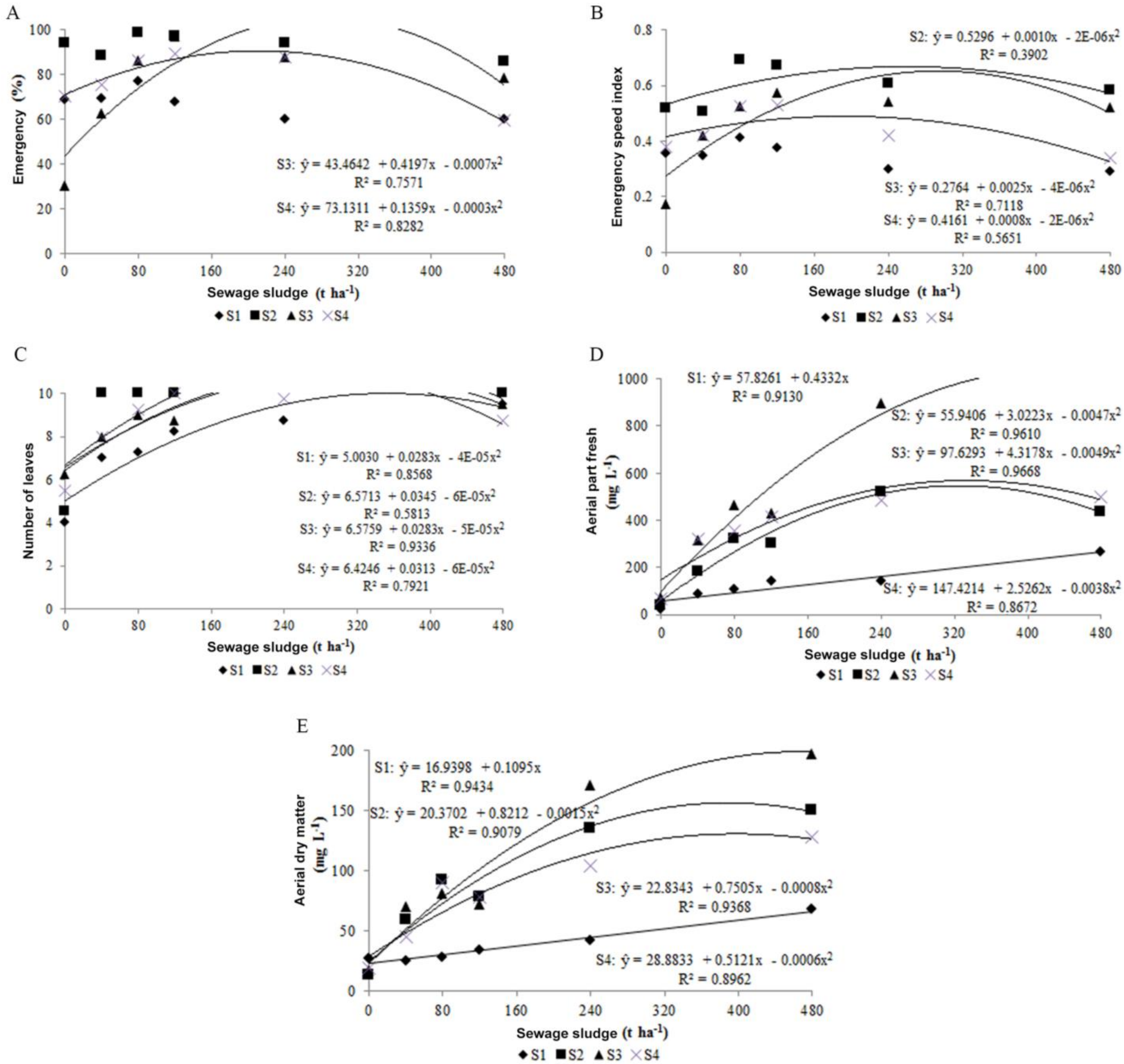

Figure 1. Emergence percentage (A), emergence speed index (B), number of leaves (C), aerial part fresh (D) and dry matter (E) of guava seedlings in substrate enriched with sewage sludge under different shade levels after 110 days. Legend: (S1) full sun without screen; (S2) coverage with one screen; (S3) two screens; (S4) three screens.

agriculture, according to Brazilian legislation.

The use of sewage sludge influenced seedling emergence. The highest averages were found at concentrations of 90 t ha^{-1} under two screens and 317 t ha^{-1} under shade obtained with three screens, with a reduction after these concentrations (Figure 1). Similar behavior was observed for the emergence speed index. Thus, it appears that, similarly, the light intensity exerted influence on the emergence and emergence speed, with lower averages under full sun (Figure 1A and B).

The highest average shoot fresh and dry weight and the highest number of leaves were observed when using substrates containing sewage sludge concentrations of 240 and 480 t ha^{-1}, under shade with two screens. However, when maintained under full sun, there was pronounced reduction in these values (Figure 1C, D and E).

Considering the root dry mass (Figure 2A and B), there

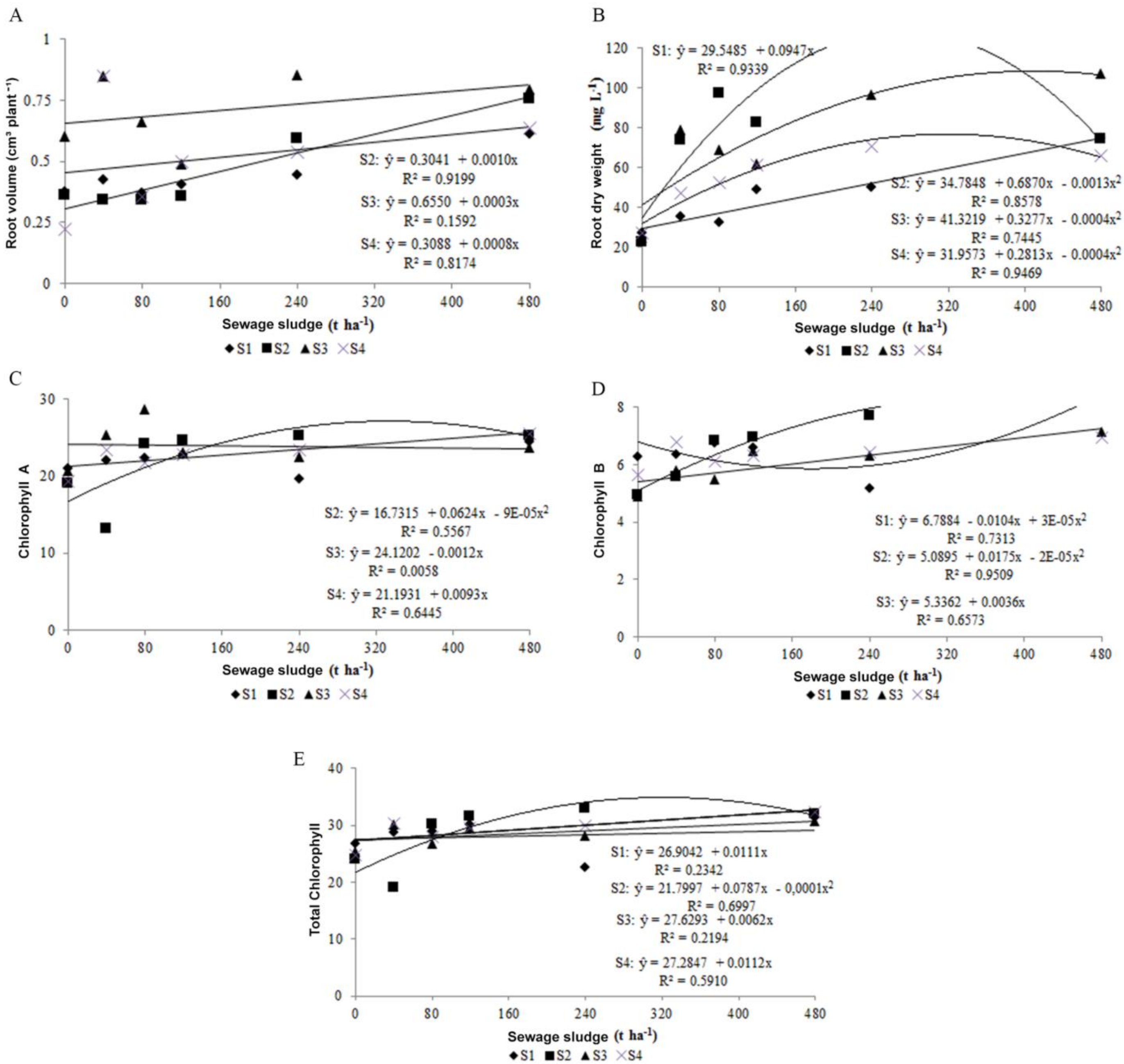

Figure 2. Root volume (A), root dry weight (B), content of chlorophyll *a* (C), chlorophyll *b* (D) and total chlorophyll (E) in guava seedlings in substrate enriched with sewage sludge under different shade levels, after 110 days. Legend: (S1) full sun without screen; (S2) coverage with one screen; (S3) two screens; (S4) three screens.

was greater accumulation using sewage sludge doses of 240 and 480 t ha^{-1}.

Regarding the chlorophyll contents, it was found that the seedlings grown in substrate with dosage of 480 t ha^{-1} of sewage sludge had higher averages of chlorophyll a, b and total. However, under different light intensities, the mean values found for the content were similar to each other (Figure 2C, D and E).

DISCUSSION

According to Taiz and Zeiger (2013), the photosynthetic properties of the leaves provide valuable information on plant adaptations to its luminous environment. Shading with two screens favored seedling emergence. The lowest index of emergence velocity of the pinhão-manso seedlings was observed in a shaded environment

(Schock et al., 2014). However, Freitas et al. (2015) evaluating the emergence and growth of sweet passion fruit seedlings, found that the highest emergence and plant emergence speed were obtained under full sun.

Light management directly affects the growth and assimilates partitioning in seedling production (Marana et al., 2015). Some species present varying behavior regarding their growth and chlorophyll content under different light intensities. In shady environments, plants develop adaptation mechanisms that enable effective use of radiation, as seen in the present study; the shaded environment provides more chlorophyll per reaction center, with thinner leaves and larger area than plants grown under full sunlight.

The use of sewage sludge influenced seedling emergence. The results are similar to those found for Lopes et al. (2005) and Freitas et al. (2015), where the sewage sludge has positive effect in initial growth seedling. These results corroborate those obtained by Caldeira et al. (2014), in a study developed with the application of sewage sludge in the formation of eucalyptus seedlings. Several studies demonstrate that the sewage sludge has the potential to provide nutrients, increasing productivity and dry matter production of crops such as castor bean (Backes et al., 2009) and sweet passion fruit (Freitas et al., 2015).

This fact is justified by the composition of the elements key to plants such as nitrogen, phosphorus and potassium, which are provided by the sewage sludge, whose analyses revealed values of 5505.00, 1,150.00, and 6.51 mg kg^{-1}, respectively. The availability of mineral elements increases the CEC. According to Trannin et al. (2008), adding a sewage sludge dose exceeding 20 t ha^{-1} increased the organic matter and effective CEC of soil composed of sand, silt and clay, in the proportions of 80, 279, and 650 g kg^{-1}, respectively. Considering growth under full sun or shade with two screens, the plants that grew in a shaded environment have more chlorophyll and these photosynthetic pigments are essential for plant growth, providing valuable information on their adaptation to a light environment (Taiz and Zeiger, 2013).

Similar results were observed by Hossain et al. (2015), who evaluated the effects of wastewater from sewage sludge biochar on growth, metal presence, and bioaccumulation of the cherry tomato, and found that treatment with the sludge had increased the number of fruits as well as their biomass. Studies verified that the use of sewage sludge in the soil provides nutrient supply and dynamics, as well as enhances microbial activity, according to Zhang et al. (2013). The fertilizer effect on the plants quality can be associated with available nutrients, which perform an important role in plant metabolism and help synthesize and accumulate carbohydrates (Taiz and Zeiger, 2013).

Different behavior was observed in Jatropha seedlings by Boechat et al. (2014), who found that the sewage sludge had no effect on root dry mass. However, several studies with the application of sewage sludge on the soil confirm the increased availability of phosphorus, organic matter, total nitrogen, and potassium (Chiaradia et al., 2009; Costa et al., 2014), providing further growth of the species. However, phosphorus is an essential nutrient in the development and becomes a significant problem due to its low mobility (Azevedo et al., 2004), the use of sewage sludge being considered as an alternative to maintenance and recycling of soil phosphorous.

Conclusions

The sewage sludge from the Espírito Santo Sanitation Company treatment plant (CESAN), in Joana D'arc, Vitória - ES, presents heavy metals levels below the limits established by CONAMA, and is considered suitable for use in agriculture. Production of guava seedlings (*P. guajava* L.) with sewage sludge should be done with dosages of 240 and 480 t ha^{-1}. Shading with two screens is recommended for the production of guava plants (*P. guajava* L.).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Azevedo WR, Faquin V, Fernandes LA, Oliveira Junior AC (2004). Disponibilidade de fósforo para o arroz inundado sob efeito residual de calcário, gesso e esterco de curral aplicados na cultura do feijão. Rev. Bras. Ciênc. Solo 28(6):995-1004.

Backes C, Lima CP, Fernandes DM, Godoy LJG, Kiihl TAM, Villas Bôas RL (2009). Efeito do lodo de esgoto e nitrogênio na nutrição e desenvolvimento inicial da mamoneira. Biosci. J. 25(1):90-98.

Baliza DP, Cunha RL, Guimarães RJ, Barbosa, JPRAD, Ávila FW, Passos AMA (2012). Physiological characteristics and development of coffee plants under different shading levels. Rev. Bras.Ciênc. Agrár. 7(1):37-43.

Boechat CL, Ribeiro MO, Ribeiro LO, Santos JAG, Accioly AMA (2014). Lodos de esgoto doméstico e industrial no crescimento inicial e qualidade de mudas de pinhão-manso. Biosci. J. 30(3):782-791.

Brasil (2006). Ministério do Meio Ambiente, Conselho Nacional de Meio Ambiente, CONAMA. http://www.mma.gov.br/port/conama/res/res06/res37506.pdf <Access em 07 set. 2016>

Caldeira MVW, Gonçalves EO, Trazzi PA, Delarmelina WM, Rocha RLF (2014). Crescimento de mudas de *Eucalyptus grandis* utilizando lodo de esgoto, fibra de coco e palha de café in natura. Floresta 44(2):195-206.

Carvalho NM, Nakagawa J (2012). Sementes: ciência, tecnologia e produção. 5.ed. Jaboticabal: Funep. 590p.

Chiaradia JJ, Chiba MK, Andrade CA, Oliveira C, Lavorenti A (2009). Produtividade e nutrição de mamona cultivada em área de reforma de canavial. Rev. Bras. Ciênc. Solo 33(3):701-709.

CONAMA (2006). Conselho Nacional do Meio Ambiente, http://www.mma.gov.br/port/conama/res/res06/res37506.pdf <Access em 27 out. 2017>.

Costa VL, Maria IC, Camargo OA, Grego CR, Melo, LCA (2014). Distribuição espacial de fósforo em Latossolo tratado com lodo de esgoto e adubação mineral. Rev. Bras. Eng. Agríc. Ambient. 18(3):287-293.

Freitas AR, Lopes JC, Alexandre RS, Venancio LP, Zanotti RF (2015). Emergência e crescimento de mudas de maracujá doce em função de lodo de esgoto e luz. Comun. Sci. 6(2):234-240.

Hossain, MK, Strezov V, Nelson PF (2015). Comparative assessment of the effect of wastewater sludge biochar on growth, yeld and metal bioaccumulation of cherry tomato. Pedosphere 25(5):680-685.

Instituto Brasileiro de Geografia e Estatística (IBGE) (2015). Instituto Brasileiro de Geografia e Estatística. http://dspace.bc.uepb.edu.br/jspui/bitstream/123456789/8181/1/PDF%20-%20Cec%C3%ADlia%20Elisa%20de%20Sousa%20Muniz.pdf/<Access em 29 mar. 2016>.

Kelessidis A, Stasinakis AS (2012). Comparative study of the methods used for treatment and final disposal of sewage sludge in European countries. Waste. Manage. 32(6):1186-1195.

Lopes JC, Ribeiro LG, Araújo MG, Beraldo MRBS (2005). Produção de alface com doses de lodo de esgoto. Hortic. Bras. 23(2):143-147.

Maguire JD (1962). Speeds of germination-aid selection and evaluation for seedling emergence and vigor. Crop Sci. 2(2):176-177.

Marana JP, Miglioranza E, Fonseca EP (2015). Qualidade de mudas de jaracatiá submetidas a diferentes períodos de sombreamento em viveiro. Ver. Árv. 39(2):275-282.

Nagajyoti PC, Lee KD, Sreekanth TVM (2010). Heavy metals, occurrence and toxicity for plants: a review. Environ. Chem. Lett. 8(1):199-216.

Nascimento AL, Sampaio RA, Zuba Junio GR, Fernandes LA, Cruz SF, Carneiro JP, Barbosa CF, Lima NN (2014). Atributos químicos do solo adubado com lodo de esgoto estabilizado por diferentes processos e cultivado com girassol. Biosci. J. 30(3):146-153.

Osório C, Carriazo JG, Barbosa H (2011). Thermal and structural study of guava (*Psidium guajava* L.) powders obtained by two dehydration methods. Química. Nova 34(4):636-640.

Pinto MAB, Fabbris C, Basso CJ, Santi AL, Girotto E (2014). Aplicação de dejeto líquido de suínos e manejo do solo na sucessão aveia/milho. Pesqui. Agropecu. Trop. 44(2):205-212.

R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Salvador JO, Moreira A, Marcante NC (2013). Waste use as substrate to yield guava seedlings. Semina 34(6):2793-2802.

Schock AA, Ramm A, Martinazzo EG, Silva DM, Bacarin MA (2014). Crescimento e fotossíntese de plantas de pinhão-manso cultivadas em diferentes condições de luminosidades. Rev. Bras. Eng. Agríc. Ambient. 18(1):3-9.

Silva PP, Barros ACSA, Pinho EVRV, Nascimento WM (2015). Physiological analysis and heat-resistant protein (LEA) activity in squash hybrid seeds during development. J. Seed Sci. 37(4):185-191.

Singh RP, Agrawal M (2008). Potential benefits and risks of land application of sewage sludge. Waste Manage. 28(3):347-358.

Taiz L, Zeiger E (2013). Fisiologia vegetal. Porto Alegre: Artmed 820p.

Taiz L, Zeiger E, Moller IM, Murphy A (2015). Plant Physiology and Development. 6ed. Sunderland:Sinauer Associates 761p.

Trannin ICB, Siqueira JO, Moreira FMS (2008). Atributos químicos e físicos de um solo tratado com biossólido industrial e cultivado com milho. Ver. Bras. Eng. Agríc. Ambient. 12(3):223-230.

Trigueiro RM, Guerrini IA (2014). Utilização de lodo de esgoto na produção de mudas de aroeira-pimenteira. Rev. Árv. 38(4):657-665.

Zhang XK, Li Q, Liang WJ, Zhang M, Bao XL, Xie ZB (2013). Soil nematode response to biochar addition in a Chinese wheat field. Pedosphere 23(1):98-102.

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Full Length Research Paper

Impact of variable NPK source on water use efficiency and growth rates of winter grasses (cereals): Wheat, rye, barley and oats

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Growth analysis [absolute growth rate (AGR), crop growth rate (CGR), and net assimilation rate (NAR)] and water use efficiency (WUE) response of four cool season C_3-cereals viz. wheat (*Triticum aestivum* L.), rye (*Secale cereale* L.), barley (*Hordeum vulgare* L.) and oats (*Avena sativa* L.) at 30, 60 and 90 days after emergence (DAE) under eight NPK sources [S_1 = 20-20-20, S_2= 20-27-5, S_3 = 7-22-8, S_4 = 10-10-10-20S, S_5 = 11-15-11, S_6 = 31-11-11, S_7 = 24-8-16, and S_8 = 19-6-12] in pot experiment. The experiment was conducted in the green house of Dryland Agriculture Institute, West Texas A&M University, Texas, USA during winter 2009-10. The results confirmed significant variations in AGR, CGR, NAR and WUE among the four crops at different growth stages and NPK source. Barley and wheat were dominant crops under each NPK source in terms of higher AGR, CGR and WUE than rye and oats at 30 DAE. The AGR, CGR and WUE at 60 DAE decreased for each crop species with application of NPK 31:11:11 and 24:8:16 having more nitrogen content. At 90 DAE, both CGR and WUE ranked first for barley with NPK 20:20:20, for wheat with 24:8:16 and NPK 10:10:10 for oats. The increase in AGR and CGR had positive impact on WUE. Interestingly, the AGR, CGR and WUE increased whereas NAR decreased with the passage of time. The S_6 NPK fertilizer, known as an acid loving fertilizer had harmful effects on the growth and WUE of different crop species in this study.

Key words: *Triticum aestivum*, *Secale cereale*, *Hordeum vulgare*, *Avena sativa*, growth stages, NPK source, absolute growth rate (AGR), crop growth rate (CGR), net assimilation rate (NAR), water use efficiency (WUE).

INTRODUCTION

Plant growth analysis provides understanding of variation in crops growth (Lambers, 1987), total dry matter accumulation and yield (Khan et al., 2013; Amanullah et al., 2014), nutrients and water use efficiencies

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(Amanullah and Stewart, 2013; Amanullah, 2014a, 2015a). The mineral nutrients application exerts pronounced influences on photosynthates and dry matter accumulation (Costa et al., 2002; Amanullah et al., 2014). There are many NPK fertilizer sources, although, there is no reported research on crop growth analysis (AGR, CGR, NAR) and water use efficiency (WUE) response of crop species grown under different NPK sources. Imbalanced nutrients application adversely affects crop growth and WUE (Amanullah and Stewart, 2013; Amanullah, 2015b). Due to the current climate scenario and water shortage, raising WUE of both irrigated and rain-fed crop production is an urgent imperative (Hamdy et al., 2003). Several strategies will be required to improve the productivity of water use in irrigated and rain-fed agriculture (Wang et al., 2002). It is hypothesized that the use of nutrients (NPK) could be one strategy to improve crop growth and increase WUE. This research project was therefore designed with to investigate the impact of different NPK sources on AGR, CGR and NAR and their relationship with WUE of winter cereal crops in pot experiment. Four crops species studied in this experiment were: wheat (*Triticum aestivum* L.), rye (*Secale cereale* L.), barley (*Hordeum vulgare* L.) and oats (*Avena sativa* L.) under eight different NPK sources (S_1 = 20-20-20, S_2= 20-27-5, S_3 = 7-22-8, S_4 = 10-10-10-20S, S_5 = 11-15-11, S_6 = 31-11-11, S_7 = 24-8-16, S_8 = 19-6-12) in pot experiment at the green house of Dryland Agriculture Institute, WTAMU, Texas, USA. The part of this research published indicates that NPK source S_6 (31: 11: 11) known as an acid loving fertilizer had negative effects on the total aerial biomass (dry matter accumulation in shoots) and below ground biomass (dry matter accumulation in roots) in the four crops species (Amanullah et al., 2015). In another paper (Amanullah, 2015b), variations in specific leaf area and specific leaf weights of the four crops (wheat, rye, barley and oats) was also observed at various growth stages and NPK source. The current paper presents the results of different NPK source sources on AGR, CGR and NAR and their relationship with WUE at 30, 60 and 90 days after emergence (DAE).

MATERIALS AND METHODS

Growth rates [absolute growth rate (AGR), crop growth rate (CGR), net assimilation rate (NAR)] and WUE response in four cool season C_3-cereals (small grains) viz. wheat (*T. aestivum* L., cv. TAM III), rye (*S. cereale* L., cv. Elbon), barley (*H. vulgare* L., cv. P919) and oats (*A. sativa* L., cv. Walker) was investigated at 30, 60 and 90 DAE under eight NPK sources [S_1 = 20-20-20 (Peter Professional by Scotts), S_2= 20-27-5 (Starter Fertilizer by Scotts), S_3 = 7-22-8 (Bedding Plant Food by FertiLoam), S_4 = 10-10-10-20S (Shake in Feed by Miracle Grow), S_5 = 11-15-11 (Gardner's Special by FertiLoam), S_6 = 31-11-11 (Acid Loving by FertiLoam), S_7 = 24-8-16 (All Purpose Plant Food by Expert Gardner) and S_8 = 19-6-12 (Slow Release by Expert Gardner)]. Each NPK source was applied at the rate of 300 mg kg^{-1} of potting soil (organic soil know as *miracle grow*) in pot experiment at Dryland Agriculture Institute, West Texas A&M University, Canyon, Texas, USA during winter 2009-2010. The fertilizer was mixed in the potting soil and the pots were filled. The experiment was performed in completely randomized design (CRD) with three repeats. There were 32 pots (treatments) per repeats and a total of 96 pots in the whole experiment. Twenty seeds of each crop species were planted in each pot, and after one week of emergence, 15 plants were maintained per pot, and then five plants were uprooted at 30, 60 and 90 DAE.

The root were washed with tap water, and the plants were then divided into three parts, that is, roots, leaves and stems. The materials was put in paper bags and then put in an oven at 80°C for 24 h. The samples were weighed by electronic balance (Sartorius Basic, BA2105) and the average data on DM of root, leaf, and stem $plant^{-1}$ was worked out. Shoot DM $plant^{-1}$ was obtained by adding leaf DM + stem DM $plant^{-1}$. The sum of the shoot + root DM $plant^{-1}$ was calculated as the total DM $plant^{-1}$. Absolute growth rate (AGR): dry matter accumulation per plant per unit time; crop growth rate (CGR): dry matter accumulation per unit pot area per unit time; and net assimilation rate (NAR): dry matter accumulation per unit leaf area per unit time, were determined using the following formulae:

$$AGR = W_2 - W_1 / t_2 - t_1 \text{ (g plant}^{-1}\text{ day}^{-1})$$

$$CGR = W_2 - W_1 / (PA)(t_2 - t_1) \text{ (g m}^{-2}\text{ day}^{-1})$$

$$NAR = CGR/LAI \text{ (g m}^{-2}\text{ day}^{-1})$$

Where, W_1 = dry weight per plant at the beginning of interval; W_2 = dry weight per plant at the end of interval; $t_2 - t_1$ = the time interval between the two consecutive samplings; PA = pot area occupied by plants at each sampling; LAI = leaf area index (leaf area per plant divided by ground area per plant).

A known amount of water in each pot was applied at 75% field capacity, and the total amount of water applied was calculated for the whole experimental period. WUE was then calculated (g L^{-1}) by dividing the total dry matter (shoot + root) produced (g) by the amount of water used (liters).

$$WUE = \text{Total dry matter produced} \div \text{Liters of water used (g L}^{-1})$$

Statistical analysis

Data were subjected to analysis of variance (ANNOVA) according to the methods described in Steel and Torrie (1980) and treatment means were compared using the least significant difference (LSD) at $P \leq 0.05$. The main effects of NPK sources and crop species at three growth stages are presented in tables. The interactive effect of NPK sources x crop species at different growth stages are presented in figures.

