African Journal of Agricultural Research
Volume 12 Number 49  7 December,  2017
ISSN  1991-637X
ABOUT AJAR

The African Journal of Agricultural Research (AJAR) is published weekly (one volume per year) by Academic Journals.

African Journal of Agricultural Research (AJAR) is an open access journal that publishes high-quality solicited and unsolicited articles, in English, in all areas of agriculture including arid soil research and rehabilitation, agricultural genomics, stored products research, tree fruit production, pesticide science, postharvest biology and technology, seed science research, irrigation, agricultural engineering, water resources management, marine sciences, agronomy, animal science, physiology and morphology, aquaculture, crop science, dairy science, entomology, fish and fisheries, forestry, freshwater science, horticulture, poultry science, soil science, systematic biology, veterinary, virology, viticulture, weed biology, agricultural economics and agribusiness. All articles published in AJAR are peer-reviewed.

Contact Us

Editorial Office: ajar@academicjournals.org
Help Desk: helpdesk@academicjournals.org
Website: http://www.academicjournals.org/journal/AJAR
Submit manuscript online http://ms.academicjournals.me/
Editors

Prof. N.A. Amusa

Dr. Panagiota Florou-Paneri
Laboratory of Nutrition, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Greece.

Prof. Dr. Abdul Majeed
Department of Botany, University of Gujrat, India, Director Horticulture, and landscaping, India.

Prof. Suleyman TABAN
Department of Soil Science and Plant Nutrition, Faculty of Agriculture, Ankara University, 06100 Ankara-TURKEY.

Prof. Hyo Choi
Graduate School
Gangneung-Wonju National University
Gangneung, Gangwondo 210-702, Korea.

Dr. MATIYAR RAHAMAN KHAN
AICRP (Nematode), Directorate of Research, Bidhan Chandra Krishi Viswaavidyalaya, P.O. Kalyani, Nadia, PIN-741235, West Bengal, India.

Prof. Hamid AIT-AMAR
University of Science and Technology, Houari Bouemdiene, B.P. 32, 16111 EL-Alia, Algiers, Algeria.

Prof. Sheikh Raisuddin
Department of Medical Elementology and Toxicology, Jamia Hamdard (Hamdard University) New Delhi, India.

Prof. Ahmad Arzani
Department of Agronomy and Plant Breeding College of Agriculture Isfahan University of Technology Isfahan-84156, Iran.

Dr. Bampidis Vasileios
National Agricultural Research Foundation (NAGREF), Animal Research Institute 58100 Giannitsa, Greece.

Dr. Zhang Yuanzhi
Laboratory of Space Technology, University of Technology (HUT) Kilonkallio Espoo, Finland.

Dr. Mboya E. Burudi
International Livestock Research Institute (ILRI) P.O. Box 30709 Nairobi 00100, Kenya.

Dr. Andres Cibils
Assistant Professor of Rangeland Science Dept. of Animal and Range Sciences Box 30003, MSC 3-I New Mexico State University Las Cruces, NM 88003 (USA).

Dr. MAJID Sattari
Rice Research Institute of Iran, Amol-Iran.

Dr. Agricola Odoi
University of Tennessee, TN., USA.

Prof. Horst Kaiser
Department of Ichthyology and Fisheries Science Rhodes University, PO Box 94, South Africa.

Prof. Xingkai Xu
Institute of Atmospheric Physics, Chinese Academy of Sciences, Beijing 100029, China.

Dr. Agele, Samuel Ohikhena
Department of Crop, Soil and Pest Management, Federal University of Technology PMB 704, Akure, Nigeria.

Dr. E.M. Aregheore
The University of the South Pacific, School of Agriculture and Food Technology Alafua Campus, Apia, SAMOA
**Editorial Board**

**Dr. Bradley G Fritz**  
Research Scientist,  
Environmental Technology Division,  
 Battelle, Pacific Northwest National Laboratory,  
902 Battelle Blvd., Richland,  
Washington,  
USA.  

**Dr. Almut Gerhardt**  
LimCo  
International, University of Tuebingen, Germany.  

**Dr. Celin Acharya**  
Dr. K.S.Krishnan Research Associate (KSRA),  
Molecular Biology Division,  
Bhabha Atomic Research Centre (BARC),  
Trombay, Mumbai-85,  
India.  

**Dr. Daizy R. Batish**  
Department of Botany, Panjab University,  
Chandigarh,  
India.  

**Dr. Seyed Mohammad Ali Razavi**  
University of Ferdowsi,  
Department of Food Science and Technology,  
Mashtad,  
Iran.  

**Dr. Yasemin Kavdir**  
Canakkale Onsekiz Mart University,  
Department of Soil Sciences, Terzioglu Campus 17100  
Canakkale,  
Turkey.  

**Prof. Giovanni Dinelli**  
Department of Agroenvironmental Science and Technology  
Viale Fanin 44 40100, Bologna  
Italy.  

**Prof. Huanmin Zhou**  
College of Biotechnology at Inner Mongolia Agricultural University,  
Inner Mongolia Agricultural University, No. 306# Zhao Wu Da Street,  
Hohhot 010018, P. R. China, China.  

**Dr. Mohamed A. Dawoud**  
Water Resources Department,  
Terrestrial Environment Research Centre,  
Environmental Research and Wildlife Development Agency (ERWDA),  
P. O. Box 45553,  
Abu Dhabi,  
United Arab Emirates.  

**Dr. Phillip Retief Celliers**  
Dept. Agriculture and Game Management,  
PO BOX 77000, NMMU,  
PE, 6031,  
South Africa.  

**Dr. Rodolfo Ungerfeld**  
Departamento de Fisiologia,  
Facultad de Veterinaria,  
Lasplases 1550, Montevideo 11600,  
Uruguay.  

**Dr. Timothy Smith**  
Stable Cottage, Cuttle Lane,  
Biddestone, Chippenham,  
Wiltshire, SN14 7DF.  
UK.  

**Dr. E. Nicholas Odongo,**  
27 Cole Road, Guelph,  
Ontario. N1G 4S3  
Canada.  

**Dr. D. K. Singh**  
Scientist Irrigation and Drainage Engineering Division,  
Central Institute of Agricultural Engineering,  
Bhopal- 462038, M.P.  
India.  

**Prof. Hezhong Dong**  
Professor of Agronomy,  
Cotton Research Center,  
Shandong Academy of Agricultural Sciences,  
Jinan 250100  
China.  

**Dr. Ousmane Youm**  
Assistant Director of Research & Leader,  
Integrated Rice Productions Systems Program,  
Africa Rice Center (WARDA) 01BP 2031,  
Cotonou,  
Benin.
### ARTICLES

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

Ogumo E. O., Kunyanga C. N., Okoth M. W. and Kimenju J.

