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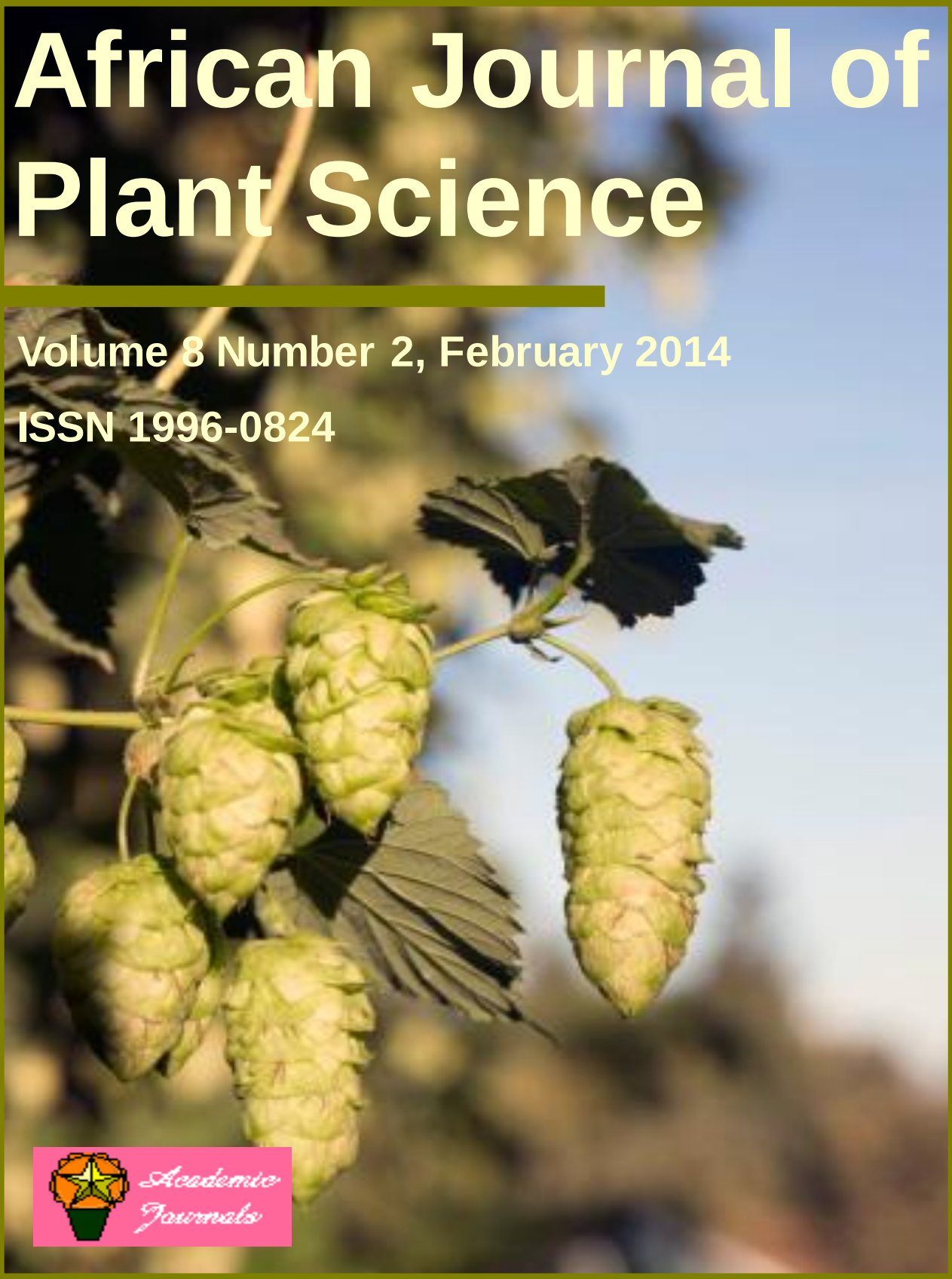

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African Journal of Plant Science

Full Length Research Paper

Influence of boron on seed germination and seedling growth of wheat (*Triticum aestivum* L.)

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A laboratory experiment was conducted at Department of Plant Science, Ambo University, Ethiopia, to study the effect of boron availability on seed germination and seedling growth of wheat (*Triticum aestivum* L. var *Danda'a*). Seeds were sown in petri dishes with varying concentrations of boron (0, 0.25, 0.5, 1, 2, 4, 8, and 16 mg/L) at room temperature (24 ± 2°C) in complete randomized design (CRD) with four replications. Germination percentage and rate, shoot and root lengths, shoot and root fresh and dry weights, root number, root - shoot ratio and seedling vigor index were found to decrease beyond 0.25 mg/L, and phytotoxicity increased significantly (except on shoot at 0.25 mg/L) with increase in the concentration of boron in the germinating medium.

Key words: Boron, germination, phytotoxicity, seedling growth, tolerance, vigor, wheat.

INTRODUCTION

Boron (B) is a micronutrient required by plants in a very small quantity (Abd El-Wahab, 2008), and its availability in soil and irrigation water is an important determinant of agricultural production (Tanaka and Fujiwara, 2007). In soil solution, boron exists primarily as boric acid (H_3BO_3), which can be easily leached under high rainfall conditions (Yan et al., 2006) leading to plant deficiencies. On the contrary, under low rainfall conditions, boron cannot be sufficiently leached and therefore may accumulate to toxic levels for plant growth (Reid, 2007). This occurs very often in arid and semi arid regions where parent material and groundwater may contain high concentrations of boron. The accumulation of boron in top soil due to evaporation of groundwater reaches toxic levels that can reduce crop yields (Tanaka and Fujiwara, 2007). Boron is often found in high concentrations in association with saline soils and saline well water (Dhankhar and Dahiya, 1980). Of all the potential sources, irrigation water is the most important contributor to high levels of soil boron (Chauhan and Power, 1978). In assessing the potential toxicity of B rich irrigation water, the physical and chemical characteristics of the soil must be considered (Rauf et al., 2007). Boron can be regenerated through the mineralisation of soil organic matter, or through weathering processes of soil minerals (Peryea et al., 1985).

Plants exposed to excess of boron have reduced vigor, retarded development, leaf burn (chlorotic and necrotic patches in older leaves), and decreased number, size, and weight of fruits (Nable et al., 1997). Boron toxicity is an important agricultural problem that limits crop productivity in different regions of the world, and can occur in B-rich soils or in soils exposed to B-rich irrigation waters, fertilizers, sewage sludge, or fly ash (Luis et al., 2012).

Wheat (*Triticum aestivum* L.) is a staple food for more

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than 35% of the world population and is also the first grain crops in most of the developing countries (Metwali et al., 2011). Bread wheat is the main food of people in many countries and about 70% calories and 80% protein of human diet is supplied from its consumption (Taregh et al., 2011). In Ethiopia, the total area put to wheat production was estimated to be 1.68 million ha with an average yield of 1827 kg/ha (CSA, 2011). Abiotic stress, especially drought stress and boron toxicity, are worldwide problems which seriously constrain global crop production (Schnurbusch et al., 2010; Pan et al., 2002). It is one of the major causes of crop loss worldwide, which commonly reduces average yield of many crops by more than 50% (Wang et al., 2003; Bayoumi, 2008). In countries like Australia, B toxicity impacts heavily on wheat production (up to 11% yield reduction) in affected areas (Moody and Rathjen, 2007).

Shoot biomass reduction of wheat was observed owing to high B addition to soil (Wimmer et al., 2003). Wheat with high B concentrations showed leaf edge burning and necrosis compared with the control treatments (Sonmez et al., 2009). Genotypic variation in susceptibility to B toxicity has been reported (Torun et al., 2006). Paull et al., (1988) reported wide range of intra-specific variation in response to B occurs in a number of crops, including bread wheat (*Triticum aestivum* L). In addition, seed germination and seedling growth are the most important phases in the life cycle of plant, and are highly responsive to the existing environment. Hence, the current experiment was conducted to investigate the effect of boron on germination and seedling growth parameters of wheat (*Triticum aestivum* L. var *Danda'a*).

MATERIALS AND METHODS

A laboratory experiment was conducted in October, 2013 at the Department of Plant Sciences, Ambo University, to investigate the effect of boron on germination and seedling growth of wheat. The experiment was arranged in completely randomized design with four replications. Cultivar *Danda'a* was treated with eight levels of boron (0, 0.25, 0.5, 1, 2, 4, 8, and 16 mg/L) for the experiment, deionized water was used for the control treatment. Boric acid (H_3BO_3) was used as a source of boron. Seeds were surface sterilized with 30% hydrogen peroxide solution for 5 min, and rinsed with deionized water. Twenty seeds were uniformly placed per Petri dish (9.5 cm diameter) using a forceps after the Petri dish were sterilized with 98% ethanol, and rinsed with deionized water. Filter papers were well soaked by adding 7 ml with the respective solutions (7 treatment solutions and the control) at an interval of 48 h as described by Naveed et al. (2001). All the Petri dishes were covered with lids and kept at room temperature (24±2°C). Germination continued for 10 days, and germinated seeds were counted daily. Germination was considered to have occurred when radicles attained a length of 2 mm. After 10 days, parameters such as percent germination and rate of germination were calculated according to ISTA (1999); and root and shoot lengths of seedling were measured using a scale. Root and shoot dry weights were recorded after oven drying for 72 h at 60°C. The seedling vigor index (SVI) was determined as Hosseein and Kasra (2011):

Seedling Vigor Index = % Germination × Seedling dry weight (g)

Tolerance index (T.I.) was determined by Iqbal and Rahmati (1992) method:

T.I. = (Mean root length in treatment solution / Mean root length in distilled water) × 100

The percentage of phytotoxicity on shoot and root of seedlings was calculated following the formula given by Chou and Lin (1976):

$$\text{Phytotoxicity of Shoot (\%)} = \frac{\text{Shoot length of control - Shoot length of treatment}}{\text{Shoot length of control}} \times 100$$

$$\text{Phytotoxicity of Root (\%)} = \frac{\text{Root length of control - Root length of treatment}}{\text{Root length of control}} \times 100$$

Relative water content (RWC) of seedling was calculated as per the formula used by (Shalaby et al., 1993):

$$\text{RWC} = \frac{\text{Fresh weight - Dry weight}}{\text{Fresh weight}} \times 100$$

Statistical analysis of the data was performed using one-way ANOVA using SAS statistical software (Version 9). Based on the ANOVA results, mean separations were performed by Duncan's multiple range test at 5% level.

RESULTS AND DISCUSSION

Seed germination and seedling growth

The germination percentage at 0.25mg/l showed no difference compared to the control (Table 1). A significant decrease in germination was observed at boron concentrations higher than 0.5 mg/L. At 8 and 16 mg /L, wheat seed failed to germinate, indicating that germination is totally inhibited at such high concentra-tions of boron (Table 1). The consistent decrease in percentage and rate of seed germination beyond 0.25 mg/L in the present study is in line with the findings of Yau and Saxena (1997) and Muhammad et al., (2013) who stated that high boron concentration reduced germination percentage of durum wheat and maize, respectively.

The shoot and root lengths, shoot and root fresh and dry weights, and seedling dry weight decreased significantly with the increase in boron concentration (Tables 1 and 2). However, the highest shoot length (5.12 cm) and root length (5.31 cm) were obtained with 0.25 mg/L boron

Table 1. Effect of boron on germination, and shoot and root lengths of wheat.

Boron Conc. (mg/L)	Germination (%)	Germination rate	Shoot length (cm)	Root length (cm)
0	96.25[a]	5.39 (3.25)[a]	14.3 (4.78)[ab]	18.8(5.31)[a]
0.25	96.25[a]	4.97(3.15)[a]	17.1 (5.12)[a]	12.7(4.56)[b]
0.5	85[ab]	2.82 (2.68)[ab]	12.6(4.55)[b]	6.1 (3.46)[c]
1	77.5[bc]	2.25(2.5)[b]	7.4 (3.72)[c]	2.2(2.46)[d]
2	68.75[c]	1.64 (2.27)[bc]	3.4(2.73)[d]	1.4 (2.14)[de]
4	38.75[d]	0.8 (1.77)[c]	0.95(1.78)[e]	0.88(1.76)[e]
8	0[e]	0 (1.00)[d]	0(1.00)[f]	0(1.00)[f]
16	0[e]	0 (1.00)[d]	0(1.00)[f]	0(1.00)[f]
SEm (±)	10.4	0.39	0.36	0.35
CV (%)	18	17.5	11.7	12.9

Means with similar letters in each column are not significant at 5% level of probability. Data in parenthesis are square root transformed.

Table 2. Effect of boron on fresh and dry weights of wheat seedling.

Boron Conc. (mg/L)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Seedling fresh weight (g)	Seedling dry weight (g)
0	0.09 (1.306)[ab]	0.008(1.089)[a]	0.22(1.373)[a]	0.007(1.081)[a]	0.31(1.56)[a]	0.015(1.39)[ab]
0.25	0.11(1.332)[a]	0.011(1.106)[a]	0.06(1.240)[ab]	0.009(1.092)[a]	0.17(1.41)[ab]	0.020(1.14)[a]
0.5	0.07(1.260)[b]	0.009(1.092)[a]	0.03(1.177)[abc]	0.007(1.082)[a]	0.10(1.32)[bc]	0.015(1.12)[ab]
1	0.03(1.1 47)[c]	0.004(1.059)[b]	0.025(1.137)[bc]	0.006(1.073)[ab]	0.055(1.23)[cd]	0.009(1.10)[b]
2	0.009(1.097)[cd]	0.002(1.037)[c]	0.019(1.136)[bc]	0.003(1.049)[bc]	0.03(1.17) [cd]	0.004(1.06)[c]
4	0.006(1.065)[d]	0.002(1.029)[c]	0.014(1.095)[bc]	0.003(1.040)[c]	0.02(1.14)[de]	0.004(1.06)[c]
8	0(1.0)[e]	0(1.0)[d]	0(1.0)[c]	0(1.0)[d]	0(1.0)[e]	0(1.0)[d]
16	0(1.0)[e]	0(1.0)[d]	0(1.0)[c]	0(1.0)[d]	0(1.0)[e]	0(1.0)[d]
SEm(±)	0.03	0.014	0.13	0.017	0.107	0.018
CV (%)	3	1.3	11.2	1.6	8.8	1.7

Means with similar letters in each column are not significant at 5% level of probability. Data in parenthesis are square root transformed.

concentration and control, respectively; and the lowest shoot and root lengths were with 8 mg/L and 16 g/L concentrations that caused a complete failure of germination. Boron inhibits root growth primarily through limiting cell elongation rather than cell division (Brown et al., 2002). Nable et al. (1997) also reported that shoot and root growth reduced when exposed to high B levels.