RESULTS

Absolute growth rate

AGR among the cool season cereals varied significantly ($P \leq 0.05$) at 30, 60 and 90 DAE as shown in Table 1. At 30 DAE, barley had the highest AGR (1.87 mg $plant^{-1}$ day^{-1}), followed by wheat (1.75 mg $plant^{-1}$ day^{-1}); while oats had the lowest AGR (0.98 mg $plant^{-1}$ day^{-1}). At 60 DAE, barley had the highest AGR (41.1 mg $plant^{-1}$ day^{-1}), followed by wheat (36.5 mg $plant^{-1}$ day^{-1}); while oats had the lowest (22.0 mg $plant^{-1}$ day^{-1}). At 90 DAE, barley had the highest AGR (70.77 mg $plant^{-1}$ day^{-1}), being at par

Table 1. Absolute growth rate (mg plant^{-1} day^{-1}) response of cool season cereals to different NPK sources at 30, 60 and 90 days after emergence (DAE).

NPK source	N-P_2O_5-K_2O	30 DAE	60 DAE	90 DAE
S_1 = PF (Scotts)	20-20-20	1.52	44.3	68.32
S_2 = SF (Scotts)	20-27-5	1.49	39.1	86.85
S_3 = BPF (Ferti. Loam)	7-22-8	1.42	49.1	48.61
S_4 = SF (Miracle Grow)	10-10-10-20(S)	1.74	31.0	27.57
S_5 = GS (Ferti. Loam)	11-15-11	1.66	32.5	62.33
S_6 = AL (Ferti. Loam)	31-11-11	1.00	4.1	31.78
S_7 = AFPF (E. Gardner)	24-8-16	1.23	18.3	58.74
S_8 = SR (E. Gardner)	19-6-12	1.56	38.0	64.73
Crops species				
Wheat (*Triticum aestivum* L.)		1.75	36.5	69.73
Rye (*Secale cereale* L.)		1.21	28.6	31.25
Barley (*Hordeum vulgare* L.)		1.87	41.1	70.77
Oats (*Avena sativa* L.)		0.98	22.0	52.71
Least significant difference				
Crops ($P \leq 0.05$)		0.11	4.2	8.36
NPK Sources ($P \leq 0.05$)		0.15	6.0	11.83
Interaction ($P \leq 0.05$)		(Figure 1)*	(Figure 2)*	(Figure 2)*

*Indicates the data is significant at $P \leq 0.05$ using LSD test.

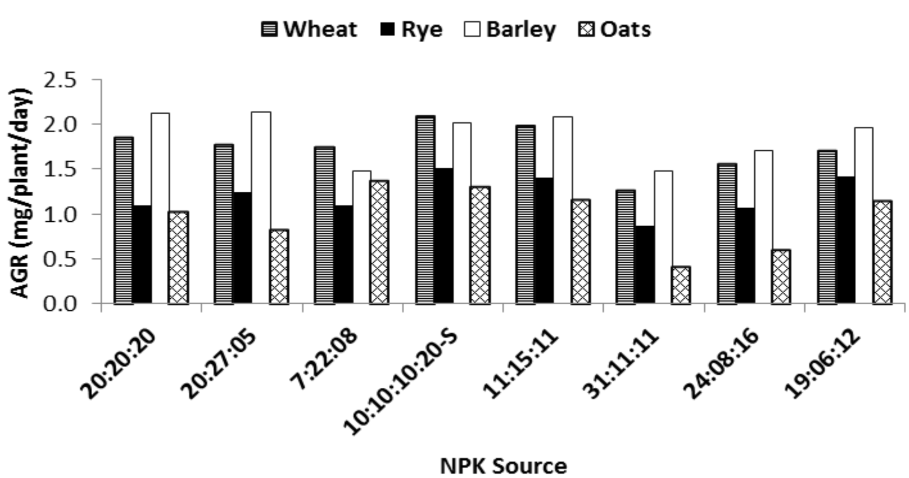

Figure 1. Absolute growth rate (mg plant^{-1} day^{-1}) response to interaction of cool season cereals with NPK sources at 30 days after emergence

with wheat (69.73 mg plant^{-1} day^{-1}); while rye had the lowest (31.25 mg plant^{-1} day^{-1}). The AGR at 30, 60 and 90 DAE among cool season cereals varied significantly ($P \leq 0.05$) when applied different NPK sources (Table 1).

At 30 DAE, the highest AGR (1.74 mg plant^{-1} day^{-1}) was noted when applied with S_4 being at par with S_5 (1.66 mg plant^{-1} day^{-1}); while the lowest AGR (1.00 mg plant^{-1} day^{-1}) was obtained when crops were grown with S_6. At 60 DAE, the highest AGR (49.1 mg plant^{-1} day^{-1}) was recorded when crops were applied S_3, being at par with S_1 (44.3 mg plant^{-1} day^{-1}); while the lowest (4.1 mg plant^{-1} day^{-1}) was obtained when the crops were applied S_6. At 90 DAE, the highest AGR (86.85 mg plant^{-1} day^{-1}) was recorded when crops were applied S_2, followed by S_1 (68.32 mg plant^{-1} day^{-1}); while the lowest (27.57 mg plant^{-1} day^{-1}) was obtained when crops were applied S_4. The interaction at 30 DAE (Figure 1) indicates that both barley and wheat are dominant crops in terms of AGR than rye and oats under different NPK sources. But at 60 DAE (Figure 2), barley growth was severely affected by the

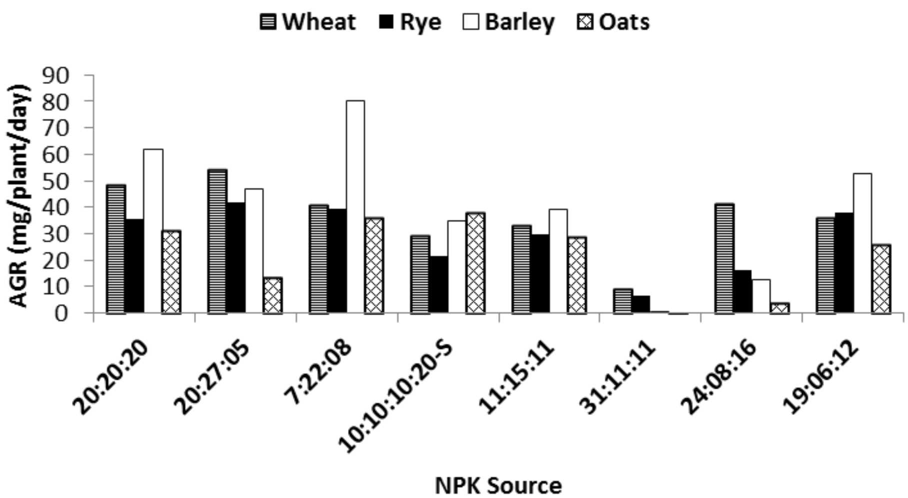

Figure 2. Absolute growth rate (mg $plant^{-1}$ day^{-1}) response to interaction of cool season cereals with NPK sources at 60 days after emergence.

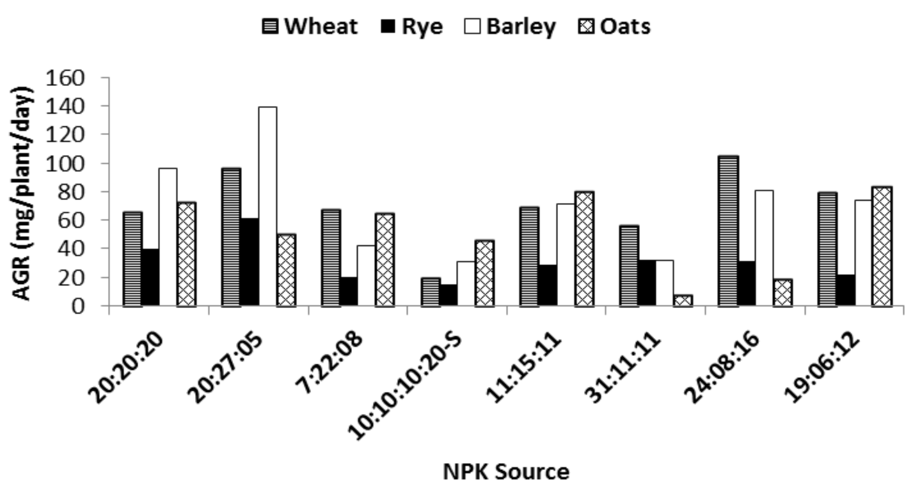

Figure 3. Absolute growth rate (mg $plant^{-1}$ day^{-1}) response to interaction of cool season cereals with NPK sources at 90 days after emergence.

two NPK sources (31:11:11 and 24:8:16) having more nitrogen. At these two sources, wheat AGR was higher than other crops. At later growth stage (Figure 3), oats show dominance in AGR than other crops under two NPK sources (11:15:11 and 19:6:12).

Crop growth rate

Crop growth rate (CGR) among the cool season cereals varied significantly ($P \leq 0.05$) at 30, 60 and 90 DAE (Table 2). At 30 DAE, barley had the highest CGR (0.84 g m^{-2} day^{-1}), followed by wheat (0.79 g m^{-2} day^{-1}); while oats had the lowest CGR (0.44 g m^{-2} day^{-1}). At 60 DAE, barley had the highest CGR (12.06 g m^{-2} day^{-1}), followed by wheat (10.68 g m^{-2} day^{-1}); while oats had the lowest (6.47 g m^{-2} day^{-1}). At 90 DAE, barley had the highest CGR (21.23 g m^{-2} day^{-1}), being at par with wheat (20.92 g m^{-2} day^{-1}); while rye had the lowest CGR (9.37 g m^{-2} day^{-1}). The CGR at 30, 60 and 90 DAE among cool season cereals varied significantly ($P \leq 0.05$) when applied different NPK sources (Table 2). At 30 DAE, the highest CGR (0.78 g m^{-2} day^{-1}) was obtained when crops were applied S_4, being at par with S_5 (0.75 g m^{-2} day^{-1}); while the lowest CGR (0.45 g m^{-2} day^{-1}) was obtained when crops received S_6. At 60 DAE, the highest CGR (14.51 g m^{-2} day^{-1}) was obtained when crops were applied S_3, being at far with S_1 (13.08 g m^{-2} day^{-1}); while the lowest CGR (1.13 g m^{-2} day^{-1}) was obtained when crops received S_6. At 90 DAE, the highest CGR (26.05 g m^{-2} day^{-1}) was calculated when crops were applied S_2, followed by S_1 (20.49 g m^{-2} day^{-1}); while the lowest CGR

Table 2. Crop growth rate (g m^{-2} day^{-1}) response of cool season cereals to different NPK sources at 30, 60 and 90 days after emergence (DAE).

NPK source	$N-P_2O_5-K_2O$	30 DAE	60 DAE	90 DAE
S_1 = PF (Scotts)	20-20-20	0.69	13.08	20.49
S_2 = SF (Scotts)	20-27-5	0.67	11.51	26.05
S_3 = BPF (Ferti. Loam)	7-22-8	0.64	14.51	14.58
S_4 = SF (Miracle Grow)	10-10-10-20(S)	0.78	9.04	8.27
S_5 = GS (Ferti. Loam)	11-15-11	0.75	9.52	18.70
S_6 = AL (Ferti. Loam)	31-11-11	0.45	1.13	9.53
S_7 = AFPF (E. Gardner)	24-8-16	0.55	5.31	17.62
S_8 = SR (E. Gardner)	19-6-12	0.70	11.16	19.42
Crops species				
Wheat (*Triticum aestivum* L.)		0.79	10.68	20.92
Rye (*Secale cereale* L.)		0.55	8.42	9.37
Barley (*Hordeum vulgare* L.)		0.84	12.06	21.23
Oats (*Avena sativa* L.)		0.44	6.47	15.81
Least significant difference				
Crops ($P \leq 0.05$)		0.05	1.27	2.51
NPK Sources ($P \leq 0.05$)		0.07	1.79	3.55
Interaction ($P \leq 0.05$)		(Figure 4)*	(Figure 5)*	(Figure 6)*

*Indicates the data is significant at $P \leq 0.05$ using LSD test.

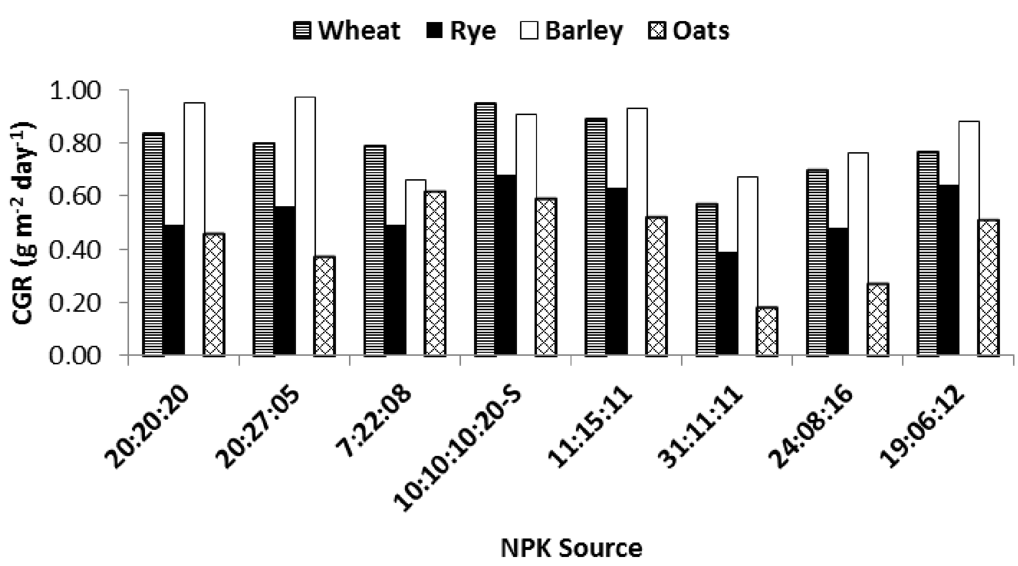

Figure 4. Crop growth rate (g m^{-2} day^{-1}) response to interaction of cool season cereals with NPK sources at 30 days after emergence.

(8.27 g m^{-2} day^{-1}) was calculated when crops received S_4. The interaction at 30 DAE (Figure 4) indicates that both barley and wheat are dominant crops in terms of CGR than rye and oats under different NPK sources. At 60 DAE (Figure 5), all crops CGR were severely affected under 31:11:11, while under 24:8:16, wheat CGR was significantly higher than other three crops. At 90 DAE (Figure 6), barley ranked first in CGR under 20:20:20 and 20:27:5; wheat ranked first under 31:11:11 and 24:8:16; oats ranked first under 11:15:11, 10:10:10 and 19:6:12. Wheat and oats had similar but more CGR than barley and rye under 7:22:8 and 19:6:12.

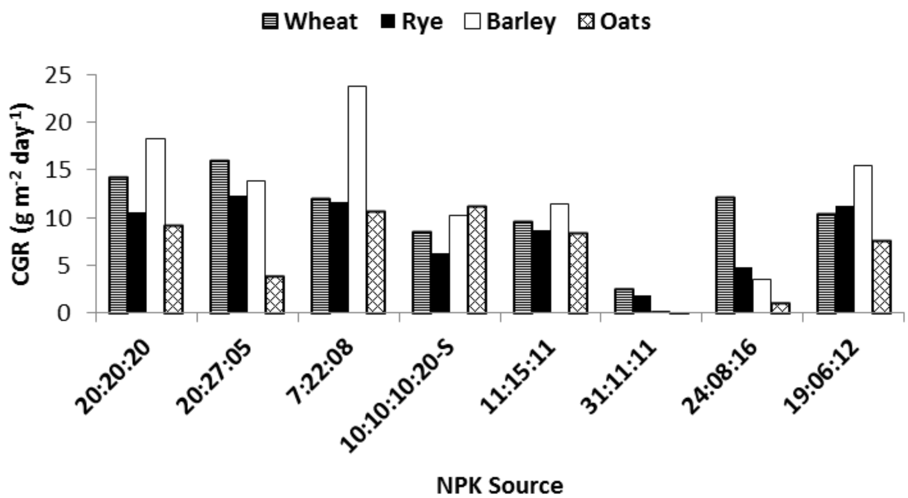

Figure 5. Crop growth rate (g m^{-2} day^{-1}) response to interaction of cool season cereals with NPK sources at 60 days after emergence.

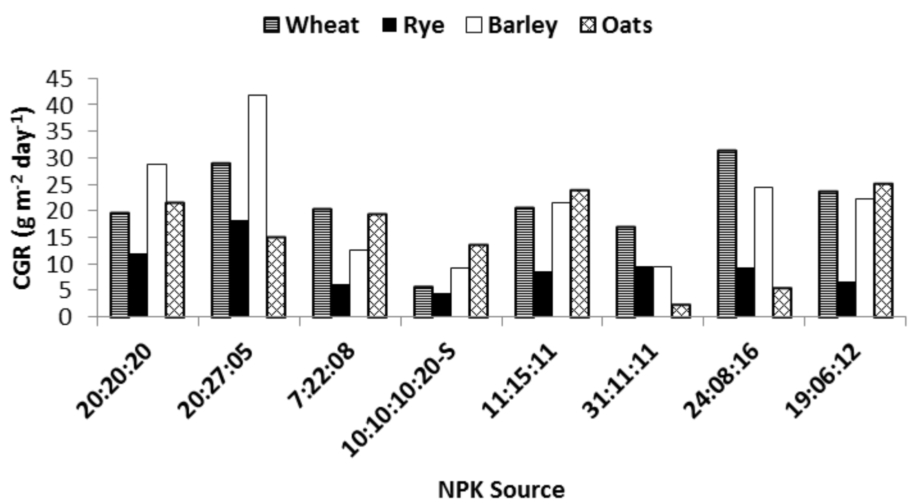

Figure 6. Crop growth rate (g m^{-2} day^{-1}) response to interaction of cool season cereals with NPK sources at 90 days after emergence.

Net assimilation rate

Net assimilation rate (NAR) among the cool season cereals varied significantly ($P \leq 0.05$) at 30 and 90 DAE, but had no significant differences at 60 DAE (Table 3). At 30 DAE, wheat had the highest NAR (22.72 g m^{-2} day^{-1}), being at par with rye (22.40 g m^{-2} day^{-1}); while oats had the lowest NAR (17.19 g m^{-2} day^{-1}). Although, the differences in NAR were not significant among the crops at 60 DAE, however, rye (16.70 g m^{-2} day^{-1}) and wheat (15.13 g m^{-2} day^{-1}) had the higher NAR as compared to barley (7.80 g m^{-2} day^{-1}) and oats (9.76 g m^{-2} day^{-1}). At 90 DAE, wheat had significantly, the highest NAR (8.83 g m^{-2} day^{-1}), followed by oats (4.46 g m^{-2} day^{-1}); while rye had the lowest NAR (3.41 g m^{-2} day^{-1}). The NAR at 30, 60 and 90 DAE among cool season cereals varied significantly ($P \leq 0.05$) when applied different NPK sources (Table 3). At 30 DAE, the highest NAR (25.38 g m^{-2} day^{-1}) was calculated when crops were applied S_6, followed by S_7 (21.67 g m^{-2} day^{-1}); while the lowest NAR (16.68 g m^{-2} day^{-1}) was calculated when crops received S_3. At 60 DAE, the highest NAR (36.28 g m^{-2} day^{-1}) was calculated when crops were applied S_6, followed by S_3 (13.87 g m^{-2} day^{-1}); while the lowest NAR (6.08 g m^{-2} day^{-1}) was obtained when crops received S_7. The NAR showed negative relationship with LAI and positive relationship with CGR. At 90 DAE, the highest NAR (9.36 g m^{-2} day^{-1}) was obtained when crops were applied S_5, followed by S_1 (6.12 g m^{-2} day^{-1}); while the lowest (3.51 g m^{-2} day^{-1}) was obtained when crops received S_8. The interaction at 30

Table 3. Net assimilation rate (g m^{-2} day^{-1}) response of cool season cereals to different NPK sources at 30, 60 and 90 days after emergence (DAE).

NPK source	$N-P_2O_5-K_2O$	30 DAE	60 DAE	90 DAE
S_1 = PF (Scotts)	20-20-20	21.09	8.47	6.12
S_2 = SF (Scotts)	20-27-5	21.26	8.25	4.93
S_3 = BPF (Ferti. Loam)	7-22-8	16.68	13.87	5.53
S_4 = SF (Miracle Grow)	10-10-10-20(S)	20.88	9.35	4.20
S_5 = GS (Ferti. Loam)	11-15-11	19.70	9.22	9.36
S_6 = AL (Ferti. Loam)	31-11-11	25.38	36.28	3.84
S_7 = AFPF (E. Gardner)	24-8-16	21.67	6.08	4.65
S_8 = SR (E. Gardner)	19-6-12	17.78	7.26	3.51
Crops species				
Wheat (*Triticum aestivum* L.)		22.72	15.13	8.83
Rye (*Secale cereale* L.)		22.40	16.70	3.41
Barley (*Hordeum vulgare* L.)		19.91	7.80	4.36
Oats (*Avena sativa* L.)		17.19	9.76	4.46
Least significant difference				
Crops ($P \leq 0.05$)		2.34	ns	1.03
NPK sources ($P \leq 0.05$)		3.30	14.63	1.46
Interaction ($P \leq 0.05$)		(Figure 7)*	(Figure 8)^{ns}	(Figure 9)*

*Indicates the data is significant at $P \leq 0.05$; ns indicates the data is not significant at $P \leq 0.05$ using LSD test.

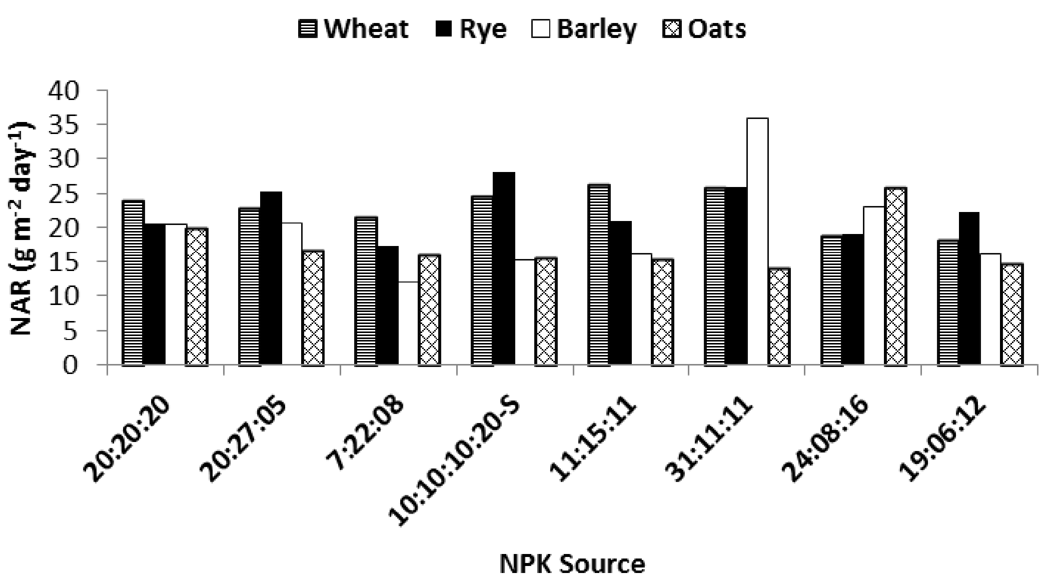

Figure 7. Net assimilation rate (g m^{-2} day^{-1}) response to interaction of cool season cereals into NPK sources at 30 days after emergence.

DAE (Figure 7) indicates that wheat had higher NAR than other crops under 20:20:20, 7:22:8, and 11:15:11; barley had higher NAR than other crops under 31:11:11; rye had higher NAR than crops under 20:27:5, 10:10:10 and 19:6:12, while oats had higher NAR under 24:8:16 than other three crops. The interaction at 60 DAE (Figure 8) indicates that except barley which had low NAR; the NAR of other three crops (wheat, rye and oats) was significantly higher with 31:11:11 (NPK). At 90 DAE (Figure 9), wheat ranked first in NAR under different NPK

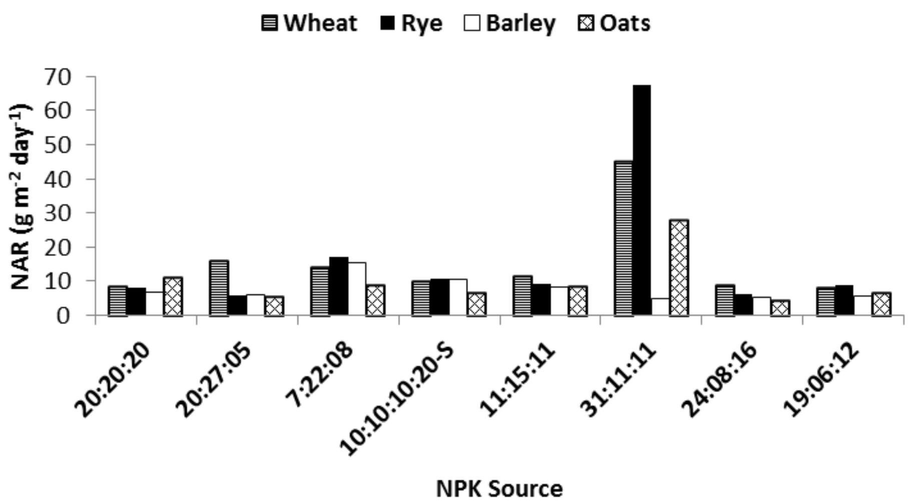

Figure 8. Net assimilation rate (g m^{-2} day^{-1}) response to interaction of cool season cereals with NPK sources at 60 days after emergence.

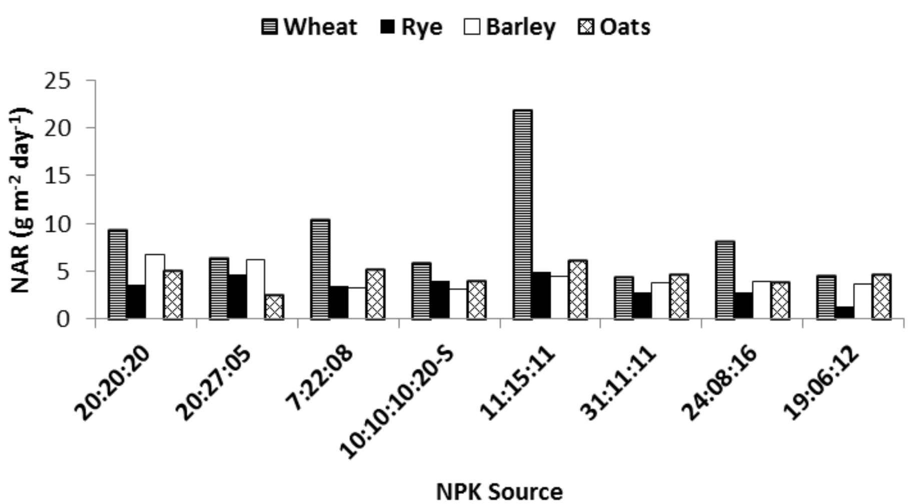

Figure 9. Net assimilation rate (g m^{-2} day^{-1}) response to interaction of cool season cereals with NPK sources at 90 days after emergence.

sources than other crops species.