Pages: 3399

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

Elisa Maria Campos Minassa, Silvério Paiva Freitas, Diego Corona Baitelle, Silvio de Jesus Freitas, Ismael Lourenço de Jesus Freitas and Claudio Luiz Melo de Souza

Pages: 3410

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

Nathália Aparecida Bragança Fávaris, Allan Rocha de Freitas, Carla Basílio Monteiro, Manoel Víctor Borges Pedrosa, Khétrin Silva Maciel, Paula Aparecida Muniz de Lima, José Carlos Lopes, Rodrigo Sobreira Alexandre

Pages: 3415

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

Amanullah Khan, B. A. Stewart, Lal K. Almas and Khalid Nawab

Pages: 3421

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

Paulo Alexandre Fernandes Rodrigues de Melo, Robson Luís Silva de Medeiros, Domingos da Costa Ferreira Júnior, Edna Ursulino Alves Alves, Sérgio Valiengo Valeri, Janaina Marques Mondego, José Ribamar Gusmão Araújo and Raimunda Nonata Santos Lemos

Pages: 3433

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

P. Elisabeth Zida, B. James Néya, W. Romain Soalla, Paco Sérémé and Ole Søgaard Lund

Pages: 3441

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

Ozlem Kalyoncu, Sener Akinci and Esin Bozkurt

Pages: 3447
Full Length Research Paper

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

Ogumo E. O. 1*, Kunyanga C. N. 1, Okoth M. W. 1 and Kimenju J. 2

1Department of Food Science, Nutrition and Technology, University of Nairobi, Nairobi, Kenya.
2Department of Plant Science and Crop Protection, University of Nairobi, Nairobi, Kenya.

Received 23 September, 2017; Accepted 26 October, 2017

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 farmers (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...
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 decay (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 thia bendazole 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.
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 data 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.

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

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.

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

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

#### Yields and main season of French bean 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°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.

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

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

<table>
<thead>
<tr>
<th>Factors considered to meet buyers demand</th>
<th>Frequency</th>
<th>% of responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fertilizer application</td>
<td>11.0</td>
<td>24.4</td>
</tr>
<tr>
<td>Control of pests and diseases</td>
<td>18.0</td>
<td>40.0</td>
</tr>
<tr>
<td>Planting certified seeds</td>
<td>9.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Proper land preparation</td>
<td>1.0</td>
<td>2.2</td>
</tr>
<tr>
<td>Irrigation in time</td>
<td>8.0</td>
<td>17.8</td>
</tr>
<tr>
<td>Harvesting and grading</td>
<td>12.0</td>
<td>26.7</td>
</tr>
<tr>
<td>Total</td>
<td>59.0</td>
<td></td>
</tr>
</tbody>
</table>

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

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

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
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

---

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

**Figure 5.** Market outlets for French beans from farmers.
Table 6. Distribution of farmers based on the use and the material for construction of charcoal coolers.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Charcoal cooler usage</td>
<td>Yes</td>
<td>36</td>
<td>80.0</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>9</td>
<td>20.0</td>
</tr>
<tr>
<td>Cemented floor</td>
<td></td>
<td>33</td>
<td>73.0</td>
</tr>
<tr>
<td>Concrete floor</td>
<td></td>
<td>2</td>
<td>4.4</td>
</tr>
<tr>
<td>Wood planks</td>
<td></td>
<td>1</td>
<td>2.2</td>
</tr>
<tr>
<td>Floor</td>
<td>Iron sheets</td>
<td>36</td>
<td>80.0</td>
</tr>
<tr>
<td>Walls</td>
<td>Charcoal wall</td>
<td>36</td>
<td>80.0</td>
</tr>
<tr>
<td>Presence of humidifiers</td>
<td>No running water</td>
<td>22</td>
<td>48.9</td>
</tr>
<tr>
<td></td>
<td>water</td>
<td>14</td>
<td>31.1</td>
</tr>
</tbody>
</table>

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

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

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
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 expected to have high productivity as they are still in their active age of agricultural production and therefore there is possibility of high productivity.

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 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.

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

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

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

**ACKNOWLEDGEMENTS**

The authors thank Oliver Okumu, Richard Sisumah, David Muiruri, Mwangangi Nzuli and Austin Okoth for their support during this study.

**REFERENCES**


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

Elisa Maria Campos Minassa1, Silvério Paiva Freitas1*, Diego Corona Baitelle2, Silvio de Jesus Freitas2, Ismael Lourenço de Jesus Freitas3 and Claudio Luiz Melo de Souza3

1Laboratory of Plant Science, Universidade Estadual do Norte Fluminense Darcy Ribeiro. End.: Av. Alberto Lamego, 2000 - Parque Califórnia, Campos dos Goitacazes, Rio de Janeiro, Brazil.
3Fundação de Apoio à Escola Técnica (FAETEC), Rua Clarimundo de Melo, 847 - Quintino Bocaiuva, Rio de Janeiro - RJ, 21311-280, Brazil.

Received 25 September, 2017; Accepted 20 October, 2017

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 sativus, 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 3x6 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 allelochemical 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, 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 Köppen, 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: 20°57'06” S, and longitude: 41°20'45” W and altitude, 250 m. The arabica coffee straw was obtained from the production, pulping and processing of grains of Catual Vermelho IAC 81 cultivar, cultivated in the city of Venda Nova do Imigrante, Espírito Santo, Brazil at Latitude: 20°20'23” S, longitude: 41°08'05” 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 species, 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 cm² 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

*Corresponding author. E-mail: silverio@uenf.br.

Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License.
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.

<table>
<thead>
<tr>
<th>VF</th>
<th>DF</th>
<th>PE (%)</th>
<th>ESI</th>
<th>FMW (mg)</th>
<th>DMW (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MS</td>
<td>F</td>
<td>MS</td>
<td>F</td>
</tr>
<tr>
<td>Mulch (M)</td>
<td>2</td>
<td>0.560</td>
<td>16.304*</td>
<td>57.458</td>
<td>30.291*</td>
</tr>
<tr>
<td>Species (S)</td>
<td>5</td>
<td>1.234</td>
<td>35.99*</td>
<td>89.987</td>
<td>47.441*</td>
</tr>
<tr>
<td>Interaction</td>
<td>10</td>
<td>0.131</td>
<td>3.815*</td>
<td>15.694</td>
<td>8.274*</td>
</tr>
<tr>
<td>Residue</td>
<td>90</td>
<td>0.034</td>
<td>1.897</td>
<td>0.484</td>
<td>0.00002</td>
</tr>
</tbody>
</table>