Shoot and root fresh and dry weights, and seedling fresh and dry weights decreased significantly with increase in boron concentration as compared to control and 0.25 mg/L (Table 2). Fresh weight and dry matter yield of the plants decreased significantly with increasing levels of applied boron (Ayvaz et al., 2012; Alpaslan and Gunes, 2001). Muhammad et al. (2013) and Turan et al. (2006) reported that shoot and root fresh and dry weights of maize and wheat decreased with the increase in the concentration of boron, respectively. Boron at 0.25 mg/L concentration resulted in the highest shoot fresh weight (1.33 g), shoot (1.1 g) and root (1.09 g) dry weights, and seedling dry weight (1.14 g). The significant increase at low concentration of boron could be due to its involvement in cell elongation or cell division and meristematic growth (Khan et al., 2006). Bonilla et al. (2004) and Farr (2010) reported that low concentrations of exogenous boric acid stimulated seed germination and seedling growth, while high concentrations showed an inhibitive effect on these parameters (Ölçer and Kocaçalışkan, 2007). Metwally et al. (2012) reported a gradual reduction in fresh and dry matter yield of shoots and roots, with increasing boron concentration in sand culture media.

Root number and root- to-shoot ratio

Root number and root-to-shoot ratio showed no significant difference up to 0.5 and 0.25 mg/L of boron concentrations, respectively. Further increase in boron concentrations significantly reduced both root and root-to shoot ratio (Figure 1). Cokkizgin (2013) reported similar

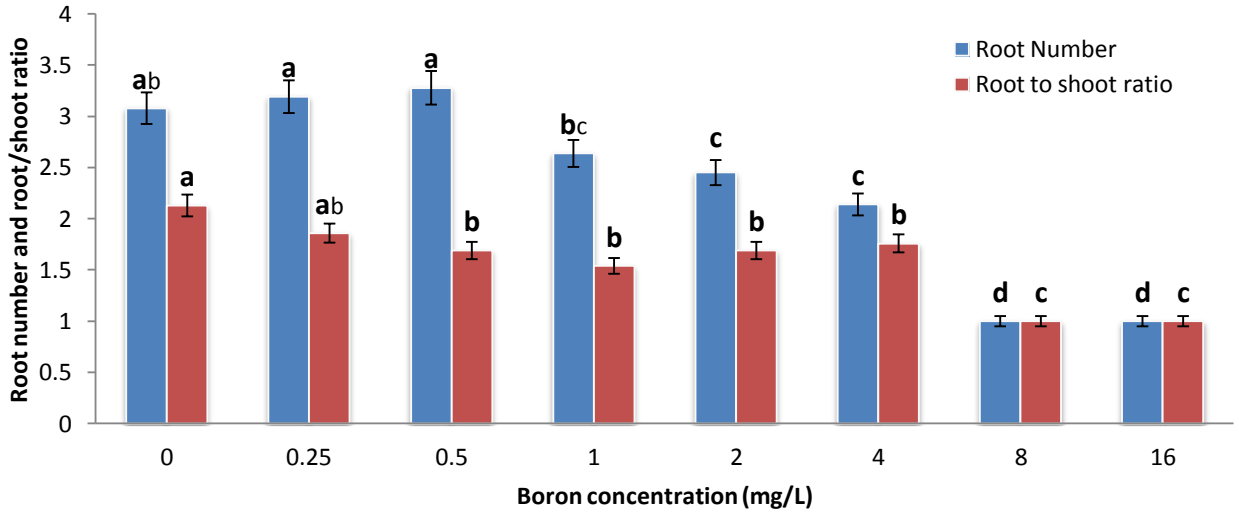

Figure 1. Effect of boron on root number and root /shoot ratio in wheat.

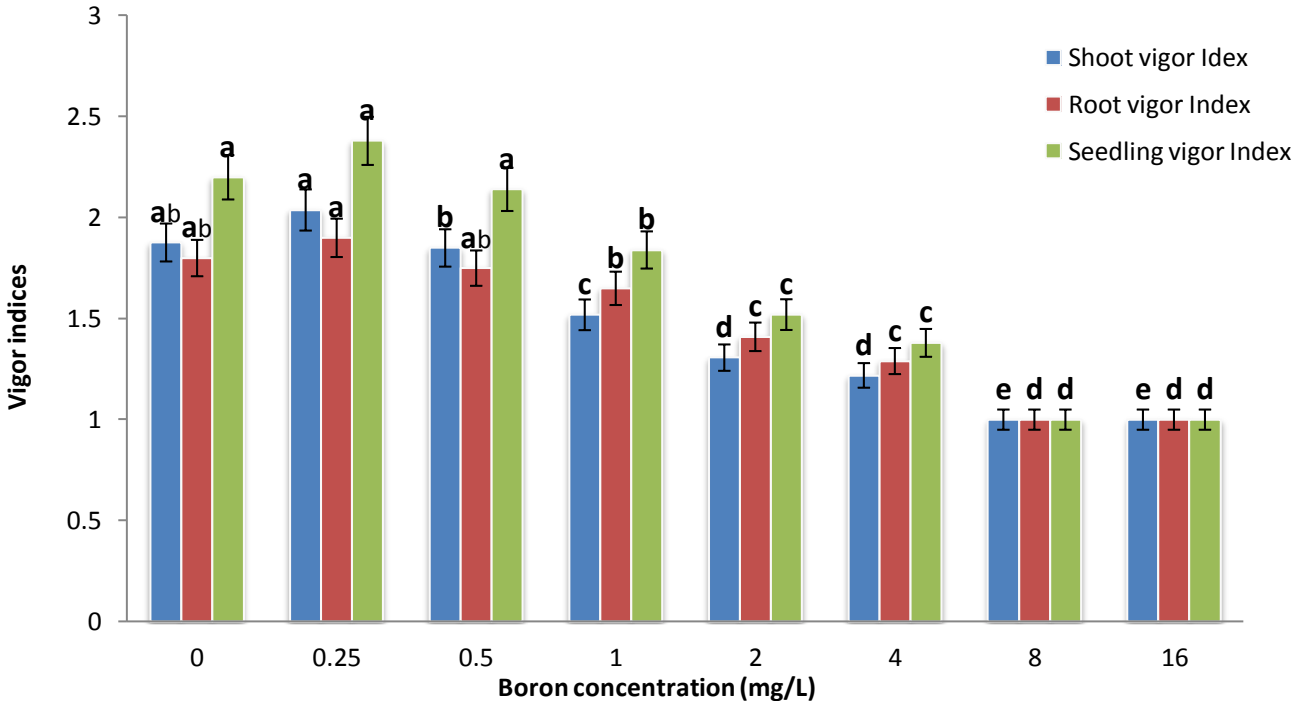

Figure 2. Effect of boron on vigor indices of Wheat.

findings on bean, and the increase in B concentration inhibited the secondary root emergence (Rehman et al., 2012).

Seedling vigour, tolerance and phytotoxicity

Boron concentrations showed a significant effect on seedling vigour index, shoot and root vigour indices, and tolerance index (Figure 2). The highest value for each trait was noticed in control treatment and 0.25 mg/L. Boron concentrations above 0.5 mg/L decreased vigour indices and tolerance index. Similar findings were reported by Mirshekari (2012) and Cokkizgin (2013), who observed a restricted, seedling vigor index of dill and

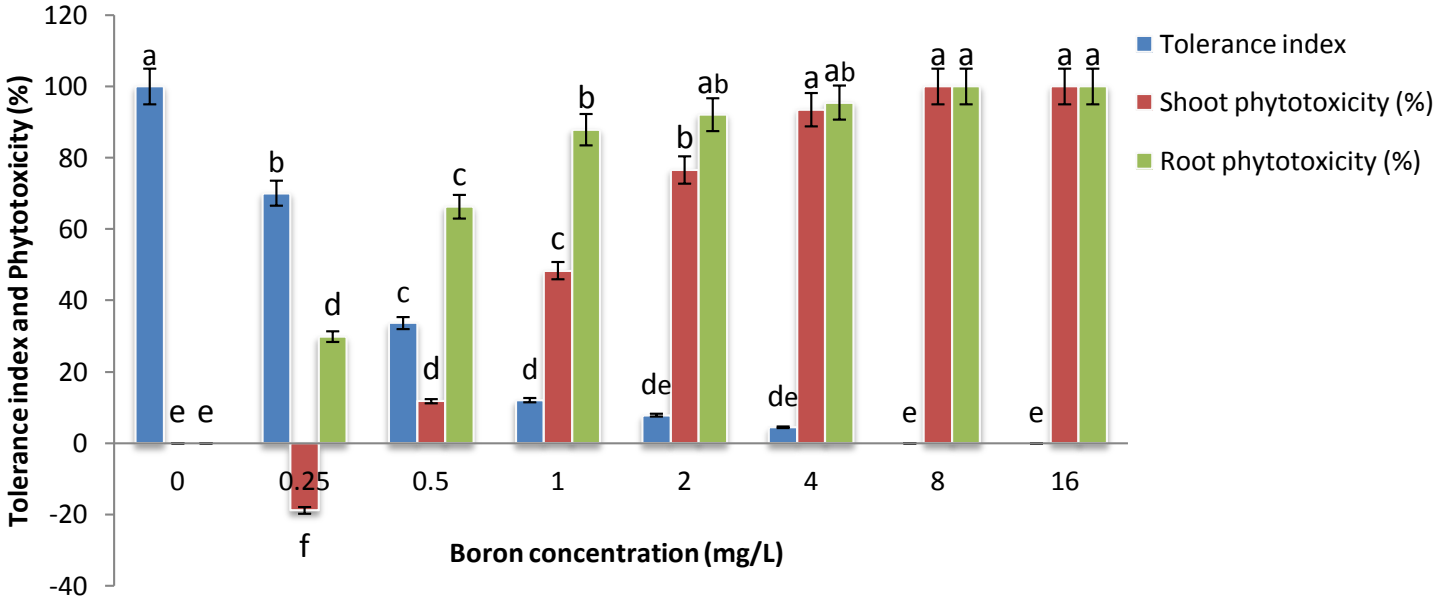

Figure 3. Effect of boron on tolerance index and phytotoxicity of wheat.

Phaseolus vulgaris at high level of boron concentrations, respectively. Ivanova et al., (2010) also reported a decrease in rapeseed seedling vigor indices with increasing micronutrient concentrations.

Boron showed a significant effect ($p<0.05$) on shoot and root phytotoxicity (Figure 3). Phytotoxicity of shoot and root increased with the increase in boron concentration. Lesser shoot and root toxicity was recorded with control treatment and lower concentration (0.25 mg/L), while it increased at higher concentration. At 0.25 mg/L B concentration shoot growth was highly promoted compared to control, hence showed a negative toxicity. Maximum phytotoxicity of boron on shoot and root was observed with ≥8 mg/L. The finding of our study is in agreement with the recent reports of Shaikh et al. (2013) and Habtamu et al. (2013) who reported that micronutrient toxicity of shoot and root decreased at lower concentration. On the other hand, it has the characteristics of promoting seedling growth at low concentrations based on crop types and varieties; however, its toxicity increased with increased in concentrations.

The tolerance index of wheat seed declined significantly with the increase in boron concentration. The maximum value of the tolerance index was obtained in the control treatment (100%) followed by 0.25 mg/L (70.1%), while a zero tolerance was attained for boron concentrations ≥8 mg/L. This result is in agreement with the recent findings of Shaikh et al. (2013) and Habtamu et al. (2013) who reported that increasing micronutrient concentrations decreased wheat and tomato tolerance index, respectively.

Relative water content

Increased boron concentrations ≥8 mg/L caused significant decrease in shoot and root relative water content of seedlings (Figure 4). In boron concentrations between 0 and 4 mg/L, the relative shoot and root water content decreased from 91.4 to 60.6%, and 85.8 to 61.4%, respectively. The highest values of relative water content of shoot and root were observed in the control, while the lowest in boron concentrations higher than ≥4 mg/L. High water content on shoot and root implies the higher dilution effect of boron that reduces its toxicity. This result was in agreement with Kinfemichael (2011) who found a drastic decrease seedling shoot and root relative water content at higher salinity level.

Conclusion

High boron concentrations caused a decrease in germination and germination rate, shoot and root lengths, shoot and root fresh and dry weights, vigor, tolerance and toxicity indices and relative water content of shoot and root in wheat. Low boron concentration (0.25 mg/L) showed the highest shoot fresh weight, shoot and root dry weights, seedling dry weight, and the lower shoot toxicity index. At higher boron concentrations, a deleterious effect on germination and seedling growth traits of wheat (*Triticum aestivum* L. var. *Danda'a*) was observed.

