African Journal of Agricultural Research

Volume 9 Number 47 20 November 2014

ISSN 1991-637X

Academic Journals

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, post harvest 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/

Editorial Board

African Journal of Agricultural Research

Table of Contents: Volume 9 Number 47 2 0 November, 2014

ARTICLES

academicJournals

Vol. 9(47), pp. 3427-3433, 20 November, 2014
DOI: 10.5897/AJAR2014. 9042
Article Number: E54B08F48589
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Assessment of knowledge gap and constraints affecting production and consumption of standardized dairy products in Wolayta Soddo, Southern Ethiopia

Shewangizaw Wolde* and Adisu Jimma

Southern Agricultural Research Institute, Areka Agricultural Research Center, Ethiopia.

Received 4 August, 2014; Accepted 10 October, 2014

A study was conducted to assess knowledge gap and identify constraints on production and consumption of standardized dairy products. Only 20, 10, 16.7, 3.3 and 6.7% had knowledge about standardized dairy products, hazard consequent of using substandard dairy products, proper standards during milk production, hazard analysis critical control point system and what to check during shopping labeled dairy products, respectively. All respondents had not consumed ghee, milk powder, ice cream and cream. About 77, 7, 60 and 67% were wash udder, use towel, check abnormal appearances, and not avoid milk from udder treated cows, respectively. Different sources of contamination of milk were less dangerous (43.3%) and not dangerous at all (30%). Milking (100%), milk handling (90%), milk processing (100%), milk marketing (90%) were done by female. During wet season, price of milk and butter were 8.00 ETB per liter and 93 ETB per Kg, respectively. During dry season, price of milk and butter were 9.00 ETB per liter and 106 ETB per Kg, respectively. Thus, knowledge was poor on standardized dairy products and awareness creation, supply of value added milk products and improved milk production technologies were needed.

Key words: Knowledge gap, standardized dairy products, production, consumption, gender role.

INTRODUCTION

The livestock subsector plays a vital role as source of food, income, services and foreign exchange to the Ethiopian economy, and contributes to 12 and 33% of the total and agricultural GDP, respectively, and accounts for 12 to 15% of the total export earnings, second in order of importance (Ayele et al., 2003).

Among the 20 major food and agricultural commodities ranked by value in 2005, whole fresh cow milk is ranked third. Milk production in the same year was estimated at 1.5 million tonnes which is equivalent to USD 398.9 million (FAOSTAT, 2007). For years or decades Ethiopia ranked first in cattle population in Africa, but the dairy industry is not developed even as compared to east African countries like Kenya, Uganda and Tanzania. Regarding dairy production, the national milk production remains among the lowest in the world, even by African standards (Zegeye, 2003).

Although, many efforts were made towards dairy

*Corresponding author. E-mail: shewanwolde@yahoo.com, Tel: +251913225191. Fax: +251465520505.

development and various research projects have been undertaken in some parts of the country, the outcome and impact have not been satisfactory. Planners should consider the relative efficiency of alternative milk marketing systems in terms of costs and marketing margins, product hygiene and quality, range and stability of services offered and stability of producers and consumer prices. To do so, policy makers, development organizations and private investors are in need of information of different aspects of the production system of the specific area, potentials and constraints. Therefore, this study was conducted to support dairy development in the region through assessment of knowledge gap and collection and documentation of information on the current practices and challenges on milk production and consumption.

MATERIALS AND METHODS

Description of the study area

The study was conducted at Kokate Kebele (rural area) and Wolayta Soddo town (urban area) of Wolayta zone, Southern Ethiopia. The Kebele and town were purposefully selected due to high milk marketing and consumption activities in Soddo town and Kokate kebele is its main milk shed. Wolayta Soddo town is located at a distance of 330 km south of the capital, Addis Ababa where as Kokate kebele is located at a distance of about 6 km north of the capital, Wolayta Soddo.

Selection of respondents and data collection

A total of 30 milk and milk products producer households at Kokate kebele, 30 milk collector households (café and restaurants) and 30 consumers at Wolayta Soddo town were selected based on milk production, collection (marketing) and consumption experiences. Therefore a total of 90 respondents were selected, interviewed and were included in the study.

Data was collected by using a pre tested semi structured questionnaire in February to April, 2011. Enumerators were selected from Areka Agricultural Research Center, Animal Science Research Case Team and orientation was given to brief them the ways of data collection. Then formal surveys were conducted by interviewing the household heads of the selected milk producing households (farmers), collectors (hotels and cafes), milk and milk products consumers.

Data analysis

The data collected were analyzed using SPSS (version 16). Descriptive statistics such as mean and percentages were used to summarize data as required.

RESULTS AND DISCUSSION

Knowledge level on standardized dairy products

Ethiopian quality and standards authority had developed milk and dairy products standards in the year 2001. These standards were then revised in 2005 to be harmonized with the common market for Eastern and Southern Africa accepted standards. However, only 20, 10, 16.7, 3.3 and 6.7% of the respondents had knowledge about standardized dairy products, hazard consequent of using substandard dairy products, proper standards during milk production, hazard analysis critical control point (HACCP) system and what to check during shopping labeled dairy products, respectively (Table 1). About 50% of the respondents had traditional dairy products standards in their area which is much higher than traditional dairy products standards (20%) in Ada'a and Lume districts of East Shewa zone of Ethiopia (Bilatu et al., 2013).

Availability and consumption trend of dairy products

As indicated in Table 2, about 70, 33, 30, 10 and 33% of respondents consumed whole fresh milk, homemade fermented milk, buttermilk, cheese and butter, respectively. However respondents consumed ghee, milk powder, ice cream and cream were nil due to unavailability. The study indicated that the consumption of milk and milk products was directly related with availability of milk and milk products. Inconsistent with the present findings, Bilatu et al. (2013) reported that standardized milk imported or locally produced such as skim milk, powdered milk and ice cream are prevalent in urban areas of Ada'a and Lume districts of East Shewa zone, Ethiopia. In close agreement with the present findings, Getachew and Gashaw (2001) indicated that about 68% of the total milk produced in rural Ethiopia is used for household consumption in the form of fresh milk, butter, cheese and yogurt. Moreover, Belete et al. (2010) reported that dairy products consumed in the household are fresh whole milk, fermented (sour) milk (locally called 'ergo'), local cheese (ayib) and buttermilk (wogemit or arera) in amhara region of Fogera woreda, Ethiopia.

In the present study, majority of respondents in Sodo town consumed whole fresh milk which is not in agreement with Ayantu (2006) in the Delbo watershed of Welayta zone and O'Connor and Zinash (1990) in Centeral highlands of Ethiopia. They observed that larger part of the produced milk processed into various products such as butter, soft cheese, fermented milk and buttermilk. In the present findings, large proportion of respondents consumed whole fresh milk might be due to large number of producers sold whole fresh milk due to short distance to urban center, access to markets and lack of traditional taboo that restricts selling of whole fresh milk (Belete et al., 2010).

In the study area, dairy farmers sold whole fresh milk for hotels and café whereas butter milk and butter were sold in the local open markets. About 60 and 47% of purchasing consumers were not concerned at all about safety of dairy products when purchased from open

Table 1. Producers' knowledge level on standardized dairy products (SDP).

Knowledge assessment	Soddo (N=30)	
	n	%
Have knowledge about SDP	6	20
Have knowledge about hazard consequent of using substandard dairy products	3	10
Have knowledge about proper standards during milk production	5	16.7
There is presence of traditional standard in the area	16	53.3
Have knowledge about HACCP	1	3.3
Knowledge about what to check during shopping labeled dairy products	2	6.7

Table 2. Consumption trend of dairy products in the study area.

Parameter	Soddo (N=30)	
Consumption of dairy products:	**n**	**%**
Consume whole fresh milk	21	70
Homemade fermented milk	10	33.3
Arera/butter milk	9	30
Cheese	3	10
Traditional butter	10	33.3

Table 3. Concern about safety of dairy products in the study area.

Parameter	Soddo (N=30)	
	n	%
Concern about safety of milk and milk products when buy from producers		
Not concerned at all	14	46.7
Somewhat concerned	3	10
Very concerned	13	43.3
Concern about safety of milk and milk products when buy from open markets		
Not concerned at all	18	60
Somewhat concerned	9	30
Very concerned	3	10

markets and producers, respectively (Table 3). To assess the perception about danger of different sources of milk contamination, consumers responded less dangerous, not dangerous, somewhat dangerous, dangerous, very dangerous were 43.3, 30, 13.3, 6.7 and 6.7%, respectively (Table 4). All respondents indicated that priority of whole milk consumption was given for children less than 3 years of age and male household head. Among the family members, children less than 3 years of age (50%) was the most vulnerable to dairy born food illness which is in agreement with Bilatu et al. (2013).

Milking practices

Milking practice is one of the most important criteria for hygienic milk production. About 93% washed their hand before milking, 76.6% washed udder with water only, 43.3% washed and dried teats with a warm sanitizing solution, 6.7% used individual clean towel, 63.3% checked udder and foremilk for mastitis/udder inflammation, 60% checked for abnormal appearances in milk, 80% used clean water, 10% used teat dip solutions after milking, 50% cooled milk immediately after milking, 40% milkier covered hair, 46.7% cutting nails often, 76.7% had no smoked, 63.3% had no dinked alcohol, 50% avoided spitting and 70% had wet hands by use of milk to facilitate milk letdown. About 67 and 80% were not avoided milk from udder treated cows and not avoided milk contamination by feed during milking, respectively (Table 5). Consistent with the present study, Zelalem and Faye (2006) reported that dairy processing

Table 4. Family members vulnerable to dairy borne food illness and perception about danger of different sources of contamination.

Parameter	Soddo (N=30)	
	n	%
Vulnerable family members to dairy borne food illness		
Babies (0-3 years)	15	50
Pregnant woman	1	3.3
Children (4-12 years)	4	13.3
Others	11	33.3
Perception about danger of contamination		
Not dangerous at all	9	30
Less dangerous	13	43.3
Somewhat dangerous	4	13.3
Dangerous	2	6.7
Very dangerous	2	6.7

Table 5. Milking practices in the study area.

Milking practice (%)	Sodo (N=30)	
	n	%
Hand wash	28	93.3
Udder wash (with water only)	23	76.7
Dry teats thoroughly	13	43.3
Use individual clean towel	2	6.7
Check for abnormal appearances	18	60
Use of clean water	24	80
Use of teat dip solutions after milking	3	10
Cool milk immediately after milking	15	50
Milkier must cover hair	12	40
Cutting nails often	14	46.7
No smoking	23	76.7
No drinking alcohol	19	63.3
Wetting hands by use of milk	21	70
Avoiding milk contamination (%)		
avoid milk from udder treated cows	10	33.3
avoid milk contamination by feed	6	20

in the country is basically limited to smallholder level and hygienic qualities of products are generally poor. The same authors also reported that about 52% of smallholder producers and 58% of large-scale producers used common towel to clean the udder and 45% did not treat milk before consumption which was in close agreement with the present findings. Contrary to the present findings, Zelalem and Faye (2006) reported that all producers do not use clean water to clean the udder and other milk utensils.

Milking and fermentation materials

Proper milk handling practice is a prerequisite prior to consumption, marketing and/or further processing purposes and therefore utensils that are used in milking, fermenting, churning, or consumption of milk must be properly and easily cleaned (Anteneh et al., 2008). About 47, 37 and 16% used clay pot, plastic bucket and stainless steel as a milking material, respectively. None of the respondents reported wooden container, metallic

Table 6. Material used for milking and fermentation.

Material used for milking	Sodo (N=30)	
	n	%
Clay pot	14	46.7
Stainless steel	5	16.6
Plastic bucket	11	36.7

Table 7. Gender analysis on milking and post-harvest activities.

Milking	Sodo (N=30)	
	n	%
Male	0	0
Female	30	100
Milk handling		
Male	3	10
Female	27	90
Milk processing		
Male	0	0
Female	30	100
Marketing		
Male	3	10
Female	27	90

container and calabash (qil) as a milking and fermenting material (Table 6). In agreement with the present study, Sintayehu et al. (2008) reported that majority of dairy producers at shashmene- Dilla areas of the same region used traditional churning material made from clay pot. This observation is also similar to the case for the central highlands where clay pot churn is mostly used (O'Mahoney and Peter, 1987), whereas it is different from the case of East Wollega where 91% of women used gourd for churning and storage of milk (Alganesh, 2002).

The role of gender in milking and post-harvest activities

In the study area, gender has a great role in milking and post harvest activities. According to the respondents, milking (100%), milk handling (90%), milk processing (100%), milk marketing (90%) were done by female household members (Table 7). In agreement with the present findings, Ayantu (2006) reported that milking and milk processing are done by female and men do not process milk and/or involve in the marketing of milk and milk products in Delbo watershed of Wolayta zone. Alganesh (2002) also reported that in Wollega area, women exclusively do milking and processing of milk into different products.

Milk and milk products marketing

All producers in the milk shed of Sodo town who own crossbred cows participated in fluid milk marketing. The dominant informal milk marketing was based on contractual agreement between the producer and the client at Sodo. Prices were negotiated and the milk was delivered on a daily basis. The producer was paid at the end of the month. During the wet season (June to November), low price of milk (8.00 Ethiopian Birr (ETB) per liter on average) and butter (93 ETB per Kg on average) was due to feed availability. On the other hand, during dry season (December to May), high price of milk (9.00 ETB per liter on average) and butter (106 ETB per Kg on average) was due to scarcity of feed for dairy animals and also in association with availability of some public festivals (Figures 1 and 2). In agreement with the present findings, Anteneh et al. (2008) reported that during the wet season, the price of milk and milk products are lower due to better roughage supply to dairy cattle, resulting in higher milk yields and higher supply of milk

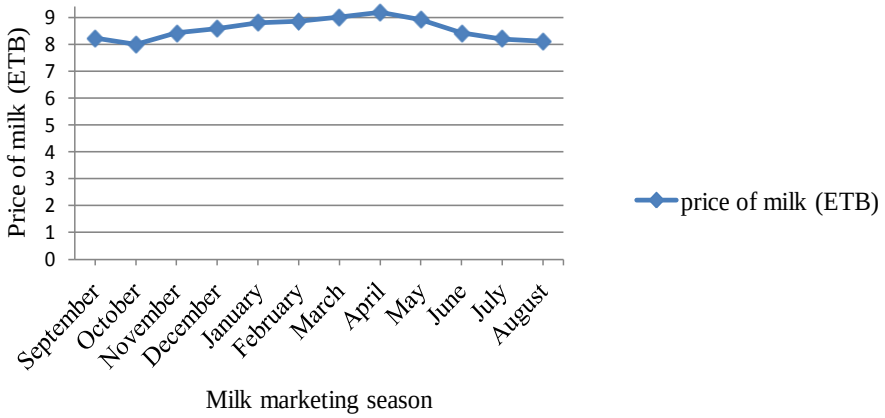

Figure 1. Price of milk in 2011/2012 (ETB).

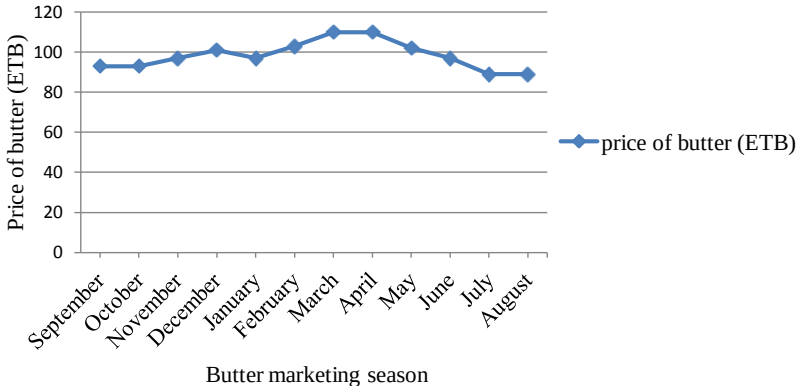

Figure 2. Price of butter in 2011/2012 (ETB).

and the contrary is true for dry season at Shashmene-Dilla areas of Ethiopia.

Conclusion

Respondents have poor knowledge on standardized dairy products. Consumption trend of ghee, milk powder and ice cream were nil due to unavailability of the products. Majority of purchasing consumers were not concerned at all about safety of dairy products when purchased from open markets. Milking practices such as use of teat dip solutions after milking and use individual clean towel were very poor. Almost all of milking and post harvest activities were done by female house hold members. Price of milk and milk products was directly depends on price and availability of feed.

Therefore, awareness creation, supply of value added milk products, milk production technologies, introduction of year-round feed production and conservation technologies were needed to improve the production and consumption trend of milk and dairy products.

Conflict of Interest

The author(s) have not declared any conflict of interests.

ACKNOWLEDGMENTS

We appreciate support from Eastern Africa Agricultural Productivity Project (EAAPP) with an equipment loan from Areka Agricultural Research Center. The terms of this arrangement have been reviewed and approved by the EAAPP and Southern Agricultural Research Institute with its policy on objectivity in research.

REFERENCES

Alganesh T (2002). Traditional milk and milk products handling practices and raw milk quality in eastern Wollega. MSc Thesis. Alemaya University. Ethiopia. P. 108.

Ayantu M (2006). Women,s role on production, processing and marketing of milk and milk products in Delbo watershed of Wolayta Zone, Ethiopia. MSc Thesis. Hawasa University. Ethiopia pp. 45-48.

Ayele S, Assegid W, Jabbar MA, Ahmed MM, Belachew H (2003).

Livestock marketing in Ethiopia: A review of structure, performance and development initiatives. Socio-economics and Policy Research Working ILRI (International Livestock Research Institute), Nairobi, Kenya. 52:35.
Bilatu A, Kassahun M, Asnaku F, Kassech M (2013). Assessment of knowledge gap and factors affecting consumption of dairy Products in Ada,a and Lume districts of East Showa Zone, Ethiopia. Afr. J. Food Sci. Technol. 3(9):2001-2010.
Belete A, Azage T, Fekadu B, Berhanu G (2010). Cattle milk and meat production and marketing systems and opportunities for market-orientation in Fogera woreda, Amhara region, Ethiopia. IPMS (Improving Productivity and Market Success) of Ethiopian Farmers Project Working ILRI (International Livestock Research Institute), Nairobi, Kenya. 19:65.
FAOSTAT (Food and Agricultural Organization of the United Nations Statistics) (2007). Online database on food and agricultural products and producers. Accessed online at: http://www.faostat.fao.org/. FAO, Rome, Italy.
Getachew F, Gashaw G (2001). The Ethiopian dairy development policy: A draft policy document. Ministry of Agriculture/AFRDRD/AFRDT Food and Agriculture Organization of the United Nations/SSFF. Addis Ababa, Ethiopia.
O'Connor CB, Zinash Z (1990). The production and utilization of milk and milk products by smallholders in the Eastern Region of Shoa, Ethiopia. Proceedings of the XXIII International Dairy Congress, Brief communications and posters Montreal, Canada 1:12.
O, Mahoney F, Peters J (1987). Options for small holder milk processing. World Anim. Rev. 62:16030.
Sintayehu Y, Fekadu B, Azage T, Berhanu G (2008). Dairy production, processing and marketing systems of Shashmene-Dilla area, South Ethiopia. P. 13.
SPSS (Statistical Procedures for Social Sciences) (2001). SPSS BI Survey Tips. Statistical Procedures for Social Sciences (SPSS) INC. Chicaco, USA.
Zegeye Y (2003). Imperative and challenges of dairy production, processing and marketing in Ethiopia. In: Jobre Y and Gebru G (eds), Challenges and opportunities of livestock marketing in Ethiopia. Proceedings of the 10th annual conference of the Ethiopian Society of Animal Production (ESAP) held in Addis Ababa, Ethiopia, 22–24 August 2002. ESAP, Addis Ababa, Ethiopia. pp. 61–67.
Zelalem Y, Faye B (2006). Handling and microbial load of cow's milk and Irgo—fermented milk collected from different shops and producers in central highlands of Ethiopia. Ethiop. J. Anim. Prod. 6(2):7–82.

academicJournals

Vol. 9(47), pp. 3434-3440, 20 November, 2014
DOI: 10.5897/AJAR2013. 8097
Article Number: 56079BD48591
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Nitrate reductase activity and corn hybrid yields intercropped with *Brachiaria brizantha* in three sowing arrangements

Orismário Lúcio Rodrigues[1], João Carlos Cardoso Galvão[2], Evander Alves Ferreira[3], Daniel Valadão Silva[2]*, Márcia Vitória dos Santos[4], Lino Roberto Ferreira[1], Rosana Cristina Pereira[3] and Rafael da Silva Felipe[2]

[1]Departamento de Agronomia, Faculdade Univértix , Matipó, Brasil.
[2]Departamento de Fitotecnia, Universidade Federal de Viçosa, Viçosa, Brasil.
[3]Departamento de Fitotecnia, Universidade Federal dos Vales do Jequitinhonha e Mucuri, Diamantina, Brasil.
[4]Departamento de Zootecnia, Universidade Federal dos Vales do Jequitinhonha e Mucuri, Diamantina, Brasil.