Water use efficiency

WUE among cool season cereals varied significantly ($P \leq 0.05$) at 30, 60 and 90 DAE (Table 4). At 30 DAE, barley had the highest WUE (0.33 g L^{-1}), followed by wheat (0.31 g L^{-1}); while oats had the lowest WUE (0.16 g L^{-1}) at 30 DAE. Barley had the highest WUE (2.19 g L^{-1}), being at par with wheat (2.02 g L^{-1}); while oats had the lowest WUE (1.16 g L^{-1}) at 60 DAE; the highest WUE (2.54 g L^{-1}), being at par with wheat (2.50 g L^{-1}) and lowest WUE (1.35 g L^{-1}) at 90 DAE. The WUE of the crops also varied significantly when applied different NPK sources at 30, 60 and 90 DAE (Table 4). At 30 DAE, the highest WUE of 0.29 g L^{-1} each was obtained when crops were applied either S_4 or S_8, being at par with S_5 (0.28 g L^{-1}). The lowest WUE (0.17 g L^{-1}) was noted when crops were grown with S_6. At 60 DAE, the highest WUE of 2.48 g L^{-1} was calculated when crops were applied either S_8, being at par with S_1 (2.18 g L^{-1}) and S_3 (2.41 g L^{-1}). The lowest WUE (0.24 g L^{-1}) was noted when crops were grown with S_6. At 90 DAE, the highest WUE of 2.63 g L^{-1} was calculated when crops were applied S_8, being at par with S_2 (2.58 g L^{-1}) and S_7 (2.61 g L^{-1}). The lowest WUE (0.75 g L^{-1}) was noted when crops were grown with S_6. The interaction at 30 DAE (Figure 10) indicates that barley

Table 4. Water use efficiency (g L^{-1}) response of cool season cereals to different NPK sources at 30, 60 and 90 days after emergence (DAE).

NPK sources	$N-P_2O_5-K_2O$	30 DAE	60 DAE	90 DAE
S_1= PF (Scotts)	20-20-20	0.26	2.18	2.31
S_2 = SF (Scotts)	20-27-5	0.25	1.93	2.58
S_3 = BPF (Ferti. Loam)	7-22-8	0.26	2.41	2.01
S_4 = SF (Miracle Grow)	10-10-10-20(S)	0.29	1.55	1.22
S_5 = GS (Ferti. Loam)	11-15-11	0.28	1.63	1.96
S_6 = AL (Ferti. Loam)	31-11-11	0.17	0.24	0.75
S_7 = AFPF (E. Gardner)	24-8-16	0.22	1.40	2.61
S_8 = SR (E. Gardner)	19-6-12	0.29	2.48	2.63
Crops species				
Wheat (*Triticum aestivum* L.)		0.31	2.02	2.50
Rye (*Secale cereale* L.)		0.20	1.54	1.35
Barley (*Hordeum vulgare* L.)		0.33	2.19	2.54
Oats (*Avena sativa* L.)		0.16	1.16	1.64
Least significant difference				
Crops ($P \leq 0.05$)		0.01	0.20	0.17
NPK sources ($P \leq 0.05$)		0.02	0.29	0.25
Interaction ($P \leq 0.05$)		(Figure 10)*	(Figure 11)*	(Figure 12)*

*Indicates the data is significant at ($P \leq 0.05$).

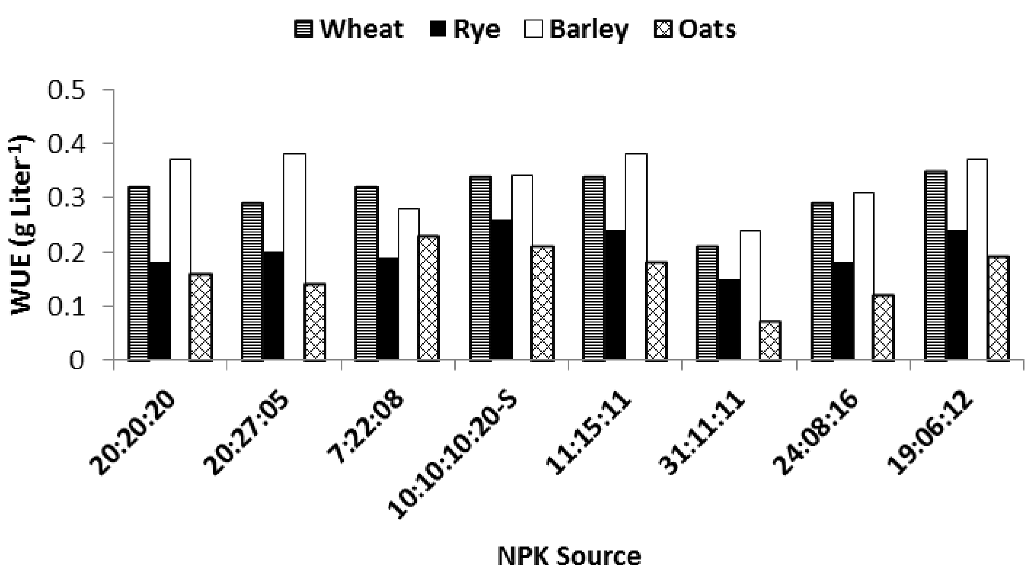

Figure 10. Water use efficiency (g $Liter^{-1}$) response to interaction of cool season cereals with NPK sources at 30 days after emergence.

and oats had higher WUE than oats and rye under different NPK sources The interaction at 60 DAE (Figure 11) indicates that barley had higher WUE than other crops under 20:20:20, 7:22:8, 11:15:11 and 19:6:12, while wheat was better in terms of WUE than other crops under 20:27:5 and 24:8:16. The WUE in all crops was significantly reduced under 31:11:11 (Figure 11). At 90 DAE (Figure 12), barley ranked first in CGR under 20:20:20, 20:27:5. 7:22:8 and 19:6:12; wheat ranked first under 31:11:11 and 24:8:16; oats ranked first under

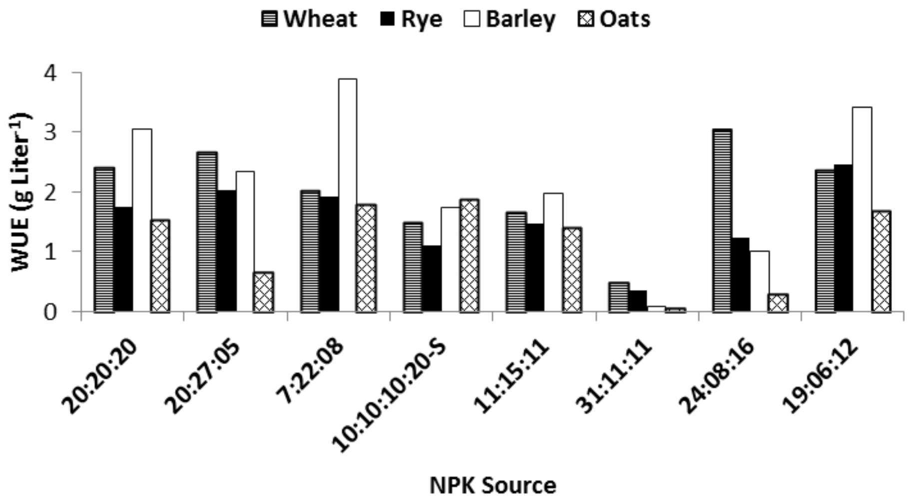

Figure 11. Water use efficiency (g L^{-1}) response to interaction of cool season cereals with NPK sources at 60 days after emergence.

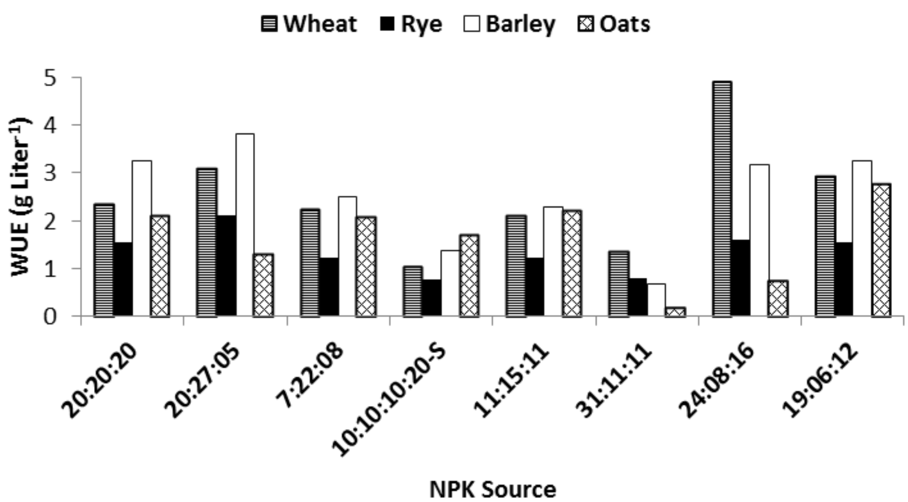

Figure 12. Water use efficiency (g L^{-1}) response to interaction of cool season cereals with NPK sources at 90 days after emergence.

10:10:10 (NPK).

DISCUSSION

Significant variations ($P \leq 0.05$) was observed in the AGR, CGR, NAR and WUE of four cool season small grains species (wheat, rye, barley and oats) at different growth stages (30, 60 and 90 DAE). The differences in the AGR, CGR, NAR and WUE of different crop species at different growth stages may be attributed to the difference in the genetic makeup of crop species (Bendichz and McCarthy, 1970). The number of chromosome in these four crops under study (wheat, barley, rye and oats) are different viz. 42, 14, 14 and 42, respectively (Dolezel et al., 2007) which may be responsible for variation in growth analysis of these crops. Variation in the growth analysis (AGR, CGR, and NAR) and WUE of different crop species depends on plant growth characteristics (leaf area, leaf area index, number of leaves, tillers and roots plant^{-1}, dry matter accumulation plant^{-1} and m^{-2}, dry matter partitioning into various plant parts, that is, roots, shoots, stems, leaf and reproductive parts, as well as root to shoot ratios etc.). Earlier, Amanullah et al. (2016) reported that C_3-cereals crops (wheat, rye, barley and oats) differ in root and shoot biomass (g plant^{-1}), root to shoot ratios and water use efficiency (g L^{-1}) when they were grown in various combinations (intercropping) and water levels. The results of Amanullah (2017) study found significant differences in these four species in terms of dry matter partitioning and accumulation at various growth stages. Differences in the WUE of wheat vs. rye was reported under different soil types (Amanullah, 2014a), and difference in the WUE was attributed to the significant variations in their shoot, root and total dry weights produced. Other research on maize genotypes (Amanullah et al., 2014) confirmed that variation in the total dry weights plant^{-1} of different genotypes was attributed to variation in mean single leaf area, number of leaves plant^{-1} and leaf area index. Differences in total biomass accumulation, root to shoot ratios (Amanullah et al., 2015), and leaf thickness (Amanullah, 2015b) in the four-crop species under different NPK sources was also reported.

Significant variations ($P \leq 0.05$) in the AGR, CGR, NAR and WUE were also observed at different growth stages (30, 60 and 90 DAE) with different NPK sources. The differences in the AGR, CGR, NAR and WUE while using different NPK fertilizers may probably be attributed to the differences in the leaf area, leaf area index, number of leaves and tillers plant^{-1}, root, shoot and total dry matter accumulation produced under different NPK sources. Amanullah (2017) found significant differences in dry matter partitioning and accumulation under different NPK sources. Increased plant growth with optimal N, P, K application provides vegetative cover, thus enhancing moisture retention, nutrient use efficiency and soil productivity (Bumb and Bannante, 1996). Hussein and Alva (2014) reported that water use efficiency responded favorably with increase in rates of N, P, K fertilizers. In previous research on maize response to different nitrogenous fertilizer sources (Amanullah et al., 2014), it was indicated that application of CAN (calcium ammonium nitrate) produced significantly higher mean single leaf area, leaves plant^{-1}, leaf area index and total dry matter accumulation than application of urea and ammonium sulphate (AS) in the first year of experiment. However, the differences in these parameters were not significant while using different N-fertilizers in the second year of experiment (Amanullah et al., 2014). Khan et al. (2013) reported that foliar application of various N-fertilizer sources (urea, CAN and AS) had produced significantly higher total biomass yield than control (water spray only). In another study, regarding maize response to phosphatic fertilizer sources (Amanullah et al., 2010), the mean single leaf area, number of leaves plant^{-1}, leaf area index and total biomass ha^{-1} was significantly higher either with application of di-ammonium phosphate (DAP) or single super phosphate (SSP) as compared to the application of nitrophos (NP) and control plots (P not applied). In the current experiment, the AGR showed positive relationship with increase in total dry weight (shoots + roots) plant^{-1}. Indicating that any NPK source that increased dry weight of shoots (leaf + stem) or roots or both (shoots + roots) resulted in higher AGR and vice versa. The increase in CGR in this experiment showed positive relationship with increase in AGR. Therefore, any NPK source that increased AGR/dry weight plant^{-1} resulted in higher CGR and vice versa. The increase in WUE showed positive relationship with increase in both AGR and CGR. This means that any NPK source which increased AGR/CGR resulted in higher WUE and vice versa. The result of Amanullah (2015) study indicated that two NPK sources viz. S_4 [(10-10-10-20(S] and S_6 (31-11-11) reduced the total weight plant^{-1} and therefore these NPK sources had negative impact on the AGR, CGR, NAR and WUE. In another greenhouse study, Amanullah (2014b) noted that higher WUE obtained under three organic soils (potting soils) was attributed to increase in shoot and root growth of wheat and rye. On the other hand, the less total dry weight per plant produced under inorganic soils (Canyon and Amarillo soils) adversely affected the growth and WUE of both wheat and rye (Amanullah, 2014b). The experiment on oats (Amanullah and Stewart, 2013) had also confirmed that increase in total dry matter accumulation per plant had positive impact on AGR, CGR, NAR and WUE. Differences in total biomass accumulation and root to shoot ratios (Amanullah et al., 2015), and leaf thickness (Amanullah, 2015b) at various growth stages under different NPK sources was also reported.

The AGR, CGR and WUE increased with the passage of time, that is, the values of all these parameters were less at the early growth stage than at the late growth stage (90 > 60 > 30 DAE). The increase in all these three parameters with advancement in crop age was attributed to the increase in the total dry matter accumulation plant^{-1} (Amanullah, 2015). Bagrintseva and Nosov (2012) reported that DM partitioning in both winter wheat and winter barley was more at grain filling > heading > tillering. Mut et al. (2006) found significant differences in the DM yield among triticale, wheat, rye and barley at early heading and dough stages. Research on maize crop (Amanullah et al., 2009) indicated that the total dry matter produced depends on plant height and leaf area plant^{-1}, and the total dry matter was more at the late (physiological maturity) than the early (silking) growth stage. In contrast, the NAR (CGR ÷ LAI) in this experiment decreased with the passage of time (90 < 60 < 30 DAE), and the decrease in NAR with advancement in crop age may be attributed to the increase in leaf area plant^{-1} and leaf area index. Earlier, Amanullah and Stewart (2013) suggested that NAR had negative relationship with increase in leaf area index and positive relationship with increase in CGR.

Conclusion

Considerable variation in AGR, CGR, NAR and WUE was observed in the four crop species at different growth stages when applied with different NPK sources. The increase in dry matter accumulation plant^{-1} was considered the best criteria to increase AGR, CGR and WUE in different crop species. The increase in AGR, CGR and WUE was observed with advancement in crop age, while on the other hand, NAR was reduced with the passage of time. The reduction in NAR with passage of time was due to the increase in leaf area index. Identification or development of crop species with higher AGR and CGR has higher WUE in different environments. Since growth rates and WUE values were determined on the average of five plants in pot experiment, more research is needed under field condition under different environmental conditions.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Amanullah K (2017). Effects of NPK source on the dry matter partitioning in cool season C3-cereals “wheat, rye, barley, and oats” at various growth stages. J. Plant Nutr. 40(3):352-364.

Amanullah K, Stewat BA (2013). Dry matter partitioning, growth analysis and water use efficiency response of oats (*Avena sativa* L.) to excessive nitrogen and phosphorus application. J. Agric. Sci. Technol. 15:479-489.

Amanullah K, Stewart BA, Hidayatullah (2015). Cool season C_3-grasses (Wheat, Rye, Barley, and Oats) differ in shoot: Root ratio when applied with different NPK sources. J. Plant Nutr. 38:1890-201.

Amanullah K, Bob AS, Lal KA (2016). Root: shoot ratio and water use efficiency differ in cool season cereals grown in pure and mixed stands under low and high-water levels. Texas J. Agric. Nat. Resour. 29: 52-65.

Amanullah K, Asif M, Nawab K, Shah Z (2010). Impacts of planting density and P-fertilizer source on the growth analysis of maize. Pak. J. Bot. 42:2349-2357.

Amanullah K, Shah P, Marwat KB (2009). Nitrogen levels and its time of application influence leaf area, height and biomass of maize planted at low and high density. Pak. J. Bot. 41:761-768.

Amanullah K, Shah S, Shah Z, Khalil SK (2014). Effects of variable nitrogen source and rate on leaf area index and total dry matter accumulation in maize (*Zea mays* L.) genotypes under calcareous soils. Turk. J. Field Crops 19:276-284.

Amanullah K (2014a). Wheat and rye differ in dry matter partitioning, shoot-root ratio and water use efficiency under organic and inorganic soils. J. Plant Nutr. 37:1885-1897.

Amanullah K (2014b). Crop growth rate differs in warm season C4-grasses grown in pure and mixed stands. Afr. J. Biotechnol. 13(30):3036-3051.

Amanullah K (2015a). Competition among warm season C_4-cereals influence WUE and competition ratios. Cogent Food Agric. 1:1011466.

Amanullah K (2015b). Specific leaf area and specific leaf weight in small grain crops wheat, rye, barley, and oats differ at various growth stages and NPK source. J. Plant Nutr. 38(11):1694-1708.

Bagrintseva VN, Nosov VV (2012). Potassium nutrition for small grains grown on chestnut soils. Better Crops 96:29-31.

Bendich AJ, McCarthy BJ (1970). DNA Comparisons among Barley, Oats, Rye, and Wheat. Genetics 65:545-565.

Bumb BI, Bannante CA (1996). The Use of Fertilizer in Sustaining Food Security and Protecting the Environment-2020. Proceedings of Conference on Agriculture and Fertilizer Use by 2010, NEDC, Islamabad. P 35.

Costa C, Dwyer LM, Zhou X, Dutilleul P, Hamel C, Reid LM, Smith DL (2002). Root morphology of contrasting maize genotypes. Agron. J. 94:96-101.

Dolezel J, Kubalakova M, Paux E, Bartos J, Feuillet C (2007). Chromosome-based genomics in the cereals. Chromosome Res. 15:51-66.

Hamdy A, Ragab R, Scarascia-Mugnozza E (2003). Coping with water scarcity: Water saving and increasing water productivity. Irrig. Drain. 52:3-20.

Hussein MM, Alva AK (2014). Growth, yield and water use efficiency of forage sorghum as affected by NPK fertilizer and deficit irrigation. Am. J. Plant Sci. 5:2134-2140.

Khan AZ, Jan A, Shah Z, Ahmad B, Khalil SK, Ali A, Ahmad F, Nawaz A. Foliar application of nitrogen at different growth stages influences the phenology, growth and yield of maize (*Zea mays* L.). Soil Environ. 32(2):135-140.

Lambers H (1987). Does variation in photosynthetic rate explain variation in growth rate and yield. Netherlands J. Agric. Sci. 35:505-519.

Mut Z, Ayan K, Mut H (2006). Evaluation of forage yield and quality at two phenological stages of triticale genotypes and other cereals grown under rainfed conditions. Bangl. J. Bot. 35:45-53.

Steel RGD, Torrie JH (1980). Principles and Procedures of Statistics. McGraw-Hill, NY, United States.

Wang HX, Liu CM, Zhang L (2002). Water-saving agriculture in China: An overview. Adv. Agron. 75:135-171.

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Full Length Research Paper

Protection of *Sideroxylon obtusifolium* seeds against *Colletotrichum* sp. with *Caesalpinia ferrea* extract

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***Sideroxylon obtusifolium* (Roem. & Schul.) Penn. is a native species from Caatinga biome, but due to disorderly exploitation for phytoteraphy industry, it is in danger of extinction. Recent researches report that the diversity of vegetal species in Brazilian semiarid regions, when meticulously assessed through methods that focus on properties of molecules from different plant structures, may present a high potential for the discovery and development of new antifungal substances. The aim of this work was to evaluate the effects of *S. obtusifolium* seeds treatment with *Caesalpinia ferrea* extract on the control of *Colletotrichum* sp. In each treatment, 100 seeds were inoculated with the pathogen through immersion in a suspension of *Colletotrichum* sp. conidia, and then subjected to the following treatments: Seeds without treatment and not inoculated (T_1), seeds infected with *Colletotrichum* sp. (T_2), infected seeds treated with captan fungicide (T_3) and infected seeds treated with *C. ferrea* extract (T_4). *C. ferrea* extract provided a higher protection to *S. obtusifolium* seeds and seedlings against *Colletotrichum sp*., indicating that it is a viable and sustainable biotechnological resource against pathogens and a promising molecule for the development of new antifungal substances.**

Key words: Antifungal activity, native species, alternative control.

INTRODUCTION

Sideroxylon obtusifolium (Roem. & Schult.) Penn is a native forestry species from Caatinga biome (Silva and Dantas, 2013). It as non-cultivated fruit tree, popularly known by local inhabitants as quixabeira, quixaba,

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sapotiaba or coronilha (Silva et al., 2012) and it is exploited in popular medicine and for industrial production of phytotherapeutic drugs (Gomes et al., 2010). This way, the development of forest recovery programs through the production of seedlings from high quality seeds and with genetic variance is necessary (Beltrão et al., 2008; Vechiato and Parisi, 2013). For this purpose, the sanitary and physiological quality of seeds is highly important, as it will determine the development of healthy seedlings in nurseries (Mondego et al., 2014).

Besides damaging the germination potential, pathogens use seeds as a dissemination vehicle and a survival shelter, involving these reproductive structures in the continuity of the biological cycle of the disease through the generations of the host plant. Thereby, researches exploring the transmission from seeds to seedlings contribute to the advancement of disease management strategies, showing that the initial inoculum of the causal agent reached the infected area through seeds or other ways of early fungal infection (Poletto et al., 2014).

Among the fungi related to seeds, there are currently 59 species of *Colletotrichum* in Brazil, infecting about 154 host plants, like several native forestry species such as *Lithraea brasiliens* March., *Myracrodrun urundeuva* Fr. All, *Tabebuia impetiginosa* (Mart. ex DC.) Standl., *Apeiba tibourbou* Aubl. and *Dalbergia nigra* (Vell.), causing seed rot, leaf spots, low germination and damages at seedling development (Vechiato and Parisi, 2013).

Nevertheless, there is still lack of studies on forestry seeds and efficiency of chemical and alternative phytosanitary products designated for the establishment of sanitary protocols for seedlings production (Mertz et al., 2009). Recent researches report that diversity of vegetal species on Brazilian semiarid region, when assessed with methods that focus on the action of molecules present in different tissues of the plant, might constitute a valuable tool for discovery of new antifungal

Among vegetal species with antifungal properties, *Caesalpinia ferrea* Mart. Ex. Tul. stand out as one of the most studied for its compounds and biological and chemical activities (Ferreira and Soares 2015). Several studies on *C. ferrea* extracts and isolated compounds with antimicrobial characteristics have been performed to control fungi such as *Aspergillus niger*, *Trichoderma viride* and *Penicillium cyclopium* (Marreiro et al., 2014; Martins et al., 2014).

New studies are still necessary to discover new bioactive molecules with highly efficient pathogen control, as it is considered more complex for pathogens to develop resistance against products derived from plant extracts due to their wide chemical constitution and modes of action (Ferreira et al., 2013). Besides that, considering the importance of seed quality and sanity for crop yield and the great potential of *S. obstusifolium* for reforestation and pharmaceutical industry (Oliveira et al., 2012), this work was carried out with the objective to evaluate the efficacy of *C. ferrea* extract in *Colletotrichum* sp. infection in *Sideroxylon obtusifolium* (Roem. & Schult.) Penn. seeds.

MATERIALS AND METHODS

Seeds obtainment

S. obtusifolium seeds were extracted from mature fruits from ten matrix trees located in Boa Vista, Paraiba State, in the first fortnight of February 2013. Trees were situated at an average altitude of 490 m and at the following coordinates: M1 6°57'6,65" S, 35°43'4,60" W; M2 6°57'6,91" S, 35°43'4,68" W; M3 6°57'6,70" S, 35°43'48,7" W; M4 6°57'6,82" S, 35°43'48,6" W; M5 6°57'7,01" S, 35°43'4,79" W; M6 6°57'6,78" S, 35°43'5,05" W; M7 6°57'6,95" S, 35°43'5,04" W; M8 6°57'7,14" S, 35° 43,48,3" W; M9 6°57'7,18" S, 35°43'46,8" W; M10 6°57'7,32" S and 35°43'44,4" W. After harvest, fruits were packed in polyethylene bags and taken to the Seed Analysis Laboratory of Federal University of Paraiba. Seeds were extracted by the natural fermentation method for 72 h, followed by washing in running water and dried on paper towel at room temperature (25 ± 2°C) (Silva et al., 2012).

Water content

Water content was determined with an oven at 105°C for 24 h (Brasil, 2009), considering four replicates of 25 seeds per matrix tree.

Inoculum and aqueous extract obtainment

Samples from each seed lots were analyzed for determination of the presence of microflora, using four replicates of 25 seeds per treatment. Seeds were surface disinfected through immersion in sodium hypochlorite (2%) for 5 min and then distributed on Petri dishes with sterilized and humidified filter paper. After a seven-day incubation period at room temperature (25 ± 2°C), fungal structures were analyzed with an optical and a stereoscopic microscope. Identification of the species was performed with an identification key (Barnett and Hunter, 1972). Then, the obtainment of *Colletotrichum* sp. isolates was proceeded following methodology proposed by Medeiros et al. (2013), incubating seeds on Petri dishes containing sterilized PDA growth media (1000 ml of deionized water, 200 g of potato, 20 g of dextrose and 17 g of agar).