*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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Control</th>
<th>Conilon coffee straw</th>
<th>Arabica coffee straw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of emergence (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactuca sativa</td>
<td>88.89±7.8cAb</td>
<td>28.70±26.6bBc</td>
<td>87.03±8.3cAb</td>
</tr>
<tr>
<td>Cucumis sativus</td>
<td>93.75±10.4aAa</td>
<td>79.17±18.8bBa</td>
<td>95.83±6.4bAa</td>
</tr>
<tr>
<td>Solanum lycopersicum</td>
<td>27.78±15.5aAd</td>
<td>25.00±27.8aAb</td>
<td>36.11±18.0aAb</td>
</tr>
<tr>
<td>Eupatorium maximilianii</td>
<td>62.50±26.7abc</td>
<td>9.17±9.16bBc</td>
<td>33.33±14.02bBb</td>
</tr>
<tr>
<td>Amaranthus hybridus</td>
<td>24.17±58.45ad</td>
<td>17.50±11.72aAb</td>
<td>18.33±23.59aAb</td>
</tr>
<tr>
<td>Bidens pilosa</td>
<td>51.39±32.23acd</td>
<td>45.83±18.81Ab</td>
<td>44.44±19.48ab</td>
</tr>
</tbody>
</table>

Emergence speed index (ESI)

<table>
<thead>
<tr>
<th>Species</th>
<th>Control</th>
<th>Conilon coffee straw</th>
<th>Arabica coffee straw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactuca sativa</td>
<td>10.24±3.72oaAb</td>
<td>1.63±1.683cab</td>
<td>6.66±0.614bAb</td>
</tr>
<tr>
<td>Cucumis sativus</td>
<td>4.75±1.21aBab</td>
<td>3.29±1.194ba</td>
<td>5.50±0.949bAb</td>
</tr>
<tr>
<td>Solanum lycopersicum</td>
<td>0.64±0.384ad</td>
<td>0.51±0.563ab</td>
<td>0.76±0.44Ab</td>
</tr>
<tr>
<td>Eupatorium maximilianii</td>
<td>2.44±1.249abcd</td>
<td>0.45±0.547bBb</td>
<td>1.03±0.491bAb</td>
</tr>
<tr>
<td>Amaranthus hybridus</td>
<td>1.30±0.46aoAbd</td>
<td>0.79±0.663ab</td>
<td>0.65±0.72Ab</td>
</tr>
<tr>
<td>Bidens pilosa</td>
<td>3.54±2.751abc</td>
<td>1.14±0.669bab</td>
<td>1.91±1.151ab</td>
</tr>
</tbody>
</table>

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 (Ps<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 Lilliefors test for normality of variance and Cochran and Bartlett 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.
Arabica coffee straw can be used as mulch for the cultivation of *C. sativa* and *S. lycopersicum* without causing damage to the emergence of the seedlings. However, conilon coffee straw harmed the emergence of *C. sativa* (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. sativa* 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. sativa*, 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. sativa* 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. sativa*. 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. sativa* 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. sativa* 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. sativa* 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. sativa*, 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. sativa* 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

<table>
<thead>
<tr>
<th>Specie</th>
<th>Control</th>
<th>Conilon coffee straw</th>
<th>Arabica coffee straw</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactuca sativa</em></td>
<td>0.048±0.014&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>0.018±0.020&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>0.063±0.012&lt;sup&gt;Abc&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Cucumis sativus</em></td>
<td>0.669±0.213&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>0.310±0.157&lt;sup&gt;Ca&lt;/sup&gt;</td>
<td>0.899±0.102&lt;sup&gt;Aa&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Solanum lycopersicum</em></td>
<td>0.109±0.016&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>0.029±0.028&lt;sup&gt;Bb&lt;/sup&gt;</td>
<td>0.144±0.037&lt;sup&gt;Ab&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Eupatorium maximilianii</em></td>
<td>0.026±0.012&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>0.031±0.039&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>0.029±0.006&lt;sup&gt;Abc&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Amaranthus hybridus</em></td>
<td>0.015±0.004&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>0.009±0.088&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>0.012±0.008&lt;sup&gt;Abc&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Bidens pilosa</em></td>
<td>0.061±0.021&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>0.027±0.010&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>0.059±0.022&lt;sup&gt;Abc&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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).
behave 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 esula*, 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. maximiliani*, 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. maximiliani* and in the reduction of the emergence speed of *A. hybridus*.

**CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

**REFERENCES**


Full Length Research Paper

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

Nathália Aparecida Bragança Fávaris, Allan Rocha de Freitas, Carla Basilio Monteiro, Manoel Victor Borges Pedrosa, Khétrin Silva Maciel, Paula Aparecida Muniz de Lima, José Carlos Lopes*, Rodrigo Sobreira Alexandre

Departamento de Agronomia, Universidade Federal do Espírito Santo, Alegre, Espírito Santo, Brazil.

Received 26 September, 2017; Accepted 8 November, 2017

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 (cm³ 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 Espírito 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

*Corresponding author. E-mail: jcufes@bol.com.br.

Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License
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 contact 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 (Ps. 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 Espirito 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 (Ps. guajava L.), obtained from orchards in the municipality of Guacuí-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 cm³ 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 Espirito 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; Ni = 5505.00; Ni = 20.3; K = 6.51; Se = 25.3; Na = 7264.00; Zn = 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 μmol 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 (cm³ plant⁻¹), number of leaves, content of chlorophyll a and b and total, with the help of ChlorofiLOG (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 cm³ capacity.

The experimental design was completely randomized in a factorial 6 x 4, consisting of six substrates and four shade levels. The data relating to the variables analyzed were transformed: emergence y = [to arcsine (x/100)]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
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
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**


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

Amanullah Khan1*, B. A. Stewart2, Lal K. Almas3 and Khalid Nawab4

1Department of Agronomy, The University of Agriculture Peshawar, Pakistan.
2Dryland Agriculture Institute, WTAMU, Canyon, Texas, United States.
3College of Agriculture and Natural Sciences, West Texas A&M University, Texas, United States.
4Department of Agriculture Extension and Communication, The University of Agriculture Peshawar, Pakistan.

Received 16 October, 2017; Accepted 20 October, 2017

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 C3-cerealsviz. 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 [S1 = 20-20-20, S2 = 20-27-5, S3 = 7-22-8, S4 = 10-10-10-20S, S5 = 11-15-11, S6 = 31-11-11, S7 = 24-8-16, and S8 = 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 S6 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
(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 the objective 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 (S1 = 20-20-20, S2 = 20-27-5, S3 = 7-22-8, S4 = 10-10-10-20S, S5 = 11-15-11, S6 = 31-11-11, S7 = 24-8-16, S8 = 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 S6 (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 C3 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 (S1 = 20-20-20 (Peter Professional by Scotts), S2 = 20-27-5 (Starter Fertilizer by Scotts), S3 = 7-22-8 (Bedding Plant Food by FertiLoam), S4 = 10-10-10-20S (Shake in Feed by Miracle Grow), S5 = 11-15-11 (Gardner’s Special by FertiLoam), S6 = 31-11-11 (Acid Loving by FertiLoam), S7 = 24-8-16 (All Purpose Plant Food by Expert Gardner) and S8 = 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 plant¹ was worked out. Shoot DM plant¹ was obtained by adding leaf DM + stem DM plant¹. The sum of the shoot + root DM plant¹ was calculated as the total DM plant¹. Absolute growth rate (AGR); dry matter accumulation per unit 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:

\[ \text{AGR} = \frac{W_2 - W_1}{t_2 - t_1} \text{ (g plant}^{-1} \text{day}^{-1}) \]

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

\[ \text{NAR} = \frac{\text{CGR}}{\text{LAI}} \text{ (g m}^{-2} \text{day}^{-1}) \]

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).