Received 15 October, 2013; Accepted 10 October, 2014

Crop-livestock systems consists in the cultivation of agricultural and foraging species in the same area, in consortium, rotation or crops succession. Among several used crops, corn and brachiaria intercropping stands out, for its importance, cultivation tradition and adaptation of species to intercropping cultivation. This study aimed to evaluate the influence of *Brachiaria brizantha* (brachiaria) sowing arrangements on yield and nitrate reductase activity in different corn hybrids. A randomized block design was used with four replications, in a 4×3 factorial scheme. Treatments constituted of four corn hybrids DKB 747, BM2202, AGN30A00 and AG2060 and three forage sowing arrangements: a) corn crop at 0.9 m spacing, two rows of *B. brizantha* in the inter-row at 0.3 m from the corn row; b), corn crop at 0.9 m spacing, one brachiaria row in the corn sowing row, mixed with starter fertilizer, and another one in the inter-row center; c) corn crop at 0.45 m spacing, with brachiaria in the corn sowing row, mixed with starter fertilizer. The spacing reduction between corn rows promoted yield increase and reduced brachiaria growth. Lower nitrate reductase activity in corn plants was found in the corn crop at 0.9 m spacing, with two *B. brizantha* rows located in the corn interrow at 0.3 m.

Key words: Brachiaria, competition crop-livestock systems, *Zea mays*.

INTRODUCTION

The establishment of forage species intercropped with annual crops, named integrated crop-livestock systems, is an efficient and economically viable technique for formation, recovery and renovation of pastures (Jakelaitis et al., 2004; Freitas et al., 2005a, b). This integration allows the development of two activities, agricultural and livestock production, which, through simultaneous or sequential crop cultivation, contributes to yield and

*Corresponding author. E-mail: danielvaladaos@yahoo.com.br, Tel: (55) 31 3899-1164.

improvement of soil physical and chemical characteristics (Amaral Filho et al., 2005).

Several crops have been cultivated with *Brachiaria* species. Among them, corn stands out, mainly, due to its cultivation tradition, great number of commercial cultivars well-adapted to different ecological regions of Brazil, numerous uses in the rural property. In addition, corn has excellent adaptation when intercropped and can be used for production of sweet corn, grains or silage (Santos and Araújo, 2011).

However, when two species are simultaneously cultivated, they are submitted to mutual interferences, which may result in competition for environmental resources, leading to losses that may make intercropping unfeasible. Among the factors that affect corn yield and pasture establishment in intercropped cultivation, the sowing arrangement of species deserves special attention.

Planting of the agricultural crop and forage grass can be carried out simultaneously or sequentially, and it is necessary to favor the early development of the crop so that there is no significant grain yield loss. Some direct seeding machines have a forage seedling device. When this equipment is not available, forage seeds can be mixed to fertilizer or broadcast by hand. Thus, forage sowing arrangements can be carried out by broadcast seeding, in the same rows or in the inter-rows of the crop (Santos and Araujo, 2011). Planting forage in the same row as corn may promote corn yield reduction, when compared with planting only in the inter-rows (Borghi and Crusciol, 2007). Reports on inter-specific competition vary according to the adopted planting arrangement: some studies reported that the forage did not interfere with corn yield, while others showed the necessity of application of herbicide subdoses to reduce forage growth and allow full corn development (Jakelaitis et al., 2005). Another factor that can influence corn yield and pasture establishment is the use of corn hybrids with different leaf architecture and life cycle, as simple hybrids have modern leaf architecture, with smaller plants and erect leaves, allowing higher light penetration in the canopy and favoring forage development, when compared with double and triple hybrids, which are larger plants with flat leaves (Jakelaitis et al., 2005). Among other factors, the period in which the forage competes for environment resources with the crop may alter crop yield, making the use of control measures necessary, like the use of sub-doses of herbicides, to avoid this interference (Jakelaitis et al., 2004). When brachiaria is mixed to fertilizer and sown in the corn row, it is deposited at a higher depth, delaying its emergence, favoring the corn crop (Kluthcouski et al., 2000). Forage emergence subsequently to corn emergence favors competitive advantage to corn, due to reduction of the competition period for water and nutrients between the species. The purpose of the present study was to evaluate the influence of *Brachiaria brizantha* cv. Marandu in diferente planting arrangement son nitrate reductase activity and yield of four corn hybrids.

MATERIALS AND METHODS

The experiment was carried out in the growing season of 2008 to 2009 at the Experimental Unit at Coimbra, of the Federal University of Viçosa, situated in the Coimbra region, Zona da Mata, MG, at 715 m altitude. Climatic data from the period of the test are shown in Figure 1. A randomized blocks design, 4 × 3 factorial arrangements, with four replications was employed. The treatments were composed of the combination of four corn hybrids and three *B. brizantha cv*. Marandu (brachiaria) sowing arrangements: a) arrangement 1, corn crop at 0.9 m spacing, with two brachiaria rows in the inter-row at 0.3 m from the corn row; b) arrangement 2, corn crop at 0.9 m spacing, with one brachiaria row in the corn sowing row, mixed to fertilizer, and another one in the interrow center; c) arrangement 3, corn crop at 0.45 m spacing, with brachiaria in the corn sowing row, mixed with fertilizer.

The used corn hybrids were DKB 747 (double hybrid, medium/large size, precocious), BM2202 (double hybrid, medium/large size, flat leaves, precocious), AGN30A00 (simple hybrid, small size, erect leaf architecture, extra-precocious) and AG2060 (double hybrid, high, flat normal leaves). Prior to experiment, soil samples were collected at 0 to 0.2 m depth, for chemical and physical analysis. The results revealed the following characteristics: 70% clay; water pH of 4.9; 8.2 mg dm^{-3} of P (Mehlich-1); 55 mg dm^{-3} of K; 0.2 $cmol_c dm^{-3}$ of Al; 1.1 $cmol_c dm^{-3}$ of Ca; 0.6 $cmol_c dm^{-3}$ of Mg; and 1.8 g dm^{-3} of MO. Nearly 30 days before sowing, desiccation was carried out in the area, using 1.8 kg ha^{-1} of glyphosate, with syrup volume of 250 L ha^{-1}

Corn and brachiaria sowings were carried out on November 21 and 22nd, by a seeding-fertilizing machine. Corn seeds were deposited at 5 cm depth. The corn population was 70.000 plants ha^{-1} for hybrid AGNA30A00 and 55.000 plants ha^{-1} for the others, according to planting recommendations of the seed producing companies. It was used 3.8 kg ha^{-1} of viable pure brachiaria seeds. In sowing arrangements where the forage was planted in the corn inter-rows, the seeds were conditioned in the machine seed deposit and planted at 3 cm depth. In arrangements in which the forage was sown in the corn row, seeds were mixed to fertilizer and conditioned in the machine fertilizer compartment, distributed at 8 cm depth, below the corn seed. The starter fertilizer application consisted of 28 kg ha^{-1} of N, 98 kg ha^{-1} of P_2O_5 and 56 kg ha^{-1} of K_2O, corresponding to 350 kg ha^{-1} of the formulated fertilizer NPK (8-28-16). When the corn crop reached the stage of four completely expanded leaves, the top dressing was carried out with 210 kg ha^{-1} of urea. The weeds were controlled at 25 days after sowing (DAS) by application of 1.5 kg ha^{-1} of atrazine herbicide, using a backpack sprayer, pressurized with CO_2, constant pressure of 200 kPa, equipped with two pulverization tips (TT 11002), and calibrated to apply 100 L it ha^{-1} of syrup. At application, weed and brachiaria plants presented an average of two leaves. The climatic conditions at application were clear sky, moist soil, wind speed inferior to 5 km h^{-1}, air temperature around 25°C and relative moisture superior to 65%. For control of corn armyworm, 129 g ha^{-1} of Metomil insecticide was used.

Each experimental unit constituted of six rows of 6 m length, totalizing an area of 32.4 m^2. The data corn and brachiaria yields were collected in the two central rows, non-considering 1.0 m in the edges. The sampling for determination of nutrient levels in the leaves was carried out when at least 50% of the corn plants exhibited tassels and style-stigma presence. In this case, the leaf opposite to the superior ear was collected, according to Malavolta et al. (1997), in 10 plants per experimental unit. At corn harvest, mean ear weigh, mean per thousand grain weight and yield, corrected to 13% moisture were evaluated. For determination of

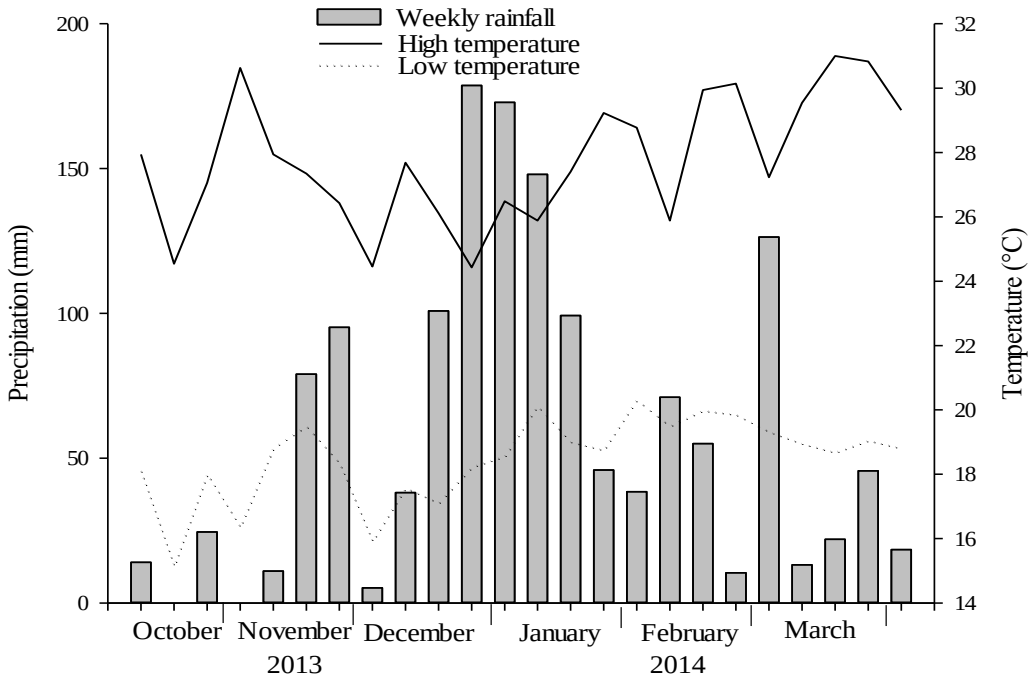

Figure 1. Rainfall and weekly average temperature during the conduct of the experiment.

forage species biomass all brachiaria plants were collected at the soil level from the two central rows of each experimental unit. This sampling was carried out at corn harvest. All collected material was dried in stove with forced air circulation, approximately 60°C, for 72 h and weighed subsequently.

The nitrate reductase (NR) activity was determined in the leaf opposite to the superior ear. Leaves were collected at 55 days after emergence, between 7 and 8 a.m. Samples were conditioned in thermal box with ice. At the laboratory, 1 cm diameter leaf discs were used, which were incubated in 10 ml phosphate buffer (KH_2PO_4 and K_2HPO_4) 0.2 M (pH 7.5), 0.25 M potassium nitrate, propanol and Triton X-100 10%. After immersion, samples were transferred to a dissecator and submitted to vacuum infiltration for one minute, three times, aiming at increasing solution penetration in the tissues. Next, incubation bottles wrapped in foil, were taken to water bath at 30°C. At previously set times of 0.5 to 1 h, 1 ml samples were withdrawn, in which 0.3 ml of 1% sulfanilamide, 0.3 ml of 0.02% naphthalenediamine and 2.4 ml of water were added. The reading was done in spectrophotometer at 540 nm. The NR activity was determined by the amount of nitrite produced, comparing the obtained values with the standard curve for this ion. The activity was expressed in µmoles of nitrite per hour per gram of fresh matter (µmoles of NW^{-2} hour/g fresh matter). The data were submitted to analysis of variance, in case of significance, means were compared by Tukey's test at 5% probability.

RESULTS AND DISCUSSION

There was significant difference in the thousand grains weight and mean ear weight between the evaluated hybrids; however, this difference did not result in statistical difference between yields (Table 1). The AGNA30A00 hybrid, though has presented lower ear weight, obtained satisfactory yield value due to higher population of plants.

The AGNA30A00 presented lower leaf area index (LAI) than the other corn hybrids (Table 1). The leaf area index that determines maximum growth rate is known as critical leaf area index, and varies according to the environment where the plant is. The critical LAI for the corn crop oscillates between values from 3 to 5, according to the region, hybrids and production system considered (Fancelli and Dourado-Neto, 2004). The simple hybrid AGN30A00 presented LAI lower than the critical value (Table 1), compromising its productive potential.

On average, the nitrate reductase activity values were 20 to 30% higher for BM2202 and AGN30A00 hybrids, in relation to the others. Despite not differing significantly, these hybrids were also the most productive (Table 1). The genotypic differences as for grain production can be attributed to the soil nitrate absorption capacity (Purcino et al., 1998) and consequently elevation of the nitrate reductase activity, since the enzyme activity varies according to the substrate concentration (Redinbaugh and Campbell, 1991).

The dry mass of the brachiaria plants aerial part did not differ statistically from the evaluated corn hybrids (Table 1). Although the AGN30A00 hybrid has presented lower plant height and smaller leaf area comparing with the others (Table 1), providing less shade in the inter-row and allowing, thus, higher brachiaria plant growth. The biomass production of this forage was not significantly higher possibly due to the inter-specific competition caused by higher population of this corn hybrid plants.

The reduction of space between corn rows increased

Table 1. Yield (YD, kg ha^{-1}), thousand grain weight (TGW, g), mean ear weight (MEW, g), leaf area index (LAI) and nitrate reductase activity (NR, µmoles of NO^{2-}/hour/g of fresh mass) of corn hybrids and dry mass of *Brachiaria brizantha cv.* Marandu (DMB, kg.ha^{-1}).

Variables	TGW (g)	MEW (g)	YD (kg^{-1})	LAI	NR (µmoles of NO_2-/hour / g M.F).	DMB (kg^{-1})
BM2202	292 ^{b}	153 ^{a}	6.918 ^{a}	3.42 ^{a}	4.0 ^{ab}	3.156 ^{a}
AG2060	260 ^{c}	140 ^{ab}	5.533 ^{a}	3.22 ^{a}	3.4 ^{b}	4.852 ^{a}
DKB747	326 ^{a}	152 ^{a}	6.206 ^{a}	3.24 ^{a}	3.3 ^{b}	3.751 ^{a}
AGN30A00	298 ^{b}	118 ^{b}	6.626 ^{a}	2.89 ^{b}	4.4 to	4.690 ^{a}
CV %	7.8	17.0	21.2	8.5	19.1	38.1

^{ns} Not significant at 5% probability. Means followed by same letter in column do not differ at 5% probability by Tukey's test. CV: Coefficient of variation.

Table 2. Corn yield (YD, kg.ha^{-1}), corn leaf area index (LAI), nitrate reductase enzyme activity (NR, µmoles of NO^{2-} /hour/g of fresh mass) and dry mass of aerial part of *Brachiaria brizantha cv*. Marandu (DMB, kg.ha^{-1}) according to sowing arrangements.

Arrangements*	YD	LAI	NR	DMB
1	5.510 ^{b}	3.0 ^{b}	3.1 ^{b}	6.284 ^{a}
2	5.568 ^{b}	3.1 ^{b}	4.2 ^{a}	3.735 ^{b}
3	8.033 ^{a}	3.5 ^{a}	4.1 ^{a}	2.318 ^{c}
CV %	21.2	8.5	19.6	38.0

Means followed by same letter in column do not differ at 5% by Tukey's test. CV: Coefficient of variation , * 1, corn cultivation at 0.9 m spacing, with two rows of *Brachiaria brizantha* (brachiaria) located in interrow at 0.3 m from corn row; 2, corn cultivation at 0.9 m spacing, with a row of brachiaria in corn sowing row, mixed to starter fertilizer, and another one in interrow center; 3, corn cultivation at 0.45 m spacing, with brachiaria in corn sowing row, mixed with starter fertilizer.

yield in 45% (Table 2). Similar results were reported by Silva et al. (2006) with reduction in space between rows from 0.8-0.1 m to 0.4-0.5 m, increasing yield in approximately 10%, in subtropical regions in the South of Brazil. In the Middle West region, space reduction from 0.9 to 0.45 m promoted growth even higher than those in the South of the country, reaching values 10 to 40% higher. The sowing space reduction between rows favors better space distribution of corn plants (Sangoi et al., 2002). This reduces intra-specific competition for the surrounding resources, stimulates crop growth at the beginning of its cycle and develops light inter-capitation, increasing its use efficiency (Andrade et al., 2002). All hybrids presented yield reduction when the spacing of 0.9 m was adopted between rows (Table 2). However, planting two rows of brachiaria in the inter-row or brachiaria in the same row and in the inter-row did not interfere with corn yield.

The spacing reduction provided higher shade for the inter-row due to the space arrangement of plants, which reduced brachiaria growth and consequently of the produced biomass (Table 2). At 0.45 m spacing, all corn hybrids presented greater leaf area, intensifying the shade effects on the brachiaria plants (Table 2). Similar results were obtained by Borghi and Crusciol. (2007).

Corn cultivation at 0.45 m spacing, with brachiaria in the corn sowing row, mixed to planting fertilizer, allowed better corn plants arrangement, as well as quick growth and yield. Besides, it provided greater shade for brachiaria plants, delaying biomass accumulation by the forage during the inter-specific competition period (Table 2).

Corn cultivation was also adopted at 0.9 m spacing, with a brachiaria row in the corn sowing row, mixed to planting fertilizer, and another one in the center of the row space, brachiaria seeds were mixed to fertilizer and deposited at 8 cm depth in the corn planting row. Under this condition, brachiaria plants were intensely shaded by corn plants and had a delayed emergence, which favored the corn development. Portes et al. (2000), while evaluating the same intercropped species used in this experiment, reported that the deposition of brachiaria seeds at 10 cm depth, with fertilizer, delayed the emergence of forage plantlets in approximately five days and weakened them. Owing to the shade provided by the corn during the intercropping period, the forage presented slow growth, mainly because both species have C4 carbon fixation.

Higher growth of brachiaria plants in the corn inter-row may cause competition for water and nutrients, reducing nitrogen availability for corn plants. Nitrogen moves in the soil by mass flow, and the exploration of the same soil

Table 3. Effect of planting arrangements on yield (YD, kg^{-1}), thousand grain weight (TGW, g), mean ear weight (MEW, g), leaf area index (LAI), and nitrate reductase (NR activity, µmoles of NO^{2-}/hour/g of fresh mass) of corn hybrids and dry mass of aerial part of *Brachiaria brizantha* cv. Marandu (DMB, kg ha^{-1}), in intercropped cultivation.