Caesalpinia ferrea leaves were used to produce the aqueous extracts. 500 g of fresh weight were immersed for 5 min in sodium hypochlorite solution (2%) and then dried at room temperature (25 ± 2°C) for 24 h. After this period, leaves were kept in an oven with forced air circulation at 40 ± 2°C, until stabilization of residual moisture, and then pulverized in a knife mill to obtain plant raw material (PRM) (Stange et al., 2009).

PRM was diluted in deionized water in a 1:3 proportion (Pedroso et al., 2011), and later, the suspension was subjected to constant agitation at 35°C for 24 h. Then, the concentrated was obtained through vacuum filtration and subsequently lyophilized (Mot et al., 2012) to turn material into powder for conservation in a freezer at -20°C.

Seed inoculation and treatment

Fungal suspension concentration was determined with Neubauer chamber, resulting in approximately 1 x 10^5 *Colletotrichum* sp.

Table 1. Germination rate (G) and germination speed index (SGI) of *Sideroxylon obtusifolium* seeds inoculated with *Colletotrichum* sp.

Matrix	G (%)		GSI	
	T1	T2	T1	T2
1	95^{aA}	79^{aB}	1.48^{aA}	0.55^{bB}
2	90^{aA}	62^{bB}	1.35^{aA}	0.71^{aB}
3	93^{aA}	$10d^{B}$	1.43^{aA}	$0.20d^{B}$
4	92^{aA}	57^{bB}	1.46^{aA}	$0.35c^{B}$
5	88^{aA}	$38c^{B}$	1.38^{aA}	$0.35c^{B}$
6	85^{aA}	$45c^{B}$	1.44^{aA}	0.55^{bB}
7	$53c^{A}$	$8d^{B}$	0.72^{bA}	$0.12d^{B}$
8	$50c^{A}$	$6d^{B}$	0.64^{bA}	$0.14d^{B}$
9	$42d^{A}$	$6d^{B}$	0.58^{bA}	$0.10d^{B}$
10	$24e^{A}$	$5d^{B}$	0.57^{bA}	$0.10d^{B}$
CV (%)	9.8			

*****Means followed by the same letter, lower case in columns and upper case in rows, do not significantly differ from each other by Scott-Knot test ($p<0.05$). T1 = non inoculated seeds; T2 = *Colletotrichum* sp. inoculated seeds.

conidia mL^{-1}. The treatments consisted of seeds without inoculation and treatment (T_1), inoculated seeds (check) (T_2), inoculated seeds treated with Captan fungicide (positive control) (T_3) and inoculated seeds treated with *C. ferrea* extract (T_4).

At room temperature, seeds were disinfected with sodium hypochlorite (2%) for 2 min, ethanol 70% for 30 s, washed two times with deionized water and dried with towel paper for 30 min (Ferraz and Calvi, 2010). Inoculation with the pathogen was performed through seed immersion in 20 mL of fungal suspension for 12 h. After inoculation, seeds were immersed for 24 h in *C. ferrea* extract solutions with concentrations of 0.075, 0.15; 0.31 and 0.62 mg mL^{-1}, as described by Flavio et al. (2014). For the positive check treatment (T_3), application of the fungicide was also after the pathogen inoculation (Mondego et al., 2014).

Physiological and sanitary quality determinations

Evaluation of the physiological potential was performed with seeds from T_1 and T_2 treatments, and for T_3 and T_4 treatments, the incidence and transmissivity rate of *Colletotrichum* sp. was also determined.

Seeds were manually scarified with sandpaper no. 80 at the opposite side of the hilum and 2 cm depth sowed in transparent plastic boxes (11.0 cm x 11.0 cm x 3.5 cm), previously disinfected with sodium hypochlorite (2%), filled with sterilized vermiculite (Silva et al., 2012) and moistened with deionized water until 60% of its water holding capacity (BRASIL, 2009). Boxes were maintained in germination chamber at 30°C with a 12 h photoperiod, equipped with fluorescent lamps (4 x 20 W).

Evaluations were carried out on alternative days, from the 15th to 30th day after sowing, with standard seedlings emergence, and the results were expressed in percentage. Seed germination rate and germination speed index (GSI) were determined according to Brasil Ministério da Agricultura, Pecuária e Abastecimento (2009) and Maguire (1962), respectively.

On the 30th day, pathogen transmissivity was assessed. Over this period, diseased seedlings with symptoms of *Colletotrichum* sp. infection in cotyledons, roots, stems and leaves and rot seeds were observed (Auer and Álvaro, 2010). To confirm pathogen etiology, seeds and seedlings shoot and root fragments were sectioned, disinfected and incubated in Petri dishes, following methodology proposed by Ferraz and Calvi (2010) and Medeiros et al. (2013), respectively. After fungal culture development, the identification was executed following an identification key of Barnett and Hunter (1972). Fungus transmission rate to seedlings was calculated using the equation (TR (%) = [IR (%)/DI(%)] × 100), adapted from Teixeira and Machado (2003), where IR = infection rate in seedlings with symptoms of *Colletotrichum* sp.; DI = disease incidence in seeds artificially inoculated. Survival rate (SR) was also calculated, according to the equation adapted from Teixeira and Machado. (2003), where SR (%) = [No. of germinated seedlings – No. of infected seeds/total of seeds] × 100).

Experimental design and statistical analysis

The experimental design was completely randomized, with treatments distributed in a 10 × 7 factorial layout, with ten matrix trees and six seed treatments (plus check). Data were subjected to variance analysis (ANOVA), regression study and comparison of means by Scott-Knot test ($p<0.05$). For variables that fit in the regression quadratic model, the higher and/or lower values were determined by derivation of the equation.

RESULTS AND DISCUSSION

A wide range of fungi genera was present in the *S. obtusifolium* seeds microbiota, which included *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus* sp., *Botrytis* sp., *Colletotrichum* sp., *Chaetonium* sp., *Cladosporium* sp., *Curvulária* sp., *Fusarium* sp., *Helminthosporium* sp., *Nigrospora* sp. and *Penicillium* sp. This is the first report on the microflora associated with this forestry species.

Evaluating the deleterious effects of *Colletotrichum* sp. due to its inoculation on *S. obtusifolium* seeds (Table 1), higher germination rates and GSI values were found in non-inoculated seeds (T1, related to all matrices, except 7, 8, 9 and 10). Meanwhile, the lowest germination

Figure 1. *Sideroxylon obtusifolium* seedling infected with *Colletotrichum* sp.

Table 2. Adjusted models through regression analysis for *Colletotrichum* sp. incidence (%) in *Sideroxylum obtusifolium* seeds artificially inoculated and treated with *Caesalpinia ferrea* extract and fungicide.

Matrix	Adjusted regression equations	R^2
1	$y = 459.92x^2 - 366.45x + 52.747$	0.65
2	$y = 371.71x^2 - 296.17x + 42.631$	0.65
3	$y = 393.83x^2 - 305.63x + 52.352$	0.56
4	$y = 504.02x^2 - 401.59x + 57.805$	0.65
5	$y = 486.76{*}x^2 - 390.15x + 57.115$	0.69*
6	$y = 424.72x^2 - 343.44x + 52.408$	0.60
7	$y = 376.23{*}x^2 - 314.20x + 52.436$	0.69*
8	$y = 500.58x^2 - 384.97x + 54.469$	0.65
9	$y = 422.12x^2 - 336.33x + 48.412$	0.65
10	$y = 485.12x^2 - 386.53x + 55.637$	0.65

R^2 = coefficient of determination. *Significant through Scott-Knot test ($P < 0.05$).

performance was associated with this same lot of seeds (7, 8, 9 and 10), although they did not statistically differ from seeds from matrix 3.

Vechiato and Parisi (2013) reported that *Colletotrichum* sp., with low or mild incidence on forestry species seeds is a potentially pathogenic fungus; however, according to these authors, it is not possible to affirm the damages this genus may cause. However, the data found in this work indicates that *Colletotrichum* sp. has a negative interference on *S. obtusifolium* seed germination, decreasing the speed and the number of emerged seedlings. These results were confirmed by observed symptoms such as necrotic lesions on cotyledons, young leaves, roots, followed by seedlings damping off (Figure 1).

These results corroborate Lopes et al. (2011), who reported that fungal infection in angico branco (*Anadenanthera colubrina* (Vell.) Brenan.) seeds drastically affected their physiological quality, even completely inhibiting germination in some cases. Santos et al. (2001) also found similar results assessing the influence of *Colletotrichum* sp. on canafistula (*Peltophorum dubium* (Sprengel) Taubert.) seeds, also Auer and Álvaro (2010) on araucaria (*Araucaria angustifólia* (Bertol.) Kuntze.) seedlings development, indicating the progression of early lesions on cotyledons to stem strangulation and seedling death.

With regards to the incidence of *Colletotrichum* sp. in *S. obtusifolium* seeds inoculated and treated with *C. ferrea* extract or captan, all the matrices fit a quadratic model (Table 2), in which a higher incidence of the pathogen is verified on check treatment (0 mg/mL) (Figure 2). However, even in the lowest extract doses (0.075 mg mL^{-1}), the pathogen incidence decreased by 92 and 96% in matrices 7 and 5, respectively, whereas the other seeds lots presented an absolute control of the fungus. Notwithstanding, the chemical treatment with fungicide reduced the occurrence of infesting microorganisms without eradicating them, with an average incidence of 69.9% among the matrices, a value indeed superior than those observed for matrices 2 and 9 without *C. ferrea* extract (Figure 2).

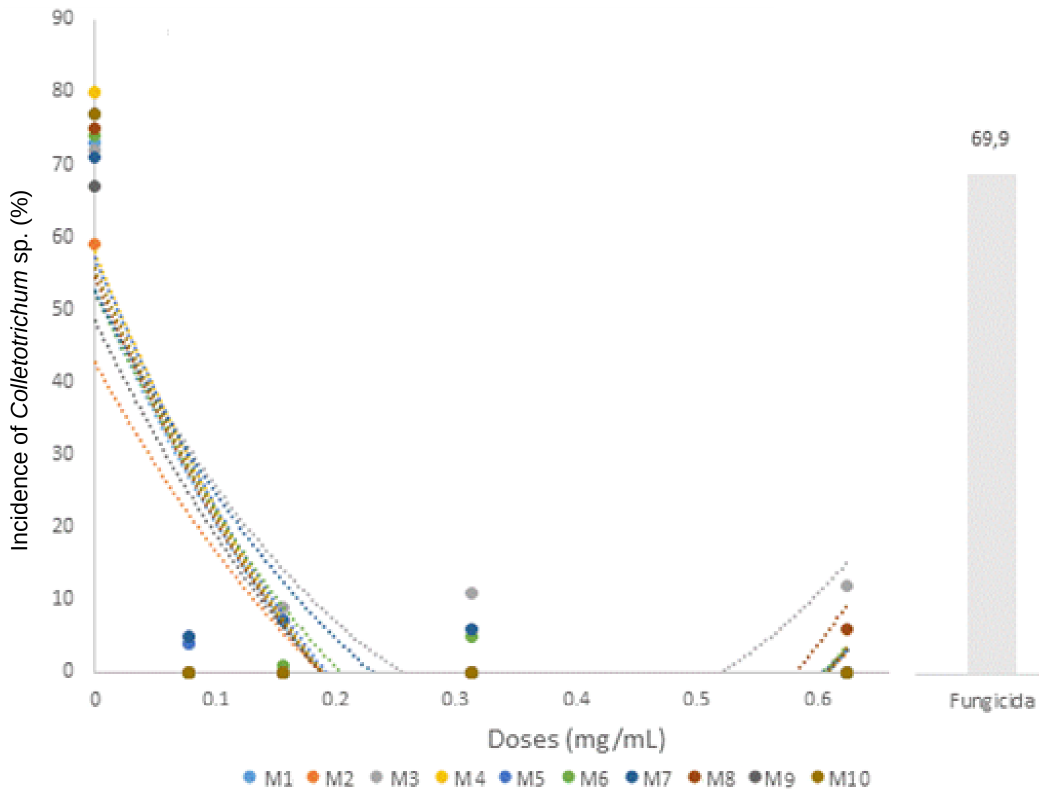

Figure 2. Incidence of *Colletotrichum* sp. in *S. obtusifolium* seeds inoculated and treated with *Caesalpinia ferrea* extract and captan fungicide.

The high fungal incidence in seeds without treatment might be due to the incubation conditions throughout the germination test which are optimal for fungi development, as in natural conditions, this pathogen occurs as low to mild incidence on forestry species (Vechiato and Parisi, 2013). According to these authors, the high occurrence of *Colletotrichum* sp. in *S. obtusifolium* matrices confirms the affinity of this pathogen for forestry species, endorsing their observations with aroeira-brava (*Lithraea brasiliens* March.), aroeira (*Myracrodrun urundeuva* Fr. All.), ipê-roxo (*Tabebuia impetiginosa* (Mart. ex DC.) Standl.), pau-de-jangada (*Apeiba tibourbou* Aubl.), cedar (*Cedrela fissilis* Vell.) and jacarandá-da-baía (*Dalbergia nigra* (Vell.).

Treatment with different doses of *C. ferrea* extract promoted significant decrease in the incidence of *Colletotrichum* sp. in *S. obtusifolium* seeds. Similar results were obtained by Lazarotto et al. (2009), who reported a reduced incidence of this fungus in cedar (*Cedrela fissilis* Vell.) seeds treated with garlic (*Allium sativum*) and boldo-brasileiro (*Plectranthus barbatus*) extracts. In seeds of other forestry species, such as amendoim-bravo (*Pterogyne nitens* Tul.) (Medeiros et al., 2013), sansão-do-campo (*Mimosa. caesalpiniaefolia* Benth.) (Leite et al., 2012) and flamboyant-mirim (*Caesalpinia pulcherrima* L.) (Medeiros et al., 2011), the adoption of vegetal extracts was also proven as an efficient tool to control seed pathogens.

The transmissivity rate (Figure 3) was negatively affected by moderate doses of *C. ferrea* extract in matrices 5, 8 and 12, which with the quadratic model, presented an initial increase of this parameter as compared to check treatment, with maximum values at the doses of 0.35, 0.30 and 0.21, respectively. Although it decreased reasonably from this point, even reaching complete suppression of transmissivity for matrices 8 and 12 (Table 3). This way, only doses higher than 0.5 mg L^{-1} for matrix 12 and the maximum evaluated dose (0.62 mg mL^{-1}) for matrix 8 were considered effective against transmission of the disease from seeds to seedlings of *S. obtusifolium*.

The other matrices likewise fit the quadratic model (Table 3). Almost all of them had a lower transmissivity rate following the increase of the extract doses, apart from matrix 13, which unexpectedly had this parameter enhanced with higher dose extracts. Fungicide treatment resulted in an average transmissivity of 20.4%, a value inferior only to matrices 2, 3, 12 and 15 without sanitary treatments (Figure 3).

Local penetration through testa is the most common mechanism for necrotrophic microorganisms, as *Colletotrichum* sp., to start their cycle (Poletto et al.,

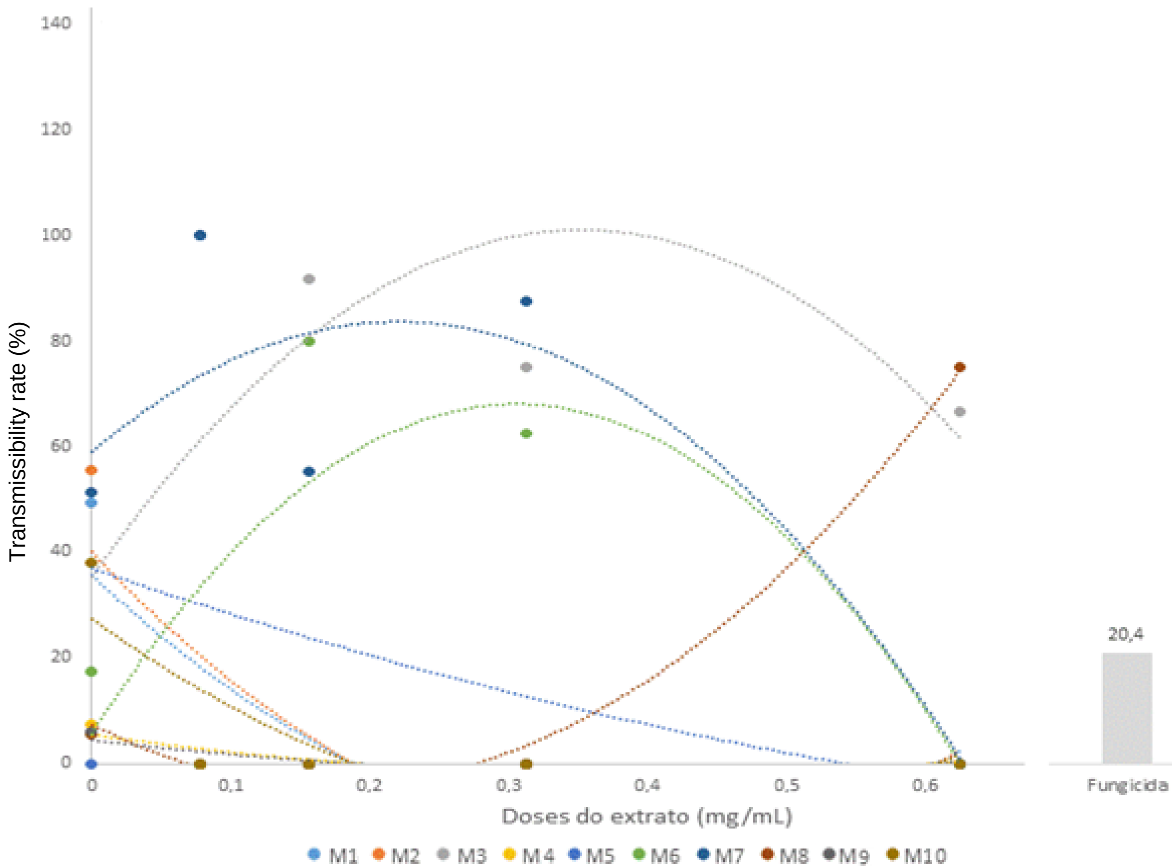

Figure 3. Transmissibility rate (%) of *Colletotrichum* sp. in seedlings of *S. obtusifolium* from inoculated seeds and treated with extract of *C. ferrea* and captana fungicide.

Table 3. Adjusted models through regression analysis for *Colletotrichum* sp. transmissivity rate (%) in *S. obtusifolium* seeds artificially inoculated and treated with *C. ferrea* extract.

Matrix	Adjusted regression equation	R^2
1	$y = 311.48x^2 - 248.18x + 35.724$	0.65
2	$y = 349.13x^2 - 278.18x + 40.041$	0.65
3	$y = - 530.39x^2 + 373.68x + 35.244$	0.42
4	$y = 47.252x^2 - 37.649x + 5.4192$	0.65
5	$y = 41.03x^2 - 90.444x + 36.934$	0.12*
6	$y = - 669.87x^2 + 409.45x + 5.529$	0.63
7	$y = - 507.38x^2 + 224.05x + 58.962$	0.74*
8	$y = 384.47x^2 - 132.94x + 7.335$	0.99
9	$y = 37.991x^2 - 30.27x + 4.3571$	0.65
10	$y = 238.95x^2 - 190.39x + 27.405$	0.65

R^2 = Coefficient of determination. *Significant through Scott-Knot test (P <0.05).

2014); this way, pathogens associated with seeds are spread by infection (establishment in internal tissues) or infestation (passive contamination on testa) of these reproductive structures. Thus, fungal transmission rate depends on the amount and localization of the inoculum in seeds (Sá et al., 2011). Due to this, the inoculation period adopted in this work might have provoked the dissemination of the pathogen to seed embryo tissues. As a result, it was observed that during *S. obtusifolium* germination, the biological cycle of *Colletotrichum* sp.

might have developed, with its spores contaminating various seedling structures, justifying the considerable transmissivity rate even in treated seeds.

Several authors have described antifungal properties of *C. ferrea* extract derived from different plant organs. However, successful results were only reported for extracts obtained from fruits (Zanin et al., 2012), seeds (Cavalheiro et al., 2009) and bark (Ferreira and Soares, 2015), and this work is the first study on the potential of leaves extract for disease control. It is also noticeable that the obtained results confirm those of Ferreira and Soares (2015), who observed antifungal properties of extracts from this species bark against *Colletotrichum lindemuthianum* and *C. truncatum*. Yet, further studies are required to elucidate *C. ferrea* potential as raw material for antifungal aqueous extracts or as a source of bioactive molecules with biotechnological relevance and environmental safety and sustainability.

Conclusion

Coletrotichum sp. infection severely inhibited germination rate and speed in almost all *S. obtusifolium* matrices, except lots 1 and 2, which demonstrated resistance to this pathogen. *C. ferrea* extract was effective in concentrations above 0.5 mg mL^{-1}, reducing both incidence and transmissivity rate of *Colletotrichum* sp. in *S. obtusifolium* seeds.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

ACKNOWLEDGEMENT

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REFERENCES

Auer CG, Álvaro FS (2010). Sistemas de produção: Cultivo de araucária. 2.ed. Colombo: EMBRAPA Florestas 25p.

Barnett HC, Hunter BB (1972). Illustrated genera of imperfect fungi. 3rd ed. Mineapolis: Burgess Publishing 241p.

Beltrão AES, Tomaz ACLA, Beltrao FAS (2008). *In vitro* biomass production of *Sideroxylon obtusifolium* (Roem & Schult). Rev. Bras. Farmacogn. 18(1):696-698.

Brasil Ministério da Agricultura, Pecuária e Abastecimento (2009). Manual de análise sanitária de sementes. Brasília: SDA/CGAL. 202p.

Cavalheiro MG, Farias DF, Fernandes GS, Nunes EP, Cavalcanti FS, Vasconcelos IM, Melo VMM, Carvalho AF (2009). Biological and enzymatic activities of aqueous extract of seeds from *Caesalpinia ferrea* Mart., Leguminosae. Rev. Bras. Farmacogn. 19(2):586-591.

Ferraz IDK, Calvi DP (2010). Teste de germinação. In: Lima-Junior, M.J.V. (Ed.). Manual de procedimentos para análise de sementes florestais. Manaus: UFAM. pp. 55-110.

Ferreira MRA, Santiago RR, Langassner SMZ, Mello JCP, Svidzinski TIE, Soares LAL (2013). Antifungal activity of medicinal plants from Northeastern Brazil. J. Med. Plants Res. 7(40):3008-3013.

Ferreira MRA, Soares LAL (2015). *Libidibia ferrea* (Mart. ex Tul.) L. P. Queiroz: a review of the biological activities and phytochemical composition. J. Med. Plants Res. 9(5):140-150.

Flavio NSDS, Sales NLP, Aquino CF, Soares EPS, Aquino LFS, Catão HCRM (2014). Health and physiological quality of sorghum seeds treated with aqueous extracts and essential oils. Semina: Ciênc. Agrár. Londrina 35(1):7-20.

Gomes R, Pinheiro MCB, Lima HÁ, Santiago-Fernandes LDR (2010). Floral biology of *Manilkara subsericea* and *Sideroxylon obtusifolium* (Sapotaceae) in restinga. Rev. Bras. Bot. 33(2):271-283.

Lazarotto M, Irardi LB, Mezzomo R, Piveta G, Muniz MFB, Blume E (2009) Tratamentos Alternativos para o Controle de Patógenos em Sementes de Cedro (*Cedrela fissilis*). Rev. Bras. Agroecol. 4(2):75-78.

Leite RP, Medeiros JGF, Nascimento LC, Neto AA, Gomes ECS, Malta AO (2012). Qualidade fisiológica de sementes de sabiá (Mimosa caesalpiniaefolia Benth) tratadas com extratos vegetais. Scient. Plena 8(4):1-5.

Lopes IS, Campelo G, Bezerra RR (2011). Avaliação antifúngica do extrato de *Allium sativum* L. no controle de fungos em sementes de *Anadenanthera colubrina*. Rev. Biol. Farmácia Campina Grande 6(1):145-150.

Maguire JD (1962). Speed of germination: aid in selection and evaluating or seedling emergence and vigour. Crop Sci. 2(2):176-177.

Marreiro RO, Bandeira MFCL, Souza TP, Almeida MC, Bendaham K, Venâncio GN, Rodrigues IC, Coelho CN, Milério PSLL, Oliveira GP, Conde NCO (2014). Evaluation of the stability and antimicrobial activity of an ethanolic extract of Libidibia ferrea. Clin. Cosmet. Invest. Dent. 6:9-13.

Martins M, Kluczkovski AM, Souza TP, Pacheco C, Savi GD, Scussel VM (2014). Inhibition of growth and aflatoxin production of Aspergillus parasiticus by guaraná (Paullinia cupana Kunth) and jucá (Libidibia ferrea Mart) extracts. Afr. J. Biotechnol. 13(1):131-137.

Medeiros JGF, Antonio Neto AC, Medeiros D, Nascimento LC, Alves EU (2013). Extratos vegetais no controle de patógenos em sementes de *Pterogyne nitens* Tul. Floresta e Ambiente, Rio de Janeiro 20(3):384-390.

Medeiros JGF, Leite RP, Nascimento LC (2011). Extratos vegetais e seus efeitos na sanidade e fisiologia de sementes de flamboyant-mirim (*Caesalpinia pulcherrima* L.). In: SEABRA G.; MENDONÇA, I. (Ed.). Educação ambiental: responsabilidade para a conservação da sóciobiodiversidade. João Pessoa: Editora Universitária. pp. 22-30.

Mertz LM, Henning FA, Zimmer PD (2009). Bioprotectors and chemical fungicides in the treatment of soybean seeds. Ciênc. Rural 39:13-18.

Mondego JM, Melo PAFR, Pinto KMS, Nascimento LC, Alves EU, Batista JL (2014). Alternative control of microflora on *Pseudobombax marginatum* seeds with essential copaiba oil (*Copaifera* sp.). Biosci. J. 30(2):349-355.

Oliveira AP, Raith M, Kuster RM, Rocha LM, Hamburger M, Potterat O (2012). Metabolite profiling of the leaves of the Brazilian folk medicine *Sideroxylon obtusifolium*. Planta Med. 78(7):703-710.

Pedroso RCR, Peralba MCR, Santos JHZ, Pizzolato TM, Fröehlich PE (2011). Development of analytical methods for HPLC-UV to the antimicrobians tetracycline, sulfa-methoxazole and trimethoprim using zirconocene-based silica as pre-concentration/extraction systems. Química nova, São Paulo 34(2):206-212.

Poletto T, Muniz MFB, Baggiotto C, Ceconi DE, Poletto I (2014). Fungos associados às flores e sementes da nogueira-pecã (*Carya illinoinensis*). Rev. Ciênc. Ambient. 8(1):5-13.