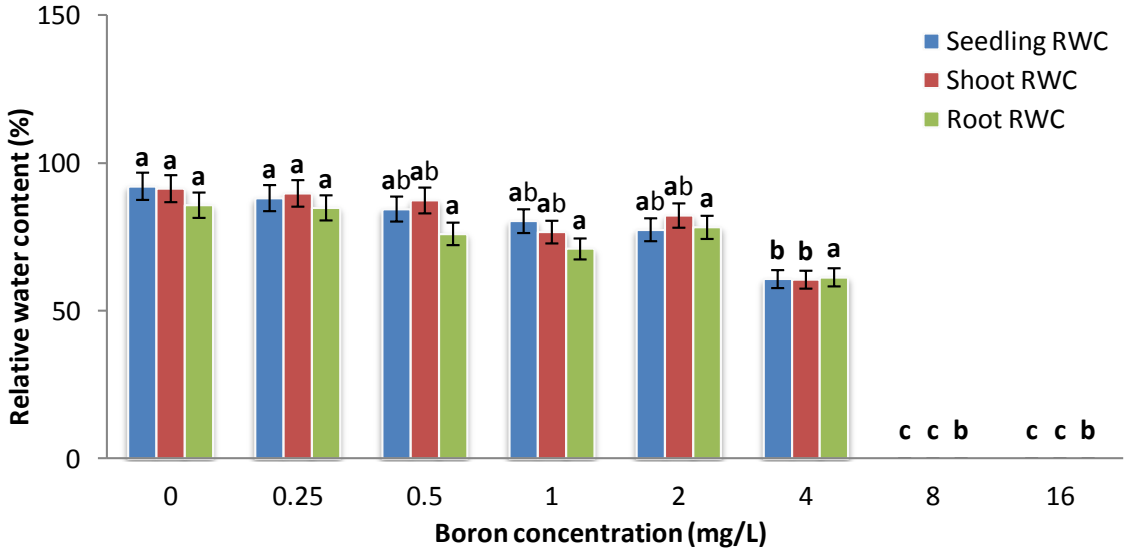

Figure 4. Effect of boron on shoot and root relative water content of wheat.

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Full Length Research Paper

In vitro potential antimicrobial activities of medicinal plants, *Cirsium arvense and Cirsium japonicum*

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Antimicrobial activities of *Cirsium* species (*Cirsium arvense and cirsium japonicum)* was evaluated against six bacterial species, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* (clinical strain/PIMS), *Enterobacter* (clinical strain/PIMS), *Staphylococcus aureus* (MRSA, clinical strain/PIMS) and six fungus *Trichophyton longifusus, Candida albicans, Aspergillus flavus, Microsporum canis, Candida glaberata* and *Fusarium solani*. The methanolic extracts of these plants were fractionated with *n*-hexane to get (F_1), chloroform (F_2), ethyl acetate(F_3), *n*-butanol(F_4) and methanolic fraction (F_5). The agar well diffusion assay and agar dilution susceptibility testing were carried out to determine the zone of inhibitions and the minimum inhibitory concentration, respectively. Ager well diffusion methods was used for antifungal activity. The zones of inhibition were determined using standard agent. The entire fraction showed some activity against these pathogens, but F_2 of both plants shows very good activity. The present study explains the potential antimicrobial properties of *C. arvense and C. japonicum*. From these observations, it is clear that *Cirsium* species contain antimicrobial constituents.

Key words: Traditional medicine, crude extract, antimicrobial activities*, Cirsium arvense*, *cirsium japonicum.*

INTRODUCTION

Throughout the history of mankind, medicinal plants have continuously been used for the treatment of multiple infections (Augustin and Hoch, 2004; Ashraf et al., 2006). Herbal medicines have made large contributions to human healthiness (Iwu et al., 1999) and provided a good source of anti-infective agents; emetine, quinine and berberine remain highly effective instruments in the fight against microbial infections. WHO reported that more than 80% of the world's population depends on traditional medicine for the treatment of their illnesses (Norman et al., 1985). In spite of development of novel drugs in modern times to combat emerging infections, increased resistance to antibiotics of many bacteria is still a global threat (Konig et al., 2000). This provoked researchers to screen plant extracts and plant compounds for antimicrobial agents (Yoshikazu et al., 2001; Norton, 2000).

Cirsium arvense and *Cirsium japonicum* (L.), *Cirsium* species, family Asteraceae are native plant of Europe, Asia (northern), and widely introduced elsewhere. They are herbaceous plants growing between 30-100 cm, associated with widespread colonies (Morishita, 1999).

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C. arvense is reported nearly in all crops including pastures and rangelands. Likewise, *C. arvense* have a role of natural host to various plants pathogens causing crop spoilage (Parendes and Jones, 2000). Livestock tend to dislike and avoid Canada thistle which may also reduce their consumption of desirable plants in the vicinity of Canada thistle colonies. The crude protein, *in vitro* digestible dry matter, micro and macro mineral concentrations of Canada is comparable to or greater than those of alfalfa (Medicagosativa) (Myers, 2000).

The vulnerary use *C. arvense* and *C. japonicum* had not previously been cited in the principal pharmacobotany texts. However, the juice of the leaves of these plants, locally applied, for healing of wounds is used in some parts of the world (Raven and Edwards, 2001; Zouhar, 2001). Fewer reports are available to address the antimicrobial potential of *C. arvense and C. japonicum* (Maria et al., 2010). No reports are available on antifungal activities of these plants to the best of our knowledge.

The present study was conducted to evaluate antimicrobial and antifungal activity of *C. arvense* and *C. japonicum* used locally as traditional medicine against multiple infections.

MATERIALS AND METHODS

Plant materials

Collection was based on information given by local inhabitants during follow up of ethno-medical and traditional uses of plant against infectious diseases used locally (Fabricant and Farnsworth, 2001). Plants were identified by Professor Farman Ullah Khan, Department of Botany Bannu, Pakistan. The specimens were deposited and voucher specimen number (117A, 117B) was obtained.

Preparation of extraction

The shade dried plants of *C. species* (11 kg) was ground and extracted with MeOH (35 L×3) at room temperature. The combined methanolic extracts were evaporated under reduced pressure to obtain a dark brown gummy material (600 g). The gummy material was suspended in water and extracted with *n*-hexane (99 g), chloroform (80 g), ethyl acetate (70 g) and *n*-butanol (50 g) soluble fractions, respectively. The fractions were then placed in a vacuum oven at not more than 40°C for about 24 h to remove any residual solvent. These fractions were screened for toxicity. The chloroform and ethyl acetate soluble fractions show highly toxicity against six bacteria and six funguses. The antimicrobial activity was carried out in the Laboratory of Life Science, Quaid-e-azam University, Islamabad, Pakistan. The activity was conformed from School of Science, Beijing University of Chemical Technology.

Preparation of fractions

The plants were cut into pieces and dried at room temperature. The dried plant materials were ground into powder. About 70 g of each plant powdered were dipped in (15LX3) methanol to get dark brown gummy material of 25 g. Extraction was performed sequentially using *n*-hexane (F_1 2g), chloroform (F_2 3g), ethyl acete (F_3 4g), *n*-butanol (F_4 4g), acetone(F_5 3g) and methanolic (F_6). These methods for the formation of extracts were used for both plants.

Antimicrobial activity

Antibacterial assay

The antibacterial activities were determined using agar well diffusion method (Boakye et al., 1977). Bacterial culture was grown in nutrient broth at 37°C for 18 to 24 h. 0.5 ml of broth culture of test organism was added by sterile pipette into molten agar (50 ml) which were then cooled to 40°C and poured into sterile Petri dish. Sterile cork borer were used to make well of 6 mm in diameter in nutrient agar plate. The wells were filled with given compounds of 100 µl and the plate was left for 1 to 2 h. The plates were incubated at 37°C for 18 to 24 h. Finally, the diameter of inhibition was measured.

Antifungal assay

The antifungal assay was carried out using agar well diffusion method (Hadacek et al., 2000). Sterile dimethyl sulfoxide (DMSO) was used to dissolve the test sample. Sabouraud dextrose agar (SDA) was prepared by mixing Sabouraud 3% glucose agar and agar-agar in distilled water. The required amount of fungal strain was suspended in 2 ml Sabouraud dextrose broth. This suspension was uniformly streaked on Petri plates containing SDA media by means of sterile cotton swab. Compounds were applied into well using same technique for bacteria. These plates were then checked for the presence of zone of inhibitor and result was noted.

Preparation of tested materials

The dilution used for all extracts was 100 mg ml^{-1}. Various extracts of CL were re-dissolved in different solvents whereby, hexane extracts were re-dissolved in hexane. Chloroform was known to be inhibitory to the growth of bacteria. Therefore, the chloroform extracts was re-dissolved in a mixture of petroleum ether and methanol (8:2 v/v).

RESULTS

Almost all the fractions of *C. arvense* and *C. jopanicum* showed activity against the bacteria and fungus. The F_2 &F_3 of both species (*C.arvense* and *C.jopanicum*) showed very good activity against *Enterobacter, Staphylococcus aureus* and *Micrococcus luteus*, while F_1, F_4 and F_5 shows moderate activity against six pathogens (Table 2).

The zone of inhibition of extracts *C. arvense* against six bacteria namely *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* (clinical strain/PIMS), *Enterobacter* (clinicalstrain/PIMS), *Staphylococcus aureus* (MRSA, clinical strain/PIMS) and *Micrococcus luteus* (clinicalstrain/PIMS) were used in antimicrobial assay. Microorganisms were incubated overnight at 37°C in Mueller-Hinton Broth (Oxoid) at pH 7.4. The result is indicated in Table 2 and Figure 2. The

Table 1. Zone of inhibition of reference antibiotics.

Antibiotic	Microorganism						
	Escherichia coli	***Klebsiella pneumoniae***	***Enterobacter***	***Trichophyton longifusus***	***Pseudomonas aeruginosa***	***Microsporum canis***	**Standard**
Ciprofloxacin	23 (±0.09)	18 (±0.06)	22 (±0.06)	21 (±0.09)	17 (±0.10)	18 (±0.11)	25 (±0.07)
Oflaxacine	21 (±0.8)	19 (±0.10)	20 (±0.05)	19 (±0.06)	16 (±0.12)	15 (±0.11)	24 (±0.18)

Table 2. Zone of inhibition of *C.arvense* plants extracts.

Bacteria	F_1	F_2	F_3	F_4	F_5
E. coli	19 (±0.03)	16 (±0.02)	14 (±0.2)	12 (±0.06)	09 (±0.05)
K. pneumoniae	17 (±0.06)	15 (±0.04)	11 (±0.02)	12 (±0.04)	10 (±0.07)
P.aeruginosa	18 (±0.03)	17 (±0.06)	15 (±0.04)	12 (±0.05)	10 (±0.09)
Enterobacter	20 (±0.11)	19 (±0.04)	16 (±0.03)	14 (±0.04)	11 (±0.11)
T. aureus	18 (±0.21)	17 (±0.14)	14 (±0.06)	18 (±0.20)	19 (±0.23)
M. luteus	20 (±0.21)	15 (±0.25)	17 (±0.20)	13 (±0.20)	15 (±0.21)

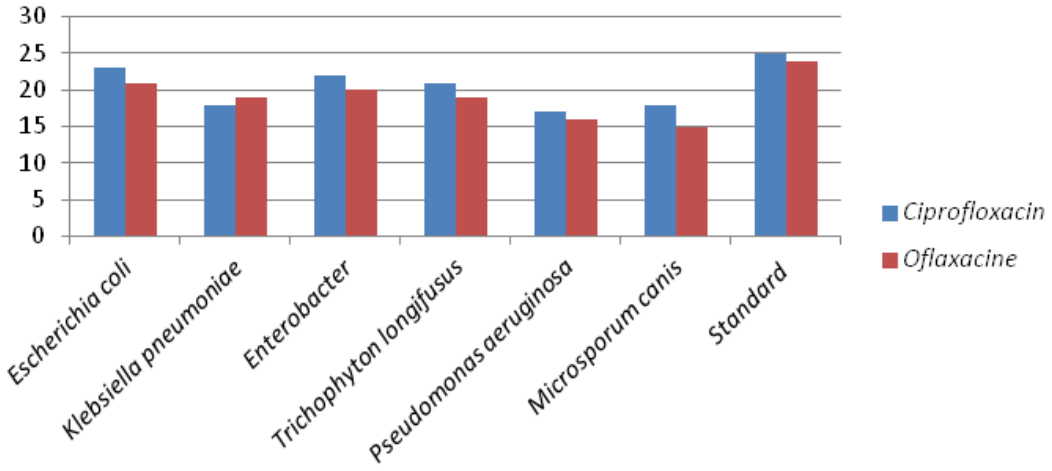

Figure 1. Zone of inhibition of reference antibiotics. Temperature, 37°C; values are inhibition zones (mm) and an average of triplicate. Concentrations used Are 1000 µg/ml.

extracts of these plants were also tested against six fungi, the result of these pathogens is showed in Table 3 and Figure 3. The result of standard drug against these extracts of the plants is summarized in Table 1, Figure 1, Table 4 and Figure 4. While the extracts of *C. japonicum* was also screened against these bacteria and fungus, as mentioned above and the result is indicated in Tables 5 to 7, Figures 5 to 7, respectively.

DISCUSSION

Mostly plants are used as medicine in different parts of the world. The present study compared the extracts of *Cirsium* species (*C. arvense* and *C. japonicum*). The whole plant extracts was gotten using different solvents to get different fractions (F_1-F_5). These fractions were used against six bacteria and six fungi. Two reference antibiotic (ciprofloxacin and ofloxicin) were used.

The chloroform and ethyl acetate (F_1 and F_2) of both plants showed good activity. Some researchers have worked on *C. arvense* and isolated some active constituents from chloroform and ethyl acetate fractions (Khan et al., 2011, 2013). The screening of bioactive agent from the plants is one of the most intensive areas of natural products research today. This is the first report that compared the extracts of the *Cirsium* species. There is little difference between the activities of the extracts of *Cirsium*

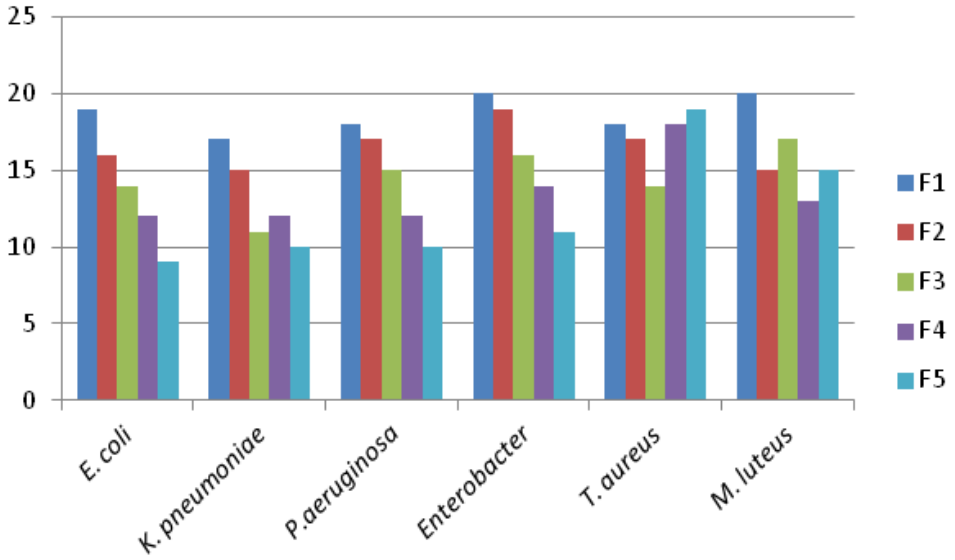

Figure 2. Zone of inhibition of *C. arvense* plants extracts.