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

Statistical analysis

Data were subjected to analysis of variance (ANOVA) 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).

<table>
<thead>
<tr>
<th>NPK source</th>
<th>N-P(_2)O(_5)-K(_2)O</th>
<th>30 DAE</th>
<th>60 DAE</th>
<th>90 DAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>S(_1) = PF (Scotts)</td>
<td>20-20-20</td>
<td>1.52</td>
<td>44.3</td>
<td>68.32</td>
</tr>
<tr>
<td>S(_2) = SF (Scotts)</td>
<td>20-27-5</td>
<td>1.49</td>
<td>39.1</td>
<td>86.85</td>
</tr>
<tr>
<td>S(_3) = BPF (Ferti. Loam)</td>
<td>7-22-8</td>
<td>1.42</td>
<td>49.1</td>
<td>48.61</td>
</tr>
<tr>
<td>S(_4) = SF (Miracle Grow)</td>
<td>10-10-10(S)</td>
<td>1.74</td>
<td>31.0</td>
<td>27.57</td>
</tr>
<tr>
<td>S(_5) = GS (Ferti. Loam)</td>
<td>11-15-11</td>
<td>1.66</td>
<td>32.5</td>
<td>62.33</td>
</tr>
<tr>
<td>S(_6) = AL (Ferti. Loam)</td>
<td>31-11-11</td>
<td>1.00</td>
<td>4.1</td>
<td>31.78</td>
</tr>
<tr>
<td>S(_7) = AFPF (E. Gardner)</td>
<td>24-8-16</td>
<td>1.23</td>
<td>18.3</td>
<td>58.74</td>
</tr>
<tr>
<td>S(_8) = SR (E. Gardner)</td>
<td>19-6-12</td>
<td>1.56</td>
<td>38.0</td>
<td>64.73</td>
</tr>
</tbody>
</table>

Crops species

- Wheat (\textit{Triticum aestivum} L.)
  - AGR (mg plant\(^{-1}\) day\(^{-1}\))
  - 30 DAE: 1.75
  - 60 DAE: 36.5
  - 90 DAE: 69.73
- Rye (\textit{Secale cereale} L.)
  - AGR (mg plant\(^{-1}\) day\(^{-1}\))
  - 30 DAE: 1.21
  - 60 DAE: 28.6
  - 90 DAE: 31.25
- Barley (\textit{Hordeum vulgare} L.)
  - AGR (mg plant\(^{-1}\) day\(^{-1}\))
  - 30 DAE: 1.87
  - 60 DAE: 41.1
  - 90 DAE: 70.77
- Oats (\textit{Avena sativa} L.)
  - AGR (mg plant\(^{-1}\) day\(^{-1}\))
  - 30 DAE: 0.98
  - 60 DAE: 22.0
  - 90 DAE: 52.71

Least significant difference

- Crops (P ≤ 0.05)
  - 0.11
- NPK Sources (P ≤ 0.05)
  - 0.15
- Interaction (P ≤ 0.05)
  - (Figure 1)*
  - (Figure 2)*

*Indicates the data is significant at P ≤ 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 ≤ 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\(_1\) 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
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$_5$ (0.74 g m$^{-2}$ day$^{-1}$); while the lowest CGR (0.44 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 par 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).

<table>
<thead>
<tr>
<th>NPK source</th>
<th>N-P(_2)O(_5)-K(_2)O</th>
<th>30 DAE</th>
<th>60 DAE</th>
<th>90 DAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>S(_1) = PF (Scotts)</td>
<td>20-20-20</td>
<td>0.69</td>
<td>13.08</td>
<td>20.49</td>
</tr>
<tr>
<td>S(_2) = SF (Scotts)</td>
<td>20-27-5</td>
<td>0.67</td>
<td>11.51</td>
<td>26.05</td>
</tr>
<tr>
<td>S(_3) = BPF (Ferti. Loam)</td>
<td>7-22-8</td>
<td>0.64</td>
<td>14.51</td>
<td>14.58</td>
</tr>
<tr>
<td>S(_4) = SF (Miracle Grow)</td>
<td>10-10-10-20(S)</td>
<td>0.78</td>
<td>9.04</td>
<td>8.27</td>
</tr>
<tr>
<td>S(_5) = GS (Ferti. Loam)</td>
<td>11-15-11</td>
<td>0.75</td>
<td>9.52</td>
<td>18.70</td>
</tr>
<tr>
<td>S(_6) = AL (Ferti. Loam)</td>
<td>31-11-11</td>
<td>0.45</td>
<td>1.13</td>
<td>9.53</td>
</tr>
<tr>
<td>S(_7) = AFPF (E. Gardner)</td>
<td>24-8-16</td>
<td>0.55</td>
<td>5.31</td>
<td>17.62</td>
</tr>
<tr>
<td>S(_8) = SR (E. Gardner)</td>
<td>19-6-12</td>
<td>0.70</td>
<td>11.16</td>
<td>19.42</td>
</tr>
</tbody>
</table>

Crops species

- Wheat (*Triticum aestivum* L.)
- Rye (*Secale cereale* L.)
- Barley (*Hordeum vulgare* L.)
- Oats (*Avena sativa* L.)

Least significant difference

<table>
<thead>
<tr>
<th></th>
<th>(P ≤ 0.05)</th>
<th>(P ≤ 0.05)</th>
<th>(P ≤ 0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crops</td>
<td>0.05</td>
<td>1.27</td>
<td>2.51</td>
</tr>
<tr>
<td>NPK Sources</td>
<td>0.07</td>
<td>1.79</td>
<td>3.55</td>
</tr>
<tr>
<td>Interaction (P ≤ 0.05)</td>
<td>(Figure 4)*</td>
<td>(Figure 5)*</td>
<td>(Figure 6)*</td>
</tr>
</tbody>
</table>