Sá DAC, Santos GR, Furtado GQ, Erasmo EAL, Nasimento IR (2011). Transporte, patogenicidade e transmissibilidade de fungos associados às sementes de pinhão manso. Rev. Bras. Sement. Londrina 33(4):663-670.

Santos AF, Medeiros ACS, Santana DLQ (2001) Fungos associados às sementes de espécies arbóreas da mata atlântica. Bol. Pesqui. Flor. Colombo 42:57-70.

Silva FFS, Dantas BF (2013). Efeito da temperatura na germinação de sementes de *Sideroxylon obtusifolium* (Sapotaceae) de diferentes procedências. Rev. SODEBRAS 8(90):40-43.

Silva KB, Alves EU, Bruno RLA, Matos VP (2012). Morphological characterization of fruits, seeds and germination of *Sideroxylon obtusifolium* (Roem e Schult.) Penn. (Sapotaceae). Rev. Árv. 36(1):59-64.

Stange VS, Gomes TD, Andrade MAD, Batitucci MDCP (2009). Evaluation of mutagenic effect of crude hydroalchoolic extract, using in vivo alive bioassays and phytochemical screening of Cecropia glaziovii Sneth (embaúba), Cecropiaceae. Rev. Bras. Farmacogn. 19(2):637-642.

Teixeira H, Machado JC (2003). Transmissibility and effect of *Acremonium strictum* in maize seeds. Ciênc. Agrotecnol. 27(5):1045-1052.

Vechiato MH, Parisi JJD (2013). Importance of sanitary quality of seeds in forestry seedling production. Rev. Inst. Biol. 75(1):27-32.

Zanin JLB, Carvalho BA, Martineli OS, Santos MH, Lago JHG, Sartorelli P, Viegas JC, Soares MG (2012). The Genus *Caesalpinia* L. (Caesalpiniaceae): Phytochemical and Pharmacological Charact. 27(7):887-902.

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Full Length Research Paper

Hydropriming of pearl millet (*Pennisetum glaucum* L.) in Northern and Central Burkina Faso applying six hours of soaking and overnight drying of seeds

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Hydropriming of pearl millet seeds was tested during two growing seasons in Burkina Faso. A total of 32 field experiments were distributed equally between two agro-ecological zones: The Northern zone receiving on average less than 600 mm annual precipitation and the Central zone receiving 600 to 900 mm. Hydropriming was performed by soaking of seeds in water for 6 h, followed by air-drying overnight. In the Northern zone, an increase of both emergence and yield was observed for hydroprimed seeds in both years of testing. This was reflected by a higher yield observed in 13 out of 16 field experiments, increased median yield (+159 kg/ha; *p*<0.0053) and an increase of the relative yield by +29% as a field average (*p*<0.000054). In contrast, in the Central zone a net negative effect on crop emergence was observed in both years, and only 5 out of 16 field experiments showed a yield increase for hydroprimed seeds. Meteorological data confirmed the difference in rainfall between the two zones. Hydropriming by 6 h of soaking and drying of seeds overnight appears as a simple method to increase yield of pearl millet significantly in the most arid out of two agro-ecological zones tested in Burkina Faso. Drying of seeds overnight is a novel agronomically feasible approach, allowing a full day for subsequent sowing.

Key words: Seed-priming, hydro-priming, Sahel, millets, location-dependent effect.

INTRODUCTION

Hydropriming has been proposed as a seed treatment to improve crop establishment and yield (Harris, 1996; Harris et al., 1999; 2001 a, b). In general, the method includes soaking of seeds in water for a number of hours followed by a short (<2 h) drying of the seeds before sowing. The method has been studied mostly in subtropical/tropical and semi-arid areas where the method has been shown to improve crop establishment particularly under constraints of water deficiency and high temperatures (Harris, 2006; Navaz et al., 2013).

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In both finger millet and pearl millet, grain yield increases of 13 to 30% have been obtained by soaking of seeds for 8 to 16 h in water (Kumar et al., 2002; Aune and Ousman, 2011; Aune et al., 2012; Jidda and Anaso, 2017). Despite the simplicity and inexpensive nature of the methodology and despite several demonstrations of yield increases in millets, the technology has only sporadically been taken up by farmers. Lack of knowledge is probably not the only reason for this. The method has some practical drawbacks in terms of organizing the field work of sowing: The drying of seeds for 2 h before sowing has the implication that the physical work of sowing (typically by hand) cannot start immediately in the morning, where working conditions in the tropics are most convenient. In order to circumvent this, we tested the possibility of drying seeds overnight (16 to 20 h). In order to further maximize the flexibility of the method we also reduced the generally recommended time for soaking (8 h) to only 6 h since laboratory studies have shown that soaking of pearl millet seeds for just 6 h is sufficient to cause a strong and positive response in seedlings (germination and seedling vigour) (Akbar et al., 2009).

Two agro-ecological zones in Burkina Faso were chosen for the testing: The Northern zone, situated North of 13°N latitude receiving less than 600 mm precipitation per year and the Central zone situated between 11 to 13°N latitude receiving 600 to 900 mm precipitation per year on average (De Longueville et al., 2016). The Northern zone (synonymous with *Sahelian* zone) is primarily characterized by dry-land agriculture including pearl millet as the main crop (INSD, 2016). The Central zone contains the capital, Ouagadougou. In the surrounding rural districts pearl millet is second to sorghum in terms of the area cultivated (INSD, 2016). It has previously been shown that the effect of hydropriming is dependent on humidity of the soil (Harris, 2006). Given the difference in annual precipitation, it appeared relevant to compare the effect of hydropriming in the two zones.

MATERIALS AND METHODS

Seed material

For each of the two years (2015 to 2016) a seed sample of cultivar, You Local, propagated during the previous year, was used for field experiments. You local is a landrace originated from the village, You (Figure 1), and is propagated by INERA (Burkina Faso).

Seed treatment

Hydropriming was conducted by soaking of seeds for 6 h in pure water at ambient temperature (around 25°C). Before sowing, seeds were subsequently air-dried over-night (16 to 20 h). Air-drying was conducted by spreading of seeds in a uniform layer of 0.2 to 0.5 cm on a piece of cloth or on a layer of blotter paper (a water absorbing material). Non-soaked seeds were used as the control.

Experimental fields

For two subsequent growing seasons, 2015 to 2016, a total of thirty two field tests were carried out comparing non-treated seeds of pearl millet to seeds hydroprimed and dried as described above (Figure 1). On each field, two plots (25 rows, 5 m long) were laid out side by side, and sown with the two types of seeds, respectively. For each of the two years, sixteen field tests were conducted – eight in Northern Zone and eight in Southern zone, respectively. In 2015, experimental fields were located near three different villages in each zone; in 2016, fields were located near 4 different villages in each zone as indicated in Figure 1. Before sowing, ploughing was done by tractor at Kamboinsé (Research station), whereas at the other locations (farmers' fields) ploughing was done by donkey or cow. Sowing of seeds was done by hand at 5 to 7 cm depth, using six to eight seeds per seed hole. Seeds were sown in rows with distances of 0.80 m between rows, and 0.60 m between holes in the same row. This corresponds to a seeding density of approximately 146.000 seeds ha^{-1} (estimated). For each village, a common time point for seed treatment and sowing was chosen within the normal sowing period (June to July) taking local weather conditions and forecasts into account.

Management and measurements

Fifteen days after sowing, the number of emerged seedlings was reduced to a maximum of 3 seedlings per seed hole. The first weeding was done 15 days after sowing, and subsequently when needed (optional). Mineral fertilizer consisting of Nitrogen-Phosphate-Potassium (NPK 14-23-14) was applied at 100 kg ha^{-1} 15 days after sowing. Urea (50 kg ha^{-1}) was applied 30 days post-emergence. Three to four weeks after sowing crop emergence was determined visually by counting the percentage of seed holes populated with emerging plants. At harvest (October-December), grain was collected from the whole plots, and sun-dried for 2 weeks before weighing to determine the yield. Data of monthly precipitation (2015 to 2016) in the Northern zone was obtained from Direction Provincial of Agriculture and Hydraulic Facilities of Loroum (DPAAH-L). Corresponding meteorological data from the Central zone was obtained from Kamboinse Environmental, Agricultural and Training Research Center (CREAF-K).

Statistics

Mean and median values were calculated for both emergence (%) and yield (kg/ha). In addition, the relative yield (%) compared to the field average (average of both treatments within each field) was also calculated. Medians were compared using Wilcoxon test for related samples ($p<0.05$). Means were compared using t-test ($p<0.05$). Statistical software PAST (version 2.17c) was applied for the analysis (Hammer et al., 2001).

RESULTS

Effect of hydropriming in Northern zone

A positive effect of hydropriming was observed in both growing seasons (Table 1, Northern zone). In total (both years), the median emergence increased from 68.1 to 75.2%, and the median yield increased from 859 to 1018 kg/ha (+ 159 kg/ha). The latter finding was statistically significant ($p<0.0053$), and reflected an increase of the

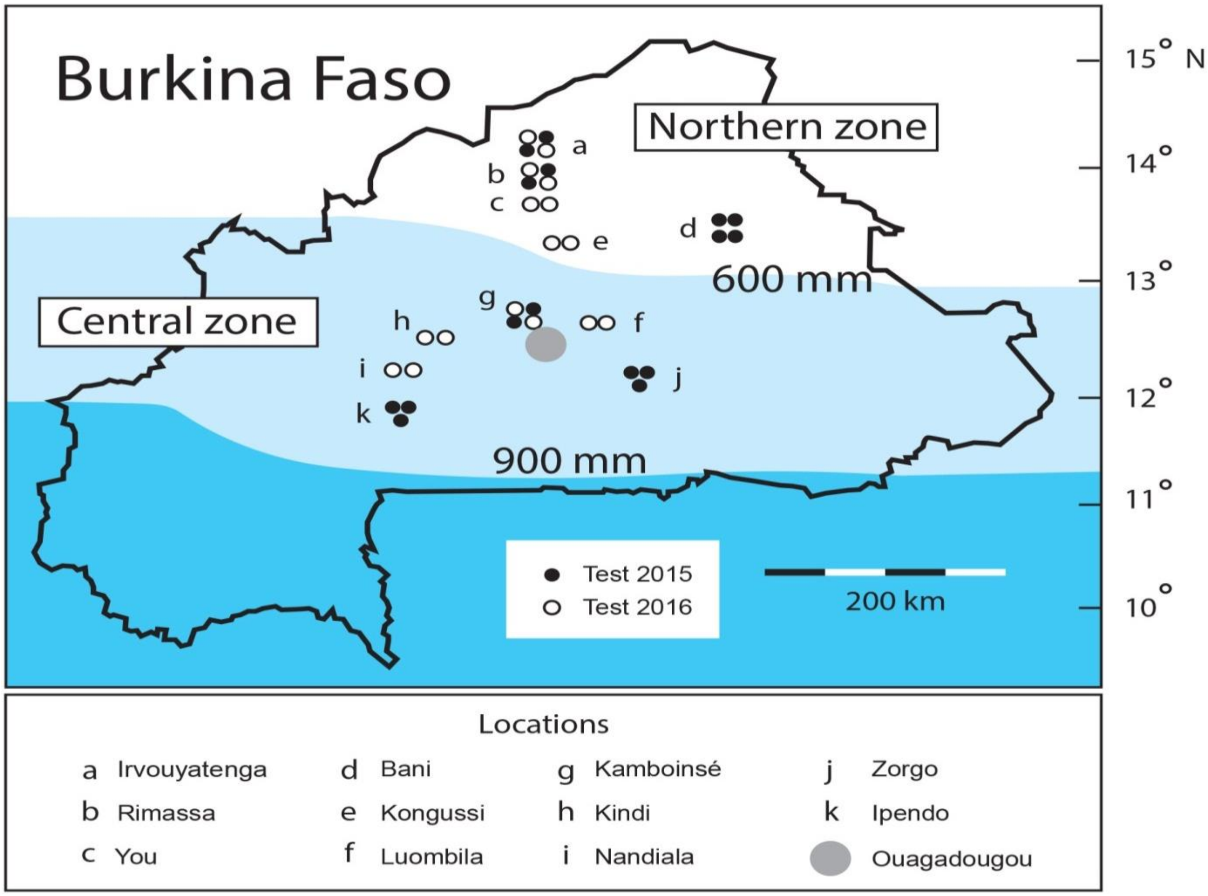

Figure 1. Field test locations 2015-2016.
Blue colors indicate average annual rainfall (De Longueville et al., 2016).

yield for hydroprimed seeds observed in 13 out of 16 fields. When yields were calculated relative to the field average, the overall effect of hydropriming was equivalent to a mean yield increase of 29% (Table 1; 112.6 relative to 87.4, p<0.000054).

Effect of hydropriming in Central zone

In the Central zone, only 4 out of 16 fields showed a positive effect of hydropriming on emergence and for the yield, an overall 7% decrease was observed (Table 1, 96.4 vs. 103.7, non-significant).

Effect of hydropriming in total (both zones) and meteorological data

By combining figures from both years and both zones (Table 1, Total), an overall yield increase of 9.4% (Table 1; 104.5 vs 95.5) was found as barely significant (*p*<0.049). No consistent change of emergence was observed (Mean emergence increased by approximately 1%, and median emergence decreased by approximately 2 %). Meteorological data obtained for 2015 and 2016 confirmed the expected difference between the two zones with regard to patterns of precipitation (Table 2). Both during crop establishment (June to July) and during the full growing season (June to October), the Northern zone received less rainfall than the Central zone in both years.

DISCUSSION

Comparison to other studies

In the present study, we found a reproducible and highly significant positive response of pearl millet to hydropriming in one of two agro-ecological zones tested: The Northern zone. In this zone, both emergence and yield were observed to increase in both years of testing (Table 1), and when data for the two years were combined, the effect on yield was very significant (Yield relative to field average was increased by 29% from 87.4 to 112.6, p<0.000054).

Table 1. Emergence and yield in field experiments (two zones, two years).

Variable	Northern zone		Central zone		Total	
	NoT	W-6	NoT	W-6	NoT	W-6
Emergence						
2015 (N=8 per zone) mean (%)	78.0	82.4	79.3	72.3	78.7	77.4
2016 (N=8 per zone) mean (%)	55.5	65.7	78.4	74.9	67.0	70.3
Total mean (%)	66.8	74.1	78.8	73.6	72.8	73.8
Total median (%)	68.1	75.2	77.6	72.4	75.1	73.4
Fields with higher emergence	ns	10 (16)	ns	4 (16)	ns	14 (32)
*p-value**	ns	ns	ns	ns	ns	ns
Yield						
Absolute (kg/ha)						
2015 (N=8 per zone) mean (kg/ha)	703	1019	1094	1213	899	1116
2016 (N=8 per zone) mean (kg/ha)	982	1247	973	841	978	1044
Total mean kg/ha	843	1133	1034	1027	938	1080
Total median (kg/ha)	859[a]	1018[b]	1064	1042	916.7	1031
Fields with higher yield	ns	13 (16)	ns	5 (16)	ns	18 (32)
*p-value**	ns	<0.0053	ns	ns	ns	ns
Relative (% of field average)						
2015 mean (%)	84.8	115.2	96.4	103.6	90.6	109.4
2016 mean (%)	90.1	109.9	110.9	89.1	100.5	99.5
Total mean (%)	87.4[a]	112.6[b]	103.7	96.4	95.5[a]	104.5[b]
*p-value***	ns	<0.000054	ns	ns	ns	<0.049

NoT = No treatment; W-6 = hydropriming (6 h).*Medians with same letters are not significantly different (Wilcoxon paired analysis, $p<0.05$). **Means with same letters are not significantly different (t-test, $p<0.05$).

Table 2. Precipitation in Northern and Central zone 2015-2016.

Variable	Precipitation (mm)			
	Early crop season (establishment) (June- July)		Whole growing season (June-October)	
	Northern zone*	Central zone**	Northern zone	Central zone
2015	211.0	346.4	629.0	837.0
2016	280.0	322.3	545.9	819.0
Average	245.5	334.4	588.0	828.0

*Climate station at Titao; ** Climate station at Kamboinsé.

In contrast, no consistent positive effect was observed in the Central zone, and the meteorological data confirmed a higher level of rainfall for this zone in the growing season (Table 2). The result of 29% yield increase obtained in the Northern zone (13 to 15°N latitude) is very similar to results obtained in two other countries in the Sahelian region with field experiments carried out at similar latitude. In neighbouring country Mali (13 to 14°N), a yield increase of 30% in pearl millet was found as a mean of 27 field experiments (Aune et al., 2012), and in Sudan (13.1 °N) a yield increase of 15% was found at a research station and 30% increase was found in farmers' fields as a mean of 25 repetitions (Aune and Ousman, 2011).

For the related cereal species, Sorghum, a recent study in Sudan (at 12°N) found a manifest effect on yield (27% to 76% increase) using hydropriming of seeds in areas having around 600 mm annual rainfall; the strongest effect was observed in plots also receiving a high dose of mineral fertilizer, and all seeds were treated with pesticide after drying (Abdalla et al., 2015). All the three aforementioned studies, have applied 8 h of soaking and

1 to 2 h for drying of seeds.

Thus, with respect to the Northern zone of Burkina Faso, the results presented here is in line with previous positive results for both millet and sorghum in the Sahelian region (receiving close to 600 mm rainfall or less per year). At the same time, this study provides a new, flexible protocol applying a shorter soaking time (6 h) and drying of seeds overnight, thereby allowing sowing to start early next day. However, the negative results obtained in the Central region clearly indicate that agro-ecological differences should be taken into account with regard to development and evaluation of seed treatment technology – including hydropriming. The reason for the negative results obtained in the Central zone is unknown at present, but the meteorological data confirmed the higher level of rainfall during the period studied in the Central zone.

Previously, it has been reported that higher soil humidity is associated with a lower effect of hydropriming in pearl millet (Harris, 2006), and in other cereal crops (wheat and sorghum) a weak response to hydropriming was reported for areas and for years with high precipitation (Harris et al., 2005; Ramamurthy et al., 2005).

Theoretical context of results

The general physiological effects of hydropriming have recently been reviewed (Lutts et al., 2016). The increase of the water potential in seeds caused by the soaking allows biochemical processes preparing the seed for germination to initiate: Mobilisation of energy and nutrients through the activity of amylases and other metabolic enzymes, and repair of cellular damage that occurred during storage of the seed (DNA, membranes, organelles, redox balances). This water-induced state of “readiness” for germination is maintained after the seeds have been dried, and phenotypically hydropriming is observed as the shortening of the time needed for the seeds to emerge after sowing.

As reviewed by Harris (2006), shortening of the time to emerge is critical to crop establishment, particularly in dry and hot areas since pre-emerged seedlings are especially susceptible to drought and heat (upper soil temperatures in Sahel may easily exceed 45°C). In addition, the risk of sun-mediated hardening of the soil surface (formation of surface crusts preventing seedlings from emerging) increases with the time needed for emergence.

Thus, the overall finding in the present study of a strong effect of hydropriming being evident in the most solar-exposed and dry zone is in full agreement with existing theory of crop physiology and seed hydropriming. However, it is still somewhat surprising that the observed effect declines to zero (or even less) in the more humid and central zone, within a distance of less than 200 km. We cannot exclude that some statistical variation contributes to this result, since we have only 16 observations in each zone. However, a clear difference between the two zones is evident.

Perspectives

Pearl millet is the dominant crop in the Northern zone of Burkina Faso, and the previous results from similar areas of Sahel (Aune and Ousman, 2011; Aune et al., 2012; Jidda and Anaso, 2017) together with the results presented here strongly encourage a participatory approach with farmers of millet in Northern Burkina Faso in order to stimulate uptake of this inexpensive and simple technology. Based on the experience from the present study, we expect the more flexible hydropriming protocol described to be appreciated by farmers as both technically feasible and as being in a good compliance with the organizing of field work in general. A participatory research approach could focus on testing hydropriming using farmers own seeds (in the present study, one large seed sample was tested per year on all fields). Similarly, with regard to management of the crop (ploughing, sowing, fertilization, weeding, etc.), farmers own practice could be followed in order to obtain results as close to the real-life situation as possible.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Abdalla EA, Osman AK, Maki MA, Nur FM, Ali SB, Aune JB (2015). The response of sorghum, groundnut, sesame and cowpea to seed priming and fertilizer micro-dosing in South Kordofan. Agronomy 5:476-490.

Akbar M, Ahmad B, Arif M, Ahmad G, Shah Z, Jianhua W (2009). Waterabsorption and priming with osmotica responses on germination of pearl millet cultivars. Sarhad J. Agric. 25:7-13.

Aune JB, Ousman A (2011). Effect of seed priming and micro-dosing of fertilizer on sorghum and pearl millet in Western Sudan. Exp. Agric. 47:419-430.

Aune JB, Traoré CO, Mamadou S (2012). Low-cost technologies for improved productivity of dryland farming in Mali. Outlook Agric. 41:103-108.

De Longueville F, Hountondji YC, Kindo I, Gemenne F, Ozer P (2016). Long-term analysis of rainfall and temperature data in Burkina Faso (1950-2013). Int. J. Climatol. 36:4393-4405.

Hammer O, Harper DAT, Ryan PD (2001). PAST: Paleontological statistics software package for education and data analysis. Palaeontol. Electr. 4(1):9.

Harris D (1996). The effects of manure, genotype, seed priming, dept and date of sowing on the emergence and early growth of Sorghum bicolor (L.) Moench in semi-arid Botswana. Soil Till. Res. 40:73-88.

Harris D (2006). Development and testing of "On-farm" seed priming. Adv. Agron. 90:129-178.

Harris D, Breese WA, Kumar Rao JVDK (2005). The improvement of crop yield in marginal environments using "on-farm" seed priming: nodulation, nitrogen fixation, and disease resistance. Austr. J. Agric. Res. 56:1211-1218.

Harris D, Joshi A, Khan PA, Gothkar P, Sodhi PS (1999). On-farm seed priming in semi-arid agriculture: development and evaluation in maize, rice and chickpea in India using participatory methods. Exp. Agric. 35:15-29.

Harris D, Pathan AK, Gothkar P, Joshi A, Chivasa W, Nyamudeza (2001a). On-farm seed priming: using participatory methods to revive and refine a key technology. Agric. Syst. 69:151-164.

Harris D, Raghuwanshi BS, Gangwar JS Singh SC, Joshi KD, Rashid A, Hollington PA (2001b). Participatory evaluation by farmers of on-farm seed priming in wheat in India, Nepal and Pakistan. Exp. Agric. 37:403-415.

Institut National de la Statistique et de la Démographie (INSD) (2016). Burkina Faso, On-line Publication: Annuaire Statistique National 2015. http://www.insd.bf/n/contenu/pub_periodiques/annuaires_stat/Annuaires_stat_nationaux_BF/Annuaire_stat_2015.pdf

Jidda MB, Anaso AB (2017). Effects of crop improvement technologies on downy mildew of pearl millet [*Pennisetum glaucum* (L.) R. Br. J. Cereals Oilseeds 8:14-20.

Kumar A, Gangwar JD, Prasad SC, Harris D (2002). On-farm seed priming increases yield of direct-sown finger millet in India. Int. Sorghum Millets Newsl. 43:90-92.

Lutts S, Benincasa P, Wojtyla L, Kubala SS, Pace R, Lechowska M, Quinet M, Garnzarska M (2016). Seed primimg: New comprensive approaches for an old empirical technique. Agricultural and Biological Sciences » "New Challenges in Seed Biology - Basic and Translational Research Driving Seed Technology". Dr. Susana Araújo (Ed.) and Alma Balestrazzi (Ed.), InTech, DOI: 10.5772/64420. https://www.intechopen.com/books/howtoreference/new-challenges-in-seed-biology-basic-and-translational-research-driving-seed-technology/seed-priming-new-comprehensive-approaches-for-an-old-empirical-technique

Navaz J, Hussain M, Jabbar A, Nadeem GA, Sajid M, Subtain MU, Shabbir I (2013). Seed priming a technique. Int. J. Agric. Crop Sci. 6:1373-1381.

Ramamurthy V, Gajbhiye KS, Venugopalan MV, Parhad VN (2005). On-farm evaluation of seed priming technology in sorghum (*Sorghum bicolor* L.). Agric. Trop. Subtrop. 38:34-40.

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The effects of humic acid on growth and ion uptake of mung bean (*Vigna radiata* (L.) Wilczek) grown under salt stress

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The study aimed to determine the effects of humic acid (HA) on seedlings of a salt-sensitive plant, mung bean (*Vigna radiata* (L.) Wilczek), grown on 50 mM (S1) and 100 mM (S2) NaCl concentrations. In controlled room conditions, mung bean seedlings were planted in pots containing torf and perlite mixture and salt effects were observed. The growth parameters were plant height, number of leaves, leaf area, leaf and stem fresh and dry weights in which all parameters significantly decreased at both salt levels. Nutrient analyses of Na, K and Ca were conducted by flame photometry (FP), and Mg, Mn, Zn were tested by inductively coupled plasma atomic emission spectrometry (ICP-AES), for the above and below ground parts of mung bean seedlings. Both salt treatments increased Na content significantly, however 10 ml addition of HA to those samples (S1HA and S2HA; 50 mM NaCl and 100 mM NaCl, respectively) caused reductions in Na contents of the above-ground parts of mung bean compared to plants watered with only Hoagland-Arnon solutions (HO). On the other hand, in the roots of mung bean seedlings, Na content rose significantly in S2 compared with control, but the amount of Na in S2HA significantly increased compared with S2 treated plants. K content was significantly decreased in both salt concentrations, while SHA1 and SHA2 caused slight increases in both above and underground parts of seedlings. In the experiment, SHA2 increased the content of K, Mg, Ca, Mn and Zn in the root of mung bean seedlings compared with both S1 and S2 treatments.

Key words: NaCl, mung bean, growth, nutrient *Vigna radiata*, humic acid.

INTRODUCTION

On a global basis, soil salinity is one of the stress factors prevalent in arid and semi-arid zones. It inhibits plant growth and development, resulting in serious reduction in yield, particularly on salt-sensitive plants (Example glycophytes) (Grattan and Grieve, 1998; Allbed and Kumar, 2003; Pessarakli and Szabolcs, 1999; Pitman and Lauchli, 2002; Asik et al., 2009; Amirjani, 2010). Salt-affected lands were first recorded in human agricultural

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history in aluvial soils of the Tigris (Mesopotamia), in present day it is Iraq (Russel et al., 1965, Pitman and Lauchli, 2000).

According to the Food and Agricultural Organization (FAO, 2005) more than 800 million ha is affected by salinity worldwide, with saline and sodic pressure affecting 23 and 37% of cultivated lands, respectively. Salt affected areas extend widely over more than 100 countries across all continents, equating to 10% of arable areas (Szabolcs, 1989). In Turkey, 1,513,645 ha of agricultural lands face salinity and alkalinity pressure due to mismanagement of irrigation and inefficient drainage systems, as well as features of water quality (Dinc at al., 1993).