Table 3. Zone of inhabitation of *C.arvense* extracts of plants.

Fungus	F_1	F_2	F_3	F_4	F_5
T. longifusus	18 (±0.20)	14 (±0.02)	11 (±0.25)	10 (±0.50)	10 (±0.08)
C. albicans	19 (±0.32)	17 (±0.07)	13 (±0.12)	15 (±0.21)	12 (±0.70)
A. flavus	17 (±0.25)	15 (±0.20)	16 (±0.24)	18 (±0.07)	14 (±0.90)
M. canis	19 (±0.21)	18 (±0.35)	19 (±0.23)	16 (±0.08)	16 (±0.20)
C. glaberata	20 (±0.17)	18 (±0.21)	12 (±0.60)	19 (±0.05)	15 (±0.25)
F. solani	19 (±0.06)	17 (±0.02)	13 (±0.40)	11 (±0.21)	17 (±0.18)

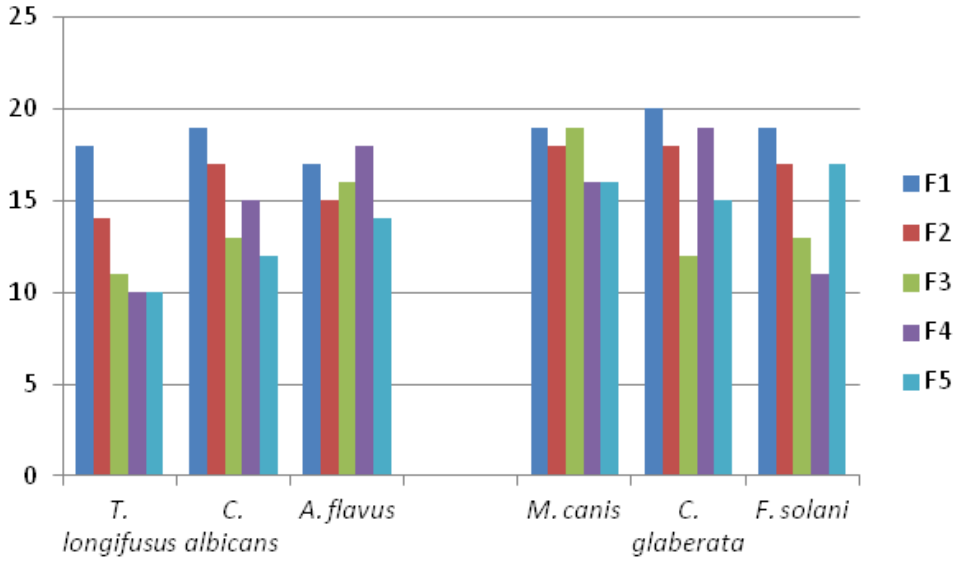

Figure 3. Zone of inhabitation of *C. arvense* extracts of plants.

Table 4. Zone of inhibition of *C. japonicum* plants extracts.

Bacteria	F_1	F_2	F_3	F_4	F_5
E. coli	18 (±0.20)	14 (±0.02)	11 (±0.25)	10 (±0.50)	10 (±0.08)
K. pneumonia	19 (±0.32)	17 (±0.07)	13 (±0.12)	15 (±0.21)	12 (±0.70)
P. aeruginosa	17 (±0.25)	15 (±0.20)	16 (±0.24)	18 (±0.07)	14 (±0.90)
Enterobacter	19 (±0.21)	18 (±0.35)	19 (±0.23)	16 (±0.08)	16 (±0.20)
T. aureus	20 (±0.17)	18 (±0.21)	12 (±0.60)	19 (±0.05)	15 (±0.25)
M. luteus	19 (±0.06)	17 (±0.02)	13 (±0.40)	11 (±0.21)	17 (±0.18)

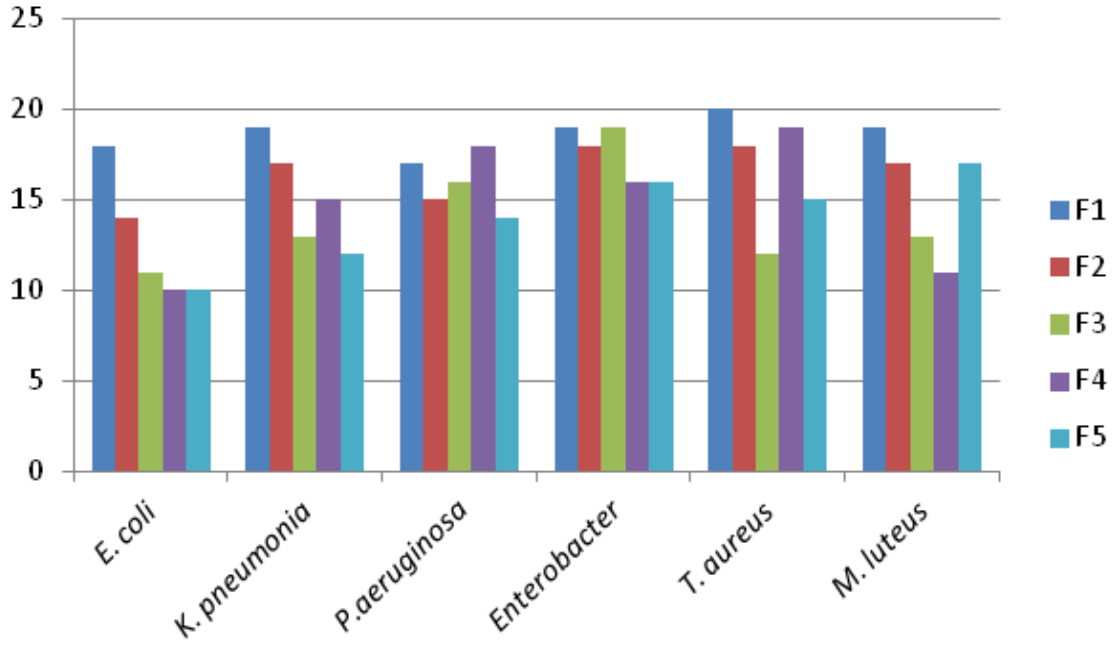

Figure 4. Zone of inhibition of *C. japonicum* plants extracts.

Table 5. Zone of inhibition of reference antibiotics.

Antibiotic	Microorganism						
	Trichophyton longifusus	***Candida albicans***	***Aspergillus flavus***	***Microsporum canis***	***Candida glaberata***	***Fusarium solani***	**Standard**
Ciprofloxacin	18 (±0.09)	16 (±0.80)	13 (±0.08)	16 (±0.10)	12 (±0.09)	12 (±0.32)	20 (±0.5)
Oflaxacine	17 (±0.8)	14 (±0.70)	16 (±0.09)	14 (±0.08)	19 (±02)	16 (±0.09)	22 (±0.19)

Table 6. Zone of inhibition *C. japonicum* plants extracts.

Bacteria	F_1	F_2	F_3	F_4	F_5
E. coli	17 (±0.34)	13 (±0.09)	11 (±0.20)	10 (±0.59)	10 (±0.10)
K. pneumonia	18 (±0.22)	16 (±0.03)	13 (±0.17)	15 (±0.25)	12 (±0.74)
P. aeruginosa	15 (±0.19)	13 (±0.22)	16 (±0.23)	18 (±0.98)	14 (±0.90)
Enterobacter	17 (±0.19)	19 (±0.33)	19 (±0.20)	16 (±0.28)	16 (±0.20)
T. aureus	16 (±0.21)	18 (±0.20)	12 (±0.43)	19 (±0.35)	15 (±0.25)
M. luteus	15 (±0.06)	16 (±0.05)	13 (±0.40)	11 (±0.51)	17 (±0.18)

Table 7. Zone of inhibition of *Cirsium jopanicum* extracts.

Fungus	F_1	F_2	F_3	F_4	F_5
T. longifusus	17(±0.18)	15(±0.10)	13(±0.20)	12(±0.14)	15(±0.10)
C. albicans	18(±0.20)	14(±0.20)	12(±0.10)	16(±0.14)	14(±0.60)
A. flavus	16(±0.20)	13(±0.22)	14(±0.09)	19(±0.18)	13(±0.90)
M. canis	17(±0.09)	16(±0.23)	17(±0.02)	16(±0.18)	12(±0.20)
C. glaberata	19(±0.12)	17(±0.25)	13(±0.08)	20(±0.09)	17(±0.25)
F. solani	17(±0.29)	14(±0.20)	10(±0.50)	13(±0.21)	18(±0.18)

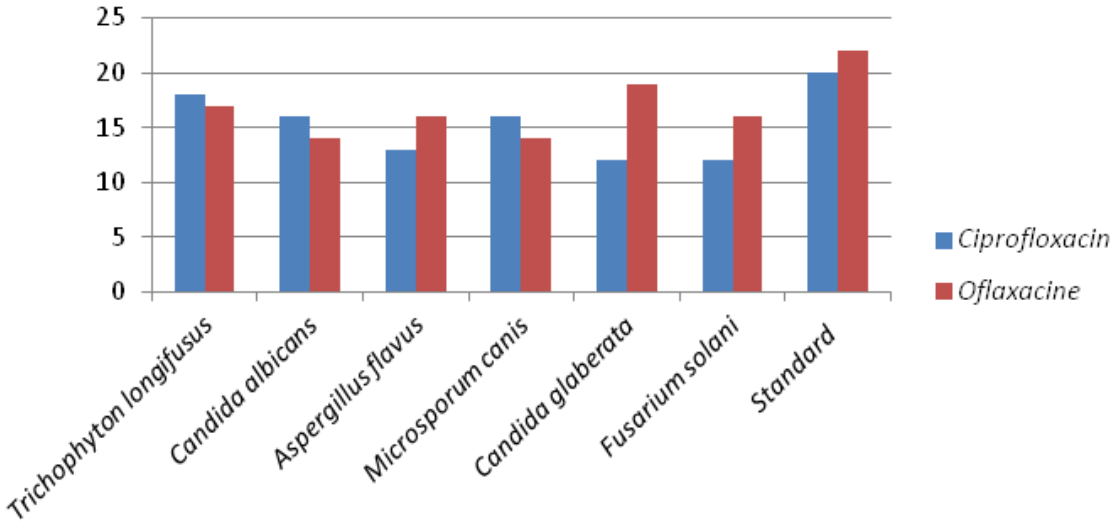

Figure 5. Zone of inhibition of reference antibiotics.