Planting arrangements*	YD	LAI	NR	DMB
AGN30A00				
1	4.094 [c]	2.65 [b]	2.61 [b]	6.492 [a]
2	6.081 [b]	2.72 [b]	5.66 [a]	4.499 [b]
3	9704 [a]	3.33 [a]	4.93 [a]	3.079 [c]
DKB 747				
1	5.605 [b]	2.98 [b]	3.04 [a]	5.017 [a]
2	5.763 [b]	3.26 [ab]	3.76 [a]	3.832 [b]
3	7.252 [a]	3.51 [ab]	3.28 [a]	1.795 [c]
AG 2060				
1	4.856 [b]	3.42 [ab]	3.46 [a]	8.100 [a]
2	4.601 [b]	2.97 [ab]	2.94 [a]	3.868 [b]
3	7.142 [a]	3.42 [a]	3.89 [a]	2.588 [c]
BM 2202				
1	6.888 [b]	3.15 [b]	3.28 [a]	5.527 [a]
2	5.829 [b]	3.43 [ab]	4.36 [a]	2.741 [b]
3	8.036 [a]	3.69 [ab]	4.49 [a]	1.200 [c]
CV %	21.0	8.5	19.1	30

In each hybrid, means followed by same letter in column do not differ at 5% by Tukey's test. CV: Coefficient of variation * 1, corn cultivation at 0.9 m spacing, with two rows of *Brachiaria brizantha* (brachiaria) in interrow at 0.3 m from corn row; 2, corn cultivation at 0.9 m spacing, with a brachiaria row in corn sowing row, mixed with starter fertilizer, and another one in interrow center; 3, corn cultivation at 0.45 m spacing, with brachiaria in corn sowing row, mixed with starter fertilizer. VC: variation coefficient.

area by the radicular system of the two crops can reduce the influx of this nutrient for the roots, causing reduction in the nitrate supply through the xylem, reducing, thus the nitrate reductase activity (Kawachi et al., 2002). Lower nitrogen availability for the plant can cause reduction in the photosynthetic rate, reduction of chlorophyll levels (Ciompi et al., 1996; Gouy et al., 2001) and reduction in leaf expansion and in the activity of some enzymes of the nitrogen reducing cycle, as nitrate reductase. The nitrate reductase enzyme activity was statistically lower in the corn crop at 0.9 m spacing, with two rows of *B. brizantha* located in the inter-row at 0.3 m of the corn row (Table 2). This result can be attributed to greater forage development, caused by higher light availability and quick emergence. The nitrate reductase activity is positively correlated with nitrate availability in the environment, corn plants, when evaluated in soils with higher nitrogen availability, present higher nitrate reductase activity, when compared with those cultivated at below N (Miranda et al., 2005; Majerowics et al., 2002; Oliveira, 2009).

The AGNA30A00 hybrid presented lower yield for corn cultivation at 0.9 m spacing, with two rows of *B. brizantha* located in the inter-row at 0.3 m of the corn row, when compared to the other arrangements (Table 3). That may have been caused by higher competition of the brachiaria plants in this arrangement. In planting arrangements in which the brachiaria was sowed in the corn row space, brachiaria was deposited at a lower depth, promoting quicker emergence and being able to compete, with the corn. The above-mentioned hybrid was shorter and presented less leaf area regarding the rest (Table 3), promoting less shade in the inter-row and allowing, thus, higher brachiaria growth. Among the evaluated corn hybrids, the AGN30A00 presented higher nitrate reductase activity (Table 1). Due to its modulating role of reduced N availability for the plants metabolism, it has been suggested that the nitrate reductase activity is associated with corn yield and/or, with its capacity of responding to nitrogen fertilizing (Araujo et al., 2009; Borges et al., 2006). This tendency was confirmed, since in planting arrangements in which the AGN30A00 hybrid did not suffer brachiaria interference it presented yield and nitrate reductase activity values superior to the others (Table 3).

The AGNA30A00 hybrid presented lower nitrate

Table 4. Effect of corn planting arrangements intercropped with brachiaria on nitrate reductase (NR) enzyme activity (µmoles of NO^-_2 g^{-1} h^{-1} of fresh mass).

Planting arrangements*	AGNA30A00	BM 2202	AG2060	DKB747
1	2.61^{Ba}	3.28^{Aa}	3.46^{Aa}	3.04^{Aa}
2	5.66^{Aa}	4.36^{Aab}	2.94^{Ab}	3.76^{Ab}
3	4.93^{Aa}	4.49^{Aab}	3.89^{Aab}	3.28^{Ab}
CV %	19.6	19.6	19.6	19.6

Means followed by same capital letter in column, and lowercase letter in row do not differ at 5% probability Tukey's test. CV: Coefficient of variation, * 1, corn cultivation at 0.9 m spacing, with two rows of *Brachiaria brizantha* (brachiaria) in interrow at 0.3 m from corn row; 2 – corn cultivation at 0.9 m spacing, with a row of brachiaria in corn sowing row, mixed with starter fertilizer, and another one in interrow center; 3, corn cultivation at 0.45 m spacing, with brachiaria in corn sowing row, mixed with starter fertilizer.

reductase activity at 0.9 m spacing, with two rows of *B. brizantha* located in the inter-row at 0.3 m of the corn row (Table 4). Lower enzyme activity may have been caused by higher competition of brachiaria plants in this arrangement. The AGN30A00 hybrid presented lower leaf rate area in this arrangement, when compared with the others (Table 3), allowing higher growth of brachiaria plants, causing greater competition for nutrients, reducing nitrogen availability, and consequently, the nitrate reductase activity. When studying the genetic and biochemical bases of nitrogen use efficiency in corn, Hirel et al. (2001) found significant correlations between physiologic characteristics (activities of NR and glutamine synthetase-GS enzymes and nitrate level in the aerial part of plants) and agronomic characteristics (yield and grain weight). Gallais and Hirel (2004) emphasized that these correlations were dependent of the quantity of nitrogenated fecundation. The activity of NR and GS enzymes in corn plants of appropriately supplied with nitrogen was superior comparing with plants submitted to stresses by nitrogen deficiency (Majerowics et al., 2002). There was no significant difference in leaves nitrogen level, regarding hybrids or adopted planting arrangements. The N level in leaves is below the ideal level for all evaluated hybrids, which is from 2.7 to 4.0 dag kg^{-1} (Ferreira et al., 2001). The lowest leaf nitrogen level was found in hybrid AGNA30A00, using 0.9 m spacing, with two rows of *B. brizantha* located in the inter-row at 0.3 m of the corn row, where lower nitrate reductase activity was also verified. For the other hybrids and spacings, the nitrogen level in leaves varied between 2.13 and 2.64 dag kg^{-1}.

Conclusions

The spacing reduction from 0.90 m to 0.45 m between corn rows increased yield and reduced growth of *B. brizantha cv*. Marandu and corn rows increased corn leaf area. Smaller corn hybrids with erect leaves were more prone to competition with brachiaria, mainly when sowing the forage in the inter-row. Lower nitrate reductase activity in corn plants occured with higher brachiaria plants growth, in the arrangement of two forage rows in the corn inter-row.

Conflict of Interest

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENTS

The authors thank the Coordination for the Improvement of Higher People Personnel (CAPES), National Council for Scientific and Technological Development (CNPq) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) for the financial support and granted scholarships.

REFERENCES

Amaral Filho JPR, Fornasieri Filho D, Farinelli R, Barbosa JC (2005). Espaçamento, densidade populacional e adubação nitrogenada na cultura do milho. Rev. Bras. Cienc Solo, 29:467-473.http://dx.doi.org/10.1590/S0100-06832005000300017

Andrade FH, Calvi-o P, Cirilo A, Barbieri P (2002). Yield responses to narrow rows depend on increased radiation interception. Agron. J. 94:975-980. http://dx.doi.org/10.2134/agronj2002.0975

Araujo EA, Oliveira TK, Rosário, A.A.S.; Oliveira FILHO, JP (Ed.). (2009); Alternativas de utilização de áreas alteradas no Estado do Acre. SEMA, Rio Branco, pp. 81-90.

Borges EA, Fernandes MS, Loss A, Silva EE, Souza SR (2006). Acúmulo e remobilização de nitrogênio em variedades de milho. Revista Caatinga, 19:278-286.

Borghi E, Crusciol CAC (2007). Produtividade de milho, espaçamento e modalidade de consorciação com Brachiaria brizantha em sistema plantio direto. Pesqui Agropecu Bra, 42:163-171. http://dx.doi.org/10.1590/S0100-204X2007000200004

Ciompi S, Gentili E, Guidi L, Soldatini G F (1996). The effect of nitrogen deficiency on leaf gas exchange and chlorophyll fluorescence parameters in sunflower. Plant Sci. 118:177-184.

Fancelli AL, Dourado Neto D (2004). Produção de milho. 2.ed. Guaíba: Agropecuária, P. 360.

Ferreira ACB, Araújo GAA, Pereira PRG, Cardoso AA (2001). Características agronômicas e nutricionais do milho adubado com nitrogênio, molibdênio e zinco. Scientia Agricola., Piracicaba, 58:131-138.

Freitas FCL, Ferreira LR, Ferreira FA, Santos MV, Agnes EL, Cardoso AA, Jakelaitis A (2005a). Formação de pastagem via consórcio de Brachiaria brizantha com o milho para silagem no sistema de plantio

direto. Planta Daninha, 23:49-58.
Freitas FCL, Ferreira LR, Ferreira FA, Santos MV, Agnes EL (2005b). Cultivo de milho para silagem com Brachiaria brizantha com o milho no sistema de plantio convencional. Planta Daninha, 23:235-244.
Gallais A, Hirel B (2004). An approach to the genetics of nitrogen of use efficiency in maize. J. Exp. Bot. 55:295-306. http://dx.doi.org/10.1093/jxb/erh006
Gouy A, Cadiou S, Retailliau C, Falque M, Gallais A (2001). Towards a better understanding of the genetic and physiological basis for nitrogen use efficiency in Maize. Plant Physiol. 125:1258-1270. http://dx.doi.org/10.1104/pp.125.3.1258
Hirel B, Bertin P, Quilleré I, Bourdoncle W, Attagnant C, Dellay C, Gouy A, Cadiou S, Retailliau C, Falque M, Gallais A (2001). Towards a better understanding of the geneticand physio logical basis for nitrogen use efficiency in maize. Plant Physiol. 125:1258-1270 http://dx.doi.org/10.1104/pp.125.3.1258
Jakelaitis A, Silva AA, Ferreira LR, Silva AF, Freitas FCL (2004). Manejo de plantas daninhas no consórcio de milho com capim brachiaria (Brachiaria decumbens). Planta Daninha, 22:553-560.
Jakelaitis A, Silva AF, Silva AA, Ferreira LR, Freitas FCL, Viana RG (2005). Influência de herbicidas e de sistemas de semeadura de Brachiaria brizantha consorciada com milho. Planta Daninha, 23:59-67. http://dx.doi.org/10.1590/S0100-83582005000100008
Kawachi TY, Shoji Y, Sugimoto T, Oji Y, Kleinhofs A, Warner RL, Ohtake N, Ohyama T, Sueyoshi K (2002). Role of xylem sap nitrate in regulation of nitrate reductase gene expression in leaves of barley (Hordeum vulgare L.) seedlings. Soil Sci. Plant Nutr. 48:79-85. http://dx.doi.org/10.1080/00380768.2002.10409174
Kluthcouski J, Cobucci T, Aidar H, Yokoyama LP, Oliveira IP, Costa JLS, Silva JG, Vilela L, Bacellos AO, Magnabosco CU (2000). Sistema Santa Fé: tecnologia Embrapa: integração lavoura-pecuária pelo consórcio de culturas anuais com forrageiras, em áreas de lavoura, nos sistemas direto e convencional. Santo Antonio de Goiás: Embrapa Arroz e Feijão, (Circular técnica, 38:28.
Majerowics N, Pereira JMS, Medici LO, Bison O, Pereira MB, Santos Jr. UM (2002). Estudo da eficiência de uso do nitrogênio em variedades locais e melhoradas de milho. Rev Bras Bot, 25:129-136. http://dx.doi.org/10.1590/S0100-84042002000200002
Miranda GV, Godoy CL, Souza LV, Santos IC (2005). Selection of discrepant maize genotypes for nitrogen use efficiency by a chlorophyl meter. Crop Breed. Appl. Biol. 5:451-459.http://dx.doi.org/10.12702/1984-7033.v05n04a11
Oliveira LR (2009). Eficiência de uso de nitrogênio e atividade da nitrato reductase e glutamina sintetase em milho. Tese (Doutorado em Fitotecnia) – Universidade Federal de Viçosa, Viçosa-MG, P. 94.
Portes TA, Carvalho SIC, Oliveira IP, Kluthcouski J (2000). Análise do crescimento de uma cultivar de brachiaria em cultivo solteiro e consorciado com cereais. Pesqui Agropecu Bra 35:1349-1358. http://dx.doi.org/10.1590/S0100-204X2000000700009
Purcino AAC, Arellano C, Athwal GS, Huber SC (1998). Nitrate effect on carbon and nitrogen assimilating enzymes of maize hybrids representing seven eras of breeding. Maydica 43:83-94.
Redinbaugh MG, Campbell WH (1991) Higher plant responses to environmental nitrate. Physiologia Plantarum. 82:640-650. http://dx.doi.org/10.1111/j.1399-3054.1991.tb02958.x
Sangoi L, Almeida ML, Silva PRF, Argenta G (2002). Bases morfofisiológicas para maior tolerância dos híbridos modernos de milho a altas densidades de plantas. Bragantia, 61:101-110. http://dx.doi.org/10.1590/S0006-87052002000200003
Santos MV, Araújo EA (2011). Integração lavoura-pecuária na recuperação e renovação de pastagens degradadas. In:
Silva PFF, Sangoi L, Argenta G, Strieder ML (2006). Arranjo de plantas e sua importância na definição da produtividade em milho. Porto Alegre: Evangraf. P. 63.

academicJournals

Vol. 9(47), pp. 3441-3447, 20 November, 2014
DOI: 10.5897/AJAR2014.9252
Article Number: AD153C048593
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Stability analysis in genetically variant oilseed *Brassica* germplasm for *Sclerotinia*-rot resistance

Rajender Singh* and Dhiraj Singh

Department of Plant Pathology, CCS Haryana Agricultural University-Hisar-India.

Received 17 October, 2014; Accepted 31 October, 2014

Ninety two genotypes of oilseed *Brassica juncea* and *Brassica napus* were evaluated over three environments for analysis of stability parameters in relation to *Sclerotinia* rot resistance with responsible characters viz: plant age at the time of inoculation, stem diameter, stem lesion length and percent plant wilted/dead. The analysis of variance for stability revealed the presence of genetic variability in studied material for all the characters. The linear component of Genotype X Environment interactions was significant for all the characters, which indicates that the major portion of interaction was linear in nature and prediction of stable genotype for *Sclerotinia*-rot resistance over the environments was possible. Therefore, genotypes AG Spectrum, RQ011, RH13 and Ringot were found stable for *Sclerotinia*-rot resistance under normal environmental conditions. However, under congenial environmental conditions only six genotypes namely JM018, Ag Outback, Monty, *Brassica juncea* 1, *Brassica juncea* 2 and *Brassica juncea* 3 were most stable for resistance, which may be utilized for further improving genetic base for *Sclerotinia*-rot resistance in oilseed *Brassica*.

Key words: *Brassica juncea, Brassica napus, Sclerotinia*-rot, stability.

INTRODUCTION

Sclerotinia stem rot is a major disease of oilseed *Brassica,* with up to 80% incidence of plants affected in the worst affected crops in the Punjab and Haryana states of Northern India (Kang and Chahal, 2000). In Haryana, 5 to 20% of plants affected by this disease are common, varying with crop growth stage and region (personal communication). Estimated yield losses from *Sclerotinia* stem rot vary throughout the world, with 13% losses in North Dakota and Minnesota (Lamey and Bradley, 2002); 20% (Fitt et al., 1992) to 50% (Pope et al., 1989) in the UK; 0.4-1.5 tonne/ha losses in Australia (Kirkegaard et al., 2006) and 70% in China (Deng and Tang, 2006). Sclerotia of *Sclerotinia sclerotiorum* can germinate either myceliogenically or carpogenically, lead to stem base infection and aerial infections, respectively. Being a ubiquitous necrotrophic pathogen with many different hosts, *Sclerotinia* stem rot is difficult to manage. Primary methods of management rely upon use of non-host crops, fungicide application and manipulation of management practices, but all have been proved unreliable and frequently of little if any economic benefit. The variation among genotypes for *Sclerotinia* stem rot under different environmental conditions was observed by Li et al. (2006). Genetic resistance to *Sclerotinia* stem rot offers the best long term prospect for making oilseed *Brassica* crops more profitable in regions prone to this

*Corresponding author. E-mail: rsb1965@gmail.com

disease. For this reason, using a field stem inoculation technique, we evaluated oilseed *Brassica napus* and *Brassica juncea* genotypes from India, China and Australia for resistance to *S. sclerotiorum* in the field. Inpresent investigation, 92 genotypes of oilseed *Brassica* were evaluated over three environments that is, three crop seasons (2009, 2010 and 2011) to identify stable genotypes with *Sclerotinia* stem rot resistance.

MATERIALS AND METHODS

Seed of accessions of *B. napus* and *B. juncea* was obtained from Australia, China and India through an Australian Centre of International Agricultural Research (ACIAR) collaborative program between these 3 countries. The *B. napus* and *B. juncea* genotypes tested are enlisted in Table 2. Ninety two genotypes of *B. napus* and *B. juncea* were tested in the field at the Chaudhary Charan Singh Haryana Agricultural University (CCSHAU), Hisar, India. The germplasms were hand sown on 23rd October, 2009, 27th October, 2010, and 25th Oct 2011 in plots with 5 x 2 m. Row to row and plant to plant spacing was maintained 45 and 15 cm, respectively. All recommended agronomic practices were followed, including 100 kg N/ha, 30 kg P_2O_5 /ha fertilizer application at sowing time, two irrigations at 45 and 90 days after sowing in sandy loam soil having pH 8.0 and electrical conductivity 3.5 mMhos/cm. Each genotype was sown in a complete randomised block design (RBD) with three replicates and used for further study. The single isolate of *S. sclerotiorum* ('CCS HAU-Hisar') used in this study was isolated from sclerotia collected from diseased *B. juncea* plants stem pith in the previous crop season. Sclerotia were surface sterilized using 0.1% mercuric chloride solution in distilled water, cut in two pieces with sterilized Gilllete platinum blade and plated cut side face down on potato dextrose agar medium in 90 mm Petri® plates under aspetic conditions in laminar flow. Thirty plants of each genotype within each replication were picked at random and inoculated at the flowering stage (when 50% of plants in the row had at least one opened flower). This was stage GS 61 BBCH on the scale of Lancashire et al. (1991) and equivalent to stage GS 41-42 on the scale of Harper and Berkenkamp (1975). Stem inoculation was carried out according to the method of Buchwaldt et al. (2005) in all crop seasons. A single 5 mm diameter agar plug disc cut from the *S. sclerotiorum* colony edge of 3 to 4 days old culture growing on a glucose rich medium (Glucose 20 g, NaOH 1 g, NH_4NO_3 2 g, $MgSO_4$ 7 H_2O 0.1 g, Malic acid 3 g, KH_2PO_4 1 g and Agar 30 g in 1 L of distilled H_2O) was used to inoculate each plant. The agar plug was placed mycelium side up on a small piece of Parafilm® (about 5 cm long). The plug was then placed on to the stem at the first internode above the middle internode of each stem (with the mycelium in contact with stem) by wrapping the Parafilm® strip around the stem to secure the plug onto the stem. A wet cotton wool swab was also wrapped around the stem just above the top of the Parafilm® strip to maintain high humidity during the infection period. The observation on four characters viz. plant age at inoculation (days) time, stem diameter (mm), stem lesion length (mm) and wilted/dead plant (%) were recorded. The statistical analysis for stability was carried out according to the method of Eberhart and Russell (1966).

RESULTS

The pooled analysis of variance (Table 1) revealed that mean sum of squares due to Genotype (G) × Environment (E) interaction tested against pooled error and was found significant for wilted plants (%). It indicated that genotypes and environments not independent in causing variation but also have involvement of G×E interaction in the expression of wilted plant (%). Non significant G×E interaction observed for plant age at inoculation, stem diameter and stem lesion length indicate that these characters are least influenced by the environments. The absence of differential response of the genotypes for plant age at inoculation, stem diameter and stem lesion length in the present investigation indicates the stable expression of these characters.

Highly significant environmental (linear) variance for all the characters suggested that variation among the environments was linear. A linear environmental variance signifies unit changes in environmental conditions. The G×E (linear) variance was non significant for plant age at inoculation, stem diameter and stem lesion length implying thereby, differential performance of genotypes under diverse environments with nearly uniform reaction norms.

On the other hand, non significant pooled deviations for all the characters suggested that performance of different genotypes non-fluctuated significantly from their respective linear path of response to environments (Table 1). In other words, the predictable environments formed the major portion of G×E interactions. Moreover, by observing the individual varietal fluctuation from linearity, it becomes clear that only a very few genotypes fluctuated significantly from linearity. The environmental index (Ij) for all the environments and for all the characters was estimated. A critical analysis revealed that E_3 that is, environmental condition prevailed during 25th Oct 2011 sown genotype expressed high environmental index for the characters viz. plant age at inoculation, stem diameter, and wilted plant (%) and E_2 that is, environmental condition prevailed during 27th Oct 2010 sown genotype for stem lesion length (Table 2).