*Indicates the data is significant at P ≤ 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.
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 \((22.72 \text{ g m}^{-2} \text{ day}^{-1})\), being at par with rye \((22.40 \text{ g m}^{-2} \text{ day}^{-1})\); while oats had the lowest NAR \((17.19 \text{ g m}^{-2} \text{ day}^{-1})\). Although, the differences in NAR were not significant among the crops at 60 DAE, however, rye \((16.70 \text{ g m}^{-2} \text{ day}^{-1})\) and wheat \((15.13 \text{ g m}^{-2} \text{ day}^{-1})\) had the higher NAR as compared to barley \((7.80 \text{ g m}^{-2} \text{ day}^{-1})\) and oats \((9.76 \text{ g m}^{-2} \text{ day}^{-1})\). At 90 DAE, wheat had significantly, the highest NAR \((8.83 \text{ g m}^{-2} \text{ day}^{-1})\), followed by oats \((4.46 \text{ g m}^{-2} \text{ day}^{-1})\); while rye had the lowest NAR \((3.41 \text{ g m}^{-2} \text{ day}^{-1})\). 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 \((25.38 \text{ g m}^{-2} \text{ day}^{-1})\) was calculated when crops were applied S6, followed by S7 \((21.67 \text{ g m}^{-2} \text{ day}^{-1})\); while the lowest NAR \((16.68 \text{ g m}^{-2} \text{ day}^{-1})\) was calculated when crops received S3. At 60 DAE, the highest NAR \((36.28 \text{ g m}^{-2} \text{ day}^{-1})\) was calculated when crops were applied S6, followed by S3 \((13.87 \text{ g m}^{-2} \text{ day}^{-1})\); while the lowest NAR \((6.08 \text{ g m}^{-2} \text{ day}^{-1})\) was obtained when crops received S7. The NAR showed negative relationship with LAI and positive relationship with CGR. At 90 DAE, the highest NAR \((9.36 \text{ g m}^{-2} \text{ day}^{-1})\) was obtained when crops were applied S5, followed by S1 \((6.12 \text{ g m}^{-2} \text{ day}^{-1})\); while the lowest \((3.51 \text{ g m}^{-2} \text{ day}^{-1})\) was obtained when crops received S8. 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).

<table>
<thead>
<tr>
<th>NPK source</th>
<th>N-P₂O₅-K₂O</th>
<th>30 DAE</th>
<th>60 DAE</th>
<th>90 DAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>S₁ = PF (Scotts)</td>
<td>20-20-20</td>
<td>21.09</td>
<td>8.47</td>
<td>6.12</td>
</tr>
<tr>
<td>S₂ = SF (Scotts)</td>
<td>20-27-5</td>
<td>21.26</td>
<td>8.25</td>
<td>4.93</td>
</tr>
<tr>
<td>S₃ = BPF (Ferti. Loam)</td>
<td>7-22-8</td>
<td>16.68</td>
<td>13.87</td>
<td>5.53</td>
</tr>
<tr>
<td>S₄ = SF (Miracle Grow)</td>
<td>10-10-10-20(S)</td>
<td>20.88</td>
<td>9.35</td>
<td>4.20</td>
</tr>
<tr>
<td>S₆ = AL (Ferti. Loam)</td>
<td>31-11-11</td>
<td>25.38</td>
<td>36.28</td>
<td>3.84</td>
</tr>
<tr>
<td>S₇ = AFPF (E. Gardner)</td>
<td>24-8-16</td>
<td>21.67</td>
<td>6.08</td>
<td>4.65</td>
</tr>
<tr>
<td>S₈ = SR (E. Gardner)</td>
<td>19-6-12</td>
<td>17.78</td>
<td>7.26</td>
<td>3.51</td>
</tr>
</tbody>
</table>

Crops species

<table>
<thead>
<tr>
<th>Crops species</th>
<th>30 DAE</th>
<th>60 DAE</th>
<th>90 DAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat (Triticum aestivum L.)</td>
<td>22.72</td>
<td>15.13</td>
<td>8.83</td>
</tr>
<tr>
<td>Rye (Secale cereale L.)</td>
<td>22.40</td>
<td>16.70</td>
<td>3.41</td>
</tr>
<tr>
<td>Barley (Hordeum vulgare L.)</td>
<td>19.91</td>
<td>7.80</td>
<td>4.36</td>
</tr>
<tr>
<td>Oats (Avena sativa L.)</td>
<td>17.19</td>
<td>9.76</td>
<td>4.46</td>
</tr>
</tbody>
</table>

Least significant difference

| Crops (P ≤ 0.05) | 2.34 | ns | 1.03 |
| NPK sources (P ≤ 0.05) | 3.30 | 14.63 | 1.46 |
| Interaction (P ≤ 0.05) | (Figure 7)* | (Figure 8)*** | (Figure 9)* |

*Indicates the data is significant at P ≤ 0.05; ns indicates the data is not significant at P ≤ 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 sources.
sources than other crop species.

**Water use efficiency**

WUE among cool season cereals varied significantly (P ≤ 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 S₄ or S₈, being at par with S₅ (0.28 g L⁻¹). The lowest WUE (0.17 g L⁻¹) was noted when crops were grown with S₆. At 60 DAE, the highest WUE of 2.48 g L⁻¹ was calculated when crops were applied either S₈, being at par with S₁ (2.18 g L⁻¹) and S₃ (2.41 g L⁻¹). The lowest WUE (0.24 g L⁻¹) was noted when crops were grown with S₆. At 90 DAE, the highest WUE of 2.63 g L⁻¹ was calculated when crops were applied S₈, being at par with S₂ (2.58 g L⁻¹) and S₇ (2.61 g L⁻¹). The lowest WUE (0.75 g L⁻¹) was noted when crops were grown with S₆. 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).

<table>
<thead>
<tr>
<th>NPK sources</th>
<th>N-P₂O₅-K₂O</th>
<th>30 DAE</th>
<th>60 DAE</th>
<th>90 DAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>S₁ = PF (Scotts)</td>
<td>20-20-20</td>
<td>0.26</td>
<td>2.18</td>
<td>2.31</td>
</tr>
<tr>
<td>S₂ = SF (Scotts)</td>
<td>20-27-5</td>
<td>0.25</td>
<td>1.93</td>
<td>2.58</td>
</tr>
<tr>
<td>S₃ = BPF (Ferti. Loam)</td>
<td>7-22-8</td>
<td>0.26</td>
<td>2.41</td>
<td>2.01</td>
</tr>
<tr>
<td>S₄ = SF (Miracle Grow)</td>
<td>10-10-10-20(S)</td>
<td>0.29</td>
<td>1.55</td>
<td>1.22</td>
</tr>
<tr>
<td>S₅ = GS (Ferti. Loam)</td>
<td>11-15-11</td>
<td>0.28</td>
<td>1.63</td>
<td>1.96</td>
</tr>
<tr>
<td>S₆ = AL (Ferti. Loam)</td>
<td>31-11-11</td>
<td>0.17</td>
<td>0.24</td>
<td>0.75</td>
</tr>
<tr>
<td>S₇ = AFPF (E. Gardner)</td>
<td>24-8-16</td>
<td>0.22</td>
<td>1.40</td>
<td>2.61</td>
</tr>
<tr>
<td>S₈ = SR (E. Gardner)</td>
<td>19-6-12</td>
<td>0.29</td>
<td>2.48</td>
<td>2.63</td>
</tr>
</tbody>
</table>

Crops species

- Wheat (*Triticum aestivum* L.)
- Rye (*Secale cereale* L.)
- Barley (*Hordeum vulgare* L.)
- Oats (*Avena sativa* L.)