Salinity plays a major role in plant growth in many ways, including physiological and biochemical mechanisms that affect cell level processes. These include inhibiting osmotic regulation, reducing stability of nutritional balance and specific ion toxicity (Neumann,1997; Yao, 1998; Alam, 1999; Jacoby, 1999; Hasegewa et al., 2000; Munns, 2002), and in the growing tissue salt impairs the supply of assimilates of photosynthesis as well as hormones (Munns, 1993).

The many salt-related studies have ranged from seed germination effects to whole plant growth, physiological to anatomical changes, biochemical composition differences to biomass and yield reduction and covered a very wide range of plant species (Niazi et al., 1987; Katerji et al., 1994; Poljakoff-Mayber et al., 1994; Villiers et al., 1994; Rogers et al., 1995; Reinhardt and Rost, 1995 a, b; Zidan and Elewa, 1995; Huang and Redmann, 1995; Wahid et al., 1998; Croser et al., 2001; Al-Mutawa, 2003; Turhan and Ayaz, 2004; Song et al., 2008; Maial et al., 2010; Ruffino et al., 2010).

Although Tavakkoli (2010) stated that most plants are thought to accumulate both Na^+ and Cl^-, Neumann et al. (1988) concluded that salt (particularly Na^+) toxicity in various plants has visible symptoms such as leaf burn, necrotic spots on leaves and limited expansion of leaf cells, particularly in salt sensitive plants. Studies have covered a very wide range of salt applications, such as NaCl, Na_2SO_4, $CaCl_2$, $MgCl_2$, individually or in various mixture compositions, looking at effects on seeds and propagule germination, commonly focusing on seed plant growth-development (Example, biomass) and physiological changes, such as nutrient composition in varied plant tissue and organs in different plant species (Jamil et al., 2005; Sagib, 2006; Turan et al., 2007a, b; Saffan, 2008; Rui, 2009; Turan et al., 2010; Memon et al., 2010).

Mung bean is a very sensitive plant to salt (Maas and Hoffman, 1977; Ashraf and Rasul, 1988) and an important crop widely grown in the Indian subcontinent, South East of Asia, Africa, South America and Australia. In South Asia, annual mung bean production is 3.1 million tonnes and it is grown on an area of 3 million ha under rainfed and irrigated conditions. It is consumed cooked, fermented, roasted etc. and has a high protein content (26.4 g/100 g dry weight) from the Fabaceae family (Ashraf and Rasul, 1988; Oplinger et al., 1990; Malik, 1994; Shangyandram, 2009; Mondal et al., 2012; Waqas et al., 2014) and 4.5 g ash, 1.75 g fat, 6.15 g crude fiber and 61.2 g carbohydrates per 100 g dry weight (El-Adawy et al., 2003).

In Turkey, mung bean is grown in South East Anatolia, particularly in Gaziantep and its environs, although there are no statistical figures on yield and cropping area (Akdag, 1995). There is some research on mung bean yield in relation to agronomical, morphological, and phenological yield components (Toker et al., 2002; Canci and Toker, 2005;2014) as well as genotypic study of its varieties (Peksen et al., 2015).

Humic substances have been widely used for agricultural research, affecting the quality of soil as well as yield quantity. Humic acids are responsible for pH adjustment, enhancing soil cation exchange capacity and extending the survival mechanism of plants grown under stress conditions such as salinity, drought and harmful effects of toxic and heavy metal elements in the soil (Fagbenro and Agboda, 1983; David et al., 1994; Stevenson, 1994; Dursun et al., 1998; Pilanali and Kaplan, 2003; Sharrif, 2002; Kolsarici et al., 2005; Fong et al., 2007; Buyukkeskin and Akinci, 2011; Khaled and Fawy, 2011)

In the present study, the salt-sensitive plant mung bean was subjected to the following treatments: a set of replicates watered only with Hoagland and Arnon (1950) solution (HO); two experimental sets exposed to 50 and 100 mM salt (NaCl) concentrations; and the remaining two sets of replicates exposed to the same two salt solutions with added 10 ml humic. The five different growth media replicates were tested for growth and physiological changes in mung bean seedlings, to determine the remediative role of humic acid on growth parameters and the mineral composition of both under and above ground parts of seedlings in all treatments.

MATERIALS AND METHODS

The study species

Superdivision: Spermatophyta; Division: Magnoliophyta; Order: Fabales; Family: Fabaceae (Leguminosae) Pea family; Genus: *Vigna* Savi (cowpea); Species:*Vigna radiata* (L.) R. Wilczek (mung bean)

Growing method under growth room conditions

The experiments were carried out in controlled growth room conditions in the department of Biology, Faculty of Arts and Sciences, Marmara University. Mung bean (*Vigna radiata* (L). Wilczek) seeds were obtained from Nickys Nursery Ltd. (SPR006) Kent, UK. The used liquid humic acid (Premium is a trade product of Turpex LTD, Turkey) contained organic, humic and fulvic acids and water soluble K_2O in ratios given in Table 1. For germination,

Table 1. Components of HA.

Components	Volume (%w/w)
Total organic substances	12
Total HA+ fulvic acid	16
Water-soluble K_2O	3

Table 2. Experimental salt and Humic acid preparations.

Experimental Groups	Contents and preparation
Control (C)	Full strength Hoagland solution (Hoagland-Arnon, 1950)
50 mM NaCl (S1)	50 mM NaCl is dissolved in HO solution and volume made up to1 L
100 mM NaCl (S2)	100 mM NaCl is dissolved in HO solution and volume made up to 1 L
50 mM NaCl + Humic acid (S1HA)	50 mM NaCl is dissolved in HO contained 10 ml humic acid and volume made up to 1 L
100 mM NaCl + Humic acid (S2 HA)	100 mM NaCl is dissolved in HO contained 10 ml humic acid and volume made up 1 L

mung bean seeds were kept overnight in a beaker containing distilled water and then transferred onto filter paper in the base of Petri dishes and covered for a week. Each germinated seed was planted in a plastic pot containing 280 g torf (Gardol) and perlite mixture in a 3:1 ratio, respectively. The five treatments were arranged in a completely randomized block design with eight replicates as follows: the control (C) contained only Hoagland and Arnon (1950); two salt concentrations (50 and 100 mM NaCl as S1 and S2, respectively); and humic treatments with 10 ml humic acid in addition to the same salt doses (S1HA and S2HA) (Table 2). The pots, with a seedling in each, were set up as blocks using a completely randomized method (Mead and Curnow, 1983) at 23±2ºC. The moisture level of the mixture was maintained at 55%±5 and sets were exposed to 4000 to 4200 lux plant fluorescence intensity for 14/10 day and night periods, respectively (Akinci et al., 2009). Growth parameters were determined at the time of harvesting, 55 days after planting, by measuring seedling heights (PH), number of leaves (NL), leaf area (LA), and fresh weights of leaves (FLW), stems (FSW) and roots (FRW). Dry weights of these parts (DLW, DSW, DRW) were evaluated after 24 h in a drying oven to fully remove tissue water in the parts.

Nutrient analyses

The dried samples of leaves and roots were prepared by the wet-ashing method after Kacar (1972). Dried parts of plants were crushed using a mortar and pestle until powdered. The powder was put in an Erlenmeyer flask and a mixture of 6 ml nitric acid + perchloric acid was added. The mixtures were digested for 30 min at 40ºC in a water-bath and the supernatant solution was removed by heating at 150 to 180ºC until 1 ml extract remained. The residue was treated with distilled water to dissolve and made up to 100 ml in coloured bottles. The elements Na, K, and Ca were determined by flame photometry, and Mn, Zn and Mg by ICP-AES.

Statistical analysis

All data from the eight randomized replicates of each treatment, including controls, were analyzed with SPSS (13.0 for Windows) for paired-sample T test. Growth and nutrient data were considered to be significant at the level of $P < 0.05$ (5% significance level for differences between means). Means are indicated with standard error (± S.E.) given in tables and shown as error bars in graphs.

RESULTS AND DISCUSSION

Effect of NaCl and HA on growth parameters and of mung bean seedlings

The results of tested growth parameters for the two levels of NaCl, controls (Hoagland) and humic acid treatments are presented in Table 3. Both salt concentrations (50 and 100 mM NaCl) significantly decreased plant height 39.9 cm and 41.7 cm, respectively compared to HO. Many studies had reported and agreed that salt stress caused similar plant height reduction in watermelon (Yetisir and Uygur, 2009); in pepper (Aktas et al., 2006); in maize (Irshad et al., 2002); in muskmelon (Carvajal et al., 1998); in tomato (Casierra-Posada et al., 2009); in common bean (Beltagi et al., 2006); in red-osier dogwood (*Cornus sericea*) (Mustard and Renault, 2006); in common bean (Gama et al., 2007).

In the study, HA caused an increase in plant heights in both salt treated plants, which were 17% and 9.8% taller in S1HA1 and S2HA2, respectively compared to S1 and S2, but these differences were not significant at the level of α=0.05 (Table 3). Numerous reports are available on the positive effect of HA on the growth of a wide variety of plants such as corn (*Zea mays* L.) (Tan and Noparmornbodi, 1979; Eyheraguibel et al., 2008), sunflower (*Helianthus annuus* L.) (Kolsarici et al., 2005), marigold (*Tagetes patula* v. Antigua Gold F1), pepper (*Capsicum annuum* grossum v. King Arthur), strawberry (*Fragaria ananassa* v. Tribute) and tomato (*Lycopersicon esculentum* v. Rutgers) (Arancon et al., 2003).

However, reports of HA addition to plants grown under salinity are limited, with positive effects on growth and

Table 3. Growth parameters of mung bean seedlings under salt stress and humic acid.

Growth parameters	Treatments				
	Control	S1	S2	S1HA	S2HA
Shoot heights (PH) cm	37.36±4.320	22.45±2.422*	21.79±1.377*	26.28±2.843	23.93±1.665
Root lenghts (RL) cm	22.10±2.096	16.34±1.255	16.33±0.943*	24.83±1.255**	23.33±1.632***
No of leaves (NL)	4.0±0.327	2.7±0.313*	2.6±0.183*	3.2±0.164	2.9±0.227
Leaf area (LA) cm^2	140.85±0.227	67.96±7.66*	61.55±12.23*	84.90±7.42	78.73±17.12
Leaves fresh weight (LFW) (g)	2.03±0.317	0.77±0.121*	0.78±0.121*	1.52±0.281**	1.16±0.227
Leaves dry weight (LDW) (g)	0.21±0.034	0.10±0.015*	0.10±0.033*	0.15±0.030	0.11±0.026
Stem fresh weight (SFW) (g)	1.52±0.289	0.70±0.098*	0.58±0.126*	0.85±0.115*	0.79±0.178
Stem dry weight (SDW) (g)	0.13±0.015	0.06±0.005*	0.05±0.008*	0.08±0.008**	0.07±0.011
Root fresh weight (RFW) (g)	0.80±0.146	0.41±0.072	0.35±0.060*	0.57±0.100	0.50±0.095
Root dry weights(RDW) (g)	0.04±0.004	0.03±0.006*	0.02±0.005*	0.04±0.008	0.03±0.006

±: Standard errors *: Significantly different from controls **: Significantly different from S1
***: Significantly different from S2

plant height reported by Unsal (2007) and Daur and Bakhashwain (2013) in chick pea; Sani (2014) in Canola; David et al. (1994) in tomato; Refaiy et al. (2016) in banana plantlets. Delfine et al. (2005) stated that in durum wheat, HA content caused increase in plant heights. Gulser et al. (2010) also stated that lower doses (1000 and 2000 mg kg^{-1}) of HA had a positive effect on pepper seedling growth under saline conditions, although higher HA application rate (4000 mg kg^{-1}) had a negative effect.

Root length of *Vigna* seedlings decreased in both salt treatments by 35.3 and 35.4% for S1 and S2 respectively, whereas the latter was significant at α=0.05 compared to controls (HO). HA treatment caused a significant increase in root length compared with S1 and S2, by 51.9 and 42.9% in S1HA and S2HA, respectively. Available reports stated that various salts caused a decrease in germination rate, root and shoot length in *Pisum sativum var., abyssinicum and Lathyrus sativus* (Tsegay and Gebreslassie, 2014), reduction of root length in tomato plants (Turkmen et al., 2002), root development inhibition in *Arabidopsis* and tobacco (Jang et al., 2007), and decrease in root length of pumpkin (Kurum et al., 2013); *Raphanus sativus* L. (Jamil et al., 2007).

However, humic acid addition caused an increase in length and dry weight of maize plant roots (Eyheraguibel et al., 2008) and root length in *H. annuus* L. (Kolsarici et al., 2005), stimulated root development in ryegrass (Bidegain et al., 2000), increased root biomass in broad bean (Buyukkeskin et al., 2015), increased root growth in pepper (Cimrin et al., 2010). These studies all agree with the present findings of an ameliorative effect of humic acid on root growth and development of mung bean.

According to David et al. (1994) humic substances promote plant growth through better-developed root systems that provide more minerals to the whole plant. Leaf number (NL) decreased in both salt treatments in the present experiment, with significant reductions compared to control plants leaf number (P= 0.002 and P= 0.014, < α= 0.05, in S1 and S2, respectively). Our results showing that NaCl has an inhibiting effect on plant growth parameters and reduction in developing parts of seedlings such as decreasing of leaves are similar to related works on corn (Irshad et al., 2002); *Pennisetum clandestinum Hochst* (Muscolo et al., 2003), tomato (Romero-Aranda et al., 2001; Lopez and Satti, 1996; Caines and Shennan, 1999), melon (Morabito et al., 1996).

On the other hand, with 50 and 100 mM humic acid addition to the salts, NL increased by 18.2 and 9.5% in S1HA and S2HA, respectively, although these did not differ statistically from the salt treatments. Similar effects of humic acid had been documented by various reseachers in agreement with the present study in plants such as tomato and eggplant (Dursun et al*.,* 1998), pepper (Cimrin et al., 2010), tomato (Turkmen et al*.,* 2004), and common bean (*Phaseolus vulgaris* L.) (Meganid et al., 2015).

Mean leaf area (LA) significantly decreased in both 50 and 100 mM NaCl treatments, with reductions of 51.8 and 56.3% ($p=0.010$; $p=0.001 < α=0.05$), respectively compared to HO. Some reports state that a reduction in LA is a result of harmful effects of salt in various plants such as eggplants (Yasar, 2003); pepper hybrids (Chartzoulakis and Klapaki, 2000); tomato (Casierra-Posada et al., 2009); *Phaseolus* (Bayuelo-Jimenez et al., 2012; Aydin et al., 2012); *R. sativus* L. (Jamil et al., 2007); *Vicia faba* L. (Qadas, 2011); and *Solanum lycopersicum* L. (Casierra-Posada et al., 2009).

The obtained data from the present experiment showed that with HA addition to the two different salt concentrations, LA of those seedlings increased 24.9 and 27.9% but neither of them was significant. A positive effect of humic acid was obtained by Aydin et al. (2012) in *Phaseolus*; Dursun et al. (1998) in tomato and eggplant; and Buyukkeskin and Akinci (2011) in broad bean grown under saline conditions.

The leaf fresh weights in the present experiment were significantly less in S1 and S2 treatments, at 0.772 g ($P=0.006 < \alpha=0.05$) and 0.784 g ($P= 0.009 < \alpha=0.05$) respectively, compared with weights in control plants of 2.031 g. For plants treated with humic acid, the fresh weights increased significantly by 98.1% in S1HA ($P=0.044 < \alpha=0.05$), while the fresh weight increase in S2HA was 48.5%, although this did not differ significantly at the level of $\alpha=0.05$. The obtained results from the present study is similar to studies on different plants such as potato (Heuer and Nadler, 1995); tomato (Lopez and Satti, 1996); pumpkin (Kurum et al., 2013); *R. sativus* L. (Jamil et al., 2007).

The mean leaf dry weights of mung bean seedlings decreased significantly in both S1 and S2, by 52.8% ($P=0.006 < \alpha=0.05$) and 51.4% ($P=0.049 < \alpha=0.05$), respectively compared to controls. This result agreed with various studies such as Dolatabadian et al. (2011) for soybean; Taban et al. (1999) for *Z. mays* L. cvs.; Rodriguez et al. (2005) for *Asteriscus maritimus*; Ramoliya and Pandey (2002) for *Salvadora oleoides*; Tuncturk et al. (2008) for *Glycine max* L. Merrill; and Reina-Sanchez et al. (2005) for tomato.

In our results, with HA addition to salt treatments LDW increased in S1HA (48%) and S2HA (10.8 %), however both increase were less than control plants without any significance at the level of $<\alpha=0.05$. Stem fresh (SFW) and dry weights (SDW) significantly decreased in both salt treatments. The decrease in fresh stem weights was 53.7% (P= 0.025) and 61.2% (P=0.013), while dry weights of mung bean leaves were 53.4% (P= 0.004) and 60.2% (P=0.003) in S1 and S2, respectively compared to HO.

Reduction in shoot or stem fresh and dry weights as a result of salinity had been reported for different plant species (Adams, 1988; Ashraf and McNeilly, 1990; Mishra et al., 1991; Zaidi and Singh, 1993; Satti and Al-Yahyai, 1995; Ashraf and O'leary, 1997; Leidi and Saiz, 1997; Caines and Shennan 1999; Ashraf et al., 2001; Kaya et al., 2001; Cakir, 2004; Jamil et al., 2007; Turkmen et al., 2008; Tuncturk et al., 2008; Qados, 2011; Kurum et al., 2013).

For plants treated with HA, both fresh and dry weights of shoots were higher in HA1 and HA2 than plants grown only in NaCl (S1, S2), and the increase in fresh weights were 20.6% in S1HA and 35.8% in S2HA. On the other hand, the dry weights of S1HA significantly differed from S1 with a 41.9% (P=-0.017) increase, and S2HA increased by 30.2% compared to S2. Regarding positive effects of HA on salt treated plants, some reports state that stem/shoot weight increase occured in eggplant (Dursun et al., 1998); in *Chrysanthemum indicum* L. (Mazhar et al., 2012); in *Linum usitatissimum* L. (Bakry et al., 2014); in banana plantlets (Refaiy et al., 2016); and in pepper (Gulser et al., 2010).

Fresh weights of roots decreased by 48.4% in S1 but did not differ significantly. However, the root fresh weight decreased (57%) significantly (P=0.018) in S2 compared to controls. A decrease in root fresh weights have been recorded in various plants grown under salinity such as wheat and barley (Adiyaman, 2005); tomato (Lopez and Satti, 1996); Pumpkin (Kurum et al., 2013). The present study revealed that for mung bean seedlings grown with salt and HA together (S1HA and S2HA), RFW increased in both HA treatments, with a 40% and 43.4% increase in S1HA and S2HA, respectively, however, these differences were not significant.

On the other hand, dry weight of root decreased similarly in both salt treatments (S1, S2) and increased with HA treatment of mung bean seedlings (S1HA, S2HA). The decrease in S1 and S2 was significant, at 40 and 55.6% (P= 0.001; P= 0.008), respectively. The findings of the present study is supported by various reports that salinity causes root dry weight reductions in different plants such as soybean (Tuncturk et al., 2008); maize (Ashraf and McNeilly, 1990); spring wheat (Ashraf and O'leary, 1997); pepper (Turkmen et al., 2008).

Regarding the remediative role of humic acids on mung bean root dry weights, although weights increased in S1HA by 33.3% and in S2HA by 70%, neither differed significantly at the level of $\alpha=0.05$. These results agree with the research articles on pepper (Cimrin et al., 2010; Gulser et al., 2010); and common bean (Aydin et al., 2012; Meganid et al., 2015).

Effects of HA on nutrient content of the mung bean seedlings

The measured nutrients, namely Na, K, Mg, Ca, Mn and Zn, were determined separately as root and above (shoot) parts of mung bean seedlings grown in C, S1, S2 and S1HA and S2HA. All results from the present study were evaluated and presented in Figures 1 and 2 as mg/g (Mn and Zn µg/g).

Sodium

Na concentration was the highest of the analyzed elements in both above and underground parts of mung bean, with higher concentrations in above ground parts than the roots.

1. **Roots:** The concentration of Na in roots increased 30.4% in S1 plants; however, this was not significant at the level of $\alpha=0.05$. The increase in S2 roots was significant ($p=0.047 < \alpha=0.05$), although the rise was slightly lower than S1 (Figure 1). Na accumulation in roots of S1HA plants was slightly increased compared to HO plants. Na content was highest in S2HA with the increase significant compared to S2.

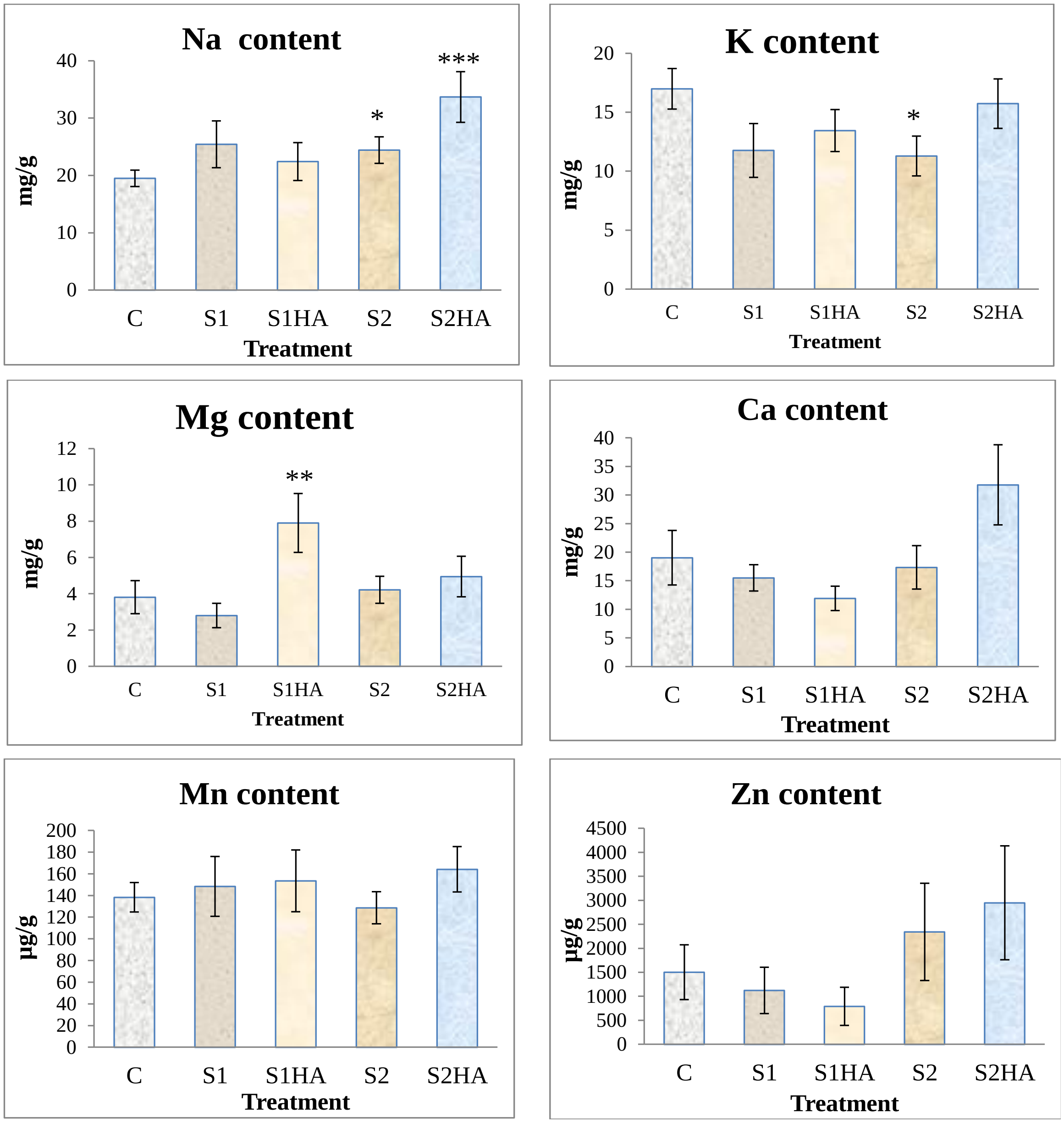

Figure 1. Mineral contents in mung bean root,.*: Significantly different from controls, **: Significantly different from S1, ***: Significantly different from S2.

2. **Shoots:** The tested Na amount in leaves and shoots together increased in all salt and humic-salt treatments compared to HO. Na content increased up to 40 and 60.7% in S1 and S2, respectively, and both were significant (p=0.01 < α=0.05). Humic addition in S1HA and S2HA caused a decrease of Na by 17.4 and 13%, however the decline was not significant in either case (Figure 2).

Potassium

K content was less than the amount of Na and was higher in upper parts of plants than in roots.

1. **Roots:** K content decreased significantly in both salt treated experimental groups by 30.8% (p=0.045 < α=0.05) and 43.6% (p=0.03< α=0.05) compared to controls (Figure1). Salt with humic treatments caused a slight increase in K in S1HA and S2HA, with rises of 14.3 and 39.4%, respectively, but neither was significant.