On the contrary, E_2 exhibited lowest value for plant age at inoculation time, and E_1 that is, environmental condition prevailed during 23rd 2009 sown genotype exhibited lowest value for stem diameter and stem lesion length (Table 2).

Plant age at inoculation (days)

On mean basis, 48 genotypes were early in plant age at inoculation (days) time and 44 genotypes were older in plant age. Out of 92 genotypes, only 8 genotypes expressed below average (b_i<1) response, 27 genotypes expressed above average (b_i>1) response and remaining 57 genotypes exhibited average response value of regression coefficient (Table 2). A consideration of the stability parameters together, 57 genotypes (30 below mean and 27 above mean) were average in response (b_i = 1) and good in stability (deviation from regression that is, S^2_{di}=0).

Table 1. Analysis of variance for stability (Eberhart and Russell, 1966).

Source of variation	d.f.	Mean sum of squares			
		Plant age at inoculation (days)	Stem diameter (mm)	Stem lesion length (mm)	Wilted plant (%)
Genotypes	91	399.852^{x}**$^{++}$	60.697^{x}**$^{++}$	2794.103^{x}**$^{++}$	878.468^{x}**$^{++}$
Environment	2	28.691^{x}**$^{++}$	10.120^{x}**$^{++}$	93.644^{x}**$^{++}$	67.068^{x}**$^{++}$
Genotype X Environment	182	0.085^{NS++}	0.061^{NS}	1.179^{NS++}	23.592**$^{++}$
Env. + (GXE)	184	0.393^{++}	0.170^{++}	2.184^{++}	24.065^{++}
Env. (linear)	1	57.382***$^{++}$	20.239***$^{++}$	187.288***$^{++}$	134.136***$^{++}$
En x Gen (linear)	91	0.123^{++}	0.030^{NS}	1.942^{++}	36.989**
Pooled deviation	92	0.041^{NS}	0.90^{NS}	0.411^{NS}	10.084**
Pooled error	546	2.686	1.083	5.086	4.917

** Significant at 1% level of significance against pooled errors, $^{++}$ Significant at 1% level of significance against pooled deviation, ^{x}Significant against Genotype (G) x Environment (E).

Stem diameter (mm)

An examination of individual stability parameter for stem diameter (Table 2) revealed that as many as 43 genotypes had above average mean performance and 49 genotypes had below average mean performance (Table 2). Further, all the genotypes were found to be stable (S^2_{di}=0). Majority of genotypes were having average response for stem diameter (b_i = 1). Only 15 genotypes exhibited below average response (b_i<1) and only one genotype that is, Rivette exhibited above average (b_i>1) response.

Stem lesion length (mm)

On the basis of mean stem lesion length (mm) it was observed that 42 genotypes exhibited below mean, 6 genotypes average mean and 50 genotypes above mean for stem lesion length. Further, all the genotypes exhibited non significant S^2_{di} value indicating the absence of non linear component of G×E interaction. Out of 92 genotypes, only 38 genotypes exhibited totally absence of G×E interaction having b_i = 1 and S^2_{di}=0, 17 genotypes (b_i > 1) and 55 genotypes (bi <1) exhibited the presence of linear component of G×E interaction (Table 2).

Considering the three stability parameters, simultaneously (high resistance/ small stem lesion, b_i = 1 and S^2_{di} = 0) RH 13, Ringot, *Brassica juncea* 1, *Brassica juncea* 2, *Brassica juncea* 3 and RQ 011 were highly resistant and stable for stem lesion length over the environments or three crop seasons (Table 2). Moreover, Ag outback was highly resistant and suitable for conducive environment (small lesion length, b_i <1, S^2_{di} = 0) for disease development against CCSHAU-Hisar isolate.

Wilted/ dead plants

An examination of data on wilted /dead plant reflected that 38, 13 and 41 genotypes were below mean, at average mean and above mean, respectively. In majority of genotypes linear components of G×E interaction was noticed, except 31 genotypes which showed the absence of linear component of G×E interaction. However, the majority of genotypes (81) exhibited the non significant for S^2_{di} value means absence of non-linear component of G×E interaction (Table 2). Out of top resistant genotypes against *Sclerotinia* stem rot, only Ag spectrum was found suitable for general environment conditions (highly resistant, b_i = 1, S^2_{di} = 0) and six other (JM018, Ag outback, Monty, *B. juncea* 1, *B. juncea* 2 and *B. juncea* 3), were suitable for conducive environment(highly resistant, b_i<1, S^2_{di} = 0) of disease development (Table 2). In contrast to this, JM 3 was highly susceptible to *Sclerotinia* stem rot; but it was also stable susceptible (S^2_{di} = 0 and b_i = 1).

DISCUSSION

On the basis of environmental index, it was found that E_3 was most conducive environment for disease development. The estimates of three stability parameters namely X, b_i and S^2_{di} (Table 2) revealed that the non significant value of S^2_{di} indicating thereby the totally absence of nonlinear component of G×E interaction in all the genotypes

Table 2. Estimates of stability parameters for *Sclerotinia* stem rot in oilseed *Brassica.*

Genotype	Plant age at inoculation (days)			Stem diameter (mm)			Steam lesion length(cm)			Wilted/dead plants (%)		
	Mean	bi	S^2_{di}	Mean	bi	S^2_{di}	Mean	bi	S^2_{di}	Mean	bi	S^2_{di}
'JN004'	81.222	1.179	-2.590	14.222	0.576*	-1.082	70.556	0.361*	-5.056	37.404	0.984	-4.891
'JN010'	79.778	0.014	-2.613	15.222	0.576*	-1.082	98.222	0.361*	-5.056	54.667	0.567*	-4.720
'JN028'	81.000	0.596*	-2.686	10.222	0.576*	-1.082	55.000	0.535*	-4.114	25.000	0.000*	-4.918
'JN031'	81.222	1.179	-2.590	11.222	0.576*	-1.082	76.556	0.249*	-4.694	34.703	-0.606*	-4.706
'JN032'	83.778	0.014	-2.613	13.333	0.922	-1.048	105.000	0.211	-4.288	44.952	1.600	-4.223
'JN033'	79.000	0.596*	-2.686	11.222	0.576*	-1.082	82.333	0.324*	-5.078	29.887	0.000*	-4.918
'JM016'	80.444	1.761	-2.324	15.222	0.576*	-1.082	96.222	0.143*	-5.054	48.144	1.969*	-4.808
'JM018'	85.000	0.596*	-2.686	12.333	0.807	-1.004	25.667	0.324*	-5.078	8.256	0.008*	-4.885
'JO008'	81.000	0.596*	-2.686	14.444	1.153	-1.079	82.889	0.249*	-4.694	27.000	-0.035*	-4.253
'JO009'	81.444	0.014	-2.613	16.222	0.576*	-1.082	97.444	0.286*	-4.957	47.397	0.773*	-4.876
'JR042'	83.111	0.887	-2.659	15.444	1.268	-0.918	92.556	0.467*	-5.012	44.033	0.177*	-4.741
'JR049'	81.222	1.179	-2.590	18.222	0.576*	-1.082	48.444	0.286*	-4.957	27.444	0.981	-4.912
'Lantern'	101.667	0.596	-2.686	23.333	0.922	-1.048	94.444	0.286*	-4.957	26.627	-0.209*	-4.824
'Ag Outback'	102.222	1.179	-2.590	16.333	0.807	-1.004	2.444	-0.038*	-5.015	1.813	-0.014*	-4.918
'Trigold'	102.222	1.179	-2.590	14.444	1.153	-1.079	98.222	0.143	-5.054	52.000	1.914	-4.036
'Monty'	101.778	0.014	-2.613	16.444	1.153	-1.079	30.111	-0.038	-5.015	6.774	-0.012*	-4.844
'Rainbow'	101.000	0.596	-2.686	14.444	1.153	-1.079	59.333	0.422	-1.894	20.111	-0.012*	-4.844
'Rivette'	100.667	0.596	-2.686	22.556	1.614*	-0.693	85.889	0.791	-4.953	34.703	-0.252	-3.227
'RQ 011'	101.222	1.179	-2.590	12.222	0.576*	-1.082	60.778	1.047	-5.021	10.000	0.000	-4.918
'Tranby'	100.889	0.305*	-2.671	16.222	0.576*	-1.082	56.444	0.505*	-5.086	19.889	0.012*	-4.844
Av Sapphire	101.333	1.193*	-2.685	16.333	0.922	-1.048	83.778	0.505*	-5.086	27.108	1.406	-4.170
'BST 702N2'	100.889	0.305*	-2.671	16.556	1.499	-1.059	65.667	0.648*	-5.052	30.370	0.599*	-4.839
'RQ 001-02M2'	102.000	0.590	-2.686	19.333	0.922	-1.048	76.556	0.467*	-5.012	40.778	0.413*	-4.648
'RR 013'	103.333	0.596*	-2.686	15.556	1.499	-1.059	107.778	0.505*	-5.086	58.999	0.933	-3.892
'RR 009'	102.111	0.887	-2.659	17.556	1.499	-1.059	89.889	0.467*	-5.012	47.700	-0.004*	-4.910
'Surpass 400'	101.889	0.902	-2.675	18.556	1.499	-1.059	115.778	0.505*	-5.086	70.949	1.387*	-4.726
'RR005'	102.111	1.484*	-2.652	21.667	0.922	-1.048	94.333	0.648*	-5.052	30.663	0.780*	-4.915
'scar'	102.222	1.179	-2.590	19.556	0.576*	-1.082	119.000	0.648*	-5.052	72.292	0.784*	-4.916
'Mystic'	101.889	0.902	-2.675	24.444	1.153	-1.079	95.889	0.678*	-3.727	67.579	-0.097	0.098
'RR 001'	102.000	1.193*	-2.685	25.111	0.231	-1.021	85.556	0.467*	-5.012	41.073	1.587*	-4.629
'Charlton'	101.778	0.887	-2.659	18.778	1.153	-1.079	83.444	1.047	-5.021	31.182	-0.126	3.492
'Skipton'	102.000	1.193*	-2.685	23.333	0.922	-1.048	110.667	0.430	-4.795	37.518	0.406*	-4.802
'Trilogy'	101.667	0.319	-2.528	19.444	1.153	-1.079	94.333	0.324*	-5.078	40.667	0.567*	-4.720
'Ag Spectrum'	102.222	1.179	-2.590	21.556	1.499	-1.059	28.889	-0.075*	-4.802	10.111	-0.012	-4.844
'TQ0055-02W2'	101.222	1.775*	-2.578	20.444	1.153	-1.079	107.000	2.711*	-3.384	44.444	1.583*	-4.720

Table 2 Contd.

‘Purler’	101.000	0.596	-2.686	21.556	1.383	-0.986	75.444	1.476*	-5.005	37.887	0.567*	-4.720
‘HNS0501’	100.889	0.902	-2.675	19.778	1.153	-1.079	79.222	1.333*	-5.075	37.888	0.768*	-4.815
‘GSL-I’	100.667	1.193	-2.685	18.444	1.268	-0.918	102.222	1.770*	-4.723	31.556	2.386*	-4.036
‘JMO 6001’	81.000	0.596	-2.686	9.556	1.499	-1.059	79.556	1.657*	-5.047	30.667	0.567*	-4.720
‘JMO 6002’	81.000	1.193*	-2.685	11.333	0.807	-1.004	73.667	0.648*	-5.052	26.889	-0.201*	-4.902
‘JMO 6003’	81.111	0.887	-2.659	9.333	0.807	-1.004	155.222	1.763	-4.671	60.778	0.768*	-4.815
‘JMO 6004’	80.889	0.902	-2.675	11.333	0.807	-1.004	127.333	0.859	-3.922	54.441	1.583*	-4.720
‘JMO 6006’	81.222	0.902	-2.675	12.444	1.268	-0.918	119.000	1.190*	-5.081	46.998	1.548*	-4.780
‘JMO 6010’	81.333	0.596*	-2.686	14.556	1.499	-1.059	93.000	1.190*	-5.081	46.144	1.011	-4.427
‘JMO 6011’	81.111	0.611	-2.623	9.556	0.576*	-1.082	85.778	1.047	-5.021	37.444	0.768*	-4.815
‘JMO 6012’	81.000	1.193*	-2.685	12.444	1.268	-0.918	85.778	0.829*	-5.077	40.889	0.969	-4.879
‘JMO 6013’	81.000	1.193*	-2.685	12.444	1.268	-0.918	82.889	1.009	-5.086	39.222	1.819	-3.003
‘JMO 6014’	81.111	0.887	-2.659	10.556	0.576*	-1.082	114.222	1.446	-4.602	38.441	2.575*	-4.292
‘JMO 6015’	81.333	1.193*	-2.685	9.222	0.576*	-1.082	115.222	1.552*	-5.024	41.516	0.902	-1.895
‘JMO 6018’	81.000	1.193*	-2.685	22.444	1.153	-1.079	133.000	1.084	-4.814	57.444	0.768*	-4.815
‘JMO 6019’	81.222	0.902	-2.675	19.444	1.153	-1.079	150.778	0.061	-1.242	60.778	0.768*	-4.815
‘JMO 6020’	80.889	0.902	-2.675	18.444	1.153	-1.079	108.556	0.467*	-5.012	41.923	1.639	-3.266
‘JMO 6021’	80.667	1.193*	-2.685	13.222	0.576*	-1.082	86.333	0.430	-4.795	38.329	1.323	-3.841
‘JMO 6026’	81.000	1.193*	-2.685	11.444	1.038	-0.802	92.000	0.430	-4.795	44.441	1.583*	-4.721
‘Loiret’	103.889	0.902	-2.675	11.444	1.153	-1.079	115.889	0.791	-4.953	38.182	1.895*	-4.906
‘Ekla’	102.333	1.193*	-2.685	11.333	0.807	-1.004	72.778	0.829*	-5.077	24.159	-0.068	-2.450
‘Montana’	102.000	1.193*	-2.685	16.444	1.153	-1.079	123.000	1.190*	-5.081	55.146	1.934*	-4.680
‘ RH13	101.667	1.193*	-2.685	11.556	1.499	-1.059	5.333	-0.113	-4.446	1.804	0.000*	-4.916
‘Ringot	102.111	1.484	-2.652	12.556	1.268	-0.474	25.111	-0.038	-5.015	1.821	-0.000*	-4.917
‘RK 2	101.222	0.902	-2.675	15.222	0.296	-0.892	59.778	1.047	-5.021	13.723	-0.026*	-4.554
‘Amora III	101.556	0.902	-2.675	15.111	0.074	1.035	140.778	0.829*	-5.077	44.887	1.298*	-4.911
RL	100.667	1.193*	-2.685	12.333	0.922	-1.048	85.556	0.685*	-5.080	40.552	1.004	-4.536
‘‘Haoyou II’	104.111	0.887	-2.659	14.556	1.499	-1.059	82.556	0.685*	-5.080	34.813	0.049	-3.633
‘Tunliuhuangiie’	104.333	2.428	-2.142	16.444	1.153	-1.079	105.889	1.770	-4.723	37.222	1.181*	-4.879
‘Qianxianjiecai’	105.222	1.775*	-2.578	19.222	0.692	-0.892	123.444	2.131*	-4.483	64.667	25.872*	93.803*
Yilihuang’	104.889	1.498*	-2.679	18.778	1.960	-0.965	111.667	2.275	-4.730	46.776	5.590	2.081
‘Hatianyoucai’	104.889	0.902	-2.675	24.556	0.692	-0.892	107.889	5.347*	-0.776	46.293	-1.024*	-3.769
‘Jinshahuang’	104.778	1.484*	-2.652	18.556	0.692	-0.892	99.667	2.056*	-5.027	41.342	8.422	14.334
‘Manasihuang’	105.111	1.484*	-2.652	16.778	1.383	-0.319	28.333	-0.113	-4.446	13.803	21.704*	74.220*
Brassica juncea 1	105.556	0.902	-2.675	16.444	1.268	-0.918	20.111	-0.038	-5.015	1.798	0.023*	-4.917
‘Brassica juncea 2’	106.000	0.319	-2.528	16.556	1.614	-0.693	25.333	-0.113	-4.446	1.801	-0.023*	-4.918
‘Brassica juncea-3’	105.889	0.902	-2.675	15.667	1.845	-0.943	30.111	-0.038	-5.015	1.802	-0.006*	-4.916

Table 2 Contd.

'Ashirwad'	78.111	1.484*	-2.652	8.222	0.576	-1.082	86.000	1.408*	-4.902	18.646	-14.810*	100.29*
'Aravali'	78.111	2.400*	-2.649	9.222	0.576	-1.082	94.667	0.866*	-5.058	29.406	-1.357*	-3.509
'Basanti'	78.556	0.902	-2.675	9.889	0.576	-1.082	55.556	0.685	-5.080	23.667	5.990	3.442
'CS 52'	77.889	0.902	-2.675	8.222	0.692	-0.892	53.333	1.732*	-4.973	20.222	-0.555	-4.404
'CS 54'	77.444	1.207	-2.632	8.333	0.807	-1.004	69.889	1.228	-4.969	24.740	-4.107	4.418
'GM 2'	77.889	0.902	-2.675	9.556	1.268	-0.474	89.222	1.770	-4.723	33.219	-5.623	7.051
'Geeta'	80.000	1.193*	-2.685	9.444	1.153	-1.079	92.889	1.228	-4.969	40.062	-1.376	2.363
'GM 3'	77.889	0.902	-2.675	8.333	0.807	-1.004	71.111	4.624*	-2.100	31.257	11.102*	38.193*
'Jagannath'	79.000	1.193*	-2.685	9.444	1.038	-0.802	94.667	0.866	-5.058	28.888	-2.896	8.285
'JM 1'	78.111	1.484*	-2.652	8.444	1.153	-1.079	96.778	2.674	-4.009	39.740	-8.336	50.132*
'JM 2'	78.222	1.179	-2.590	8.444	1.153	-1.079	105.889	1.228	-4.969	60.443	-13.906*	60.912*
'JM 3'	78.778	0.611	-2.623	11.444	1.153	-1.079	123.222	1.770	-4.723	72.618	-2.427	3.509
'Laxmi'	77.889	1.498*	-2.679	9.333	0.807	-1.004	109.222	1.770	-4.723	66.518	8.343*	16.277*
'Maya'	77.889	0.902	-2.675	12.556	1.383	-0.986	110.778	4.300*	-1.771	61.814	2.854*	-3.412
Pusa Mahak	76.667	1.512	-2.557	9.222	0.576	-1.082	98.667	0.866	-5.058	52.811	9.886*	25.915*
RGN 13'	76.889	0.902	-2.675	12.556	1.499	-1.059	90.000	1.408	-4.902	42.933	5.308	5.747
'Swaran Jyoti'	75.778	1.803*	-2.641	9.333	0.807	-1.004	95.222	2.854*	-3.821	36.257	5.276	3.971
'Vasundra'	75.889	0.902	-2.675	11.222	0.461	-0.834	69.333	1.951*	-4.610	33.394	3.447*	-3.403
'Kranti'	75.889	0.902	-2.675	9.556	1.268	-0.474	99.222	1.770	-4.723	33.083	-1.266	-4.710
Urvashi	77.778	0.611	-2.623	12.222	0.461	-0.834	102.556	0.685*	-5.080	47.619	-11.534*	45.434*

S^2_{di} =deviation from regression, a parameter of stability, b_i=regression coefficient, a parameter of stability test.

However, only linear component of G×E interaction was noticed which is expressed by significant value of regression coefficient ($b_i = 1$) only in 35 genotypes for this characters. Rivette exhibited above average ($b_i>1$) response showing their adaptability to favourable environmental conditions of disease development.

Out of 92 genotypes, only 16 genotypes were found to have b_i significant values indicating thereby the presence of linear component of G×E interaction in these genotypes only. Moreover, S^2_{di} value for all the genotypes were found non significant indicating totally absence of non-linear component of G×E interaction for stem diameter. On stability parameters basis (high resistance/ small stem lesion, $b_i = 1$ and $S^2_{di} = 0$) RH 13, Ringot, *B. juncea* 1, *B. juncea* 2, *B. juncea* 3 and RQ 011 were highly resistant and stable for small stem lesion length over the environments. Ag spectrum was found suitable for general environment conditions (small lesion length, $b_i = 1$, $S^2_{di} = 0$) for disease development. Six genotypes that is, JM018, Ag outback, Monty, *B. juncea* 1, *B. juncea* 2 and *B. juncea* 3, were stable and suitable for conducive environment of disease development (highly resistant, $b_i<1$, $S^2_{di} = 0$). In contrary to it, JM 3 was highly susceptible to *Sclerotinia* stem rot, but it was also stable susceptible ($S^2_{di} = 0$ and $b_i = 1$).