Least significant difference

- Crops (P ≤ 0.05)
- NPK sources (P ≤ 0.05)
- Interaction (P ≤ 0.05)

*Indicates the data is significant at (P ≤ 0.05).

![Wheat, Rye, Barley, Oats](image)

**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...
DISCUSSION

Significant variations (P ≤ 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 C₃-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. $S_1$ [(10-10-20(S) and $S_5$ (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 (siling) 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.

**ACKNOWLEDGEMENTS**

The authors are thankful to Dr. B. A. Stewart, Director and Distinguished Professor of Dryland Agriculture Institute, West Texas A&M University Canyon, Texas USA, for providing the opportunity to complete this research project. Many thanks are extended to Prof. Dr. Paigham Shah, Agricultural University Peshawar (Retired) for the statistical analysis of the data. This study is part of post doctorate research financed by the Higher Education Commission of Pakistan, Islamabad.

**REFERENCES**


Full Length Research Paper

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

Paulo Alexandre Fernandes Rodrigues de Melo¹, Robson Luis Silva de Medeiros²*, Domingos da Costa Ferreira Júnior³, Edna Ursulino Alves Alves⁴, Sérgio Valiengo Valeri², Janaina Marques Mondego⁵, José Ribamar Gusbão Araújo¹ and Raimunda Nonata Santos Lemos¹

¹Professores adjunto do programa de Pós-Graduação em Agroecologia, Universidade Estadual do Maranhão – UEMA, CEP. 65055-310 São Luis, Maranhão, Brazil.
²Departamento de Produção Vegetal, Faculdade de Ciências Agrárias e veterinárias, Universidade Estadual Paulista, Via de Acesso Prof. Paulo Donato Castellane, s/n, CEP 14.884-900, Jaboticabal, São Paulo, Brasil.
³Departamento de Biologia Aplicada à Agropecuária, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Via de Acesso Prof. Paulo Donato Castellane, s/n, CEP 14.884-900, Jaboticabal, São Paulo, Brasil.
⁴Centro de Ciências Agrárias, Universidade Federal da Paraíba, CEP: 58397-000, Areia, Paraíba, Brasil.
⁵Pós Doutoranda da Universidade Estadual do Maranhão – UEMA, CEP. 65055-310 São Luis, Maranhão, Brazil.

Received 9 October, 2017; Accepted 8 November, 2017

*Sideroxylon obtusifolium* (Roem. & Schult.) Penn. is a native species from Caatinga biome, but due to disorderly exploitation for phytoteryphy 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 (*T₁*), seeds infected with *Colletotrichum* sp. (*T₂*), infected seeds treated with captan fungicide (*T₃*) and infected seeds treated with *C. ferrea* extract (*T₄*). *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,

*Corresponding author. E-mail: robsonluissm@gmail.com

Author(s) agree that this article remain permanently open access under the terms of the [Creative Commons Attribution License 4.0 International License](https://creativecommons.org/licenses/by/4.0/).
sapotibia 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; Vechiatto 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 brasiliensis March., Myracrodruon 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 (Vechiatto 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. obtusifolium 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°57'8,22" 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 Paraíba. 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 10⁵ Colletotrichum sp.
Table 1. Germination rate (G) and germination speed index (GSI) of Sideroxylon obtusifolium seeds inoculated with Colletotrichum sp.

<table>
<thead>
<tr>
<th>Matrix</th>
<th>G (%)</th>
<th>GSI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>1</td>
<td>95**A</td>
<td>79aB</td>
</tr>
<tr>
<td>2</td>
<td>90**A</td>
<td>62bB</td>
</tr>
<tr>
<td>3</td>
<td>93**A</td>
<td>10dA</td>
</tr>
<tr>
<td>4</td>
<td>92**A</td>
<td>57abB</td>
</tr>
<tr>
<td>5</td>
<td>88**A</td>
<td>38cA</td>
</tr>
<tr>
<td>6</td>
<td>85**A</td>
<td>45cA</td>
</tr>
<tr>
<td>7</td>
<td>53cA</td>
<td>8dA</td>
</tr>
<tr>
<td>8</td>
<td>50cA</td>
<td>6dA</td>
</tr>
<tr>
<td>9</td>
<td>42dA</td>
<td>6dA</td>
</tr>
<tr>
<td>10</td>
<td>24eA</td>
<td>5dA</td>
</tr>
<tr>
<td>CV (%)</td>
<td>9.8</td>
<td></td>
</tr>
</tbody>
</table>

*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.

Physiological and sanitary quality determinations

Evaluation of the physiological potential was performed with seeds from T1 and T2 treatments, and for T3 and T4 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 Álvare, 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(%)] x 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] x 100.

Experimental design and statistical analysis

The experimental design was completely randomized, with treatments distributed in a 10 x 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., Chaetomium 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.

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Adjusted regression equations</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>( y = 459.92x^2 - 366.45x + 52.747 )</td>
<td>0.65</td>
</tr>
<tr>
<td>2</td>
<td>( y = 371.71x^2 - 296.17x + 42.631 )</td>
<td>0.65</td>
</tr>
<tr>
<td>3</td>
<td>( y = 393.83x^2 - 305.63x + 52.352 )</td>
<td>0.65</td>
</tr>
<tr>
<td>4</td>
<td>( y = 504.02x^2 - 401.59x + 57.805 )</td>
<td>0.65</td>
</tr>
<tr>
<td>5</td>
<td>( y = 486.76x^2 - 390.15x + 57.115 )</td>
<td>0.69*</td>
</tr>
<tr>
<td>6</td>
<td>( y = 424.72x^2 - 343.44x + 52.408 )</td>
<td>0.60</td>
</tr>
<tr>
<td>7</td>
<td>( y = 376.23x^2 - 314.20x + 52.436 )</td>
<td>0.69*</td>
</tr>
<tr>
<td>8</td>
<td>( y = 500.58x^2 - 384.97x + 54.469 )</td>
<td>0.65</td>
</tr>
<tr>
<td>9</td>
<td>( y = 422.12x^2 - 336.33x + 48.412 )</td>
<td>0.65</td>
</tr>
<tr>
<td>10</td>
<td>( y = 485.12x^2 - 386.53x + 55.637 )</td>
<td>0.65</td>
</tr>
</tbody>
</table>

\( R^2 = \) 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 angustifolia (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.), *ipê-roxo* (*Tabebuia impetiginosa* (Mart. ex DC.) Standl.), *pau-de-jangada* (*Apeiba tibourbou* Aubl.), *cedar* (*Cedrela fissilis* Vell.) and *jacarandá-da-baia* (*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.