2. **Shoots:** The amount of K decreased with increasing salt concentration. The decreases were 28.8 and 38.9% in S1 and S2, respectively (Figure 2). K reduction in both salt treatments was significant at the levels of p=0.009 <

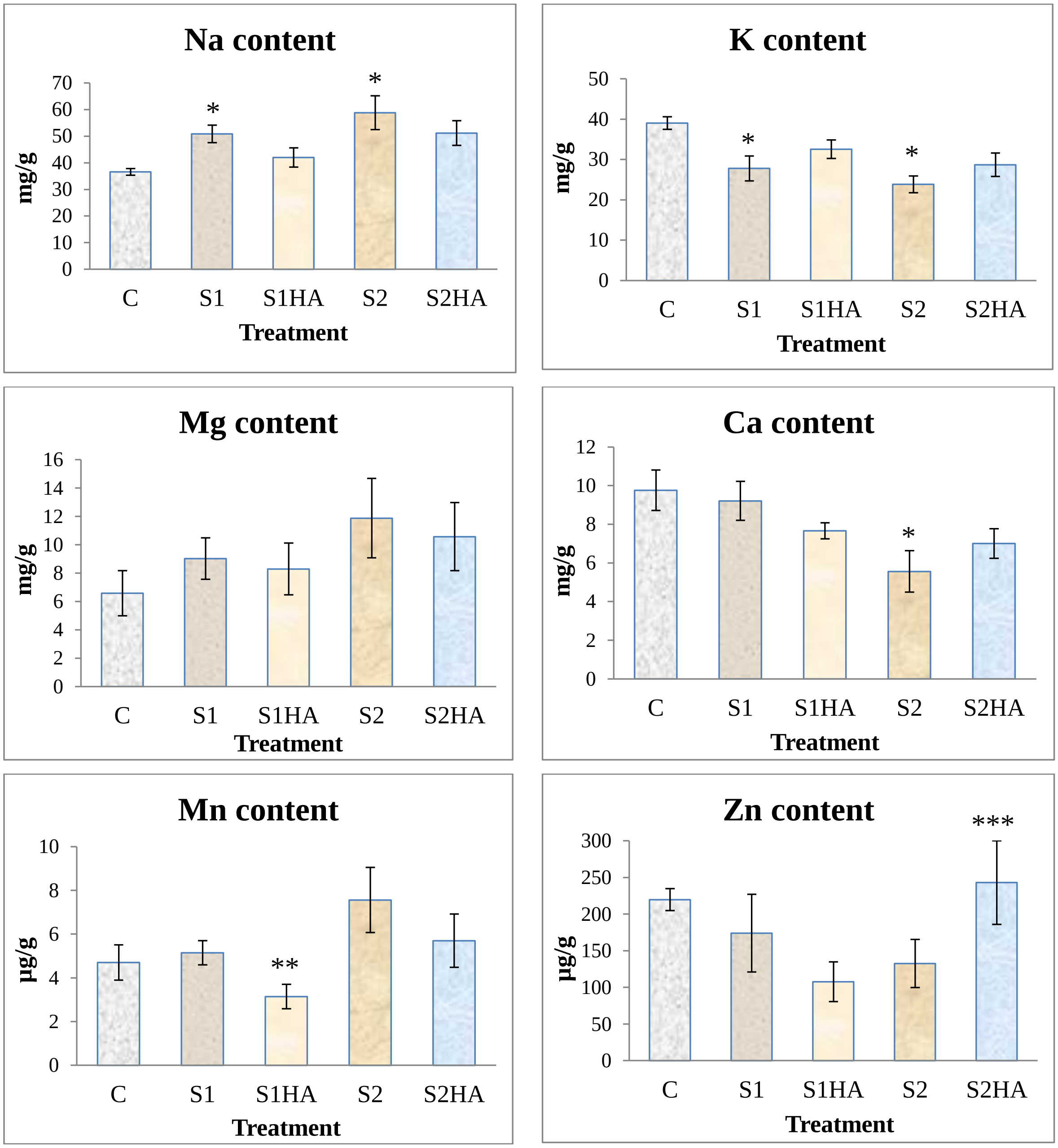

Figure 2. Mineral contents of above part of mung bean, *: Significantly different from controls, **: Significantly different from S1, ***: Significantly different from S2

α=0.05 for S1 and p=0.001< α=0.05 for S2. Humic acid addition to both salt treatments caused the K content to increase in S1HA and S2HA compared to S1 and S2 but both humic plus salt treatments decreased K compared to control values without any statistical significance.

Magnesium

Mg content was the lowest of the elements tested, after Zn and Mn, in both root and above parts of *Vigna* seedlings.

1. **Roots:** The Mg content varied in both salt and salt with humic treatments compared to controls. It decreased by 36.5% in S1 while it was increased in S2 (10.7%) without any significance. However, adding Humic acid increased the Mg content significantly in S1HA by 182.2% (p=0.008< α=0.05) compared to S1. In S2HA, the increase in Mg was only 17.3% above S2 root (Figure 1).

2. **Shoots:** Unlike roots, Mg content increased in all

treatments without any of these being significant. Increase in Mg was 37.1% and 80.3% in S1 and S2 respectively (Figure 2). HA treatment caused a slight decrease in Mg of 8.2% in S1HA and 11% in S2HA compared to both salt treated plants with 50 and 100 M NaCl.

Calcium

Ca as a proportion from all treatments of *Vigna* seedlings was quite close to K content present in root parts, however its ratio was lower than the content of K in above ground parts for all salt and salt-humic treated seedlings.

1. **Roots:** Ca content in both salt treatments and in S1HA was decreased but the latter differed significantly compared to 50 mM salt treated *Vigna* seedling root ($p=0.03 < \alpha=0.05$). Although, the treatment of S2HA increased the amount of Ca by 83.2%, this increase was not significant at the level of 5% (Figure 1).

2. **Shoots:** The proportion of Ca in all treatments including S2HA -contrary to root- decreased compared to HO values. The decrease of 43% in S2 was significant ($p=0.036 < \alpha=0.05$). The humic acid treatment with 100 mM salt caused a slight increase in Ca compared to plants treated with only 100 mM NaCl (S2) (Figure 2).

Manganese

Mn content showed fluctuations in both roots and shoot samples compared to controls. The ratio was found to be higher in roots than in shoots.

1. **Roots:** The changes in Mn concentrations in the root were higher in S1, S1HA and S2HA compared to controls (Figure 1). Both humic acid addition to salts increased Mn level by 3.4% in S1HA and 27.6% in S2HA compared to S1 and S2, respectively, but neither differed significantly.

2. **Shoots:** Mn content increased in both salt treatments by 9.4 and 60.7% compared to controls (HO), but without significance, while humic addition caused reductions in S1HA and S2HA when compared to salt-only treated samples (S1 and S2, respectively). The decrease in Mn concentration was 38.9% in S1HA (Figure 2) which was significant compared to 50 mM salt treatment ($p=0.034 < \alpha=0.05$).

Zinc

Zn is an essential micronutrient for crop production. It appeared as a high proportion of mung bean seedlings compared to Mn which is also a micronutrient measured in micrograms. Zn content in all treatments showed similar behaviour in both root and shoot samples.

1. **Root:** Zn content decreased in S1 by 25.2% compared to controls and in S1HA declined by 29.6% compared to controls. Zn concentration increased in S2 by 55.8% over HO and in S2HA also rose by 25.8% compared to S2 values. However, none of these changes showed any statistical difference at the level of $\alpha=0.05$ (Figure 1).

2. **Shoot:** As in roots, the Zn concentration decreased in S1 and S1HA by 20.8 and 38.1% compared to controls and S1 samples, respectively (Figure 2). The Zn content also decreased in S2 by 39.7%; however, humic addition to 100 mM NaCl caused a significant increase of 83.4% compared to S2 samples ($p=0.046 < \alpha=0.05$). The investigation above mainly focused on better understanding of physiological responses of six main crop nutrients, namely Ca, K, Na, Mg, Mn and Zn, to understand their uptake mechanism under salt stress. It is clear that salinity is one of the major stress factors that drastically affect many areas in the world, causing serious crop production impacts for salt sensitive plants particularly.

The study revealed the impact on growth of two NaCl levels on a very salt sensitive cultivar of mung bean (Ashraf and Ras, 1988) and the effective remediative role of humic acid when added to both salt treatments. It is well known that salinity has a drastic effect on general plant growth through osmotic stress and ion toxicity (Grattan and Grieve, 1998; Munns, 2002; Hanumantha et al., 2016), and causes a decline in the rates of net photosynthesis and decrease in nutrient uptake (Parida and Das, 2005; Cha-Um and Kirdmanee, 2009).

According to Dash and Panda (2001) and Delgado and Sanchez-Raya (2007) the decrease in root and shoot development was brought about by the inhibition of cytokinesis and cell expansion as a result of salt stress. Salinity may also cause a reduction in rhizobial nodulation directly or indirectly in legumes (Hanumantha et al., 2016) resulting in reduced N accumulation (Alam, 1994; Turan et al., 2007 a,b).

The present study revealed that the lower S1 salt concentration significantly affected the measured growth parameters except root lengths and fresh weights compared to control plants. However, the increased salt concentration of 100 mM in the S2 treatment caused a significant reductions in all parameters compared to control values. The findings for mung bean agree with reports for other Fabaceae species, including plant growth retardation in soybean (Grattan and Maas, 1988; Elsheikh and Wood, 1995), chickpea (Elsheikh and Wood, 1990), pea and faba bean (Delgado et al., 1994), shoot and root weight reduction in broad bean (Yousef and Sprent, 1983; Zahran and Sprent, 1986) and relative growth rate in bean (Gama et al., 2007).

Under salinity, many plant species have developed various mechanisms to cope with the osmotic and ionic effects and their inbition of plant growth (Munns and Tester, 2008), such as a reduction in osmotic potential by accumulation of ions (Na^+ Cl^- and K^+) or organic solutes (Example. soluble carbohydrates, glycinebetaine, proline) (Rodriguez et al., 1997; Azevedo Neto et al., 2004; Hasegawa et al., 2000; Lacerda et al., 2001, Bhaskaran et al., 2013), or increasing total soluble proteins (Ashraf and O'Leary, 1999) and free amino acids, particularly in salt sensitive plants (Azevedo Neto et al., 2009).

Humic substances have been known to have an enhancing effect on plant shoot and root growth, through effects on cell membranes by solubilization of macro and micro mineral nutrients, promoting photosynthesis, regulating enzyme activity, behaving like plant hormones, reducing toxic elements and increasing microbial populations (Chen and Aviad, (1990); Fagbenro and Agboda, (1993); David et al., (1994); Seyedbagheri, (2010); Gholami et al., (2013). Although enhanced stress resistance is demonstrated, its physiological mechanism has not been well established (Delfine et al., 2005).

Humic acids have been used to counter abiotic stress factors such as drought, cold, high-salinity and heat. It is believed that their application alleviates stress effects; in other words, it has positive effects on stress tolerance by stimulating nutrient uptake and improving the antioxidant system (Fernandez-Escobar et al., 1996; Buyukkeskin et al., 2011; Aydin et al., 2012). The humic acid preparation used was the same as from previous studies (Buyukkeskin et al., 2011), namely 10 ml/1L Hoagland – Arnon solution. The new mixture of solutions with humic acids and 50 and 100 mM NaCl concentrations was applied to the seedlings to assess the alleviative effects on a salt sensitive plant *V. radiata* (L.) Wilczek compared to salt treated control plants.

Nearly all growth parameters declined under salt treatments as expected, with salt causing reductions in plant growth and development, with the effect increasing with salt concentration (Table 3). Growth and development effects are mostly related to the excessive uptake of potentially toxic ions (Grattan and Grieve, 1998). Salt may cause restriction of water absorption and impact on the biochemical processes employed in plant growth, particularly a decline in the rate of net photosynthesis due to negative effects on the CO_2 influx into leaves, as well as excessive decrease in mineral nutrients in the roots (Parida and Das, 2005; Cha-Um and Kirdmanee, 2009).

Reduced dry weight on treatment with a low level of NaCl was reported by Al-Karaki (1997) and Taban et al. (1999). The results obtained was that the current study were similar to these findings for 50mM salt; however, 100 mM NaCl continued to inhibit growth parameters of mung bean seedlings except leaf fresh and dry weights (Khan et al., 2000; Asik et al., 2009). Application of humic acid to each salt level (50 and 100 mM) clearly revealed that the humic treatment mitigated the detrimental effect of salts on salt sensitive plants such as mung bean tested in this study.

The humic acid treatment affected all growth parameters compared to salt-only treatments (that is, S1 to S1HA and S2 to S2HA), with significant increases in S1HA and S2HA root length, but leaf fresh and weight increased in S1HA only, compared to S1 (Table 3). These findings supports reports by Turkmen et al., (2004) in tomato; Masciandaro et al., (2002) in maize; Pilanali and Kaplan (2002) in strawberry; Turkmen et al. (2005) and Gulser et al. (2010) in pepper. Aydin et al. (2012) reported that HA ameliorated soil and mitigated unfavorable effects on growth and development of beans grown in salty conditions. Meganid et al. (2015) found significant increases in plant height, number of leaves, root length, shoot and root fresh weights of common bean grown under salt stress following application of HA.

Francois and Maas (1999) reported that solubility of micronutrients is particularly low in plants exposed to salt stress which show mineral deficiencies. The same report stated that the detrimental effects of salt are observed at the whole plant level. Studies of mineral uptake by plants under saline conditions by various researchers report reduction of some nutrients such as N (Alam, 1994; Turan et al., 2007a,b), P (Navarro et al., 2001) and K (Lopez and Satti, 1996). Yermiyahu et al. (1997) stated that Na had an antagonistic effect on the uptake of Ca and Mg by reason of displacement of those elements by Na in roots. On the other hand, according to Kurum et al. (2013) NaCl caused Na, K and Ca increases in pumpkin varieties exposed to salt stress.

The mitigatitive role of humic acids on detrimental effects of salt on plants may be evaluated by growth parameters and mineral nutrients uptake together. HA effects on nutrient uptake in unstressed plants have been much studied, with varied HA quantites, growth conditions and species of plant. For instance, HA prolonged uptake of P, K, Na, Mg, Ca, Mn, Fe, Zn and Cu in tomato and eggplant (*Solanum melongena* L.) plants (Dursun et al.,1999); in maize N, P, K, Ca, Cu, Mn, Zn and Fe (Eyheraguibel et al., 2008; Sharif et al., 2002); N, P, K in maize and tomato stem (Abdel-Mawgoud et al., 2007) and in melon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai leaves (Salman et al., 2005) and P, K, Ca, Mn, Fe, Mg and Zn in tomato stem (David et al., 1994); P, K, Mg, Na, Cu and Zn in foliar application of humic in corn (Khaled and Fawy, 2011).

Foliar HA and soil application of humus increased the uptake of P, K, Mg, Na, Cu and Zn in wheat (Asik et al., 2009). Bakry et al. (2014) stated that using humic acids on previously salt stressed flax varieties increased the absorption of Fe and P as well as other nutrients to improve nutritional status of plants. In terms of nutrient availability, the current experiment evaluated mung bean root and shoot content of Na, K, Mg, Ca, Mn and Zn for controls (C), two levels of salt treatment (S1, S2), as well as HA treatments to previously salt stressed plants (S1HA, S2HA). Both HA treatments appeared to mitigate the effect of salt, enhancing growth parameters previously reduced by Na in shoots.

However, whilst S1HA decreased Na uptake in roots, S2HA increased uptake of Na when compared to S1 and S2 salt levels, respectively. The detrimental effect of Na was seen in shoots, where HA decreased the Na content in both salt treated plants. The findings agree with those of Cimrin et al. (2010) in which increasing HA doses decreased Na content in both root and shoot of pepper seedlings. Jarošová et al. (2016) stated that HA application to salt exposed cultured barley plants reduced Na accumulation in both above-and below-ground tissues.

In the present study, in mung bean root, HA continued to decrease Na in S1HA treatment, however, S2HA Na content increased significantly compared to samples grown in 100 mM salt stress (Figure 1). This increase may be related to excessive flow of Na cations accumulated in roots penetrating cell membranes, since humic acid stimulates root growth and increases membrane permability (Valdrigh et al., 1996). According to Khaled and Fawy (2011), application of humic acid 0.1% by foliar caused an increase in Na content, as well as N, P, K, Ca, Mg, Fe, Zn, and Mn, in corn. Asik et al. (2009) also stated that soil humus and foliar HA application increased Na uptake in wheat and significant Na increase was also reported by Liu and Cooper (2002) in creeping bentgrass.

K content in both root and shoot of mung bean, showed the opposite of Na, decreasing significantly in all salt treatments. Nevertheless, humic acid treated S1 and S2 (that is, S1HA and S2HA), increased K concentration in both root and shoot samples. These results agree with the studies carried out by Khaled and Fawy (2011) using foliar application of humic acid on corn, and Jarošová et al. (2014) in spring barley, who stated that K accumulation was found under saline and HA treatment. Ashraf et al. (1994), Turkmen et al. (2000) and Sensoy et al., (2007) found that increasing K has an important role in making plants more tolerant to salt stress.

In terms of magnesium content in mung bean root, the increase compared to HO on addition of HA to previously salinity-treated samples agree with reported Mg increase by Khaled and Fawy (2011) in corn, Liu and Cooper (2012) in creeping bentgrass, and Cimrin et al. (2010) in pepper shoot.

Shoot Mg content was slightly higher than it was in root, and while Mg accumulated in both salt treatments compared with controls, HA decreased the Mg content compared to previously salt exposed samples (Figure 2). HA seemed to regulate the Mg in the shoot, thereby influencing growth parameters and decreasing stress under salinity. Mg may bind to HA, which is a polydentate ligand with high chelating capacity but this binding mostly depends on the number of available dentates and pH of the medium.

Although Ca content in root was relatively higher than in shoot, both root and shoot Ca concentrations behaved similarly. Ca was slightly decreased by 50 mM NaCl (S1) in root and shoot, while HA treatment caused a reduction in this decrease in both root and shoot, with the former significant compared with S1. The Na:Ca ratio in mung bean shoots was 5.5 in S1 and 10.5 in S2 plants. Simiarly, after HA treatment, the ratio increased to 5.4 and 7.3 in S1HA and S2HA, respectively. These Figures agree with previous studies showing that plant Ca level is usually between 5 and 10 mM under salt stress, depending on the salinity level and genotype (Cramer, 2002).

In root, Ca content in S2 was 8.9% less than HO values; however, in S2HA Ca increased by 83.2% compared with S2, although this was not significant. Similarly, shoot Ca content of S2 decreased significantly by 5.6% compared to controls (HO), while HA treatment resulted in an increase of 28.2% compared with S2. Since HA has carboxy and phenol groups, HA has the ability to form a calcium complex. Since Ca atomic diameter is larger than that of Na, with higher valence, Ca may bind to HA and be absorbed more easily by the roots. The obtained results from the present study of Ca increase in S2HA are supported by other studies stating that Ca and other nutrients increase in pepper (Cimrin et al., 2010) and where HA and $CaSO_4$ were used together in maize (Mohamed, 2012).

In the present study, Mn concentration in roots was nearly 20 times higher than in shoots. The finding of a higher amount of HA in previously salt treated root may relate to the chelating capacity of HA, which binds Mn tightly. The rapid decrease of Mn content in shoots supports previous Mn labelling experiments on young wheat (*Triticum aestivum* cv. Arina) plants which found fast Mn transport via xylem from root to shoot, but particularly that it is immobile in phloem (Page and Feller, 2005).

The same method on white lupin plants (*Lupinus albus*) gave similar results, with the presence of a large amount of labelled Mn in the root system, hypocotyls and stem, but release of Mn into xylem after a certain time (7 days) and a large amount reaching photosynthetically active young expanded leaves (Page et al., 2006). The accumulation of Mn in rhizosphere and subsequent uptake depends on pH changes, but HA may stabilize the soil pH near root area thus, facilitating absorption of available Mn and other nutrients.

The Zn content of root was 6 to 17-fold than in shoot. These findings agree with the report by Gupta et al. (2016), who stated that Zn levels are higher in roots than aerial parts because the photosynthetic shoots are protected against toxic effects of Zn. The finding from the present study can be explained by HA binding Zn similarly to Mn, with the high chelation capacity of humic acids holding Zn with the COOH unit. However, Zn was gradually released from Zn-HA complexes for plant consumption, which in cases of high concentration of Zn causes toxic effect (Bednerak et al., 2011).

Conclusions

The present study revealed that HA application caused bio-mass increase of mung bean root, which may lead to greater uptake of minerals such as K. The obtained results from the study also showed that humic acid mitigated the effects of moderate salt stress (50 mM NaCl) on mung bean growth, in other words it increased the resistance to salinity by decreasing the negative effect of Na. However, HA application in severe salt stress (100 mM NaCl) showed mixed results in terms of uptake of the nutrients tested, seemingly having no clear role in decreasing the detrimental effect of salt and uptake of those nutrients. Whilst HA increased K uptake Ca, Mg, Mn and Zn showed no significant changes, indicating that more detailed investigations are required to fully elucidate the role of HA on membrane permeability.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

References

Abdel-Mawgoud, ASA, Gameh MA, Abdel-Aziz SH, El-Sayed MM (2007). Wheat water relations at various irrigation regimes with modern irrigation systems under climatic condition of Assiut governorate, Upper Egypt. J. Agric. Sci. Mansoura Univ. 32(7):6051-6066.

Adams P (1988). Some responses of tomatoes grown in NFT to sodium chloride. In Proceedings of Seventh International Congress on Soilless Culture, 59-70. Wageningen, the Netherlands: ISOSC.

Akdag C (1995). The determining of sowing date effects on mas bean [*Vigna radiata* (L.) Wilczek] seed yield and others some features in Tokat conditions. Gaziosmanpasa Univ. J. Agric. Fac. 12:135-140.

Akinci S, Buyukkeskin T, Eroglu A, Erdogan BF (2009). The effect of humic acid on nutrient composition in broad bean (*Vicia faba* L.) roots. Notulae Sci. Biol. 1(1):1-8.

Aktas H, Abak K, Cakmak I (2006). Genotypic variation in the response of pepper to salinity. Sci. Hortic.110:260-266.

Alam SM (1994). Nutrient uptake by plants under stress conditions. In: Handbook of plants and crop stress. Edited by M. Pessarakli. Mercel Dekker, Inc. New York.

Alam SM (1999). Nutrient uptake by plants under stress conditions. In: Pessarakli M (ed), Handbook of plant and crop stress. Marcel Dekker, Inc. New York, USA. pp. 285-314.

Al-Karaki GN (1997). Barley response to salt stress at varied levels of phosphorus. J. Plant Nutr. 20:1635-1643.

Allbed A, Kumar L (2013). Soil Salinity Mapping and Monitoring in Arid and Semi-Arid Regions Using Remote Sensing Technology: A Review. Adv. Remote Sens. 2(4):373-385.

Al-Mutawa MM (2003). Effect of salinity on germination and seedling growth of chick pea (*Cicer arietinum* L.) genotypes. Inter. J. Agric. Biol. 5:227-229.

Amirjani MR (2010). Effect of salinity stress on growth, mineral composition, proline content, antioxidant enzymes of soybean. Plant Physiol. 5(6):350-360.

Arancon NQ, Lee S, Edwards CA, Atiyeh R (2003). Effects of humic acids derived from cattle, food and paper-waste wermicompost on growth of greenhouse plants. Pedobiologia 47:741-744.

Ashraf M, McNeilly T (1990). Improvement of salt tolerance in Maize by selection and breeding. Plant Breed. 104:101-107.

Ashraf M, Nazir N, McNeilly T (2001). Comparative salt tolerance of amphidiploid and diploid *Brassica* species. Plant Sci. 160:683-689.

Ashraf M, O'leary JM (1997). Ion distribution in leaves of salt–tolerant and salt–sensitive lines of spring wheat under salt stress. Acta Bot. Neerl. 46(2):207-217.

Ashraf M, Rasul E (1988). Salt tolerance of mung bean [*Vigna radiata* (L.) Wilczek] at two growth stages. Plant Soil. 110:63-67.

Ashraf M, Zafar ZU, Cheema ZA (1994). Effect of Low Potassium Regimes on Same Salt and Drought Tolerant Lines of Pearl Millet. Phyton. Horn. 34(2):219-227.

Asik BB, Turan MA, Çelik H, Katkat AV (2009). Effects of humic substances on plant growth and mineral nutrients uptake of wheat (*Triticum durum* cv. Salihli) under conditions of salinity. Asian J. Crop Sci. 1(2):87-95.

Aydin A, Kant C, Turan M (2012). Humic acid application alleviates salinity stress of bean (*Phaseolus vulgaris* L.) plants decreasing membrane leakage. Afr. J. Agric. Res. 7:1073-1086.

Azevedo Neto AD, Prisco JT, Ene´as-Filho J, Lacerda CF, Silva JV, Costa PHA, Gomes-Filho E (2004). Effects of salt stress on plant growth, stomatal response and solute accumulation of different maize genotypes. Braz. J. Plant. Physiol. 16(1):31-38.

Azevedo Neto AD, Prisco JT, Gomes Filho E (2009). Changes in soluble amino-N, soluble proteins and free amino acids in leaves and roots of salt-stressed maize genotypes. J. Plant Interact. 4:137-144.

Bakry BA, Taha MH, Abdelgawad ZA, Abdallah MMS (2014). The Role of Humic Acid and Proline on Growth, Chemical Constituents and Yield Quantity and Quality of Three Flax Cultivars Grown under Saline Soil Conditions. Agric. Sci. 5:1566-1575.

Bayuelo-Jimenez JS, Jasso-Plata N, Ochoa I (2012). Growth and physiological responses of Phaseolus species to salinity stress. Int. J. Agron. pp. 1-13.

Beltagi MS, Ismail MA, Mohamed FH (2006). Induced salt tolerance in common bean (*Phaseolus vulgaris* L.) by gamma irradiation. Pak. J. Biol. Sci. 6:1143-1148.

Bhaskaran J, Paul AI, Paneerselvam R (2013). Compatible solute accumulation, osmoticum maintanance and growth in *Panicum miliaceum* L. exposed to salinity. Int. J. Pharm. Biol. Sci. 4(2):933-941.

Bidegain RA, Kaemmerer M, Guiresse M, Hafidi M, Rey F, Morard P, Revel JC (2000). Effects of humic substances from composted or chemically decomposed poplar sawdust on mineral nutrition of ryegrass. J. Agric. Sci. 134:259-267.

Buyukkeskin T, Akinci S (2011). The Effects of Humic Acid on Above-Ground Parts of Broad Bean (*Vicia faba* L.) Seedlings under Al^{3+} Toxicity. Fresenius Environ. Bull. 20(3):539-548.

Buyukkeskin T, Akinci S, Eroglu AE (2015). Effects of humic acid on root development and nutrient uptake of *Vicia faba* L. (broad bean) seedlings grown under aluminum toxicity. Commun. Soil Sci. Plant Anal. 46:277-292.

Caines AM, Shennan C (1999). Interactive effects of Ca^{2+} and NaCl salinity on the growth of two tomato genotypes differing in Ca^{2+} use efficiency. Plant Physiol. Biochem. 37(7-8):569-576.

Cakir R (2004). Effect of water stress at different development stages on vegetative and reproductive growth of corn. Field Crop Res. 89:1-16.

Canci H, Toker C (2005). Mas fasulyesinde [*Vigna radiata* (L.) Wilczek] verim ve verim kriterlerinin belirlenmesi icin genis anlamda kalitim derecesi tahminleri. GAP IV. Tarım Kongresi, 21-23 Eylül 2005, Sanliurfa.

Canci H, Toker C (2014). Yield components in mungbean [*Vigna radiata* (L.) Wilczek]. Turkish J. Field Crops 9(2):258-261.

Carvajal M, Del Amor FM, Fernandez-Ballester G, Martinez V, Cerda A (1998). Time course of solute accumulation and water relations in muskmelon plants exposed to salt during different growth stages. Plant Sci. 138:103-112.

Casierra-Posada F, Rodriguez CA, Fischer G (2009). Reducing negative effects of salinity in Tomato (*Solanum lycopersicum* L.) plants by adding leonardite to soil. Acta Hortic. 821:133-140.

Chartzoulakis K, Klapaki G (2000). Response of two greenhouse pepper hybrids to NaCl salinity during different growth stages. Sci. Hortic. 86:247-260.

Cha-Um S, Kirdmanee C (2009). Effect of salt stress on proline accumulation, photosynthetic ability and growth characters in two maize cultivars. Pak. J. Bot. 41:87-98.