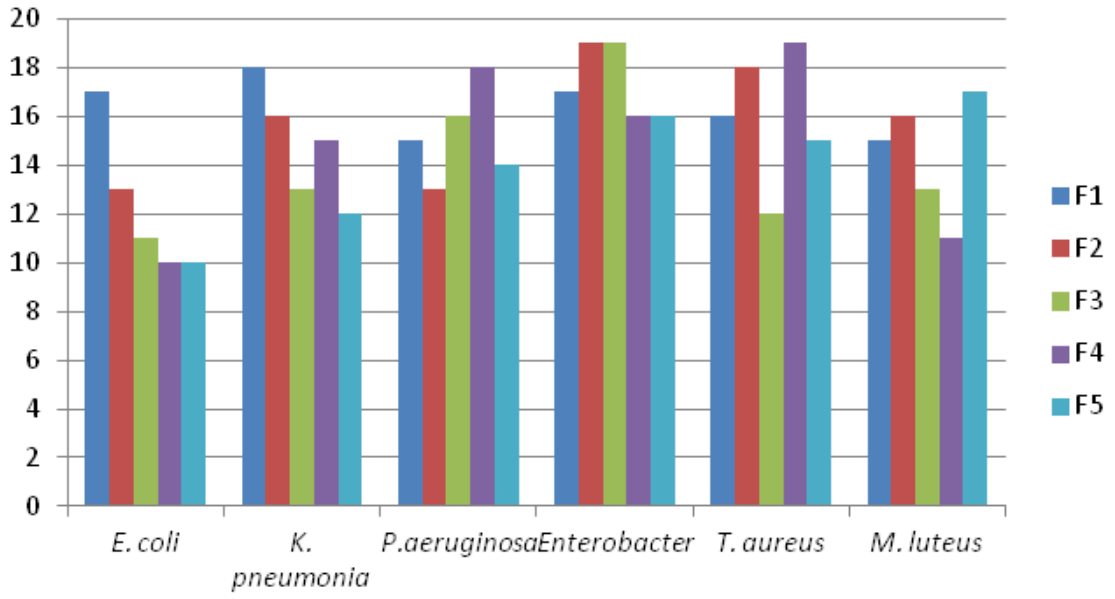

Figure 6. Zone of inhibition *C. japonicum* plants extracts

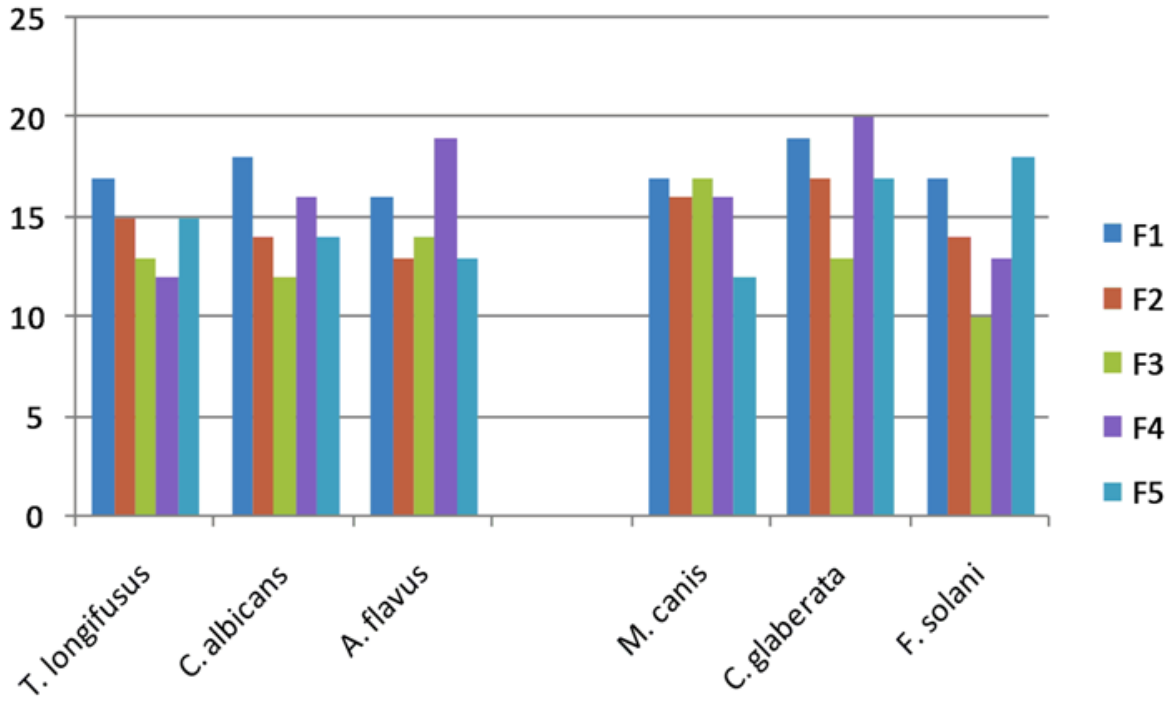

Figure 7. Zone of inhibition of *Cirsium jopanicum* plants extracts.

species. The F_3, F_4 and F_5 of both plants extracts showed moderate activity against six pathogens.

The plants extracts showed good antifungal activity. Like bacteria, F_2 and F_3 showed good antifungal activity. F_4 of both medicinal plants show very good antifungal activity. While fraction F_5 showed moderate activity. This was comparatively less than the reports of a recently published research (Maria et al., 2010).

This variation may be due to geographical location of plant. The present study shows that *Cirsium* species have potential antimicrobial activity and further study have been recommended to show the medical properties of these species.

Conclusion

The result shows that *Cirsium* species contain active ingredients against bacteria and fungus. Several antimicrobial activity constituents have been isolated from these plants; these plants are used as folk-medicine in different parts of the words. From this, it is clear that *Cirsium* species have potential antimicrobial activity against different pathogens. Several flavonoid and phenolic compounds have been isolated from these genuses. We highly recommend the family species for further research to exploit the hidden medicinal properties of these plants, to best of our knowledge; it will contain highly active alkaloids.

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Seasonal availability and palatability of native flora of Santh Saroola Kotli Sattian, Rawalpindi, Pakistan

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The purpose of this study was to document the palatable indigenous flora of Santh Saroola Kotli Sattian, Rawalpindi. A total of 169 plant species belonging to 126 genera and 56 families have been identified during 2009-2010. Of them, 106 species are noted as highly palatable with the percentage of 62.72%, followed by moderately palatable plants (37 species; 21.89%), whereas, only small proportion was found as are less palatable species (26 species; 15.3%). Poaceae family contributed good forage grasses (21 species, 12.42%), followed by Asteraceae (19 species; 11.24%), Fabaceae (15 species; 8.87%), Euphorbiaceae, Lamiaceae (7 species; 4.14% each). With reference to plant parts, leaves were fairly used as fodder/forage purpose (68 species; 41.97%), followed by whole plants (61 species; 37.65%) and aerial parts (33 species; 20.37%). During the month of April, most of the forage was available (110 species; 65.09%), followed by May and March (99 and 96 species, respectively). Maximum species (103 species, 44.98%) were found palatable to all domesticated animals such as goat, sheep, cows and donkey. Goat was found best suited to the climatic conditions which preferred 64 species (60.95%). Sheep was found attached with 34 species (37.87%), whereas, cows alone utilized 24 species (20.12%).

Key words: Kotli Sattian, **Santh Saroola**, palatable, Rawalpindi, animal preference, palatable.

INTRODUCTION

The total land area of Pakistan is 88 million hectare (ha) and about 65% of the area is marked as rangelands. The country is divided into five different ecological zones (Khan and Mohammad, 1987). These rangelands are providing major feed source to the domesticated animals as well as wildlife. Pakistan being an agricultural country has 154.7 million heads of livestock that contribute about 11.3% GDP (Anonymous, 2008a).

Different zones are endowed with peculiar vegetation and unique floral diversity for feeding livestock of the area in question. Therefore, there is need to identify and document this natural plant wealth which serve the livestock of local communities. Previously, few studies were carried out in Pakistan to report native fodder/forage species and their palatability. Wahid (1990) carried a survey and reported that sheep and goats diet comprised 53 to 81% shrubs from different rangelands of Balochistan. Hussain and Mustafa (1995) recorded 131 species of 42 families in pastures of Nasirabad Valley, Hunza, Pakistan during summer season. They reported that 27 species were found to be highly palatable, 68 species moderately palatable, 20 less palatable and 4 species rarely palatable. Seasonal pattern of forage production was evaluated by Omer et al. (2006) who

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reported that forage species was high during spring in dry temperate rangeland in Northern areas of Pakistan. Likewise, Hussain and Durrani (2009) studied the seasonal availability, palatability and animal preferences of forage plants from Harboi arid rangeland, Kalat, Pakistan. They documented 129 palatable species including 50.4% (65 species) highly palatable, 41.1% (53 species) mostly palatable, 4.65% (6 species) less palatable and 3.87% (5 species) rarely palatable species in the area. Few other studies include that of Hussain and Chughtai (1984), Khan (1996), Hussain and Durrani (2007, 2008).

The study area is recently included in the National Park (Murree-Kotli Sattian-Kahuta National Park) and no study is previously reported in documenting palatable plant species, therefore it was worthwhile to carry out such type of study that can be used in management and planning for fodder species. The purpose of this study was to document the palatable indigenous flora of Santh Saroola Kotli Sattian, Rawalpindi.

MATERIALS AND METHODS

Study area

Santh Saroolais located between 33°-04° and 34°- 01°north latitude and 72°-38° and 73°-37° east longitude. This is a hilly area and transitional zone in between subtropical to temperate resulting in unique floral biodiversity. The environment of the area is severe in winter and mild in summer. The area receives 990 mm annual rainfall. The temperature ranges were 117-25°F (Anonymous, 2008b). The livelihood of local community is dependent on livestock rearing; therefore there is a trend to increase livestock population. This rangeland is full of nutritious and palatable species of grasses, herbs, shrubs and trees. Keeping in view, it was felt worthwhile to document inventory of palatable species, their seasonal availability and animal preference from the study area.

Seasonal availability of forage species

The whole study area was surveyed from October, 2009 to May, 2010 to document data of forage species. During the period, plant growth of species such as grasses, herbs, shrubs and trees were identified. The biennial and perennial species and their seasonal availability were also noted.

Differential palatability of plant parts and animal preference

The degree of palatability for each plant species was noted in the field and the local people and shepherd involved in livestock keeping were asked. The palatable species were further categorized by animal preference (goats, sheep, cow and camel) and parts grazed (whole plant, leaves, aerial parts, etc.). Based on frequency use, the documented plants were grouped as: 1). Highly palatable (HP), species highly preferred by the most grazing animals; 2) moderately palatable (MP), species with an average likeness by the livestock; 3) Less palatable (LP), species with less preference. Likewise, plants were classified by animal preferences, parts used and seasonal availability.

Specimen collection and identification

Plant specimens were collected, pressed, dried and identified with the help of various floras (Nasir and Ali, 1970-1989; Ali and Nasir 1990-1991; Ali and Qaiser, 1993-2009).

RESULTS AND DISCUSSION

During the survey, a total of 169 plant species belonging to 126 genera and 56 families were identified as forage source in the study area (Table 1). The palatability of all species is summarized in Figure 1 which reveals that highest number of species were found highly palatable (106 species; 62.72%), followed by moderately palatable plants (37 species; 21.89%), whereas, only small proportion was found as less palatable species (26 species; 15.3%).

The least palatable species include *Ajuga bracteosa, Adaintum capillus-veneris, Berberis lyceum, Calotropis procera*, *Carissa opaca, Coniogramme rosthornii, Rubus fruticosus, Tagetes minuta* and *Verbascum thapsus*. These species have less palatability and mostly avoided by the livestock, resultantly dominating large area. Overgrazing has reduced the populations of palatable and desired species, ultimately resulting in the replacement with non-preferred species. Many studies concluded that over grazing reduces pala-table cover and species diversity (Khan, 1996; Liu et al., 1996; Hickman et al., 1996; Makulbekova, 1996; Hussain and Chughtai, 1984; Hussain and Durrani, 2007, 2008).

Poaceae family (Table 2) contributed good forage grasses (21 species; 12.42%), followed by Asteraceae (19 species; 11.24%), Fabaceae (15 species; 8.87%), Euphorbiaceae, Lamiaceae (7 species; 4.14% each). Three plant parts such as whole plant, aerial parts and leaves were selected by the individual animals for grazing/browsing. Different plant parts were preferred by individual animals and shown in Figure 2. Out of these, leaves were fairly used as fodder/forage purpose (68 species; 41.97%), followed by whole plants (61 species; 37.65%) and aerial parts (33 species; 20.37%).

The study area is located in humid climate and forage species were found available in different months. Month-wise data of forage species is provided in Figure 3. Maximum species were available during the month of April (110 species; 65.09%), followed by May (99 species; 58.58%), June (76 species; 44.97%), August (74 species; 43.79%), September (72 species; 42.60%), whereas, December and January months were noted as drier in terms of providing forage to the cattle. During these months, people utilized stored forage for feeding their livestock. In this season, most of the livestock were forced to graze/browse less palatable as well as dried plants. Our results are in agreement with that of Hussain and Durrani (2009) who reported decreased productivity of rangelands during winter in the Harboi rangeland, Kalat (Pakistan).

Table 1. Inventory of native flora along with local names, family, part used, palatability, availability and animal preference.

Plant species	Local name	Growth form	Family	Parts used			Palatability			Availability			Animal preference
				WP	AP	Lv	LP	MP	HP	C	VC	R	
Abutilon bidentatum Hochst. ex Rich.	Kanghi Buti	Sh	Malvaceae	X	√	X	X	√	X	X	X	√	Goat
Arabis himalaica (Edgew.) O.E. Schulz		H	Brassicaceae	X	√	X	X	X	√	X	X	√	Goat, cow
Acacia modesta Wall.	Phulai	T	Mimosaceae	X	X	√	X	X	√	√	X	X	Goat, sheep
Achyranthus aspera L.	Put Kanda/Kanda	Sh	Amaranthaceae	√	X	X	X	X	√	X	√	X	Goat, sheep, cow
Adiantum capillus-veneris L.	Persiaon shan	H	Adiantaceae	√	X	X	X	X	√	X	√	X	Goat
Ailanthus altissima (Mill.) Swingle	Durawia	T	Simarubaceae	X	X	X	√	X	X	√	X	X	Goat
Ajuga bracteosa Wall. ex Bth.	Guchi	H	Lamiaceae	√	X	X	√	X	X	√	X	X	Goat, sheep
Ajuga parviflora Bth.	Kauri Buti	H	Lamiaceae	√	X	X	√	X	X	√	X	X	Goat, sheep
Albizzia lebbeck (L.) Benth.	Shirin	T	Mimosaceae	X	X	√	√	X	X	X	X	√	All
Alternanthera pungens Kunth.	Lundri	H	Amaranthaceae	X	X	√	X	√	X	X	X	√	Goat, cow, donkey
Amaranthus hybridus L.	Choleri	H	Amaranthaceae	√	X	X	X	X	√	√	X	X	Goat, sheep, cow
Amaranthus spinosus L.	Khardar Cholai	H	Amaranthaceae	√	X	X	X	X	√	√	X	X	All
Amaranthus viridis L.	Cholai	H	Amaranthaceae	√	X	X	X	X	√	X	√	X	All
Anagalis arvensis L.	Billi buti	H	Primulaceae	√	X	X	X	X	√	√	X	X	All
Argyrolobium helleborifolium Sonott.		H	Fabaceae	√	X	X	X	X	√	X	X	√	All
Argyrolobium roseum (Camb.) Jaub & Spach		H	Fabaceae	X	√	X	X	X	√	X	X	√	All
Aristida cyanatha Nees ex Steud.		G	Poaceae	X	√	X	X	√	X	√	X	X	All
Arundo donax L.	Narra, Sukna, Kana	G	Poaceae	X	X	√	X	√	X	X	X	√	Goat, sheep, donkey
Asphodelus tenuifolius Cavan.	Bhagat/Piazi	H	Liliaceae	X	X	√	X	X	√	√	X	X	All
Astragalus squarrosus Bunge	Kikri	Sh	Fabaceae	X	X	√	X	√	X	X	X	√	Goat, sheep
Avena fatua L.	Jangli Jai	G	Poaceae	X	X	√	X	X	√	√	X	X	All
Barleria acanthoides Vahl		H	Acanthaceae	X	√	X	X	√	X	X	X	√	All
Barleria cristata L.	Bansa Siah	H	Acanthaceae	X	X	√	X	√	X	X	X	√	All
Berberis lyceum Royle.	Sumbulu	Sh	Berberidaceae	X	X	√	√	X	X	X	√	X	Goat
Boerhavia procumbens Banks ex Roxb.	Itsit	H	Nyctaginaceae	√	X	X	X	X	√	X	√	X	All
Calotropis procera (Willd.) R. Br.	Aak, Madar	Sh	Asclepiadaceae	X	X	√	√	X	X	X	X	√	Goat
Cannabis sativa L.	Bhang	H	Cannabinaceae	X	√	X	√	X	X	X	√	X	Goat
Capsella bursa-pastoris (L.) Medik	Shepherd's Purse	H	Brassicaceae	√	X	X	X	X	√	X	√	X	All
Cardiospermum halicacabum L.	Kan Phuti	C	Sapindaceae	X	√	X	X	√	X	X	X	√	Goat
Carissa opaca Stapf ex. Haines	Granda	Sh	Apocynaceae	X	X	√	√	X	X	√	X	X	Goat
Carthamus oxycantha M. Bieb	Pholi	H	Asteraceae	X	√	X	X	√	X	X	X	√	All
Cenchrus ciliaris L.	Barshok	G	Poaceae	X	√	X	X	X	√	√	X	X	All
Cenchrus pennisetiformis Hochst. & Steud.		G	Poaceae	√	X	X	X	X	√	√	X	X	All
Cenchrus setigerus Vahl		G	Poaceae	X	√	√	X	X	√	√	X	X	All
Chenopodium album L	Bathu	H	Chenopodiaceae	X	X	X	X	X	√	√	X	X	All

Table 1. Contd.