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Adjusted regression equation</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$y = 311.48x^2 - 248.18x + 35.724$</td>
<td>0.65</td>
</tr>
<tr>
<td>2</td>
<td>$y = 349.13x^2 - 278.18x + 40.041$</td>
<td>0.65</td>
</tr>
<tr>
<td>3</td>
<td>$y = -530.39x^2 + 373.68x + 35.244$</td>
<td>0.42</td>
</tr>
<tr>
<td>4</td>
<td>$y = 47.252x^2 - 37.649x + 5.4192$</td>
<td>0.65</td>
</tr>
<tr>
<td>5</td>
<td>$y = 41.03x^2 - 90.444x + 36.934$</td>
<td>0.12*</td>
</tr>
<tr>
<td>6</td>
<td>$y = -669.87x^2 + 409.45x + 5.529$</td>
<td>0.63</td>
</tr>
<tr>
<td>7</td>
<td>$y = 419.99x^2 - 224.05x + 58.962$</td>
<td>0.74*</td>
</tr>
<tr>
<td>8</td>
<td>$y = 384.47x^2 - 132.94x + 7.335$</td>
<td>0.99</td>
</tr>
<tr>
<td>9</td>
<td>$y = 37.991x^2 - 30.27x + 4.3571$</td>
<td>0.65</td>
</tr>
<tr>
<td>10</td>
<td>$y = 238.95x^2 - 190.39x + 27.405$</td>
<td>0.65</td>
</tr>
</tbody>
</table>

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

In 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 linemuthianum* 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**

*Colletotrichum* 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**

The authors acknowledge the Foundation for Scientific Research and Development of Maranhão – FAPEMA.

**REFERENCES**


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.


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

P. Elisabeth Zida¹, B. James Néya¹, W. Romain Soalla¹, Paco Sérémé¹ and Ole Søgaard Lund²*

¹Institut de l’Environnement et de Recherches Agricoles (IN.E.R.A.), P.O. Box 8645, Ouagadougou 04, Burkina Faso.
²Department of Plant and Environmental Sciences, University of Copenhagen, Højbakkegård Allé 13, 2630 Taastrup, Denmark.

Received 15 September, 2017; Accepted 20 October, 2017

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).

*Corresponding author. E-mail: osl@plen.ku.dk.

Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License
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 hydroproming is dependent on humidity of the soil (Harris, 2006). Given the difference in annual precipitation, it appeared relevant to compare the effect of hydroproming 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

Hydroproming 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 hydropromed 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 Kamboinsé 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 hydroproming in Northern zone

A positive effect of hydroproming 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
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).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Northern zone</th>
<th>Central zone</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NoT W-6</td>
<td>NoT W-6</td>
<td>NoT W-6</td>
</tr>
<tr>
<td><strong>Emergence</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2015 (N=8 per zone) mean (%)</td>
<td>78.0</td>
<td>82.4</td>
<td>79.3</td>
</tr>
<tr>
<td>2016 (N=8 per zone) mean (%)</td>
<td>55.5</td>
<td>65.7</td>
<td>78.4</td>
</tr>
<tr>
<td>Total mean (%)</td>
<td>66.8</td>
<td>74.1</td>
<td>78.8</td>
</tr>
<tr>
<td><strong>Total median (%)</strong></td>
<td>68.1</td>
<td>75.2</td>
<td>77.6</td>
</tr>
<tr>
<td>Fields with higher emergence</td>
<td>ns</td>
<td>10 (16)</td>
<td>ns</td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td>ns</td>
<td>&lt;0.0053</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Yield</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute (kg/ha)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2015 (N=8 per zone) mean (kg/ha)</td>
<td>703</td>
<td>1019</td>
<td>1094</td>
</tr>
<tr>
<td>2016 (N=8 per zone) mean (kg/ha)</td>
<td>982</td>
<td>1247</td>
<td>973</td>
</tr>
<tr>
<td>Total mean kg/ha</td>
<td>843</td>
<td>1133</td>
<td>1034</td>
</tr>
<tr>
<td><strong>Total median (kg/ha)</strong></td>
<td>859&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1018&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1064</td>
</tr>
<tr>
<td>Fields with higher yield</td>
<td>ns</td>
<td>13 (16)</td>
<td>ns</td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td>ns</td>
<td>&lt;0.0053</td>
<td>ns</td>
</tr>
<tr>
<td>Relative (% of field average)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2015 mean (%)</td>
<td>84.8</td>
<td>115.2</td>
<td>96.4</td>
</tr>
<tr>
<td>2016 mean (%)</td>
<td>90.1</td>
<td>109.9</td>
<td>110.9</td>
</tr>
<tr>
<td>Total mean (%)</td>
<td>87.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>112.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>103.7</td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td>ns</td>
<td>&lt;0.000054</td>
<td>ns</td>
</tr>
</tbody>
</table>

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

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

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

<table>
<thead>
<tr>
<th>Precipitation (mm)</th>
<th>Early crop season (establishment) (June- July)</th>
<th>Whole growing season (June-October)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Northern zone*</td>
<td>Central zone**</td>
</tr>
<tr>
<td>2015</td>
<td>211.0</td>
<td>346.4</td>
</tr>
<tr>
<td>2016</td>
<td>280.0</td>
<td>322.3</td>
</tr>
<tr>
<td>Average</td>
<td>245.5</td>
<td>334.4</td>
</tr>
</tbody>
</table>

*Climate station at Titao; ** Climate station at Kamboinsé.
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 hydroproming. 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 hydroproming in pearl millet (Harris, 2006), and in other cereal crops (wheat and sorghum) a weak response to hydroproming 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 hydroproming 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 hydroproming 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 hydroproming being evident in the most solar-exposed and dry zone is in full agreement with existing theory of crop physiology and seed hydroproming. 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 hydroproming 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 hydroproming 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.

ACKNOWLEDGEMENT

The technical assistance of Séni Bilgo, Solange Zida and Marcellin Tiemtoré is gratefully acknowledged and we thank the European Union for the grant no. DCI-FOOD/2012/304-690 under the Europe AID program: Increasing yields of Sorghum and Millet by a new and sustainable seed technology developed in Sahel. We further thank the “Fonds National de la Recherche et de l’Innovation pour le Développement (FONRID)” and the National Extension Service in Burkina Faso for the grant no. AP3/PC 005/12/2013 and for fruitful collaboration, respectively.

REFERENCES


Full Length Research Paper

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

Ozlem Kalyoncu¹, Sener Akinci²* and Esin Bozkurt³

¹Institute of Pure and Applied Sciences, Marmara University, Ziverbey/Kadikoy, Istanbul, Turkey.
²Faculty of Arts and Sciences, Marmara University, Ziverbey/Kadikoy, Istanbul, Turkey.
³Faculty of Engineering, Environmental Engineering, Marmara University, Ziverbey/Kadikoy, Istanbul, Turkey.

Received 18 September, 2017; Accepted 20 October, 2017

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-Arnold 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; Alibed 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
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 et 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; Hasegawa 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; Neumann et al., 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, Na2SO4, CaCl2, MgCl2 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 (Toktor 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 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.) R. 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 K2O in ratios given in Table 1. For germination,
Table 1. Components of HA.