The genotypes showing resistance against *Sclerotinia* stem rot as indicated by lesser number of wilted / dead plant were also exhibited short stem lesion length, wider stem diameter and older plant age for inoculation. Similar observations have also been reported by Li et al. (2006) that there is significant positive linear relationship between plant death and stem lesion length. Hence, the identified stable resistance genotypes could be utilized directly as cultivar after evaluation over time and space if found suitable. Moreover, these should be incorporated in resistance breeding programme to enhance the

genetic level of resistance in future cultivars against recalcitrant necrotroph.

Conflict of Interest

The authors have not declared any conflict of interest.

REFERENCES

Buchwaldt L, Hegedus D, Li R, Lydrate D, Rimmer R (2005). Priminary mapping of physiological resistance to *Sclerotinia sclerotiorum* in Brassica napus: (In) Proceeding of 13th International Sclerotinia Workshop, California. P. 45.

Deng HM, Tang H (2006). Study on the regulatory of outbreak and control of rapeseed stem white rot. China Plant Prot. 26:16-18.

Eberhart SA, Russell WA (1966). Stability parameters for comparing varieties. Crop Sci. 6:36-40. http://dx.doi.org/10.2135/cropsci1966.0011183X000600010011x

Fitt B, McCartney HA, Davies JML (1992). Strategies for control of Sclerotinia. Agronomist 1:12-13.

Harper FR, Berkenkamp B (1975). Revised growth stage key for *Brassica campestris* and *B. napus*. Canadian J. Plant Sci. 55:657-658.http://dx.doi.org/10.4141/cjps75-103

Kang IS, Chahal SS (2000). Prevalence and incidence of white rot of rapeseed and mustard by *Sclerotinia sclerotiorum* in Punjab. Plant Dis. Res. 15:232-233.

Kirkegaard JA, Michael AE, Hamblin RP, Strange S (2006). Effect of blackleg and Sclerotinia stem rot on canola yield in high rainfall zone of Southern New South Wales. Aust. J. Agric. Res. 57:201-212. http://dx.doi.org/10.1071/AR05179

Lamey A, Bradley CA (2002). Sclerotinia rot of canola. Biology and management. NDSU Extention Service North Dakota State University. PMCid:PMC1769595

Lancashire PD, Bleiholder H, Van Den Boon T, Langeluddek P, Stauss R. Elfriede W, Witzenberger A (1991). A uniform decimal code for growth stages of crops and weeds. Ann. Appl. Biol. 119:561-601. http://dx.doi.org/10.1111/j.1744-7348.1991.tb04895.x

Li C, Sivasthamparam K, Fu TD, Li YC, Li SY, Barbetti MJ (2006). Expression of field resistance under western Australian conditions of Sclerotinia sclerotiorum in Chinese and Australia of *Brassica napus* and *Brassica juncea* germplasm and its relation with stem diameter. Australian J. Agric. Res. 57:1131-1135. http://dx.doi.org/10.1071/AR06066

Pope SJ, Vansey FL, Sweet JB (1989). Susceptibility of cultivars of oilseed rape to *Sclerotinia sclerotiorum* and effect of infection on yield. Aspects Appl. Biol. 23: 451-57.

academicJournals

Vol. 9(47), pp. 3448-3457, 20 November, 2014
DOI: 10.5897/AJAR2013.7074
Article Number: 28C1EC948595
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Bio activity of some botanicals on *Helminthosporium infestans* L. and *Solanum aethiopicum* L. (host)

Mbadianya J. I., Echezona B. C. and Ugwuoke K. I.

Department of Crop Science, University of Nigeria, Nsukka, Enugu State, Nigeria.

Received 18 March, 2013; Accepted 10 October, 2014

Some botanicals were assayed in two laboratory trials for their relative toxicities to African eggplant (*Solanum aethiopicum* L.) and *Helminthosporium infestans* causing leaf spots disease at the Department of Crop Science, University of Nigeria, Nsukka. In the first experiment on the toxicity of the protectants to *S. aethiopicum*, three concentrations (10, 20 and 30 g/ml) were used. Four concentrations (0.03, 0.06, 0.12 and 0.25 g/ml) were used in the second experiment on the toxicity of the botanicals (*Azadirachta indica* leaves, *Gongronema latifolia* leaves, *Garcinia kola* seeds, *Zingiber officinale* stems and *Carica papaya* leaves) to *H. Infestans*. Distilled water served as control in each case. *Solanum* seeds dressed with *G. kola* germinated faster and attained 100% germination 14 days after incubation (DAI). On the other hand, *Z. officinale* inhibited the seed germination most, resulting in the least germination being recorded on seeds treated with *Z. officinale*. Similarly, all the plant extracts assayed inhibited the growth of *H. Infestans* to varying degrees relative to check at 3 days after inoculation. Anti-fungal activity of extracts on *H. Infestans* decreased in the following order: *G. kola* at 0.12 g/ml (90.00%) > *C. papaya* at 0.25 g/ml (59.54%) > *G. kola* at 0.06 g/ml (59.15%) > *G. kola* at 0.03 g/ml (55.15%) > *A. Indica* at 0.06 g/ml (53.67%) > *Z. officinale* at 0.03 g/ml (52.65%) > control (7.71%). The seeds extracts of *G. kola* at 0.12 g/ml therefore proved more effective in the suppression of *H. infestans* relative to other botanicals and concentrations and were be recommended as a good alternative in its control in the field.

Key word: African eggplant anti-fungal, inhibition, protectants, toxicity.

INTRODUCTION

The African eggplant (*Solanum aethiopicum* L.*)*, known as garden egg (Hausa: Dauta; Igbo: añara; Yoruba: Igbagba) is one of the important vegetable crops grown almost worldwide and are highly valued constituents of Nigerian foods and an indigenous medicine.The name eggplant is derived from the shape of the fruits of some varieties which are white and have the shape of chicken eggs. It is commonly consumed almost on daily basis by both rural and urban families (Tindall, 1965). Eggplant can be grown in all parts of Nigeria all the year round. It is grown commercially as an annual crop; the plant is a short- lived perennial branching herb with a height of 0.5 to 1.5 m. It forms part of the traditional sub-sahara African culture are said to represent blessings, fruitfulness and are offered as a token of goodwill during visits, marriages and other social events (Osei et al., 2010)

*Corresponding author. E mail: janembadianya@yahoo.com

.The fruits can be eaten in various forms without the need for an elaborate preparation. It is eaten raw, cooked or used to season other foods. Eggplant supplements starchy foods. It is also a cheap source of protein, minerals and vitamins (Lombin and Yayock., 1988). The tender green leaves of some species are also used as vegetables or eaten raw in African salads, *ugba*. It can be eaten as appetizer.

The African eggplant (*S. aethiopicum* L.*)* is affected by several fungal diseases which inflict heavy losses in its production. One of such fungal disease is the leaf spot disease. The disease can infect seedlings but generally is a problem of older plants. Lower leaves are attacked first and then the disease progresses upwards. Dark-brown spots with concentric rings develop on the leaves, which give target board effect, the most characteristic symptom of the disease. In humid weather, affected areas coalesce and form dark-brown patches. In severe attacks, affected leaves shrivel and fall down pre-maturely resulting in early defoliation. On the leaves, the spots appear as small angular scattered, light-brown in colour, progressing between veins towards the leaflet margin. The severely infected leaflets curl and dry out pre-maturely. Subsequently, the pathogen invades the adjoining healthy leaflets and gradually progresses on the foliage upwards from the infected leaves. Elongated dark brown lesion also appears on the stem and branches (Gupta and Barnerjee, 1970). Severely infected leaves drop off prematurely resulting in the reduction of yield. Due to environmental concerns, great emphasis has been laid on alternative measures other than chemicals, to control this fungal disease. The use of botanicals and antimicrobial agents of plant origin is a time –honored practice for control of plant diseases and pests. The necessity to develop a non-toxic, safe and biodegradable alternative to synthetic fungicides has in recent years led to a concerted effort at developing new control measures from plant parts. The humid especially the rainforest ecological zones are endowed with abundant flora of families of plants and herbs with untapped pesticides potentials (Amadioha, 2003, 2004). Stoll (2000) listed an array of plant families and genera possessing antimicrobial properties, amongst which were *Azadirachta indica, Zingiber officinale, Garcinia kola, Carica papaya, Gongronema latifolium* and host of others. Amadioha (2003), Kumar and Pamar (1996) and Prakash and Roa (1997) listed some of the advantages of plant extracts over synthetic chemicals to include possession of low mammalian toxicity, minimal health hazards and environmental pollution. There is practically no reported risk of developing pest resistance to these products when used in their natural forms. Also, no side effect on plant growth, seed viability or food quality has been reported. Botanicals are less expensive and easily available because of their natural occurrence. Synthetic fungicides are expensive and inaccessible to indigenous farmers who are the bulk producers of eggplant in Nigeria (Amadioha, 1998; Onuegbu et al., 2001). A natural plant product with fungicidal properties could be more environmental friendly than synthetic fungicides.

Aqueous extracts of some plants have been used in laboratory bioassays (John and James, 2004). These plants include *Allium cepa* (onion), a biennial herb of Liliaceae family used commonly as spice for flavoring food. *Allium sativum* L. (garlic), another biennial herb of Liliaceae family and the second most widely use *Allium* after *A. cepa;* it is used as condiments for flavoring foods. Stoll (1998) reported the bactericidal properties of *Azadirachta indica* A Juss (neem), a fast growing tree of the family Meliaceae and also a medicinal plant with insecticidal, nematicidal, antifungal and bactericidal properties. It occupies a foremost status among all the plants exploited so far for bio-efficacy against pests and diseases (Kumar and Pamar, 1996). The primary antimicrobial constituents are Azadirachtin A and B. In addition, Neem contains a good number of other chemical substances which include Salannin, Meliantriol, Azadirachtannin A, Cinnamoyl, Isoazadirohide, Nimbin/Nimbidin, which seem to have anti- viral effects as well as Vilasinim as isolated from the leaf and Azadirone from the seed. *Garcinia kola* Henkel (bitter cola) is a perennial tree in the family Guttiferae with whorled leathery leaves. The seeds are chewed as stimulants and for other various medicinal values. Traditionally, the seeds are believed to repel snakes. *Zingiber officinale* Rose (Ginger) is rhizome of the family Zingiberaceae. The rhizome yields essential oil, oleoresin, consisting 1 to 3% volatile of which serve as the active ingredient against microorganisms and pests (Benjilali et al., 1984).

Medicinal plant materials have been successfully used for the treatment of fungal and bacterial infections in humans (Akinyosoye and Oladunmoye, 2000), suggesting that some plant materials may also possess antifungal and antibacterial constituents which are useful in controlling plant diseases (Amadioha, 1998). Previous reports (Akpomedaye and Ejechi, 1998; Ejechi and Ilondu, 1999; Ejechi and Akpomedaye, 1999) show that spices, herbs and other plant materials possess antifungal activity. Akinyosoye and Oladunmoye (2000) have reported the antifungal efficacy of stem and leaf-extracts of *Mirabilis jalapa* L. in reducing mycelia growth of four different strains of fungi. The legendary medicinal qualities of the neem tree have been known for a long time and the aqueous leaf extract have systemic action (Egunjobi and Onoyerni, 1981; Sowunmi and Akinusi, 1983). The toxic effects of some plant extracts on fungal activities is an indication that such plants could be used as fungicides especially by the peasant farmers who cannot afford the costly synthetic agrochemicals to control fungal diseases that attack their crops.

The research was therefore to determine the bio-activity of plant extract on eggplant and disease organism (*Helminthosporium infestans*).

MATERIALS AND METHODS

Two laboratory experiments were carried out at the Department of Crop Science, University of Nigeria, Nsukka. Nsukka is located in the derived Savannah Zone (06° 52' N, 07° 24'E and altitude of 447.26 m above sea level).

Determination of the bio activity of plant extract on eggplant

In this experiment, 5 locally available plants were evaluated for their ability to inhibit the growth of the fungus responsible for the leaf spot found on the leaves of the eggplants. The 5 test plants selected for the assessment were as follows (*A. indica* leaves, *G. kola* seeds, *Z. officinale* stems, *G. latifolia* leaves and *C. papaya* leaves). Distilled water served as a control.

To determine if botanicals could be harmful to plants at certain concentrations thought to be toxic to the pathogens, bio activity test was carried out. The botanicals selected for use as antimicrobial agents were assessed for bio-activity effect at germination stage of the plant.

Sources of plant materials

The fresh leaves of *A. indica* was obtained from the botanical garden, Department of Botany, *G. latifolia* and *C. papaya* were obtained from Department of Crop Science farm, University of Nigeria, Nsukka while *Z. officinale* stems and *G. kola* seeds were bought from Ogige Main Market, Nsukka.

Preparation of the plant extracts

Fresh leaves (the lower leaves) of the tested vegetable species were washed separately under tap water, rinsed with sterile distilled water and allowed to dry in a glasshouse. The dried leaves, stems and seeds were mashed and ground using electric milling machine to a fine powder.

Hot water extraction

Dried fine powder of each plant materials (5 g) were put in beaker separately and 200 ml of distilled water was added. The mixtures were heated on a hot plate with continuous stirring at 30 to 40°C for 20 min, allowed to cool and filtered through cheese cloth. The filtrates obtained were used for the phytochemical analysis. The water extract was kept in refrigerator for further use.

Ethanol extraction

Each powder (200 g) was soaked in 600 ml of analytical ethanol. These mixtures were left to stand for 24 h after which they were filtered with cheesecloth and the supernatant obtained were concentrated to dryness in an oven (100 to 105°C). The dry supernatant of each was used as the crude plant extracts.

Germination test

Twenty seeds of Eggplant were placed in a sterile Petri dish with sterile filter paper (Whatman No.1) and replicated 3 times so that each treatment had 20 seeds. Using sterile syringe, 1 ml each of the 5 plant extracts was applied at different concentrations (10, 20, 30 100 g/L). The filter paper was previously moistened with sterile water before adding the extracts. The extracts where applied in the morning. There was a control, which was treated with distilled water. The Petri dishes had their lids covered and incubated for 14 days at room temperature. At 7 and 14 days of incubation, percentage seed germination was recorded.

In vitro control of the organism using plant extracts and synthetic fungicides

Assay of plant extracts

The efficacy of the tested extracts against the fungi; *H. Infestans* followed the hyphal growth inhibition technique (Palanichamy and Nagarajan., 1990). Then, 1 g (100 mg) of each tested extracts was dissolved in 4 ml of dimethysulfoxide (DMSO) and mixed thoroughly to obtain the test solution (250 mg/ml per each extracts). From the test solution, serial 2-fold dilution were made using three different test tubes and concentrations (0.03125 mg/ml, 0.0625 mg/ml, 0.125 mg/ml and 0.25 g/ml) were obtained from each extract respectively. Aliquots of 1 ml of each concentration of the different plant extracts were separately and asceptically introduced into the conical flasks containing 19 ml of cool, sterilized PDA. Two drops of streptomycin were added to the mixture, which was gently swirled to obtain even distribution of the plant extracts, PDA and the antibiotics. This mixture was poured on the sterilized 9 cm Petri dishes and allowed to stand for 24 h. Discs (5 mm), taken from the advancing margins of a pure culture of *H. infestans*, with the aid of a sterilized spatula were placed on the centre of each Petri dishes.

Assay of synthetic fungicides

The fungicides used were bought from an agrochemical dealer and they were;

(1) Ridomil Plus 66 – WP [(both systemic and contact fungicide) - 12% metalaxyl- M and 60% copper (1) oxide)]
(2) Conti-zeb “5” to 80% WP (Contact fungicide – 80% mancozeb)
(3) Total 5% SC (Systemic fungicide – 5 % hexaconazole)
(4).Kocide 2000 (contact fungicide - 53.8% Copper hydroxide)

The same procedures and set ups employed in the plant extracts assay were used here except that the DMSO used in dissolving the plant extracts was replaced with distilled water. Thus, 4 ml of distilled water was used to dissolve 1 g (100 mg) of Ridomil Plus 66-WP, Conti-zeb “5” to 80% WP, Kocide 2000 and Total 5% SC to obtain 250 mg/ml concentration. Two fold dilutions were also obtained and concentrations (0.03125, 0.0625, 0.125 and 0.25 g/ml) were obtained, respectively. Every other procedure remained the same as that of the plant extracts assay. Cultured plate with neither plant extract nor synthetic fungicides is the control. All inoculated plates were incubated at 28°C. Data on mycelial growth in terms of colony diameter of the pathogenic fungus were taken after 3, 7 and 14 days after inoculation. The percentage growth inhibition or the minimum inhibitory rate were assessed and recorded. Growth inhibitions were obtained by measuring the colony growth diameter, taken as mean of the widest and the shortest diameter. The percentage growth inhibitions were determined using the formula adopted by Amadioha (2003, 2004) as follows:

$$\text{Percentage growth inhibition} = \frac{dc-dt}{dc} \times \frac{100}{1}$$

Where, dc = colony diameter of control. dt = colony diameter of treated plates.

Table 1. Effect of plant extracts concentrations on mean days to seed germination.

Plant extracts	Concentration (g/L)			Plant extracts means
	10	20	30	
G. kola	4.67	4.67	4.67	4.67
Z. officinale	5.00	12.67	7.33	8.33
A. indica	5.33	5.33	6.67	5.78
C. papaya	6.33	7.00	14.67	9.33
G. latifolia	9.00	8.33	7.67	8.33
Water	5.33	5.33	5.33	5.33
Conc. Means	5.94	7.22	7.72	6.96

F-LSD $_{(0.05)}$ for comparing any 2 plant extract means =1.95; F-LSD $_{(0.05)}$ for comparing any 2 comparing concentration means = NS; F-LSD $_{(0.05)}$ for comparing any 2 plant extract x concentration means = 3.37.

Table 2. Effects of plant extract concentrations on mean days to 50 percent seed germination.

Plant extracts	Concentration (g/L)			Plant extract means
	10	20	30	
G. kola	7.67	8.33	8.00	8.00
Z. officinale	17.33	21.00	16.67	18.33
A. indica	8.00	11.00	14.00	11.00
C. papaya	12.33	11.00	16.67	13.33
G. latifolia	10.67	11.00	11.33	11.00
Water	7.33	7.33	7.33	7.33
Conc. Means	10.56	11.61	12.33	11.50

F-LSD $_{(0.05)}$ for comparing any 2 plant extract means =3.34; F-LSD $_{(0.05)}$ for comparing any 2 comparing concentration means= NS; F-LSD $_{(0.05)}$ for comparing any 2 plant extract x concentration means = 5.78.

The experimental design was a 9 × 4 factorial (four plant extracts + four fungicides + distilled water x four concentrations) in a completely randomized design (CRD). Data on the colony growth diameter were transformed to their respective square root value prior to statistical analysis, as the residuals were not normally distributed$\sqrt{X+0.5}$; where X is the colony growth diameter (Bartlett, 1937). Means were later compared using Fisher's LSD procedure as outlined by Obi (2002). Data were analyzed using GENSTAT 5.0 Release 4.23 DE (GENSTAT, 2003). Percentage growth inhibitions were angular transformed (Arc Sin √ Percentage) before analysis of variance (ANOVA).

RESULTS

Bio activity of the different plant extracts on eggplant seeds

Plant extracts concentrations on mean days to seed germination and 50 percent seed germination

The effect of the three concentrations (10, 20, 30 g l^{-1}) of the 5 plant extracts and distilled water on days to seed germination showed that there was significant effect ($p<0.05$) on the plant extracts and the interaction between plant extracts and concentrations (Table 1). The data revealed that *G. kola* (4.67 days) treated seeds germinated faster than other plant extracts and distilled water at all concentrations, those treated with while *C. papaya* (12.67 days) at 20 g/L concentration delayed seed germination. No statistical significant effect was obtained in different concentrations. The effect of the three concentrations (10, 20, 30 g l^{-1}) of the 5 plant extracts and distilled water on days to 50% seed germination showed that the plant extracts and their combinations with the various concentrations significantly ($p<0.05$) influenced 50% seed germination (Table 2). The result revealed that seeds soaked with distilled water (7.33 days) and *G. kola* (8.00 days) were statistically similar ($p<0.05$) in seed germination when compared to other plant extracts. The seeds took 7 to 8 days to attain 50% germination while those treated with *Z. officinale* took up to 18 days to reach 50% seed germination. On the interactions, *G. kola* at 10 g/L concentration took 8 days to reached 50% seed germination, while *Z. officinale* at 20 g/L concentration took 21 days to attain 50% seed germination. However, no significant effect was seen on the different concentrations.