Chenopodium ambrosioides L.	Chandan Bathu	H	Chenopodiaceae	X	X	√	X	X	√	√			Goat
Chrysopogon aucheri (Boiss.) Stapf		G	Poaceae	X	X	√	√	X	X	X	X	√	All
Cirsium arvense (L.) Scope.	Leh	H	Asteraceae	√	X	X	X	X	√	√	X	X	All
Clematis montana Buch.		C	Rananculaceae	X	X	√	√	X	X	X	X	√	Cow, goat, sheep
Clematis napaulensis Royle		C	Rananculaceae	X	√	X	√	X	X	X	X	√	Cow, goat, sheep
Colchicum aitchisonii (Hook. f.) E. Nasir	Suranjan	H	Liliaceae	X	X	√	X	X	√	√	X	X	Goat
Colebrookia oppositifolia Sm.	Shakar Dana	Sh	Lamiaceae	X	X	√	√	X	X	√	X	X	Goat
Coniogramme rosthornii Hieron.	Fern	H	Coniogrammaceae	√	X	X	√	X	X	√	X	X	Goat
Convolvulus arvensis L.	Lehli	C	Convolvulaceae	√	X	X	X	X	√	√	X	X	All
Conyza aegyptica Ait.	Gider buti	H	Asteraceae	X	X	√	X	√	X	√	X	X	Goat, sheep, cow
Conyza bonariensis L.	Gider buti	H	Asteraceae	X	X	√	X	√	X	√	X	X	Goat, sheep, cow
Conyza canadensis L.	Gider buti	H	Asteraceae	X	X	√	X	√	X	√	X	X	Goat, sheep, cow
Coronopus didymus (L.) Sm.	Jangli Haloon	H	Brassicaceae	√	X	X	X	√	X	√	X	X	Goat, sheep, cow
Crotolaria medicagnea Lam.		H	Fabaceae	X	√	X	X	X	√	√	X	X	All
Cuscuta reflexa Roxb. s	Akash Bail/ Baleri	P	Cuscutaceae	√	X	X	X	X	√	√	X	X	Goat, sheep, cow
Cyperus rotundus L.	Dela	Se	Cyperaceae	X	√	X	X	X	√	√	X	X	All
Dactyloctenium aegyptium L.	Gandeel	G	Poaceae	X	X	√	X	X	√	X	X	√	All
Debregeasia salicifolia (D. Don) Rendle	0	Sh	Rhamnaceae	X	√	X	√	X	X	√	X	X	Cow, goat
Desmostachya bipinnata (L.) *S*tapf	Dab Ghaa	G	Poaceae	X	X	√	X	√	X	√	X	X	All
Dicanthium annulatum (Forssk.) Stapf	Murgha Ghaas	G	Poaceae	X	X	√	X	X	√	√	X	X	All
Dicliptera roxburghiana Nees	Somni	H	Acanthaceae	√	X	X	X	X	√	X	√	X	All
Dioscorea deltoides Wall. ex Kunth	0	H	Dioscoreaceae	X	X	√	X	X	√	√	X	X	Goat
Diospyros lotus L.	Amlok	T	Ebenaceae	X	X	√	X	X	√	√	X	X	Goat
Echinochloa crus-galli (L.) P. Beauv.	Bara sawank	G	Poaceae	√	X	X	X	X	√	√	X	X	All
Echinops echinatus Roxb.	Kandiara	H	Asteraceae	X	√	X	X	√	X	√	X	X	All
Eclipta prostrate (L.) L.	Bhangra	H	Asteraceae	X	X	X	X	X	√	X	X	√	All
Eragrostis ateroviens (Desf.) Trin. ex Nees		G	Poaceae	X	X	√	X	X	√	√	X	X	All
Eragrostis minor Host.	Kusum	G	Poaceae	X	√	X	X	X	√	X	√	X	All
Eruca sativa L.	Tara Meera	H	Brassicaceae	X	X	√	X	√	X	X	X	√	All
Euphorbia clarkeana Hkf.	Dudhi	H	Euphorbiaceae	X	√	X	X	X	√	√	X	X	All
Euphorbia granulates Forssk.	Sheer Bar	H	Euphorbiaceae	X	√	X	X	X	√	√	X	X	All
Euphorbia helioscopia Mewski.	Chattri dodak	H	Euphorbiaceae	X	X	√	X	X	√	X	√	X	All
Euphorbia hirta L.	Dudhi	H	Euphorbiaceae	X	X	√	X	X	√	√	X	X	All
Euphorbia indica (Lam.)	Dudhi Kalan	H	Euphorbiaceae	X	X	√	X	X	√	√	X	X	All
*Euphorbia prostrata (*L.) Ait	Dudhi	H	Euphorbiaceae	√	X	X	X	X	√	√	X	X	All
Euphrasia himalayica Wettst.		H	Scrophulariaceae	X	√	X	X	√	X	X	√	X	All
Ficus carica L.	Anjeer/Phuwari/Phagwari	T	Moraceae	X	X	√	X	√	X	X	√	X	Goat

Table 1. Contd.

Ficus palmate Forssk.	Phagwara	T	Moraceae	X	X	√	X	√	X	X	X	√	Goat
Ficus roxburghii Wall. ex Brand.	Dusi	T	Moraceae	X	X	√	X	√	X	X	X	√	Goat
Flacourtia indica (Burm. f.) Merrill	Kakoh	T	Flacourtiaceae	X	X	√	X	X	√	√	X	X	Goat
Foeniculum vulgare Miller	Soonuf	H	Apiaceae	√	X	X	X	X	√	√	X	X	All
Fumaria indica (Hausskn.) H.N. Pugsley	Shahtrah	H	Fumariaceae	√	X	X	X	X	√	X	√	X	All
Gallium aparine L.		H	Rubiaceae	X	√	X	X	√	X	√	X	X	All
Geranium rotundifolium L.		H	Geraniaceae	X	√	X	X	X	√	√	X	X	Cow, Goat, Sheep
Grewia optiva Drum. ex Burret.	Taman	T	Tiliaceae	X	√	X	X	X	√	X	√	X	All
Heliotropium crispum Stocks		H	Boraginaceae	X	X	√	√	X	X	X	X	√	Goat, Sheep
Imperata cylindrical (L) Raeuschel	Dab Ghaa	G	Poaceae	√	X	X	X	√	X	√	X	X	All
Indigofera himalayensis Ali		B	Fabaceae	X	X	√	X	X	√	√	X	X	All
Indigofera linifolia (L. f.) Retz.	Torki	H	Fabaceae	√	X	X	X	X	√	√	X	X	All
Indigofera sessiliflora DC.		H	Fabaceae	√	X	X	X	X	√	√	X	X	All
Ipomoea hederacea (L.) Jacq.		C	Convolvulaceae	√	X	X	X	X	√	√	X	X	All
Ipomoea nil (L.) Roth		C	Convolvulaceae	√	X	X	X	X	√	√	X	X	All
Kickxia ramosissima (Wall.) Janchen		H	Scrophulariaceae	X	X	√	X	X	√	X	X	√	Goat, Sheep
Lactuca auriculata (Wall. ex Dc.)		H	Asteraceae	X	X	X	X	X	√	√	X	X	All
Lactuca dissecta D. Don.		H	Asteraceae	X	X	√	X	X	√	√	X	X	All
Lactuca serriola L.		H	Asteraceae	√	X	X	X	X	√	√	X	X	All
Lathyrus aphaca L.	Jangali matar	H	Fabaceae	√	X	X	X	X	√	√	X	X	All
Launaea procumbens (Roxb.) Ram. & Rajgo.	Dodak	H	Asteraceae	√	X	X	X	X	√	√	X	X	All
Lepidium sativum L.	Haleon	H	Brassicaceae	√	X	X	X	X	√	√	X	X	All
Lotus corniculatus (Wald. & Kit. ex Willd.) Briq. & Rech. F.		H	Fabaceae	X	√	X	X	X	√	X	√	X	All
Mallotus philipensis (Lam.) Muell.	Kamela	T	Euphorbiaceae	X	X	√	X	√	X	X	√	X	Goat, Sheep, Cow
Malva neglecta Waller.	Sonchal	H	Malvaceae	X	X	√	X	X	√	√	X	X	All
Malvastrum coromendelianum L.	Yard Sonchal	H	Malvaceae	√	X	X	X	√	X	√	X	X	All
Maytenus royleanus (Wall. ex Lawson) Cufodontis	Patakhi	Sh	Celastraceae	√	X	X	X	X	√	√	X	X	Goat, Sheep
Medicago denticulate Willd.	Maina	H	Fabaceae	√	X	X	X	X	√	X	√	X	All
Medicago laciniata (L.) Mill.	Maina	H	Fabaceae	√	X	X	X	X	√	X	√	X	All
Medicago polymorpha L.	Maina	H	Fabaceae	√	X	X	X	X	√	X	√	X	All
Melilotus indica Lour.		H	Fabaceae	X	X	X	X	X	X	√	X	X	All
Mentha longifolia (L.) Huds.	Sufaid Poodina	H	Lamiaceae	X	X	√	X	X	√	√	X	X	Goat
Micromeria biflora (Ham.) Bth.		H	Lamiaceae	X	√	X	X	√	X	X	X	√	All
Morus alba L.	Shehtoot	T	Moraceae	X	X	√	X	X	√	√	X	X	All
Morus nigra L.	Tut	T	Moraceae	X	X	√	X	X	√	√	X	X	All
Myrsine africana L.	Khokhal/Khokhan	Sh	Myrsinaceae	√	X	X	X	X	√	√	X	X	Goat

Table 1. Contd.

Olea europaea L.	Koh	T	Oleaceae	X	X	√	X	√	X	√	X	X	All
Olea ferruginea Royle	Kahu	T	Oleaceae	X	X	√	X	√	X	√	X	X	All
Origanum vaulgare L.		H	Lamiaceae	X	X	√	√	X	X	√	X	X	Goat
Otostegia limbata (Benth.) Boiss.	Chitti Bui	Sh	Lamiaceae	√	X	X	X	√	X	X	X	√	Goat, Sheep
Oxalis corniculata L.	Khati Buti	H	Oxalidaceae	X	X	√	X	X	√	X	√	X	Goat, Sheep
Parthenium hytserophorus L.		H	Asteraceae	X	X	X	√	X	X	√	X	X	Goat, Sheep
Phalaris minor Retz.	Dumbi sitti	G	Poaceae	X	X	√	X	X	√	√	X	X	All
Physalis minima L.	Wild cherry	H	Solanaceae	√	X	X	X	X	√	√	X	X	All
Plantago lanceolata L.	Ispaghol	H	Plantginaceae	√	X	X	X	X	√	√	X	X	All
Plantago major L.	Ispaghol	H	Plantginaceae	√	X	X	X	X	√	√	X	X	All
Plantago ovate Frossk.	Ispaghol	H	Plantginaceae	√	X	X	X	X	√	√	X	X	All
Polygonum barbatum L.		H	Polygonaceae	X	√	X	X	√	X	X	X	√	Goat, Sheep, Cow
Polygonum plebejum R. Br.		H	Polygonaceae	√	X	X	X	X	√	X	√	X	All
Polypogon fugax Nees ex Steud.		G	Polygonaceae	√	X	X	X	X	√	√	X	X	All
Populus deltoides Bartram ex Marsh.	Sufaid poplar	T	Salicaceae	X	X	√	√	X	√	√	X	X	Goat, Sheep, Cow
Pteridium aquilinum (L.) Kuhn		H	Pteridaceae	√	X	X	X	X	√	X	X	√	Goat, Sheep, Cow
Punica granatum L.	Druna/Druni	T	Punicaceae	X	X	√	X	X	√	X	√	X	Goat, Sheep, Cow
Quercus dilatata Lindl.	Barungi	T	Fagaceae	X	X	√	√	X	X	X	X	√	All
Quercus incana Roxb.	Rein, Shah0e0baloot	T	Fagaceae	X	X	√	√	X	X	X	X	√	All
Rananculus sceleratus L.	Jal Dhania	H	Rananculaceae	X	√	X	√	X	X	√	X	X	All
Ranunculus arvensis L.		H	Rananculaceae	X	√	X	√	X	X	√	X	X	All
Rhynchosia minima (L.) DC.		H	Fabaceae	√	X	X	X	X	√	√	X	X	All
Rosa brunonii Lindll.	Jangli gulab	Sh	Rosaceae	X	√	X	X	√	X	X	X	√	Goat
Rubia cordifolia L.	Surkh Majeth	C	Rubiaceae	X	√	X	X	√	X	X	√	X	Goat
Rubus ellipticus Smith	Aakhra	C	Rubiaceae	√	X	X	X	√	X	√	X	X	Goat
Rumex dentatus L.	Jangli palak	H	Polygonaceae	X	X	√	X	X	√	X	√	X	All
Rumex hastatus D. Don	Khatimber/Chuki	H	Polygonaceae	X	X	√	X	X	√	X	√	X	Goat
Rumex nepalensis Spreng		H	Polygonaceae	X	X	√	X	√	X	√	X	X	Goat
Saccharum bengalense Retz.	Kana	G	Poaceae	X	X	√	X	√	X	X	X	√	Cow, Donkey
Saccharum spontaneum L.	Kanna	G	Poaceae	X	X	√	X	√	X	√	X	X	Cow, Donkey
Saussurea albescens (DC.) Schr. Bip.		H	Asteraceae	X	√	X	X	X	√	√	X	X	All
Saussurea atkinsonii (Clarke)		H	Asteraceae	X	√	X	X	X	√	√	X	X	All
Saussurea heteromalla DC.		H	Asteraceae	√	X	X	X	X	√	√	X	X	All
Setaria glauca (L.) P. Beauv	Ban0Kangni	G	Poaceae	X	X	√	X	X	√	X	X	√	All
Sida cordata (Burm. f.) Borss.0Waalkes		H	Poaceae	X	X	√	X	X	√	X	X	√	Goat, Sheep, Cow
Silene conoidae L.		H	Malvaceae	X	√	X	X	X	√	√	X	X	All

Table 1. Contd.