Chen Y, Aviad T (1990). Effect of Humic Substances on Plant Growth. In: MacCarthy P, Clapp CE, Malcolm RL, Bloom PR (Eds.). Humic substances in soil and crop sciences: selected reading. Soil Science Society Am, Madison. pp. 161-187.

Cimrin KM, Onder T, Turan M, Burcu T (2010). Phosphorus and humic acid application alleviate salinity stress of pepper seedling. J. Afr. Biotechnol. 9:5845-5851.

Croser C, Renault S, Franklin J, Zwiazk J (2001). The effect of salinity on the emergence and seedling growth of *Picea mariana*, *Picea glanca* and *Pinus barksiana*. Environ. Pollut. 115:6-16.

Dash M, Panda SK (2001). Salt stress induced changes in growth and enzyme activities in germinating *Phaseolus mungo* seeds. Biol. Plantarum. 44:587-589.

Daur I, Bakhashwain AA (2013). Effect of humic acid on growth and quality of maize fodder production. Pak. J. Bot. 45(1):21-25.

David PP, Nelson PV, Sanders DC (1994). A Humic Acid Improves Growth of Tomato Seedling in Solution Culture. J. Plant Nutr. 17:173-184.

De Villiers AJ, Van Rooyen MW, Theron GK, Van de Venter HA (1994). Germination of three Namaqualand Pioneer species, as influenced by salinity, temperature and light. Seed Sci. Technol. 22:427-433.

Delfine S, Tognetti R, Desiderio E, Alvino A (2005). Effect of foliar application of N and humic acids on growth and yield of durum wheat. Agron. Sustain. Dev. 25:183-191.

Delgado MJ, Ligero F, Lluch C (1994). Effects of salt stress on growth and nitrogen fixation by pea, faba-bean, common bean and soybean plants. Soil Biol. Biochem. 26:371-376.

Delgado IC, Sanchez-Raya AJ (2007). Effects of sodium chloride and mineral nutrients on initial stages of development of sunflower life. Commun. Soil Sci. Plant Anal. 38:2013-2027.

Dinc U, Senol S, Kapur S, Atalay I, Cangir C (1993). Turkiye Topraklari. Cukurova Universitesi Ziraat Fakultesi Genel Yayin, 2, 51.

Dolatabadian A, Modarressanavy SAM, Ghanati F (2011). Effect of salinity on growth, xylem structure and anatomical characteristics of soybean. Not. Sci. Biol. 3:41-45.

Dursun A, Guvenc I, Turan M (1999). Macro and Micro Nutrient Contents of Tomato and Eggplant Seedlings and Their Effects on Seedling Growth in Relation to Humic Acid Application. Improved Crop Quality by Nutrient Management, Martin-Prevel, Kluwer Academic Publishers, Dordrecht, Boston, London.

El-Adawy TA, Rahma EH, El-Bedawey AA, El-Beltagy AE (2003). Nutritional potential and functional properties of germinated mung bean, pea and lentil seeds. Plant Foods Hum. Nutr. 58:1-13.

Elsheikh EAE, Wood M (1990). Effect of salinity on growth, nodulation and nitrogen yield of chickpea (*Cicer arietinum* L.). J. Exp. Bot. 41:1263-1269.

Elsheikh EAE, Wood M (1995). Nodulation and N_2 fixation by soybean inoculate with salt tolerant rhizobia or salt sensitive bradyrhizobia in saline soil. Soil Biol. Biochem. 27:657-661.

Eyheraguibel B, Silvestre J, Morard P (2008). Efects of humic substances derived from organic waste enhancement on the growth and mineral nutriton of maize. Bioresour. Technol. 9:4206-4212.

Fagbenro JA, Agboda AA (1993). Effect of different levels of humic acid on the growth and nutrient uptake of teak seedlings. J. Plant Nutr. 16:1465-1483.

FAO (2005). Global network on integrated soil management for sustainable use of salt-affected soils. Rome, Italy: FAO Land and Plant Nutrition Management Service. http://www.fao.org/ag/agl/agll/spush.

Fernandez-Escobar R, Benlloch M, Barranco D, Duenas A, Guterrez Ganan J (1996). Response of olive trees to foliar application of humic substances extracted from leonardite. Sci. Hortic. 66:191-200.

Fong SS, Seng L, Mat HB (2007). Reuse of Nitric Acid in the Oxidative Pretreatment Step for Preparation of Humic Acids from Low Rank Coal of Mukah, Sarawak. J. Braz. Chem. Soc. 18:41-46.

Francois LE, Maas EV (1999). Crop response and management on salt-affected soils. In M. Pessarakli (ed.), Handbook of Plant and Crop Stress. Marcel Dekker Press Inc. New York pp. 169-201.

Gama PBS, Inanaga S, Tanaka K, Nakazawa R (2007). Physiological response of common bean (*Phaseolus vulgaris* L.) seedlings to salinity stress. Afr. J. Biotechnol. 6(2):79-88.

Gholami H, Samavat S, Ardebili ZO (2013). The alleviating effects of humic substances on photosynthesis and yield of *Plantago ovata* in salinity conditions. Int. Res. J. Appl. Basic Sci. 4:1683-1686.

Grattan SR, Grieve CM (1998). Salinity-mineral nutrient relations in horticultural crops. Sci. Hortic. 78:127-157.

Grattan SR, Mass EV. (1988). Effect of salinity on phosphate accumulation and injury in soybean. 1. Influence of CaCl/NaCl ratios. Plant and Soil. 105:25-32.

Gulser F, Sonmez F, Boysan S (2010). Effects of calcium nitrate and humic acid on pepper seedling growth under saline condition. J. Environ. Biol. 31(3):873-876.

Hanumantha RB, Nair, RM, Nayyar H (2016). Salinity and High Temperature Tolerance in Mungbean [*Vigna radiata* (L.) Wilczek] from a Physiological Perspective. Front. Plant Sci. 7:957.

Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ (2000). Plant cellular and molecular responses to high salinity. Annu. Rev. Plant Mol. Plant Physiol. 51:463-499.

Heuer B, Nadler A (1995). Growth and development of potatoes under salinity and water deficit. Aust. J. Agric. Res. 46:1477-1486.

Hoagland DR, Arnon DI (1950). The water-culture method for growing plants without soil. California Agric. Exp. Stn. Circ. 347:32.

Huang J, Redmann RE (1995). Salt tolerance of *Hordeum* and *Brassica* species during germination and early seedling growth. Can. J. Plant Sci. 75:815-819.

Irshad M, Yamamoto S, Eneji AE, Endo T, Honna T (2002). Urea and manure effect on growth and mineral contents of maize under saline conditions. J. Plant Nutr. 25(1):189-200.

Jacoby B (1999). Mechanisms involved in salt tolerance of plants. In: Pesarakli, M. (ed.), Handbok of Plant and Crop Stress (2nd ed.). Marcel Deker, New York pp. 97-123.

Jamil M, Lee CC, Rehman S, Lee DB, Ashraf M, Rha ES (2005). Salinity (NaCl) tolerance of *Brassica* species at germination and early seedling growth. Electron. J. Environ. Agric. Food Chem. 4:970-976.

Jamil M, Rehman S, Lee KJ, Kim JM, Kim HS, Rha ES (2007). Salinity reduced growth, PS2 photochemistry and chlorophyll content in radish (*Raphanus sativus* L.). Sci. Agric. 64:111-118.

Jang JY, Lee SH, Rhee JY, Chung GC, Ahn SJ, Kang H (2007). Transgenic *Arabidopsis* and tobacco plants overexpressing an aquaporin respond differently to various abiotic stresses. Plant Mol. Biol. 64:621-632.

Jarošová M, Klejdus B, Kováčik J, Babula P, Hedbavny J (2016). Humic acid protects barley against salinity. Acta Physiol. Plant 38:161.

Jarošová M, Klejdus B, Kovacik J, Hedbavny J (2014). The impact of humic substances on oxidative stress and plant growth of spring barley exposed to NaCl. Mendelnet 2014:463-468.

Kacar B (1972). Chemical Analyses of Soil and Plant. Press of Ankara Univ. Ag. Fac. 53 (In Turkish).

Katerji N, Van Hoom JW, Hamdy A, Karam F, Mastrordli M (1994).Effect of Salinity on Emergence and on Water Stress and Early Seedling Growth of Sunflower and Maize. Agric. Water Manage. 26:81-91.

Kaya C, Higgs D, Kirnak H (2001). The effects of high salinity and supplementary phosphorus and potassium on physiology and nutrition development of spinach. Bulg. J. Plant Physiol. 27(3-4):47-59.

Khaled H, Fawy HA (2011). Effect of different levels of humic Acids on the nutrient content, plant growth and soil properties under conditions of salinity. Soil Water Res. 6(1):21-29.

Khan MA, Ungar IA, Showalter AM (2000). Effects of salinity on growth, water relations and ion accumulation of the subtropical perennial halophyte *Atriplex griffthii* var. *stocksii.* Ann. Bot. 85:225-232.

Kolsarici O, Kaya, MD, Day S, Ipek A, Uranbey S (2005). Effects of humic acid doses on emergence and seedling growth of sunflower (*Helianthus annuus* L.). Akd. Uni. J. Agric. Fac. 18(2):151-155.

Kurum R, Ulukapı K, Aydınşakir K, Onus AN (2013). The influence of salinity on seedling growth of some pumpkin varieties used as rootstock. Not. Bot. Hortic. Agrobot. 41(1):219-225.
Lacerda CF, Cambraia J, Cano MAO, Ruiz HA (2001). Plant growth and solute accumulation and distribution in two sorghum genotypes, under NaCl stress. Rev. Bras. Fisiol. Veg. 13:270-284.
Leidi EO, Saiz JF (1997). Is salinity tolerance related to Na accumulation in upland cotton (*Gossypium hirsutum*) seedlings? Plant Soil 190:67-75.
Liu C, Cooper RJ (2002). Humic acid application does not improve salt tolerance of hydroponically grown creeping bentgrass. J. Am. Soc. Hortic. Sci. 127(2):219-223.
Lopez MV, Satti SME (1996). Calcium and potassium-enhanced growth and yield of tomato under sodium-chloride stress. Plant Sci. 114:19-27.
Maas EV, Hoffman GJ (1977). Salt crop tolerance–current assessment. Irrig. Drain. Div. Am. Soc. Civil Eng. 103:115-134.
Malik BA (1994). Grain Legumes. In: Nazir S, Bashir E, Bantel R. Eds. Crop Production. National Book Foundation. Islamabad pp. 277-228.
Masciandaro G, Ceccanti B, Ronchi V, Benedicto S, Howard L (2002). Humic substances to reduce salt effect on plant germination and growth. Commun. Soil Sci. Plant Anal. 33:365-378.
Mazhar AM, Shedeed SI, Abdel-Aziz NG, Mahgoub MH (2012). Growth, Flowering and Chemical Constituentsof *Chrysanthemum indicum* L. Plant in Response to Different Levels of Humic Acid and Salinity. J. Appl. Sci. Res. 8:3697-3706.
Mead R, Curnow RN (1983). Statistical methods in agricultural and experimental biology. Chapman and Hall, London.
Meganid AS, Al-Zahrani HS, EL-Metwally MS (2015). Effect of Humic Acid Application on Growth and Chlorophyll Contents of Common Bean Plants (*Phaseolus vulgaris* L.) Under Salinity Stress Conditions. Int. J. Innov. Res. Sci. Eng. Technol. 4(5):2651-2660.
Memon SA, Hou X, Wang LJ (2010). Morphological analysis of salt stress response Pak Choi. Electron. J. Environ. Agric. Food Chem. 9(1):248-254.
Mishra SK, Subrahmanyam D, Singhal GS (1991). Interrelationship between Salt and Light Stress on Primary Processes of Photosynthesis. J. Plant Physiol. 138:92-96.
Mohamed WH (2012). Effects of humic acid and calcium forms on dry weight and nutrient uptake of maize plant under saline condition. Aust. J. Basic Appl. Sci. 6:597-604.
Mondal MMA, Puteh AB, Malek MA, Ismail MR, Rafii MY, Latif MA (2012). Seed Yield of Mungbean [*Vigna radiata* (L.) Wilczek] in Relation to Growth and Developmental Aspects. Sci. World J. 212:1-7.
Morabito D, Jolivet Y, Prat D, Dizengremel P (1996). Differences in the physiological responses of two clones of *Eucalyptus microtheca* selected for their salt tolerance. Plant Sci. 114:129-139.
Munns R (1993). Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. Plant Cell Environ. 16:15-24.
Munns R (2002). Comparative physiology of salt and water stress. Plant Cell Environ. 25:239-250.
Munns R, Tester M (2008). Mechanisms of salinity tolerance. Ann. Rev. Plant Biol. 59:651-681.
Muscolo A, Panuccio MR, Sidari M (2003). Effects of salinity on growth, carbohydrate metabolism and nutritive properties of kikuyu grass (*Pennisetum clandestinum* Hochst). Plant Sci. 164:1103-1110.
Mustard J, Renault S (2006). Response of red-osier dogwood (*Cornus sericea*) seedling to NaCl during the onset of bud break. Can. J. Bot. 84(5):844-851.
Navarro JM, Botella MA, Cerda A, Martinez V (2001). Phosphorus uptake and translocation in salt-stressed melon plants. J. Plant Physiol. 158:375-381.
Neumann P (1997). Salinity resistance and plant growth revisited. Plant Cell Environ. 20:1193-1198.
Neumann PM, Van Volkenburgh E, Cleland RE (1988). Salinity stress inhibits bean leaf expansion by reducing turgor, not wall extensibility. Plant Physiol. 88:233-237.
Niazi MLK, Mahmood K, Malik KA (1987). Salt tolerance studies in different cultivars of barley (*Hordeum vulgare* L.). Pak. J. Bot. 19:17-27.
Oplinger ES, Oelke EA, Kaminski AR, Kelling KA, Doll JD, Durgan BR, Schuler RT (1990). Spelt Alternative Field Crops Manual. University of Wisconsin, Madison, WI.
Peksen E, Toker C, Ceylan FO, Aziz T, Muhammad F (2015). Determination of promising high yielded mungbean [*Vigna radiata* (L.) Wilczek] genotypes under Middle Black Sea Region of Turkey Anadolu. J. Agric. Sci. 30:169-175.
Pessarakli M, Szabolcs I (1999). Soil Salinity and Sodicity as Particular Plant/Crop Stress Factors. In: Handbook of Plant and Crop Stress, 2nd Edition, Revised and Expanded (M. Pessarakli, Ed.), Marcel Dekker, Inc. New York pp. 1-15.
Pilanali N, Kaplan M (2002). Cilegin meyve rengi ile farkli formlarda uygulanan humik asit ve topragin bazi bitki besin maddesi kapsamlari arasindaki iliskilerin belirlenmesi. Yuzuncu Yil Universitesi, Ziraat Fakultesi, Tarim Bilimleri Dergisi 12(1):1-5.
Pilanali N, Kaplan M (2003). Investigation of effects on nutrient uptake of humic acid applications of different forms to strawberry plant. J. Plant Nutr. 26:835-843.
Pitman MG, Läuchli A (2002). Global impact of salinity and agricultural ecosystem. In Salinity: Environment-Plants-Molecules. A. Läuchli and U. Lüttge. Eds. Kluwer Academic. Dodrecht. Netherlands pp. 3-20.
Pitman MG, Läuchli A (2002). Global impact of salinity and agricultural ecosystems. *In* A Läuchli. E Lüttge. eds. Salinity: Environment-Plants-Molecules. Kluwer Academic Publishers. Dordrecht. Netherlands. pp. 3-20.
Poljakoff-Mayber A, Somers GF, Werker E, Gallagher JL (1994). Seeds of *Kosteletzkya virginica* (Malvaceae): their structure, germination, and salt tolerance. II. Germination and salt tolerance. Am. J. Bot. 81:54-59.
Qados AMSA (2011). Effect of salt stress on plant growth and metabolism of bean plant. *Vicia faba* (L.). J. Saudi Soc. Agric. Sci. 10:7-15.
Ramoliya PJ, Pandey AN (2002). Effect of salinization of soil on emergence, growth and survival of seedlings of *Acacia nilotica*. Bot. Comp. 26:105-119.
Refaiy A, S. EL-Kosary S, El- Khawaga AS, El-Sherbeny NR (2016). Effect of Potassium Humate on Plant Growth and Chemical Contents of Banana Plantlets Grown *in vitro* under Salinity Stress. Middle East J. Agric. Res. 5(1):45-49.
Reina-Sánchez A, Romero-Aranda R, Cuartero J (2005). Plant water uptake andwater use efficiency of greenhouse tomato cultivars irrigated with saline water. Agric. Water Manage. 78:54-66.
Reinhardt DH, Rost TL (1995a). On the correlation of primary root growth and tracheary element size and distance from the tip in cotton seedlings grown under salinity. Environ. Exp. Bot. 35:575-588.
Reinhardt DH, Rost TL (1995b). Primary and lateral root development of dark-and light- grown cotton seedlings under salinity stress. Bot. Acta. 108:457-465.
Rodríguez M, Canales E, Borrás-Hidalgo O (2005). Molecular aspects of abiotic stress in plants. Biotecnol. Appl. 22:1-10.
Rogers ME, Noble GM, Halloran GL, Nicolas ME (1995). The effect of NaCI on the germination and early seedling growth of white clover population selected for high and low salinity tolerance. Seed Sci. Technol. 23:277-287.
Romero-Aranda R, Soria T, Cuartero S (2001). Tomato plant-water uptake and plant-water relationships under saline growth conditions. Plant Sci. 160:265-272.
Ruffino AMC, Rosa M, Hilal M, Gonzalez JA, Prado FE (2010). The role of cotyledon metabolism in the establishment of quinoa (*Chenopodium quinoa*) seedlings growing under salinity. Plant Soil 326:213-224.
Rui L, Wei S, Mu-xiang C, Cheng-jun J, Min W, Bo-ping Y (2009). Leaf anatomical changes of *Burguiera gymnorrhiza* seedlings under salt stress. J. Trop. Subtrop. Bot. 17(2):169-175.
Russel JC, Kadry L, Hanna AB (1965). Sodic soils in Iraq. Agrokomia ES Talajtan. Tom 14:91-97.
Saffan SE (2008). Effect of salinity and osmotic stresses on some economic plants. Res. J. Agric. Biol. Sci. 4(2):159-166.
Salman SR, Abou-Husein SD, Abdel-Mawgoud AMR, El-Nemr MA (2005). Fruit yield and quality of watermelon as affected by hybrids and humic acid application. J. Apl. Sci. Res. 1(1):1-58.
Sani B (2014). Foliar Application of Humic Acid on Plant Height in Canola. APCBEE Procedia 8:82-86.

Saqib M, Zorb C, Schubert S (2006). Salt-resistant and sensitive wheat genotypes do not show very different biochemical reaction at the level of proteins in the first phase of their response to NaCl salinity. J. Plant Nutr. Soil Sci. 169:542-548.

Satti SME, Al-Yahyai RA (1995). Salinity tolerance in tomato: Implications of potassium, calcium, and phosphorus. Commun. Soil Sci. Plant Anal. 26(17-18):2749-2760.

Sensoy S, Demir S, Turkmen O, Erdinc C, Savur OB (2007). Responses of Some Different Pepper (*Capsicum annuum* L.) Genotypes to Inoculation with Two Different Arbuscular Mycorrhizal Fungi. Sci. Hortic. 113:92-95.

Seyedbagheri M (2010). Influence of humic products on soil health and potato production. Potato Res. 53:341-349.

Shanmugasundaram S, Keatinge JDH, Hughes J (2009). Counting on Beans: Mungbean Improvement in Asia. In: Spielman, D.J. and Pandya-Lorch, R., Eds., Millions Fed: Proven Successes in Agricultural Development, IFPRI, Washington DC.

Sharif M, Khattak RA, Sarir MS (2002). Effect of different levels of lignitic coal derived humic acid on growth of maize plants. Commun. Soil Sci. Plant Anal. 33:3567-3580.

Song WY, Zhang ZB, Shao HB, Guo XL, Cao HX, Zhao HB, Fu ZY, Hu XJ (2008). Relationship between calcium decoding elements and plant abiotic-stress resistance. Int. J. Biol. Sci. 4(2):116-125.

Stevenson FJ (1994). Humus Chemistry: Genesis, Composition, Reactions, 2nd. Edition, John Wiley and Sons, Inc, New York P. 285.

Szabolcs I (1989). Salt Affected Soils, CRC Press, Boca Raton, Florida, USA. P. 274.

Taban S, Gunes A, Alpaslan M, Ozcan H (1999). Sensibility of various maize (*Zea mays* L. cvs.) varieties to salinity. Turk. J. Agric. For. 23:625-633.

Tan KH, Nopamornbodi V (1979). Effect of different levels of humic acids on nutrient content and growth of corn (*Zea mays* L.). Plant Soil 51:238-287.

Tavakkoli E, Fatehi F, Coventry S, Rengasamy P, McDonald GK (2011). Additive effects of Na+ and Cl- ions on barley growth under salinity stress. J. Exp. Bot. 62:2189-2203.

Toker C, Canci H, HAQ MA, Çagirgan MI. (2002). Evaluation for agronomic, morphologic and phenologic characters of mung bean [*Vigna radiata* (L.) Wilczek] genotypes in the lowland of the West Mediteranean Region of Turkey. Turk. J. Field Crops 7(2):78-83.

Tsegay BA, Gebreslassie B (2014). The effect of salinity (NaCl) on germination and early seedling growth of *Lathyrus sativus* and *Pisum sativum* var. *abyssinicum.* Afr. J. Plant Sci. 8(5):225-231.

Tuncturk M, Tuncturk R, Yasar F (2008). Changes in micronutrients, dry weight and plant growth of soybean (*Glycine max* L. Merrill) cultivars under salt stress. Afr. J. Biotechnol. 7(11):1650-1654.

Turan MA, Asik BB, Katkat AV, Celik H (2011). The effects of soil-applied humic substances to the dry weight and mineral nutrient uptake of maize plants under soil salinity conditions. Not. Bot. Hort. Agrobot. Cluj. 39(1):171-177.

Turan MA, Elkarim AHA, Taban N, Taban S (2010). Effect of salt stress on growth and ion distribution and accumulation in shoot and root of maize plant. Afr. J. Agric. Res. 5:584-588.

Turan MA, Katkat V, Taban S (2007b). Variations in proline, chlorophyll and mineral elements contents of wheat plants grown under salinity stress. J. Agron. 6:137-141.

Turan MA, Türkmen N, Taban N (2007a). Effect of NaCl on stomatal resistance and proline, chlorophyll, Na, Cl and K concentrations of lentil plants J. Agron. 6:378-381.

Turhan H, Ayaz C (2004). Effect of salinity on seedling emergence and growth of sunflower (*Helianthus annuus* L.) cultivars. Int. J. Agric. Biol. 6(1):149-152.

Turkmen O, Demir S, Sensoy S, Dursun A (2005). Effects of Mycorrhizal Fugus and Humic Acid on the Seedling Development and Nutrient Content of Pepper Grown under Saline Soil Conditions. J. Biol. Sci. 5:568-574.

Turkmen O, Dursun A, Turan M, Erdinc C (2004). Calcium and Humic Acid Affect Seed Germination, Growth, and Nutrient content of Tomato (*Lycopersicon esculentum* L.). Seedlings in saline Soil Conditions. Acta Agric. Scand. Sect. B, Soil Plant Sci. 54:168-174.

Turkmen O, Sensoy S, Demir S, Erdinc C (2008). Effects of two different AMF species on growth and nutrient content of pepper seedlings grown under moderate salt stress. Afr. J. Biotechnol. 7(4):392-396.

Turkmen O, Sensoy S, Erdal I, Kabay T (2002). Effects of calcium on the emergence and seedling growth of tomatoes grown in salty grown media conditions. Yuzuncu Yil Universitesi, Ziraat Fakultesi, Tarim Bilimleri Dergisi 12(2):53-57.

Turkmen O, Sensoy S, Erdal I (2000). Effect of Potassium on Emergence and Seedling Growth of Cucumber Grown in Salty Conditions. Yuzuncu Yil University. J. Agric. Sci. 10:113-117.

Unsal H (2007). The effect of humic acid and Zn applications on yield and N, P, K contents of two different chickpea varieties in alchaline soils. MSc Thesis Yuzuncu Yil University, Van, Turkey.

Valdrigh MM, Pera IA, Agnolucci M, Frassinetti S, Lunardi D, Vallini G (1996). Effects of compost-derived humic acids on vegetable biomass production and microbial growth within a plant (*Cichorium intybus*) soil system: a comparative study. Agric. Ecosyst. Environ. 58:133-144.

MaiaI JM, VoigtI EL, MacêdoI CEC, Ferreira-SilvaII SL, Silveira JAG (2010). Salt-induced changes in antioxidative enzyme activities in root tissues do not account for the differential salt tolerance of two cowpea cultivars. Braz. J. Plant. Physiol. 22:2.

Wahid A, Javed IUH, Ali I, Baig A, Rasul E (1998). Short term incubation of sorghum caryopses in sodium chloride levels: changes in some pre- and post-germination physiological parameters. Plant Sci. 139:223-232.

Waqas M, Ahmad B, Arif M, Munsif F, Latif Khan A, Amin M, Kang S, Kim Y, Lee I (2014). Evaluation of Humic Acid Application Methods for Yield and Yield Components of Mungbean. Am. J. Plant Sci. 5:2269-2276.

Yao AR (1998). Molecular biology of salt tolerance in the context of whole plant physiology. J. Exp. Bot. 49:915-929.

Yasar F (2003). Tuz Stresi Altindaki Patlican Genotiplerinde Bazi Antioksidant Enzim Aktivitelerinin *in vitro* ve *in vivo* Olarak Incelenmesi. (Doktora Tezi, basilmamis), Yuzuncu Yil Universitesi Fen Bilimleri Enstit usu, Van P. 138.

Yermiyahu U, Nir S, Ben-Hayyim G, Kafkafi U, Kinraide B (1997). Root elongation in saline solution related to calcium binding to root cell plasma membranes. Plant Soil 191:67-76.

Yetisir H, Uygur V (2009). Plant growth and mineral element content of different gourd species and watermelon under salinity stress. Turk. J. Agric. For. 33:65-77.

Yousef AN, Sprent JI (1983). Effects of NaCl on growth, nitrogen incorporation and chemical composition of inoculated and ammonium nitrate fertilized *Vicia faba* L. plants. J. Exp. Bot. 34:941-950.

Zahran HH, Sprent JI (1986). Effects of sodium-chloride and polyethylene-glycol on root-hair infection and nodulation of *Vicia faba* L. plants by Rhizobium leguminosarum. Planta 167:303-309.

Zaidi PH, Singh BB (1993). Dry matter partitioning and yield attributes of soybean as affected by soil salinity and growth regulators. Legume Res. 16:139-143.

Zidan MA, Elewa MA (1995). Effect of salinity on germination, seedling growth and some metabolic changes in four plant species (Umbelliferae). Ind. J. Plant Phys. 38:5-16.

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