Table 3. Effects of plant extract concentrations on mean days to 100% seed germination.

Plant extracts	Concentration (g/L)			Plant extract means
	10	20	30	
G. cola	12.00	12.67	12.00	12.22
Z. officinale	19.00	21.00	19.00	19.67
A. indica	12.00	13.00	16.00	13.67
C. papaya	18.67	14.00	21.00	17.89
G. latifolia	16.67	14.33	18.00	16.33
Water	12.00	12.00	12.00	12.00
Conc. Means	15.06	14.50	16.33	15.30

F-LSD $_{(0.05)}$ for comparing any 2 plant extract means = 2.40; F-LSD $_{(0.05)}$ for comparing any 2 comparing concentration means = NS;F-LSD $_{(0.05)}$ for comparing any 2 plant extract x concentration means = 4.16.

Table 4. Effects of plant extract concentrations on mean days to leaf formation.

Plant extracts	Concentration (g/L)			Plant extract means
	10	20	30	
G. kola	7.67	7.67	7.67	7.67
Z. officinale	7.00	15.00	9.67	10.56
A. indica	8.00	7.67	8.33	8.00
C. papaya	8.33	9.33	16.67	11.44
G. latifolia	11.33	10.00	9.33	10.22
Water	7.00	7.00	7.00	7.00
Conc. Mean	8.22	9.44	9.78	9.15

F-LSD $_{(0.05)}$ for comparing any 2 plant extract means = 1.66; F-LSD $_{(0.05)}$ for comparing any 2 comparing concentration means = NS; F-LSD $_{(0.05)}$ for comparing any 2 plant extract x concentration means = 2.87.

Plant extract concentrations on mean days to 100 percent seed germination and leaf formation

The effect of the three concentrations (10, 20, 30 g l^{-1}) of 5 plant extracts and distilled water on days to 100 percentage germination showed that plant extracts significantly ($p<0.05$) affected the days on 100% seed germination (Table 3). There were 100% germination on the plates treated with *G. kola* (12.22 days) and distilled water (12.00 days) at the 12th day of incubation. It took up to 20 days for the whole seeds in the ginger plates to germinate. No statistical effect was obtained on the different concentrations. Seeds treated with *G. kola* and distilled water was statistically the same ($p\leq0.05$). On the interactions, *G. kola* at 10 g/L concentration took 12 days to attain 100% seed germination while *Z. officinale* at 20 g/l concentration took 21 days to attain 100 seed germination.

The effect of the three concentrations (10, 20, 30 g l^{-1}) of the 5 plant extracts and distilled water on days to leaf formation showed that that the plant extracts and their combinations with the various concentrations significantly ($p<0.05$) influenced leaf formation (Table 4). The first leaf formation was seen on the seeds soaked with distilled water (7.00 days) at all concentrations. Although, it was significantly the similar with those treated with *G. kola* (7.67 days). Seeds in *C. papaya* extracts were the last to form leaves (11.44 days). On the interactions, *Z. officinale* at 20 g/L concentration delayed leaf formation (15.00 days). No significant effect was seen on the different concentrations.

Plant extract concentrations on mean percentage seed germination at 7 and 14 days of incubation

The effect of the three concentrations (10, 20, 30 g l^{-1}) of the 5 plant extracts and distilled water on percentage germination at 7 days (Table 5) showed that that the plant extracts and their combinations with the various concentrations significantly ($p<0.05$) influenced the percentage seed germination at 7 days of incubation. Distilled water (68.30%) and *G. kola* had the highest percentage germination (53.30%) at all the concentrations. The distilled water and *G. kola* were significantly higher ($p<0.05$) than other plant extracts.

Table 5. Effects of plant extract concentrations on mean percentage seed germination at 7 days of incubation.

Plant extracts	Concentration (g/L)			Plant extract means
	10	20	30	
G. cola	53.30	46.70	53.30	51.10
Z. officinale	5.00	0.01	20.00	8.30
A. indica	46.07	15.00	10.00	23.90
C. papaya	5.00	3.30	1.70	3.30
G. latifolia	26.70	3.30	31.70	20.60
Water	68.30	68.30	68.30	68.30
Conc. mean	34.20	22.80	30.80	29.30

F-LSD $_{(0.05)}$ for comparing any 2 plant extract means =20.47; F-LSD $_{(0.05)}$ for comparing any 2 comparing concentration means = NS.F-LSD $_{(0.05)}$ for comparing any 2 plant extract x concentration means = 35.46.

Table 6. Effects of plant extract concentrations on mean percentage seed germination at 14 days of incubation.

Plant extracts	Concentration (g/L)			Plant extract means
	10	20	30	
G. kola	100.00	100.00	100.00	100.00
Z. officinale	41.70	11.70	45.00	32.80
A. indica	100.00	88.30	63.30	83.90
C. papaya	58.30	86.70	25.00	56.70
G. latifolia	73.30	91.70	68.30	77.80
Water	100.00	100.00	100.00	100.00
Conc. Mean	78.90	79.70	66.90	75.20

F-LSD $_{(0.05)}$ for comparing any 2 plant extract means =19.11; F-LSD $_{(0.05)}$ for comparing any 2 comparing concentration means= NS;F-LSD $_{(0.05)}$ for comparing any 2 plant extract x concentration means = 32.10.

Also, the interaction between the plant extracts and concentrations showed significant difference ($p<0.05$). Seeds treated with *G. kola* at 10 g/L concentration had the highest seed germination (53.30%) while seeds treated with *Z. officinale* (0.01%) at 20 g/L concentration had the lowest seed germination. No significant effect was seen on the different concentrations. The effect of the three concentrations (10, 20, 30 g l^{-1}) of the 5 plant extracts and distilled water on percentage germination at 14 days of incubation (Table 6) showed that the plant extracts and their combinations with the various concentrations significantly ($p<0.05$) affected percentage seed germination at 14 days of incubation. Distilled water (100%) and *G. kola* (32.80%) attained 100% seed germination at all concentrations on the 14th day of incubation while *Z. officinale* recorded the lowest percentage germination. The distilled water and *G. kola* at 10 to 30 g/L concentrations were significantly higher ($p<0.05$) than other plant extracts. The interactions between the plant extracts and the concentrations showed significant effects. Only 12 seeds germinated on the plate treated with *Z. officinale* at 20 g/L concentration. No statistical effect was seen on the different concentrations.

Number of dormant seeds after 14 days of incubation

The effect of the three concentrations (10, 20, 30 g l^{-1}) of the 5 plant extracts and distilled water on number of dormant seeds at 14 days of incubation (Table 7) showed that there was significant effect ($p<0.05$) among the plant extracts on the dormant seeds. The number of dormant seeds after 14 days were highest in seeds treated with *Z. officinale* extracts (13 .44). No dormant seed was seen on the plates treated with *G. kola* and distilled water at all concentrations after 14 days of incubation. No significant effect was seen on the different concentrations.

Table 7. Effect of plant extracts concentrations on the mean number of dormant seeds at 14 days of incubation.

Plant extracts	Concentration (g/L)			Plant extract mean
	10	20	30	
G. kola	0.00 (0.71)	0.00 (0.71)	0.00(0.71)	0.00(0.71)
Z. officinale	11.67(3.41)	17.67(4.26)	11.00(3.39)	13.44(3.73)
A. indica	0.00(0.71)	2.33(1.68)	7.33(2.80)	3.22(1.93)
C. papaya	7.33(2.80)	2.67(1.76)	15.00(3.94)	8.33(2.97)
G. latifolia	5.33(2.41)	1.67(1.47)	6.33(2.61)	4.44(2.22)
Water	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)
Conc. Mean	4.06(2.14)	4.06(2.14)	6.61(2.67)	4.91(2.33)

F-LSD $_{(0.05)}$ for comparing any 2 plant extract means = 3.78; F-LSD $_{(0.05)}$ for comparing any 2 comparing concentration means = NS F-LSD $_{(0.05)}$ for comparing any 2 plant extract x concentration means=6.54 Figures in parentheses were square root transformed data and mean separation were based on them.

Table 8. Mean percentage inhibition of the plant extracts and synthetic fungicides 3 days after inoculation.

Treatment	Concentration				Treatment means
	0.03	0.06	0.12	0.25	
G. cola	68.4(55.80)	73.7(59.15)	100.0(90.00)	59.6(50.53)	75.4(60.27)
Z.officinale	63.2(52.65)	57.8(49.49)	57.8(49.49)	61.4(51.59)	60.1(50.83)
A. indica	57.9(49.54)	64.9(53.67)	52.6(46.49)	49.1(44.48)	56.1(48.50)
C.papaya	61.4(51.59)	52.6(46.49)	57.9(49.54)	74.3(59.54)	61.6(51.71)
Cu $(OH)_2$	57.9(49.54)	100.0(90.00)	82.5(65.27)	70.2(56.91)	77.6(61.68)
Metalaxyl-M	73.7(59.15)	79.0(62.72)	66.7(54.76)	100.0(90.00)	79.8(63.36)
Hexaconazole	100.0(90.00)	100.0(90.00)	100.0(90.00)	100.0(90.00)	100.0(90.00)
Mancozeb	100.0(90.00)	100.0(90.00)	100.0(90.00)	100.0(90.00)	100.0(90.00)
Control	1.75(7.71)	17.5(24.73)	17.5(24.73)	00.00(0)	9.21(17.66)
Conc. Means	64.9(53.67)	71.7(57.23)	70.6(57.17)	68.3(55.73)	68.9(56.11)

F-LSD $_{(0.05)}$ for comparing any 2 treatment means = 8.13; F-LSD $_{(0.05)}$ for comparing any 2 comparing concentration means = 6.55; F-LSD $_{(0.05)}$ for comparing any treatment x concentration means = 11.54; Mean separation were based on transformed data; Figures in parentheses were the arc-sine transformed data.

The mean percentage (%) inhibition of the plant extracts and synthetic fungicides 3 days after inoculation

All the plant extracts assayed inhibited the growth of the fungus to varying degrees when compared with the untreated control 3 days after inoculation (Table 8). Anti-fungal activity of *H. infestans* was highest with seed extracts of *G. kola* (90.00%) at 0.0120 g/ml concentrations followed by leaf extracts of *C. papaya* (59.54%) at 0.250 g/ml concentrations, then, 0.060 g/ml (59.15%) and 0.030 g/ml (55.80%) concentrations of *G. kola*. At these concentrations, leaves and seeds of *G. kola* and *C. papaya* respectively were significantly ($p<0.05$) more toxic to *H. infestans* than the other plant extracts tested. The result also indicated that these extracts were significantly ($p<0.05$) more efficacious in inhibiting the growth of *H. infestans* than the other plant extracts evaluated. The stem extracts of *Z. officinale* (52.65) and leaf extracts of *A. indica* (53.67) at 0.030 and 0.060 g/ml concentrations, respectively were also significantly higher ($p<0.05$) than the untreated control (17.66%). The fungicides; Hexaconazole (90%) and Mancozeb (90%) were very effective at all concentrations.

Mean percentage inhibition of the plant extracts and synthetic fungicides 7 days after inoculation

The data obtained showed that all the plant extracts inhibited the growth of the pathogen *H. infestans* to varying degree. The results indicate that the different plant extracts used in this study (Table 9) showed

Table 9. Mean percentage inhibition of the plant extracts and synthetic fungicides 7 days after inoculation.

Treatment	Concentration (g/ml)				Treatment mean
	0.030	0.060	0.120	0.250	
G. kola	75.00(60.00)	75.00(60.00)	76.00(60.67)	68.00(55.55)	73.50(59.08)
Z.officinale	66.00(54.33)	79.00(62.72)	63.00(52.53)	69.00(56.17)	69.25(56.35)
A. indica	64.00(53.13)	64.67(53.55)	65.33(53.91)	60.67(51.18)	63.67(52.95)
C.papaya	67.00(54.94)	58.33(49.78)	66.67(54.70)	75.00(60.00)	66.75(54.82)
CU (OH) $_2$	61.00(51.35)	61.00(51.35)	61.00(51.35)	59.00(50.18)	60.50(51.06)
Metalaxyl	60.00(50.77)	61.00(51.35)	61.00(51.35)	74.00(59.34)	64.00(53.13)
Hexaconazole	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)
Mancozeb	67.00(54.94)	89.00(70.630	70.00(56.79)	80.00(63.44)	76.50(61.00)
Control	0.00(0.0)	0.00(0.0)	0.00(0.0)	0.00(0.0)	0.00(0.0)
Conc. Mean	62.22 (52.06)	65.33(53.91)	62.56(52.30)	65.07(51.94)	63.80(53.01)

F-LSD $_{(0.05)}$ for comparing any 2 treatment means = 6.29; F-LSD $_{(0.05)}$ for comparing any 2 comparing concentration means = 5.23; F-LSD $_{(0.05)}$ for comparing any treatment x concentration mean = 8.91; Mean separation were based on transformed data; Figures in parentheses were the arc sine transformed data.

significant difference ($p \leq 0.05$) in the mean percentage inhibition of mycelia growth of the fungus at 7 days after inoculation. The highest (62.72%) mean percentage inhibition rate were recorded on the stem extracts of *Z. officinale* at 0.06 concentrations. Also the seed extracts of *G. kola* had higher inhibitory effects (60.67%) at 0.12 and 0.06 (60.00%) concentrations, followed by the concentrations of 0.30 to 0.060 of the same material. The stem extracts of *Z. officinale* (56.35%) and the leaf extracts of *A. indica* (52.95%) and *C. papaya* (54.82%) at all concentrations also inhibited the growth of pathogen and were more effective than the fungicide; $Cu(OH)_2$ (51.06%).

Mean percentage inhibition of the plant extracts and synthetic fungicides 14 days after inoculation

The results indicate that the different plant extracts used in this study (Table 10) showed significant difference ($p<0.05$) in the mean percentage inhibition of mycelia growth of the fungus at 14 days after inoculation. The data revealed that the highest mean percentage inhibition of mycelia growth of the fungus (51.88%) were seen on the plates treated with *G. kola* while *A. indica* has the lowest percentage inhibition (30.26%). It also proved that the seeds extracts of *G. kola* (51.88%) was still very effective than fungicide Cu $(OH)_2$ (49.31%) even after 14 days. On the interactions, *G. kola* at 0.3 g/ ml concentration had the highest phytotoxic effect while *A. indica* at 0.03 g/ml concentration was the least. The fungicide hexaconazole (0.0) was the best treatment even after 14 days of inoculation, it had 100 percentage inhibitions. The inhibitory effect of the fungicide metalaxyl on mycelia growth increased with increase in concentration.

DISCUSSION

The effect of plant extracts concentration (10, 20, 30 g/ml) and distilled water on eggplant seeds showed no significant difference on the concentrations. However, differences existed among plant extracts and their interactions. Seeds treated with *G. kola* germinated faster and attained 100% seed germination at the 14th day of incubation, while seeds with *Z. officinale* had the least seed germination percentage. This could be attributed to high levels of flavonoid in *G. kola* which are health promoting in action (Ferguson, 2001). Again, *G. kola* gave the best protection against the pathogen *H. infestans* and that may also be the reason why it germinated faster than the other extracts tested. This observation of high germination percentage by *G. kola* agreed with the findings by Opara and Wokocha (2008) on the phytotoxicity test of the plant extracts on tomato seedlings. The use of plant extracts in disease control is eliciting much interest in developing countries due to high cost of synthetic pesticides and their hazardous effects on the environment (Schmutterer, 1990, Tovingan et al., 2001 and Salako, 2002). In the *in vitro* control experiment, the 4 plant extracts tested inhibited the growth of the pathogen, *H. infestans* to varying degree when compared with the untreated control. The anti-fungal activities to *H. infestans* were highest with the seed extracts of *G. kola* followed by the stem extracts of *Z. officinale* then leaf extracts of *C. papaya* and *A. indica*. The result also indicated that these plant extracts assayed were significantly ($p<0.05$) more toxic and more active than the fungicide; Cu(OH) $_2$ between 3 and 7 days of inoculation. The seed extracts of *G. kola* still proved more phytotoxic and effective than the other three plant extracts and fungicide $Cu(OH)_2$ even after 14 days. This observation of high phytotoxicity response by *G. kola*

Table 10. Mean percentage inhibition of the plant extracts and synthetic fungicides 14 days after inoculation.

Treatment	Concentration (g/ml)				
	0.030	0.060	0.120	0.250	Treatment means
G. kola	75.00(60.00)	61.000(51.35)	53.00(46.72)	58.67(50.010	61.92(51.88)
Z. officinale	44.00(41.55)	61.00(51.35)	52.00(46.15)	40.00(39.23)	49.33(44.60)
A. indica	0.00(0.0)	33.00(35.06)	48.00(43.85)	20.67(26.99)	25.42(30.26)
C. papaya	48.67(44.25)	35.67(36.69)	42.67(40.80)	56.00(48.45)	45.75(42.59)
Cu $(OH)_2$	58.00(49.60)	58.00(49.60)	58.00(49.60)	56.00(48.45)	57.50(49.31)
Metalaxyl	59.67(50.59)	59.67(50.67)	59.00(50.18)	74.00(59.34)	63.08(52.53)
Hexaconazole	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)
Mancozeb	56.00((48.45)	89.00(70.63)	67.00(54.94)	80.00(63.44)	73.00(58.69)
Control	0.00(0.0)	0.00(0.0)	0.00(0.0)	0.00(0.00	0.00(0.0)
Conc. Mean	49.04(44.43)	55.26(48.04)	53.30(46.89)	53.96(47.24)	52.89(46.66)

$F\text{-}LSD_{(0.05)}$ for comparing any 2 treatment means =7.05; $F\text{-}LSD_{(0.05)}$ for comparing any 2 comparing concentration means = 5.65; $F\text{-}LSD_{(0.05)}$ for comparing any treatment x concentration means = 9.81;Mean separation were based on transformed data; Figures in parentheses were the arc –sine transformed data.

is consistent with the findings by Okereke and Wokocha (2006) on the effect of some tropical plant extracts, *Trichoderma harzianum* and Captan on the damping off of tomato induced by *Sclerotium rolfsii*. Amadioha (2002) reported that the differences in toxicity of different plant extracts were due to the presence of different active compounds in the plant materials. The active material in *A. indica* is *azadiratin*; *Z. officinale* consisted of linalool, imonene, zingeberl, zingerene, camphene, oleoresin (gingerol and shogoal), phenol (gingerol and zingibain), vitamin B_6 and vitamin C, calcium, magnesium, phosphorus, and potassium and linoelic acid (Kikuzaki and Nakatani.,1993). Sridhar et al. (2002) reported that the stem extracts of *Z. officinale* ground into paste and mixed with water and soap, sprayed thoroughly on the infected plant parts were effective in the control of American boll worm, aphids, plant hoppers and thrips. In *G. kola*, the active compounds responsible for anti-microbial, anti-viral and anti-inflammatory properties were bio-flavonoids, xanthenes and benzophenomes (Amadioha, 2003). The quantity phytochemical analysis done in this study showed that *G. kola* had the highest quantity of flavonoid (1.70 mg/100 g) than the other plant extracts tested. The anti-fungal activity observed in *C. papaya* may be due to the action of proteolytic enzyme papain which is the major component of paw-paw latex. These enzyme acts in adverse manner of the protein components of the fungal cell wall thereby hindering growth of these fungi. Igboko (1983) reported the presence of several chemical compounds (steroids, flavonoids, glucosides and protein) known to perform physiological activities against micro-organisms in *G. kola*. Ilondu (2011) observed the anti-fungal properties of crude leaf extracts of *C. papaya* in paw-paw fruits rot. Several researchers (Amadioha, 1998; Wokocha and Okereke, 2005; Wokocha, 2006) also reported the fungicidal activities of extracts of *A. indica, Z. officinale, C. papaya, G. kola* and other plant materials on *Erysiphe cichoracearum*, *Collectotrichum capsici* and *Protomycopsis phaseoli,* which compared favourably with the chemical pesticides; benlate and ridomil. Akpa et al. (1991) reported a significant inhibitory property of *A. indica* extracts on mycelia growth of *Collectotrichum graminicola*. Nzanza and Mashela (2012) in their field experiments with tomato found out that fermented plant extracts of neem and wild garlic, alone or in combination, have insecticidal properties to maintain lower population densities of whitefly and aphid.