Silybum marianum (L.) Gaertin	Kandiari	H	Caryophyllaceae	X	X	√	√	X	X	X	X	√	Goat, Sheep, Cow
Sisymbrium irio L.	Khub Kalan	H	Asteraceae	X	X	X	X	X	√	X	√	X	All
Solanum nigrum L.	Peelan/Kach mach	H	Brassicaceae	√	X	X	X	X	√	√	X	X	All
Solanum surattense Burm.f.	Kandiali	H	Solanaceae	√	X	X	X	X	√	√	X	X	Goat, Sheep
Solanum villosum (L.) Moench	Peelan/Kach mach	H	Solanaceae	√	X	X	X	X	√	X	√	X	All
Sonchus asper (L.) Hill.	Dodak Machal	H	Solanaceae	X	X	X	X	X	√	√	X	X	All
Sorghum bicolor (L.) Moench.	Jawar/Chari	G	Asteraceae	X	X	√	X	X	√	√	X	X	All
Sorghum halepense (L.) Bern.	Baru	G	Poaceae	X	X	√	√	X	X	X	X	√	Goat
Stellaria media (L.) Cyr.	Chickweed	H	Caryophyllaceae	√	X	X	X	X	√	X	√	X	All
Taraxacum officinale Weber.	Dodak	H	Asteraceae	X	X	√	X	X	√	X	√	X	All
Taraxcum wallichii DC.		H	Asteraceae	√	X	X	X	X	√	√	X	X	All
Themeda anathera (Nees) Hack	Loonder, Lunji	G	Poaceae	√	X	X	X	X	√	X	√	X	All
Trianthema portulacastrum L.	It Sit	H	Aizoaceae	√	X	X	X	√	X	X	X	√	All
Tribulus terrestris L.	Bhakhra	H	Zygophyllaceae	√	X	X	X	X	√	√	X	X	All
Trichodesma indicum (L.) R. Br.	Gao Zeban	H	Boraginaceae	X	X	√	√	X	X	X	X	√	Goat, Sheep
Valeriana wallichii DC.		H	Valerianaceae	X	√	X	X	X	√	X	√	X	Goat, Sheep, Cow
Verbascum thapsus L.	Pahari Tambaku	H	Scrophulariaceae	√	X	X	X	X	√	√	X	X	Goat, Sheep, Cow
Verbena officinalis L.		H	Verbenaceae	√	X	X	X	X	√	X	√	X	All
Vicia faba L.	Rewari	H	Fabaceae	√	X	X	X	X	√	√	X	X	All
Viola cancescens Wall. ex Roxb.	Banafsha	H	Violaceae	√	X	X	X	X	√	√	X	X	All
Withania somnifera (L.) Dunal.	Asghand/Aksan	Sh	Simarubaceae	√	X	X	X	X	√	√	X	X	Goat
Woodfordia fruticosa (L.) S. Kurz	Tavi	Sh	Vitaceae	X	X	√	X	X	√	X	√	X	All
Zanthoxylum alatum Roxb	Timbar/Timar	Sh	Rutaceae	X	X	√	X	√	X	X	√	X	Goat, Sheep
Zizyphus mauritiana Mill.	Beri	T	Rhamnaceae	X	X	√	X	X	√	√	X	X	All
Zizyphus oxyphylla Edgew.	Ber maloki	Sh	Rhamnaceae	X	X	√	√	X	X	X	X	√	All
		Sh		61	33	68	26	37	106	97	34	38	

Whole plant (WP), area parts (AP), leaves (Lv), highly palatable (HP), moderately palatable (MP), less palatable (LP), common (C), very common; (VC), rare (R), bush (B), climber (C), grass (G), herb (H), paeasite (P), sadge (Se), shruberb (Sh), tree (T). √ (present), X (absent).

In the area, four domesticated animals viz., goat, sheep, cows and donkey were recorded and animal preference for fodder species is given in Figure 4. Maximum species (103 species, 44.98%) were found palatable for all the animals. Besides, goat was found suited to the climatic conditions as browser which preferred 64 species (60.95%) as selective ones. Sheep was found attached to 34 species (37.87%), whereas, cows alone utilized 24 species (20.12%).

With reference to growth form of the native flora, 8 life spans are determined (Figure 5). Herbs were dominating in the area and very frequently used as fodder forage (101 species; 59.41%), followed by grasses (22 species; 12.94%), trees (19 species; 11.18%), shrubs (17 species; 10.00%) and climbers (8 species; 4.71%), whereas rest of the forms were found nominal.

Conclusion

The present work reported seasonal availability of fodder/forage species, differential palatability by

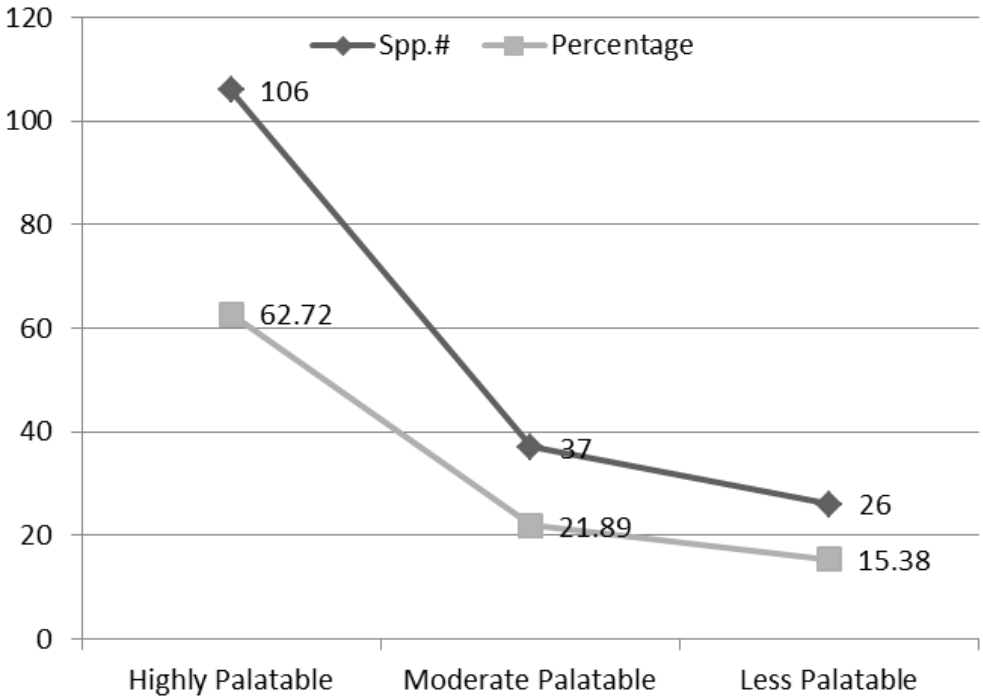

Figure 1. Palatability of native plant species.

Table 2. Contribution of different families in forage flora of Santh Saroola.

Family	Species no.	Percentage
Poaceae	21	12.43
Asteraceae	19	11.24
Fabaceae	15	8.88
Euphorbiaceae	7	4.14
Lamiaceae	7	4.14
Brassicaceae	6	3.55
Polygonaceae	6	3.55
Amaranthaceae	5	2.96
Moraceae	5	2.96
Malvaceae	4	2.37
Rananculaceae	4	2.37
Rutaceae	4	2.37
Solanaceae	4	2.37
Acanthaceae	3	1.78
Convolvulaceae	3	1.78
Plantginaceae	3	1.78
Rhamnaceae	3	1.78
Scrophulariaceae	3	1.78
Boraginaceae	2	1.18
Caryophyllaceae	2	1.18
Chenopodiaceae	2	1.18
Fagaceae	2	1.18
Lamiaceae	2	1.18
Mimosaceae	2	1.18

Table 2. Contd.

Oleaceae	2	1.18
Simarubaceae	2	1.18
Verbenaceae	2	1.18
Adiantaceae	1	0.59
Aizoaceae	1	0.59
Apiaceae	1	0.59
Apocynaceae	1	0.59
Asclepiadaceae	1	0.59
Berberidaceae	1	0.59
Cannabinaceae	1	0.59
Celastraceae	1	0.59
Coniogrammaceae	1	0.59
Cuscutaceae	1	0.59
Cyperaceae	1	0.59
Dioscoreaceae	1	0.59
Ebenaceae	1	0.59
Flacourtiaceae	1	0.59
Fumariaceae	1	0.59
Geraniaceae	1	0.59
Myrsinaceae	1	0.59
Nyctaginaceae	1	0.59
Oxalidaceae	1	0.59
Primulaceae	1	0.59
Pteridaceae	1	0.59
Punicaceae	1	0.59
Rosaceae	1	0.59
Salicaceae	1	0.59
Sapindaceae	1	0.59
Tiliaceae	1	0.59
Violaceae	1	0.59
Vitaceae	1	0.59
Zygophyllaceae	1	0.59

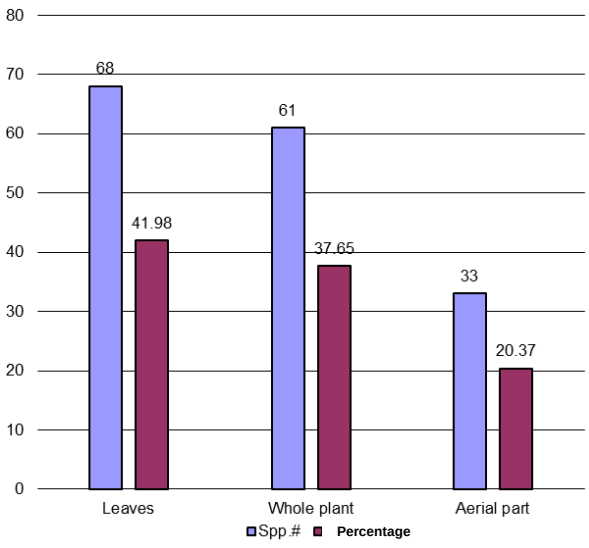

Figure 2. Parts used as forage in Santh Saroola Kotli Sattian, Rawalpindi.

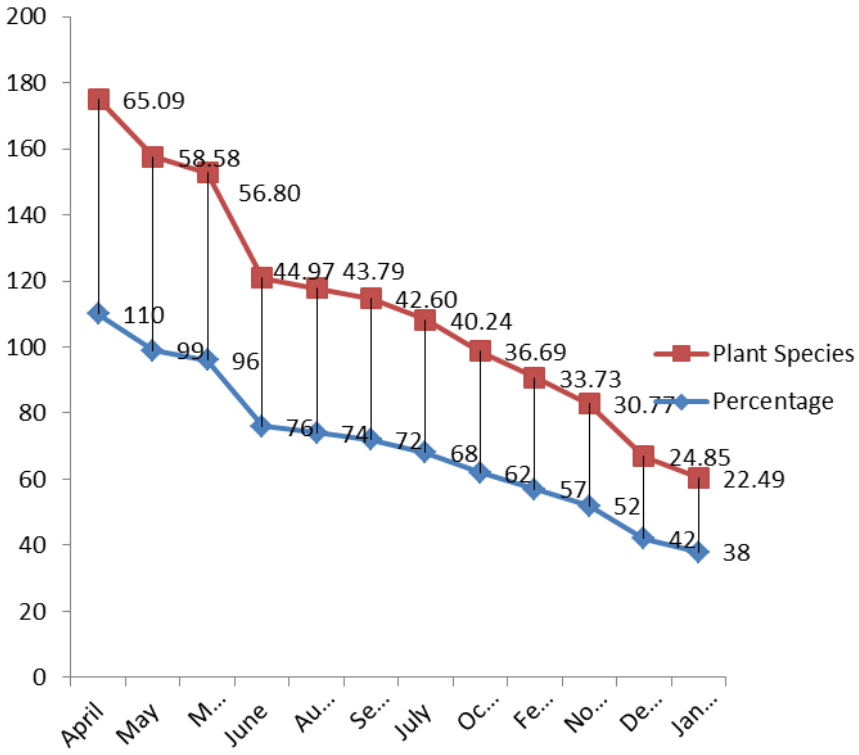

Figure 3. Availability of fodder species through out the year.