<table>
<thead>
<tr>
<th>Components</th>
<th>Volume (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total organic substances</td>
<td>12</td>
</tr>
<tr>
<td>Total HA + fulvic acid</td>
<td>16</td>
</tr>
<tr>
<td>Water-soluble K2O</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 2. Experimental salt and Humic acid preparations.

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>Contents and preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (C)</td>
<td>Full strength Hoagland solution (Hoagland-Aron, 1950)</td>
</tr>
<tr>
<td>50 mM NaCl (S1)</td>
<td>50 mM NaCl is dissolved in HO solution and volume made up to 1 L</td>
</tr>
<tr>
<td>100 mM NaCl (S2)</td>
<td>100 mM NaCl is dissolved in HO solution and volume made up to 1 L</td>
</tr>
<tr>
<td>50 mM NaCl + Humic acid (S1HA)</td>
<td>50 mL NaCl is dissolved in HO contained 10 ml humic acid and volume made up to 1 L</td>
</tr>
<tr>
<td>100 mM NaCl + Humic acid (S2 HA)</td>
<td>100 mL NaCl is dissolved in HO contained 10 ml humic acid and volume made up to 1 L</td>
</tr>
</tbody>
</table>

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 torg (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±2°C. 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 40°C in a water-bath and the supernatant solution was removed by heating at 150 to 180°C until 1 mL extract remained. The residue was treated with distilled water to dissolve and made up to 100 mL in colored 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 (Carvalhal 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 (Lycoopersicon esculentum v. Rutgers) (Arancion et al., 2003).

However, reports of HA addition to plants grown under salinity are limited, with positive effects on growth and
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 \(\alpha=0.05\) 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 (Cimirin 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 \(P=0.014\), \(< \alpha=0.05\), 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 researchers in agreement with the present study in plants such as tomato and eggplant (Dursun et al., 1998), pepper (Cimirin 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\% (\(p=0.010\); \(p=0.001\) \(<\alpha=0.05\)), 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

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

<table>
<thead>
<tr>
<th>Growth parameters</th>
<th>Control</th>
<th>S1</th>
<th>S2</th>
<th>S1HA</th>
<th>S2HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoot heights (PH) cm</td>
<td>37.36±4.320</td>
<td>22.45±2.422*</td>
<td>21.79±1.377*</td>
<td>26.28±2.843</td>
<td>23.93±1.665*</td>
</tr>
<tr>
<td>Root lengths (RL) cm</td>
<td>22.10±0.996</td>
<td>16.34±1.255</td>
<td>16.33±0.943*</td>
<td>24.83±1.255*</td>
<td>23.33±1.632***</td>
</tr>
<tr>
<td>No of leaves (NL)</td>
<td>4.0±0.327</td>
<td>2.70±0.313*</td>
<td>2.60±0.183*</td>
<td>3.20±0.164*</td>
<td>2.90±0.227*</td>
</tr>
<tr>
<td>Leaf area (LA) cm(^2)</td>
<td>140.85±2.27</td>
<td>67.96±1.766*</td>
<td>61.55±12.23*</td>
<td>84.90±7.42</td>
<td>78.73±17.12</td>
</tr>
<tr>
<td>Leaves fresh weight (LFW) (g)</td>
<td>2.03±0.317</td>
<td>0.77±0.121*</td>
<td>0.78±0.121*</td>
<td>1.52±0.281**</td>
<td>1.16±0.227</td>
</tr>
<tr>
<td>Leaves dry weight (LDW) (g)</td>
<td>0.21±0.034</td>
<td>0.10±0.015*</td>
<td>0.10±0.033*</td>
<td>0.15±0.030</td>
<td>0.11±0.026*</td>
</tr>
<tr>
<td>Stem fresh weight (SFW) (g)</td>
<td>1.52±0.289</td>
<td>0.70±0.098*</td>
<td>0.58±0.126*</td>
<td>0.85±0.115*</td>
<td>0.79±0.178*</td>
</tr>
<tr>
<td>Stem dry weight (SDW) (g)</td>
<td>0.13±0.015</td>
<td>0.06±0.005*</td>
<td>0.05±0.008*</td>
<td>0.08±0.008**</td>
<td>0.07±0.011*</td>
</tr>
<tr>
<td>Root fresh weight (RFW) (g)</td>
<td>0.80±0.146</td>
<td>0.41±0.072</td>
<td>0.35±0.060*</td>
<td>0.57±0.100</td>
<td>0.50±0.095*</td>
</tr>
<tr>
<td>Root dry weights(RDW) (g)</td>
<td>0.04±0.004</td>
<td>0.03±0.006*</td>
<td>0.02±0.005*</td>
<td>0.04±0.008</td>
<td>0.03±0.006*</td>
</tr>
</tbody>
</table>

\(\pm\): Standard errors *: Significantly different from controls **: Significantly different from S1 ***: Significantly different from S2
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 <α=0.05) and 0.784 g (P=0.009 <α=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 <α=0.05), while the fresh weight increase in S2HA was 48.5%, although this did not differ significantly at the level of α=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 <α=0.05) and 51.4% (P=0.049 <α=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 <α=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 control.

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 occurred 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 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 α=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 α=0.05. The increase in S2 roots was significant (p=0.047 < α=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.
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 \((p=0.01 < \alpha=0.05)\). 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% \((p=0.045 < \alpha=0.05)\) and 43.6% \((p=0.03 < \alpha=0.05)\) compared to controls (Figure 1). 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 \(p=0.009 <\)
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 < α=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 < α=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 < α=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 α=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 < α=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-Ray (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 inhibition 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 Turkmens 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 mitigative 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 quantities, 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 (Eyheraguibelt et al., 2008; Sharif et al., 2002); N, P, K in maize and tomato stem (Abdel-Mawgoud et al., 2007) and in melon (Citrus latan) (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 (Ask 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. Jarosová 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 permeability (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 Jarosová 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. Similarly, 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 CaSO₄ 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. Árinya) 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


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


Khan MA, Unger IA, Showalter AM (2000). Effects of salinity on growth, water relations and ion accumulation of the subtropical perennial *Helianthus annuus* L. Agric. Water Manage. 26:81-91.


Sani B (2014). Foliar Application of Humic Acid on Plant Height in Canola. APCBEE Procedia 8:82-86.


African Journal of Agricultural Research

Related Journals Published by Academic Journals

- African Journal of Environmental Science & Technology
- Biotechnology & Molecular Biology Reviews
- African Journal of Biochemistry Research
- African Journal of Microbiology Research
- African Journal of Food Science
- African Journal of Biotechnology
- African Journal of Pharmacy & Pharmacology
- African Journal of Plant Science
- Journal of Medicinal Plant Research
- International Journal of Physical Sciences
- Scientific Research and Essays