To summarize, *G. kola* seeds extracts gave the highest phytotoxicity response to *H. infestans* which incites the leaf spot found on the leaves of eggplant. Again, they were seen to germinate faster and attained 100% seed germination at the 14^{th} day of incubation. It may be possible therefore to use *G. cola* as fungicide to control leaf spot in eggplant fields because of their availability, eco-friendliness and high levels of flavonoid that promotes health.

Conflict of Interest

The authors have not declared any conflict of interest.

REFERENCES

Akinyosoye FA, Oladunmoye MK (2000). Effect of extracts of Mirabilis Jalapa on some selected fungi. Niger. J. Microbiol. 14:91-94.

Akpa AD, Musa B, Paswall AT (1991). Effect of neem extracts and mycelia growth of sorghum anthracnose pathogen *Collectotrichum graminicola*. Proceedings of 211st Annual Conference of Nigeria Soc. Plant Protect.10-13:47.

Akpomedaye DE, Ejechi BO (1998). The hurdle effect of mild heat and two tropical spices extracts on growth of three fungi in fruit juices. Food Res. Int. 31:339-341. http://dx.doi.org/10.1016/S0963-9969(98)00052-0

Amadioha AC (1998). Control of Powdery Mildew in pepper (*Capsicurn annum* L.) by leaf extracts of papaya (*Carica papaya* L.) J. Herbs. Spices. Med. Plants 6(2):41-47. http://dx.doi.org/10.1300/J044v06n02_05

Amadioha AC, Obi VI (1998). Fungitoxic Activity of Extracts from *Azadirachta indica* and *Xylopia aethiopica* on *Collectotrichum lindemuthianum* in cowpea. J. Herbs Spices Plants 6(2):33-40. http://dx.doi.org/10.1300/J044v06n02_04

Amadioha AC (2003). Evaluation of some plant leaf extracts against *Colletotrichum lindemuthianum* in cowpea. Acad. J. Phytopathol. Enthomol. Hungarica 38:259-265.

Amadioha AC (2004). Control of black rot of potato caused by Rhyzoctonia bataticola using some leaf extracts. Arch. Phytopathol. Plant Prot. 37:111-117.

Bartlett MS (1937). The square root transformation in the analysis of variance. Supplementary J. Royal Stat. Soc. 3:68-78. http://dx.doi.org/10.2307/2983678

Benjilali B, Tantaoui-Elaraki A, Hilal M (1984). Method to study antimicrobial effect of essential oils. Application to the antifungal activity of six moraccan essences. J. Food Prot. 47:748–752.

Egunjobi OA, Onoyerni SO (1981). The efficacy of water extract of neem (*Azadirachta indica* L.) leaves as a systemic nematicide. Niger. J. Plant Prot. 5:70-74.

Ejechi BO, Akpomedaye DE (1999). Wood protection potential of *Acalypha hispida* (Euphorbiaceae) leaf phenolic extract against degradation by two Basidiomycetes. In: Book of Abstracts, 27th Annual Conference of Nigerian Society of Plant Protection, NIFOR, Benin-city 26-30 Sept. pp. 23-35.

Ejechi BO, Ilondu ME (1999). Control of yam tuber (*Dioscorea rotundata*) rot agent Sclerotium rolfsii with camwood (*Baphia nitida* L.) sawdust extract. Afr. J. Root Tuber Crop 3(2):13-15.

Ejechi BO, Nwafor OE, Okoko FI (1999). Growth inhibition of tomato-rot fungi by phenolic acid and essential oil extracts of pepper fruit (*Dennetia tripetala*). Food Res. int. 32:395-399. http://dx.doi.org/10.1016/S0963-9969(99)00057-5

Ferguson LR (2001). Role of plant polyphenols in genomic stability. Mutat. Res. J. 475: 9-111.

Gen Stat (2003). Gen Stat for windows. Released 4. 23 DE discovery edition VSN international limited. Hemel Hempstead, Uk.

Gupta S, Barnerjee AB (1970). Rapid method of screening antifungal antibiotic producing agents. Indian J. Exp. Biol. 18:148-149.

Igboko AO (1983). Phytochemical studies on Garcinia cola Heckel. M.Sc. Thesis, University of Nigeria, Nsukka.

Ilondu ME (2011). Evaluation of aqueous plant extracts to control rot fungi in paw paw. J. Appl. Biosci. 37:2419-2424.

John JB, James C L (2004). Effects of botanicals on incidence of sheath rot of rice. J. Food Chem. 44:2147-21150.

Kikuzaki H, Nakatani N (1993). Anti oxidant effects of ginger constituents. J. Food Sci. 58(6):1407-1410. http://dx.doi.org/10.1111/j.1365-2621.1993.tb06194.x

Kumar J, Parmar BS (1996). Physiochemical and chemical variation in neem oils and some bioactivity, against Spodoptera lituru F. J. Agric. Food Chem. 44:2137-2143.

Lombin G, Yayock JY (1988). Crop Science and production in Warm Climates. Macmillan Publisher Ltd., London. pp. 210-211.

Nzanza B, Mashela PW (2012). Control of whiteflies and aphids in tomato (*Solanum lycopersicum* L.) by fermented plant extracts of neem leaf and wild garlic. Afr. J. Biotechnol. 11(94):16077-16082.

Obi IU (2002). Statistical methods of detecting different between treatment means and research methodology issues in laboratory and field experiments AP Express Publishers Ltd Nsukka. Nigeria. PMCid:PMC2732465

Okereke VC, Wokocha RC (2006). Effects of some tropical plant extracts, *Trichoderma harzianum* and captan on the damping-off of tomato induced by *Sclerotium rolfsii*. Agric. J. 1(2)52-54.

Onuegbu. B. A, Ibe, A. E. and. Onwugbuta-Enyi, J. A. (2001). Effects of water-extract of flowers of Balsam (*Impatiens balsamina*) on in-vitro germination and growth of fungus (*Sclerotium rolfsii* Sacc.) Niger. J. Horticult. Sci. 5:114-118.

Osei MK, Banfull B, Osei CK, Oluoch MO (2010). Characterization of African eggplant for morphological characteristics. J. Agric. Sci. Technol. 4(3):33-37.

Palanichamy S, Nagarajan S (1990). Antifungal activity of Cassia alata leaf extract. J. Ethnopharmacol. 29:337-340. http://dx.doi.org/10.1016/0378-8741(90)90043-S

Prakash A, Roa J (1997). Botanical Pesticides in Agriculture Lewis Publishers, London P. 416.

Rippon, J.W. (1988). Medical Mycology. 3rd Edition. W.B. Saunders Co.

Salako EA (2002). Plant protection for the Resource poor farmers. Paper presented at the 30th annual conference of Nigeria Society of Plant Protection, UNAAB Abeokuta. pp. 1-4.

Schmutterer H (1990). Properties and potentials of natural pesticides from neem tree. Ann. Rev. Entomol. 35:271-298. http://dx.doi.org/10.1146/annurev.en.35.010190.001415PMid:24057 1

Sridhar S, Arumugasamy S, Saraswathy H, Vijayalakshi K (2002). Organic vegetable gardening. Centre for Indian Knowledge System. Chennal.

Stoll G (1998). Natural Crop Protection in the tropics and sub-tropics. Agrecol, Switzerland P. 188.

Stoll G (2000). Natural crop protection in the Tropics; Letting Information come to life. Margraf Verlag 101-139.

Tindal HD (1965). Fruits and Vegetables in West Africa, London: Oxford University Press. 2nd edition 5(8):105.

Tovignan S, Vodoulie SD, Dinhan B (2001). Cotton Pesticides Cause more deaths in Benin. Pesticide Newsl. 52:l 2-4.

Wokocha RC, Okereke VC (2005). Fungicidal activity of extracts of some medicinal plants on Sclerotium rolfsii, causal organism of the basal stem rot disease of tomato. Niger. J. Plant Prot. 22:106-111.

academicJournals

Vol. 9(47), pp. 3458-3464, 13 November, 2014
DOI: 10.5897/AJAR2013.8411
Article Number: 39AB30C48597
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Efficacy of imazapic, halosulfuron and sulfentrazone for *Cyperus rotundus* L. control in response to weed tuber density

Bruna Pires da Silva[1]*, Renan Oliveira de Almeida[1], Tiago Pereira Salgado[2] and Pedro Luis da C. A. Alves[1]

[1]FCAV-UNESP - Departamento de Biologia Aplicada à Agropecuária, Jaboticabal-SP, Brazil.
[2]HERBAE – Consultoria e Projetos Agrícolas, Jaboticabal- SP, Brazil.

Received 18 December, 2013; Accepted 10 October, 2014

This study determined the efficacy of sulfentrazone, halosulfuron and imazapic applied in preemergence conditions for the control of purple nutsedge (*Cyperus rotundus* L.) in response to weed tuber density. The tubers were planted in 5 L pots at densities of 6, 18, 24, 30 and 36 tubers/pot (133.3, 399.9, 533.2, 665.5 and 799.8 tubers/m^2, respectively), and sulfentrazone (800.0 g a.i.ha^{-1}), halosulfuron (112.5 g a.i. ha^{-1}) or imazapic (147.0 g a.i.ha^{-1}) was applied 24 h after planting. The experimental design was the Completely Randomized Design (CRD) in a 5×4 factorial scheme with 4 replications. At the end of the experimental period (60 days after planting), the number of dead and viable tubers, dry biomass of viable tubers, and number and dry biomass of epigean manifestations were determined. The data were analyzed using the F test, and the means were compared using Tukey's test at 5% probability. Independently of tuber density, sulfentrazone performed better than either imazapic or halosulfuron in controlling nutsedge epigean manifestations and tubers.

Key words: Purple nutsedge, chemical control, herbicides, preemergence.

INTRODUCTION

Sugarcane is a crop that can be extremely affected by competition with weeds, often budding and exhibiting slow initial growth in response to this stress (Procópio et al., 2003). The weeds on sugarcane are responsible for up to 80% of production losses (Azania et al., 2008). Among the various weed species present in Brazilian sugarcane, *Cyperus rotundus* L. (nutsedge) is one of the most difficult to control, as few herbicides are recognized to provide effective control (Jakelaitis et al., 2003).

This weed grows mainly in the tropical and subtropical areas of the world (Brecke et al., 2005), resulting in significant losses in agricultural income (Bangarwa et al., 2008, Wang et al., 2008). Nutsedge is primarily characterized by its rapid growth rate (Lati et al., 2011) a single tuber can expand to an area of 5.5 m^2, yielding 750 tubers within 24 weeks (Webster, 2005). The deleterious effect of this weed on sugarcane is a function of its underground structure; its underground weight is five to ten times greater than that of its shoots (Singh, 1968). Densities of nutsedge bulbs and tubers equal to or

*Corresponding author. E-mail: brunapiresagro@yahoo.com.br. Tel: + 55 16 3209 2620. Fax: + 55 16 3209 2600.

Table 1. Macronutrient contents and fertility and physical parameters, Jaboticabal – SP, Brazil.

Chemical analysis									
pH	P res	O.M.	K	Ca	Mg	H+Al	SB	T	V
($CaCl_2$)	mg dm^{-3}	g dm^{-3}	$mmol_c$ dm^{-3}						%
5.3	35	26	3.2	36	11	34	50	84	60

Physical analysis				
Clay	Silt	Sand		Textural class
		Fine	Coarse	
g kg^{-1}				
320	70	320	290	Medium

greater than 1.0 kg/m² cause a significant deleterious effect on the sprouting of some sugarcane varieties (Bacchi et al., 1984). Because of the plant's ramified structure, efficient underground system and vegetative reproduction (bulbs, rhizomes and tubers), nutsedge can cause up to a 45% reduction in the production of sugarcane stalks when it occurs partially or entirely throughout the cycle of the crop (Keeley, 1987). Nutsedge competes for environmental resources (water, light and nutrients) (Durigan et al., 2005) and releases allelopathic compounds. Kuva et al. (2000) observed a 20% reduction in sugarcane productivity of sugarcane when the weed occurred throughout the crop cycle. Moreover, according to those authors, nutsedge control for only 22 days after sugarcane planting was enough to ensure that the crop reached 95% of its maximum productivity.

The mechanisms of tuber dormancy play an important role in weed dispersion (Nesser et al., 1997). Under satisfactory weather conditions, nutsedge tuber density in sugarcane can reach 3,000 tubers/m^2, producing up to 2000 shoots/m^2, which, after weeding, can grow from one to three centimeters per day (Lorenzi, 1983). A nutsedge tuber density of 815.5 tubers/m^2 is commonly found in moderate infestations of sugarcane (Kuva et al., 2000).

Higher weed density in an area leads to a greater number of individuals competing for the same resources in the environment and, therefore, a stronger deleterious impact on the crop (Pitelli, 1985). Additionally, because the action of herbicides on the nutsedge tubers is directly related to their density, higher numbers of tubers in the soil mean that less herbicide is available for each tuber, which may hinder pre-emergence control.

Thus, as the control of weeds in sugarcane is essentially dependent on herbicide application, the present study evaluated the efficacy of sulfentrazone, halosulfuron and imazapic applied in pre-emergence conditions for the control of nutsedge (*C. rotundus*) in response to weed tuber density.

MATERIALS AND METHODS

The experiment was conducted under partially controlled conditions (without water restriction) at experimental area located at 21°15'22" south latitude, 48°18'58" west longitude, and an altitude of 595 m. According to the global climate classification system of Köppen (1948), the climate is Cwa; the wet season is characterized by summer rains, and the winter is relatively dry.

For the experiment, 5 L pots were filled with soil collected from the surface soil of a red latosol, for which the chemical and physical characteristics are shown in Table 1. Based on the results of this chemical analysis, soil fertility was corrected prior to planting.

Nutsedge tubers were planted in the pots at densities of 6, 18, 24, 30 and 36 tubers/pot corresponding to 133.3, 399.9, 533.2, 665.5 and 799.8 tubers/m^2, respectively; the maximum tuber density was similar to the density observed by KUVA et al. (2000) for weed tubers in sugarcane. The tubers were placed at a depth of 3 cm, and the pots were then watered and left untouched for 24 h before pre-emergence herbicide application. The doses used in this study followed those recommended for the control of *C. rotundus* in sugarcane: Sulfentrazone at 800 g a.i ha^{-1}, halosulfuron at 112.5 g a.i ha^{-1} and imazapic at 147 g a.i ha^{-1}. After herbicide application, the pots were watered as necessary.

Herbicide application was performed on wet soil using an XR 8002 backpack sprayer with 4 spouts under constant pressure (CO_2), adjusted to a spraying consumption rate of 200 L ha^{-1}. During the application, the temperature was 32.5°C, the RH was 47%, and the temperature of the soil (5 cm depth) was 36.8°C, with no wind or clouds.

A 5x4 factorial completely randomized design (CRD) was employed in this study, with 4 replications: the main factors were the 5 tuber densities and the 4 herbicide treatments, including the untreated control.

At the end of the experimental period (60 days after planting), the final number of epigean manifestations (shoots), dead tubers (rotten, blackened or voids) and viable tubers with the potential for reinfestation were evaluated. Tuber viability was assessed using the tetrazolium test. For the determination of dry weight, the shoots and viable tubers were cut, washed, bagged and placed to dry in an oven with forced air circulation at 70°C until they reached constant weight.

The data were submitted to analysis of variance using the F test, and the means were compared using Tukey's test at 5% probability.

RESULTS AND DISCUSSION

At the end of the experimental period, sulfentrazone decreased the total number of tubers per plant compared to the initial number of tubers regardless of density; the herbicide inhibited the multiplication of *C. rotundus* tubers from the beginning of the experiment. Average increases of 108, 40.8, 120.83, 63.33 and 47.92% for the initial

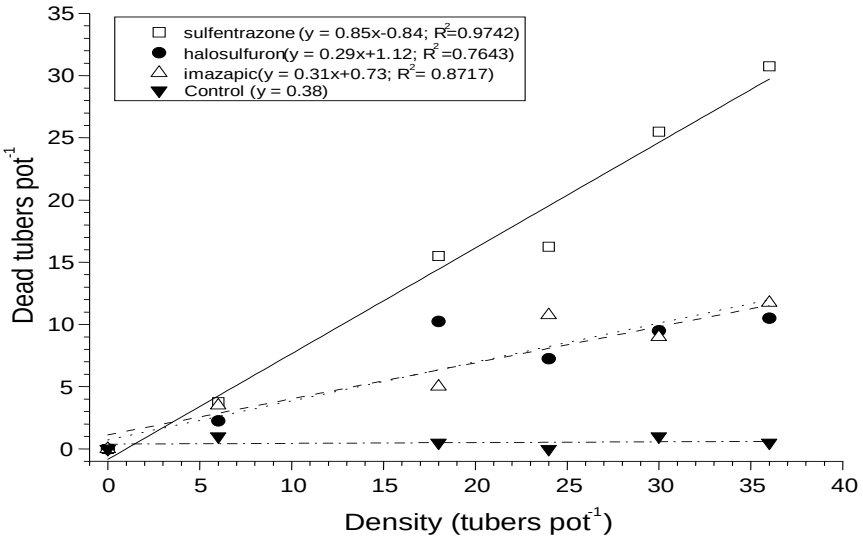

Figure 1. Effect of herbicides on the number of dead tubers nutsedge due the initial density of tubers at 60 days after application. Jaboticabal/SP, Brazil.

tuber densities of 6, 18, 24, 30 and 36 tubers/pot, respectively, were observed in the halosulfron treatment. The average increases observed in the imazapic treatment for the initial tuber densities of 6, 18, 24, 30 and 36 tubers/pot were 208.33, 62.50, 53.13, 45.00 and 10.42%, respectively. For the untreated control, the increases were considerably higher than those observed for the herbicide treatments: 716.67, 444.44, 457.20, 357.50 and 302.08% for the respective increasing densities (data not shown). The low rates of tuber multiplication observed at higher densities should not be attributed solely to the initial action of the herbicides imazapic and halosulfuron. At a density of 6 tubers/pot, where the availability of the herbicide to each tuber was higher, the rate of tuber multiplication was higher compared to that of the other densities. The same behavior was observed in the control treatment. Thus, in the treatments with the highest number of tubers per area, intraspecific competition occurred for environmental resources (water and space), which caused a reduction in the rate of tuber multiplication.

The application of a pre-emergence herbicide for the control of nutsedge is expected that to quickly reduce tubers or even prevent the multiplication of nutsedge in the soil, preventing reinfestation. The reduction in tuber multiplication rate is related to the efficacy of the herbicide in promoting the death of the tubers. Under the conditions of this study, the herbicide sulfentrazone increased the number of dead tubers linearly with increasing initial density of tubers, at a rate of 0.85 dead tubers per tuber planted, whereas with imazapic and halosulfuron, this increase in mortality was only 0.30. For the untreated control, the mortality of tubers was 0.38 for all densities (Figure 1).

The number of viable tubers decreased exponentially with increasing initial density of tubers; this effect was greatest for halosulfuron (61.21), followed by imazapic (14.36) and the untreated control (8.31). For sulfentrazone, the number of viable tubers was the same (2.37) for all densities and substantially lower than the values of the other treatments (Figure 2).

No significant differences in the herbicide effects on the number of viable tubers were observed at densities of 6 and 18 tubers/plant. Overall, sulfentrazone was superior to the other herbicides in decreasing the number of viable tubers. In general, imazapic and halosulfuron yielded statistically similar effects regarding the number of viable tubers. However, all herbicide treatments differed from the control for this parameter. Similarly, regarding tuber mortality, sulfentrazone achieved a control rate ranging from 79 to 97% among the different densities. The herbicides halosulfuron and imazapic also promoted the mortality of tubers, with average values ranging from 13 to 41% for halosulfuron and from 18 to 43% for imazapic. For the untreated control, the mortality rate of tubers did not exceed 2%, indicating that all the herbicides had an effect on the control of tubers. For this parameter, the density was significant for the effect of the herbicide at a density of 6 tubers/plant. However, a direct relationship between the interaction of density and herbicide on tuber mortality could not be obtained (Table 2).

The dry weight of those tubers that remained viable or capable of promoting multiplication and reinfestation was reduced in all herbicide treatments compared to the untreated control at all densities. At a density of 6 tubers/pot, the results of the sulfentrazone, imazapic and halosulfuron treatments were similar. At a density of 18 tubers/pot, the biomass of viable tubers in the halosulfuron treatment was similar to that in the sulfentrazone and imazapic treatments, with

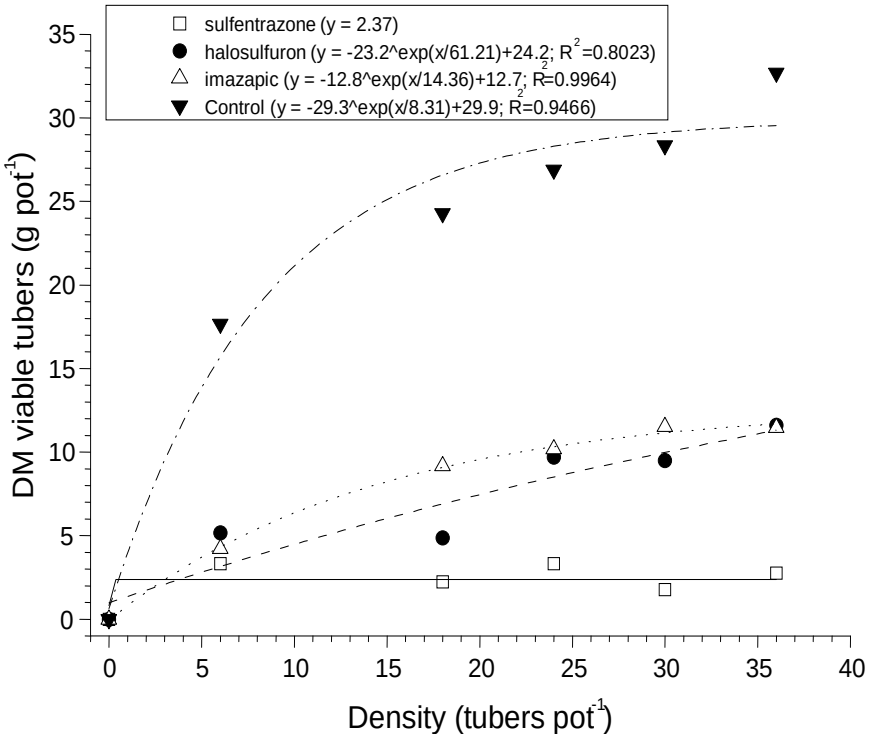

Figure 2. Effect of herbicides on the dry matter (DM) of tubers alive nutsedge due the initial density of tubers at 60 days after application. Jaboticabal/SP, Brazil.

sulfentrazone yielding a lower biomass of viable tubers than imazapic. For other densities (24, 30 and 36 tubers/pot), sulfentrazone also promoted a lower biomass of viable tubers than did halosulfuron and imazapic, which yielded similar results (Table 3).

As the control of tuber production and sprouting is the most important effect of an herbicide used to manage this weed for a longer period of time (Durigan et al., 1991), sulfentrazone proved to be more effective than the other herbicides. Other authors have also obtained excellent results for nutsedge control using sulfentrazone (Langbeck et al., 2004). The efficacy of sulfentrazone for the control this species has been evaluated in several studies and its suppressor potential has been highlighted by Wehtje et al. (1997), Werlang et al. (2004) and Rahnavard et al. (2010).

Treatment with sulfentrazone also promoted fewer epigean manifestations (Table 4) compared to the other treatments: At densities of 6, 18 and 30 tubers/pot, the sprouting of tubers was completely inhibited by sulfentrazone. At densities of 6, 30 and 36 tubers/pot, halosulfron did not promote the efficient control of the epigean manifestations, presenting results similar to those of the untreated control (no herbicide). At all densities, the imazapic treatment caused a reduction in sprouting tubers compared to the control but was less effective than sulfentrazone. At densities of 6 and 30 tubers/pot, the imazapic and halosulfuron treatments were similar to the untreated control in their effects on the sprouting of nutsedge tubers. The number of epigean manifestations per area is closely related to the production of rhizomes and tubers (Williams, 1978) and, consequently, to the competitive abilities of the plant. A greater number of epigean manifestations leads to a greater rate of weed multiplication and spread over an area. If a herbicide does not promote tuber inviability, the chemical control of nutsedge will not be effective.

For the dry biomass of epigean manifestations (Table 3), the three herbicide treatments yielded results that differed statistically from those of the untreated control regardless of density, except for imazapic at a density of 30 tubers/pot; these results demonstrated significant effects of the herbicides on this characteristic at the evaluated densities. Except for the density of 36 tubers/pot, at which no significant differences were observed among the herbicides, sulfentrazone achieved lower values for the dry biomass of epigean manifestations than those obtained by imazapic, halosulfuron and the untreated control, demonstrating its high potential for the control of nutsedge. At densities of 18 and 24 tubers/pot, significant differences were observed between halosulfuron and imazapic, with halosulfuron achieving better control of tuber sprouting. When a tuber remains viable in the soil, it uses its energy reserves to issue new epigean manifestations under favorable environmental conditions. Durigan et al. (2005)

Table 2. Effect of interaction between tuber density and herbicides on the number of tubers viable and dead at 60 days after application. Jaboticabal/SP, Brazil.

Herbicides	Density of tubers pot^{-1}					
	6	18	24	30	36	F
	Number of viable tubers pot^{-1}					
Sulfentrazone	1.00^{Ba}	0.50^{Ba}	2.00^{Ca}	1.50^{Ca}	2.50^{Ca}	0.0124^{ns}
Halosulfuron	10.25^{Bc}	14.75^{Bbc}	47.75^{Ba}	39.50^{Bab}	42.75^{Bab}	5.5475**
Imazapic	15.00^{Ba}	24.25^{Ba}	26.00^{BCa}	34.50^{Bab}	28.00^{BCa}	0.9857^{ns}
Untreated control	48.00^{Ac}	97.50^{Ab}	133.75^{Aa}	136.25^{Aa}	144.25^{Aa}	31.6886**
F	8.2875**	37.0582**	65.2991**	66.6488**	76.4874**	
$F_{DENSITY}$ = 19,3643**; $F_{HERBICIDE}$ = 228,5913**; F_{DxH} = 6,2899**; standard deviation = 14,2197; CV (%) = 33,5370						
	Number of dead tubers pot^{-1}					
Sulfentrazone	3.75^{Ac}	15.50^{Ab}	16.25^{Ab}	25.50^{Aa}	30.75^{Aa}	55.5282**
Halosulfuron	2.25^{Ab}	10.50^{Aa}	7.25^{Bab}	9.50^{Ba}	10.50^{Ba}	6.2386**
Imazapic	3.50^{Ab}	5.00^{Bb}	10.75^{Bab}	9.00^{Bab}	11.75^{Ba}	6.6900**
Untreated control	1.00^{Aa}	0.50^{Ba}	0.00^{Ca}	1.00^{Ca}	0.50^{Ca}	0.0903^{ns}
F	0.8275^{ns}	21.9559**	23.8877**	54.3794**	82.2891**	
$F_{DENSITY}$ = 34,0236**; $F_{HERBICIDE}$ = 137,3084**; F_{DxH} = 11,5078**; Standard Deviation = 2,7846; CV (%) = 31,8699						

Ns, Not significant by F test at 5% probability. **, significant by F test at 1% probability. Means followed by the same uppercase in the column and lowercase in the row do not differ significantly at 5% probability by Tukey test.

Table 3. Effect of interaction between tuber density and herbicides on dry biomass of viable tubers at 60 days after application. Jaboticabal/SP, Brazil.

Herbicides	Dry biomass of viable tubers (g pot^{-1})					
	Density of tubers pot^{-1}					
	6	18	24	30	36	F
Sulfentrazone	3.3335^{Ba}	2.2271^{Ca}	3.3321^{Ca}	1.7783^{Ca}	2.7720^{Ca}	0.26^{NS}
Halosulfuron	5.1577^{Bb}	4.8713^{BCb}	9.6983^{Bab}	9.4873^{Bab}	11.5657^{Ba}	4.98**
Imazapic	4.2068^{Bb}	9.1808^{Bab}	10.1764^{Ba}	11.5094^{Ba}	11.4467^{Ba}	5.08**
Untreated control	17.6641^{Ac}	24.2904^{Ab}	26.8977^{Ab}	28.3401^{Aab}	32.6926^{Aa}	17.31**
F	25.60**	54.48**	56.93**	70.20**	90.90**	
$F_{DENSITY\ (D)}$, 16.56**; $F_{HERBICIDE\ (H)}$, 283.35**; F_{DxH}, 3.68**; DMS_D, 2.66; DMS_H, 2.23; CV(%), 23.19						

Ns, Not significant by F test at 5% probability. **, significant by F test at 1% probability. Means followed by the same uppercase in the column and lowercase in the row do not differ significantly at 5% probability by Tukey test.

evaluated the effects of nutsedge density (*Cyperus rotundus* L.) on its chemical control in sugarcane, observing that the herbicides sulfentrazone and imazapic, when applied pre-emergence, provided control levels of 79.6 and 70 6%, respectively. In another study, Durigan et al. (2004) studied the control of nutsedge (*Cyperus rotundus* L.), observing that in the absence of straw, the herbicides sulfentrazone and imazapic achieved a control level of over 80% within 90 days.

However, in the present work, imazapic was not as effective as sulfentrazone. This result may be explained by the acidity of imazapic (pKa = 3.9), resulting in the lower sorption of the herbicide and a higher potential for leaching to lower soil layers, a phenomenon previously observed by Monquero et al. (2010).

Another important factor that may have contributed to the leaching of the herbicide is soil texture, which facilitates the percolation of water in the profile, allowing the greater downward movement of herbicide by mass flow. Sandy soils exhibit greater leaching of herbicide than do silt or clay soils (Rossi et al., 2005). Thus, the leaching of imazapic likely affected the activity of the herbicide and thereby reduced its potential for tuber control at the evaluated densities.

The same reasoning also applies for halosulfuron, which has a pKa of 3.5 and consequently may have also leached into the soil profile. Halosulfuron is recommended for the postemergence control of weeds,

Table 4. Effect of interaction between tuber density and herbicides on number and dry biomass of epigean part per plant at 60 days after application. Jaboticabal/SP, Brazil.

Herbicides	Density of tubers/pot					
	6	18	24	30	36	F
	Number of epigean part pot^{-1}					
Sulfentrazone	0.000^{Ca}	0.000^{Ca}	0.433D^{a}	0.000^{Ca}	0.500^{Ca}	0.26^{NS}
Halosulfuron	3.523^{ABb}	4.080^{Bb}	6.292^{Ba}	6.684^{ABa}	7.592^{Aa}	2.18**
Imazapic	1.878^{Bb}	3.745^{Bab}	4.349^{Ca}	4.960^{Ba}	3.551^{Bab}	5.32**
Untreated control	4.499^{Ac}	6.146^{Abc}	8.288^{Aa}	7.923^{Aab}	8.287^{Aa}	11.09**
F	15.49**	26.07**	44.65**	48.20**	52.88**	
$F_{DENSITY\ (D)}$, 19.72**; $F_{HERBICIDE\ (H)}$, 175.11**; $F_{DxH,}$ 3.04**; DMS_D, 0.99; DMS_H, 0.84; CV(%), 22.24						
	Dry biomass of epigean part/tuber pot^{-1}					
	6	18	24	30	36	F
Sulfentrazone	0.000^{Ca}	0.000D^{a}	0.118^{Ca}	0.000^{Ca}	0.320^{Ba}	0.51^{NS}
Halosulfuron	1.196^{Ba}	0.770^{Ca}	1.019^{Ba}	0.921^{Ba}	0.931^{Ba}	0.63^{NS}
Imazapic	1.126^{Ba}	1.581^{Ba}	1.309^{Aba}	1.186^{ABa}	0.919^{Ba}	1.55^{NS}
Untreated control	2.958^{Aa}	2.439^{Aab}	1.802^{Ab}	1.864^{Ab}	1.880^{Ab}	6.50**
F	38.61**	28.51**	12.94**	15.41**	10.76**	
$F_{DENSITY\ (D,}$ 1.99$^{ns.}$; $F_{HERBICIDE\ (H)}$, 96.65**; $F_{DxH,}$ 2.39*; DMS_D, 0.39; DMS_H, 0.33; CV(%), 35.20						

Ns, Not significant by F test at 5% probability. **, significant by F test at 1% probability. Means followed by the same uppercase in the column and lowercase in the row do not differ significantly at 5% probability by Tukey test.

as 1 to 20% of the herbicide is absorbed by leaves while only 0.1 to 5% is taken up by the root system (Mascarenhas et al., 1995). However, even when the herbicide is applied preemergence, as in this work, its control potential is equal to that of imazapic, especially at higher tuber densities.

Conversely, sulfentrazone has a low dissociation in water and behaves as a weak acid (pKa = 6.56), existing in the soil solution primarily in its nonionized form (FMC, 1995). Rossi et al. (2005) evaluated the mobility of sulfentrazone in Red Latossol in terms of rainfall, characterizing sulfentrazone as very mobile, remaining on the soil surface regardless of the rainfall measured. This reported low mobility of the herbicide corroborates the observations of Alves et al. (2004), who reported that the mobility and adsorption capacity of sulfentrazone in the soil is heightened when the soil pH is below the pKa of the herbicide; this situation reduces the efficiency of the herbicide in the field. In this study, the pH of the soil solution and the pKa of the herbicide were similar. As the nutsedge tubers in this study were placed at a depth of 3 cm beneath the soil surface, this observation explains the superiority of the control promoted by this herbicide at all tuber densities over that of halosufuron and imazapic.

In summary, under the conditions of this study, treatment with sulfentrazone caused a greater mortality rate of *C. rotundus* tubers and greater consequent reductions in the numbers of viable tubers and epigean manifestations than did treatment with halosulfuron or imazapic, demonstrating that this herbicide was more effective for the pre-emergence control of the weed. Importantly, this herbicide inhibited the multiplication rate of the tubers from the point of its application. In general, treatments with lower tuber density showed a higher mortality rate of tubers and, consequently, a lower number of viable tubers, confirming the hypothesis that the availability of the herbicide for each reproductive structure is increased at low densities. To optimize the control of *C. rotundus*, further studies should evaluate dose adjustments of halosulfuron and imazapic for application on sandy soils, especially in areas with a history of high infestation of this weed.

Conflict of Interest

The author(s) have not declared any conflict of interest.

REFERENCES

Alves PLCA, Marques Junior J, Ferraudo AS (2004). Soil attributes and the efficiency of sulfentrazone on the control of nutsedge (*Cyperus rotundus*). Sci. Agric. 61(3):319-325. http://dx.doi.org/10.1590/S0103-90162004000300014

Azania CAM, Rolim JC, Azania AAPM (2008). Plantas daninhas. In: Dinardo-Miranda LL, Vasconcelos ACM, Landell MGA (Ed.). Cana-de-açúcar. Campinas: Inst. Agron. pp. 465-490.

Bacchi OOS, Rolim JC, Christoffoleti PJ (1984). Efeito da tiririca (*Cyperus rotundus* L.) sobre brotação da cana-de-açúcar (Saccharum spp.) APC, São Paulo 7(32):44-48.

Bangarwa SK, Norsworthy JK, Jha P, Malik MS (2008). Purple nutsedge (Cyperus rotundus) management in an organic production system. Weed Sci. 56:606–613. http://dx.doi.org/10.1614/WS-07-187.1

Brecke BJ, Stephenson DO, Unruh JB (2005). Control of nutsedge (*Cyperus rotundus*) with herbicides and mowing. Weed Technol. 19:809–814.http://dx.doi.org/10.1614/WT-04-254R1.1

Durigan JC (1991). Manejo da Tiririca (*Cyperus rotundus* L.) antes e durante a implantação da cultura da cana-de-açúcar (Saccharum spp.). Jaboticabal, São Paulo, Universidade Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias, Campus de Jaboticabal, (Tese de Livre-Docência). P. 336.

Durigan JC, Timossi PC, Leite GJ (2004). Controle químico da tiririca (*Cyperus rotundus*), com e sem cobertura do solo pela palha de cana-de-açúcar. Planta Daninha 22(1):127-135. http://dx.doi.org/10.1590/S0100-83582004000100016

Durigan JC, Timossi PC, Correia NM (2005). Densidades e manejo químico da tiririca na produtividade de cana-de-açúcar. Planta Daninha 23(3):463-469. http://dx.doi.org/10.1590/S0100-83582005000300010

Jakelaitis A, Ferreira LR, Silva AA, Agnes EL, Miranda GV, Machado AFL (2003) Efeitos de sistemas de manejo sobre a população de tiririca. Planta Daninha 21:89-95. http://dx.doi.org/10.1590/S0100-83582003000100011

Keeley PE (1987). Interference and interation of and yellow nutsedges (*C. rotundus* and *C. esculentus*) with crops. Weed Technol. 1:74-81.

Köppen W (1948). Climatología con un estudio de los climas de la Tierra. Mexico: Ed. Fondo de Cultura Económica-Pánuco.

Kuva MA, Pitelli RA, Christoffoleti PJ, Alves PLCA (2000). Períodos de interferência das plantas daninhas na cultura da cana-de-açúcar. I-Tiririca. Planta Daninha 18(2):241-251. http://dx.doi.org/10.1590/S0100-83582000000200006

Langbeck FM, Novo MCSS, Lago AA, Deuber R (2004). Viabilidade de tubérculos de *Cyperus rotundus* L. tratados com sulfentrazone. Arquivos do Instituto Biológico, São Paulo 71:372-375.

Lati RN, Filin S, Eizenberg H (2011). Temperature and radiation based models for predicting spatial growth of nutsedge (*Cyperus rotundus*). Weed Sci. 59:476–482. http://dx.doi.org/10.1614/WS-D-11-00007.1

Lorenzi H (1983). Plantas daninhas e seu controle na cultura de cana-de-açúcar. In: REUNIÃO TÉCNICA AGRONÔMICA. Piracicaba, 1983. Anais. Piracicaba, COOPERSUCAR pp. 59-73.

Mascarenhas MAT, Galli AJB, Viana MCM, Macêdo GAR, Lara JFR (1995). Eficácia do halosulfuron no controle de tiririca (*Cyperus rotundus*) na cultura da cana-de- açúcar. Planta Daninha 13(2):69-80.http://dx.doi.org/10.1590/S0100-83581995000200002

Monquero PA, Silva PV, Silva Hirata AC, Tablas DC, Orzari I (2010). Lixiviação e persistência dos herbicidas sulfentrazone e imazapic. Planta Daninha 28(1):185-195. http://dx.doi.org/10.1590/S0100-83582010000100022

Nesser C, Aguero R, Swanton CS (1997). Survival and dormancy of nutsedge (*Cyperus rotundus*) tubers. Weed Sci. 45:784-790.

Pitelli RA (1985). Interferência das plantas daninhas em culturas agrícolas. Inf. Agropec. 11(129):16-27.

Procópio SO, Silva AA, Vargas L, Ferreira FA (2003). Manejo de plantas daninhas na cultura da cana-de-açúcar. Viçosa, Universidade Federal de Viçosa P. 150.

Rahnavar A, Ashrafi ZY, Rahbari A, Sadeghi S (2010). Effect of different herbicides on control of nutsedge (*Cyperus rotundus* L.). Pak. J. Weed Sci. Res. 16(1):57-66.

Rossi CVS, Alves PLCA, Marques Junior J. Mobilidade do sulfentrazone em Latossolo Vermelho e em Chernossolo. Planta Daninha 23(4):701-710. http://dx.doi.org/10.1590/S0100-83582005000400019

Singh SP (1968). Presence of a growth inhibitor in the tubers of nutgrass (*Cyperus rotundus* L.) Proc. Indian Acad. Sci. Bangalore, 67:18-23.

Wang G, McGiffen ME, Ogbuchiekwe EJ (2008). Crop rotation effects on *Cyperus rotundus* and *C. esculentus* population dynamics in southern California vegetable production. Weed Res. 48:420–428. http://dx.doi.org/10.1111/j.1365-3180.2008.00649.x

Webster TM (2005). Patch expansion of nutsedge (Cyperus rotundus) and yellow nutsedge (*Cyperus esculentus*) with and without polyethylene mulch. Weed Sci. 53:839–845. http://dx.doi.org/10.1614/WS-05-045R.1

Wehtje GR, Walker RH, Grey TL, Hancock HG (1997). Response of nutsedge (*Cyperus rotundus*) and yellow nutsedge (C. esculentus) to selective placement of sulfentrazone. Weed Sci. 45:382-387.

Werlang RG, Silva AA, Reis MR dos, Jakelaits A (2004). Manejo de plantas daninhas na cana-de-açúcar plantio de ano. In: CONGRESSO BRASILEIRO DE CIÊNCIA DAS PLANTAS DANINHAS, 24. 2004, São Pedro. Resumos. São Pedro: SBCPD P. 163.

Williams RD (1978). Photoperiod effects on the reproductive biology of nutsedge (*Cyperus rotundus* L.). Weed Sci. 26(6):539-542.

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 Pure & Applied Chemistry*
- *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*

academicJournals