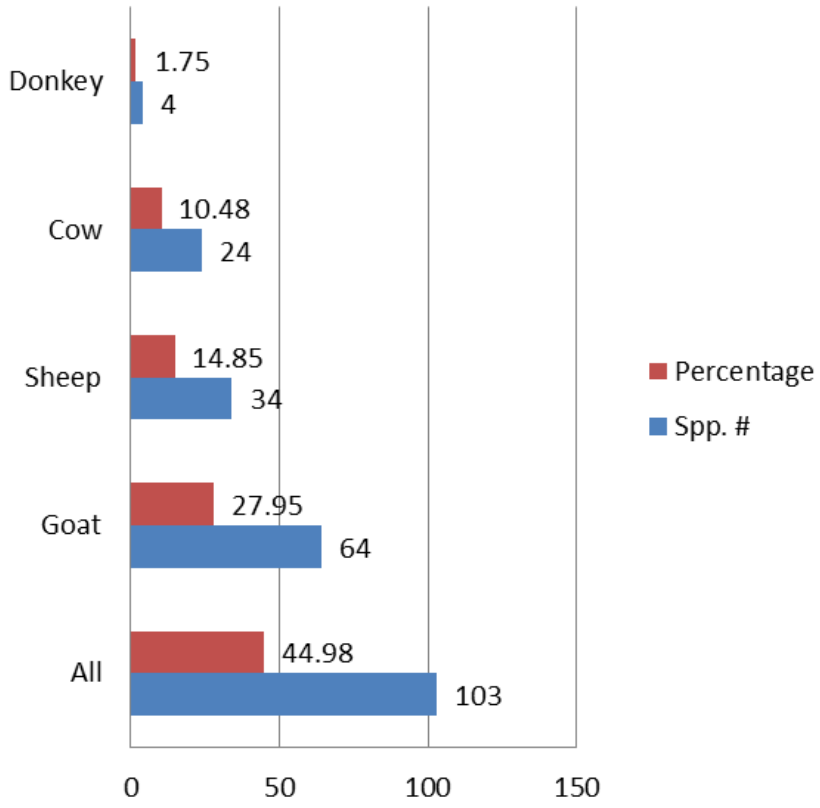

Figure 4. Number of plant species preferred by the domesticated animals.

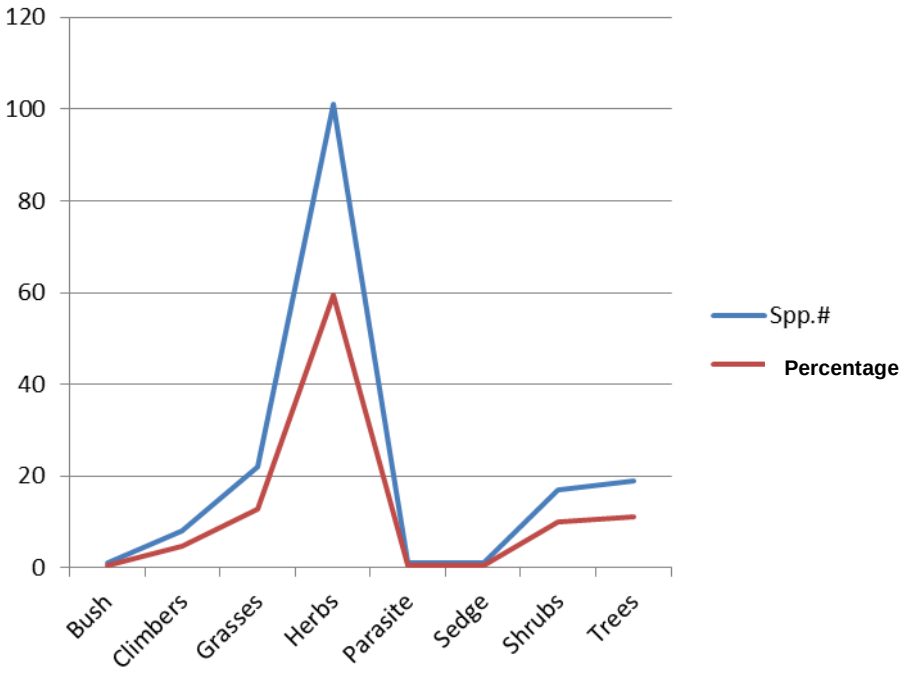

Figure 5. Growth form of the palatable flora of the study area.

parts and forage preferences by grazing animals. The area was found to have large amount of forage species which are grazed throughout the year. During the dry period, some of the species are harvested and stored for feeding of the domesticated animals, and stored fodder materials are mostly trees and subshurbs. The findings of this study will serve as benchmark for the development of fodder species and their varieties. The study will be helpful to range ecologists for the rehabilitation ofover-grazed areas of this rangeland. Further study is required to evaluate the nutritional composition and mineral status of the reported plant species.

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Full Length Research Paper

Comparative effects of NPK fertilizer, cowpea pod husk and some tree crops wastes on soil, leaf chemical properties and growth performance of cocoa (*Theobroma cacao* L.)

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A nursery experiment was carried out in Akure (rain forest zone), south-western Nigeria to study relative effect(s) of some organic wastes as fertilizers on growth performance, soil and leaf chemical composition of cocoa seedlings (*Theobroma cacao* L.) in the nursery. The experiment comprised of five treatments: Cowpea Pod Husk (CPH) (2.5 t/ha), cocoa pod husk ash (CPHA) (2.5 t/ha), kola pod husk (KPH) (2.5 t/ha), NPK15-15-15 (2.5 t/ha) and control (no fertilizer application). Each treatment was applied to 2.5 kg of soil filled polythene bags containing cocoa seedlings. The experiment was arranged in completely randomized design (CRD) with three replications. The organic wastes increased significantly (P>0.05) the plant height, stem diameter, leaf area, number of leaves, fresh root and shoot weights and dry root and shoot weights of cocoa seedlings. The treatments also increased significantly (P>0.05) soil and leaf N, P, K, Ca, Mg, Na, soil pH and organic matter (OM) content relative to the control. Kola pod husk (KPH) was the most effective in improving cocoa growth, leaf and soil chemical composition.

Key words: Cocoa, growth, organic matter, NPK, cowpea pod husk, cocoa pod husk ash, kola pod husk.

INTRODUCTION

Cocoa is one of the most important tropical crops (FAOSTAT, 2006). West Africa contributes about 70% of the world's cocoa production. The crop significantly contributed to the economies of countries in this sub-region, as well as economics of many other countries in Central America and South East Asia. Nigeria is the fourth largest producer of cocoa in the world with an estimated production of 485,000 metric tons in 2006 (FAOSTAT, 2006). Cocoa is therefore a major commodity crop cultivated in Nigeria and is a major raw material used in the production of cocoa powder (for beverage drink), various chocolate based products, biscuits and confectioneries. Processed cocoa bean is also used to make sweets, sweetening products, cocoa butter (used in making chocolate), perfume, and in pharmaceuticals. Locally, cocoa bean is used in cooking soup that has resemblance of okra and in treating various abdominal problems or ailments (Opeke, 2005). The production of cocoa in Nigeria has witnessed a downward trend since the early 1970s due to numerous factors like ageing trees, ageing farmers, wrong application of recom-mended agronomic techniques by farmers, effects

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of pests and diseases and deficiencies in macro and micro nutrients in the soils (Adejobi et al., 2011a).

Previous studies have attributed this yield decline essentially to soil nutrients imbalance (Ojeniyi et al., 1981). One way of combating this problem is the use of fertilizer. However, African farmers use very little fertilizer (8 kg/ha) compared to their counterparts in other parts of the agrarian world; hence, Africa's soils are increasingly depleted of nutrients (IFDC, 2008/2009). This is particularly true with cocoa farmers in Nigeria.

Ogunlade et al. (2009) reported that more than 85% of cocoa farmers in Nigeria do not use fertilizers on cocoa. Reasons for this low usage of fertilizers vary from lack of farmers' knowledge of the nutrients status of their soils to scarcity and high cost of fertilizers where available. The need to pay attention to soil fertilization is now almost as important as the control of capsids and black-pod disease in cocoa. Ayanlaja (2002), Adejobi et al. (2011a, b, c, d) and Moyin-Jesu (2008) reported the use of organic residues such as animal manures, urban refuse, agro-industrial processing wastes, animal dungs, refuse dump compost, pit latrine compost, foot of the hill compost, mulching, passive refuse dump in home gardens and alley cropping with appropriate nitrogen fixing shrubs, have been found capable of increasing and balancing soil nutrients with consequential increase in yield and crop performance.

The main objective of this study therefore was to examine the influence of different organic wastes on soil, leaf chemical composition and growth performance of cocoa seedlings.

MATERIALS AND METHODS

The experiment was carried out at Federal college of Agriculture, Akure between 2010 and 2011. Akure is located in the sub-humid region with distinct dry and wet seasons. The annual rainfall ranges from 1100 to 1300 mm per annum, temperature ranges between 24 and 30°C and relative humidity is about 85%.

Soil sampling and analysis before planting

Soil samples were randomly collected from 0 to 15 cm depth on the site. The soils were bulked, air dried and made to pass through a 2 mm sieve for chemical analysis. The soil pH (1:1 soil/water) was measured using pH meter. Organic matter was determined by the Murphy blue coloration and determined on a spectronic 20 at 882 um (Murphy and Riley, 1962). Soil potassium (K), calcium (Ca) and Magnesium (Mg) were extracted with $lMNH_4$ OAC, PH_7 and were determined with flame photometer; Mg was determined with an atomic absorption spectrophotometer. The total nitrogen (N) was determined by the Microkjedahl method (AOAC, 1990).

Processing of the organic residues used for the experiment

Cocoa pod husk ash (CPHA) and kola pod husk (KPH) were both obtained from cocoa and kola processing departments of Cocoa Research Institute of Nigeria (CRIN), Ibadan. Cowpea pod husk (CPH) was obtained from near-by farm in Ibadan, Oyo State while NPK 15-15-15 fertilizer was obtained from Mikky Farm Limited, Akure, Ondo State. Cocoa pod husk, cowpea pod husk and kola pod husk were sun dried for 32 h. Only cocoa pod husk was bunt to ash and allowed to cool for another 32 h, bagged and kept in a dry place. Kola and cowpea pod husk were ground with heavy mortal, bagged and kept in a dry place.

Chemical analysis of the organic material used

Two (2) grams each of the processed forms of the organic material used were analysed for nutrient composition using the standard procedure as described by Udo and Ogunwale (1986).

Nursery experiment

Mature, disease-free and ripe cocoa pods were harvested from cacao plantation of the Cocoa Research Institute of Nigeria (CRIN). The pods were broken and the beans were hand- scooped for planting. The bulked soil taken from the site (0 to 15 cm depth) of the experiment was sieved to remove stones and plant debris and 2.5 kg of the sieved soil was placed into a polythene bag (25 × 13 cm). There were 5 treatments: 2.5 t/ha cowpea pod husk (CPH), 2.5 t/ha cocoa pod husk ash (CPHA), 2.5 t/ha kola pod husk (KPH), 2.5 t/ha NPK 15-15-15 and the control (no fertilizer application). Two cocoa beans were sown per polythene bag arranged in completely randomized design (CRD) and later thinned to one seedling per polythene bag. The amount of the treatments were applied using spot method a month after sowing, the parameters such as plant height, number of leaves, leaf area, stem girth and number of branches were recorded from 8 to 32 weeks after planting. At 32 weeks after planting in the nursery, the seedlings were carefully removed from the polythene bags for the measurement of shoot and root lengths, fresh shoot and root weights; then oven dried and both dry shoot and root weights were taken before they were finally analysed for N, P, K, Ca and Mg contents.

At the time of taking the shoot weight, soil samples were taken from each of the polythene bag, air dried and sieved for analysis of major elements (soil N, P, K, Ca, Mg, pH and OM) as described earlier.

RESULTS AND DISCUSION

The result of the initial physico-chemical properties of the soil used for the experiment is presented in Table 1. The soil which was classified as an Affisol belonging to Akure series (Soil Survey Staff, 1999) had pH (H_2O) of 5.40, Organic matter (0.52%), total N (0.11%), available P (6.05 mg/kg), exchangeable K, Ca, and Mg being 1.20, 1.42 and 0.95 mole/kg, respectively. The values for organic matter, N, P, and Mg were generally low and fell below the critical level required for optimal performance of most tree crops in Nigeria (Egbe et al., 1989). With low N, P, K, Ca, Mg and organic matter, it is quite obvious that the soil is inherently low in fertility and therefore expected to show positive response to soil amendment. The insufficient levels of the major nutrients in the soil showed that the soil is depleted and would not be able to meet the nutritional needs of the cocoa plants unless external nutrients supply is made for the soil to be able to support optimum growth of cocoa plants. The soil particle size distribution indicated that the overall mean sand, silt

Table 1. Soil physiochemical composition before planting cocoa.

Soil properties	Value
Physical properties	
Sand	76.02%
Silt	16.25%
Clay	7.73%
Textural class	Sandy loam
Chemical properties	
Soil pH (H_2O)	5.40
Organic matter	0.52%
Organic carbon	0.25%
Nitrogen	0.11%
Available P	6.05 mg/kg
Exchangeable bases	
K^+	1.20 cmol/kg
Ca^{2+}	1.42 cmol/kg
Mg^{2+}	0.95 cmol/kg
Mn^{2+}	0.89 cmol/kg
Exchangeable acidity	
Al^{3+}	1.39 cmol/kg
H^+	0.12 cmol/kg
ECEC	6.97

and clay contents of the soil were 76.02, 16.25 and 7.73%, respectively. The clay + silt values were generally below 32% estimated to be adequate for soils considered to be ideal for tree crop production especially cocoa plant (Egbe et al., 1989).

Table 2 presents data on the nutrient composition of the organic materials used for raising the cocoa seedlings; cowpea pod husk (CPH) contained 4.02% OM, 22.93 mg/kg P, 8.25 mg/kg Mg, 4.91 mg/kg Ca and 4.19 mg/kg Na. Kola pod husk (KPH) on the other hand had 2.6% N, 3.21% OM, 6.51 mg/kg P, 1.09 mg/kg Mg, 2.66 mg/kg Ca and 2.61 mg/kg Na. On the contrary, cocoa pod husk ash (CPHA) contained 2.0% OM, 1.02% N, 4.02 mg/kg P, 5.31 mg/kg K, 1.08 mg/kg Mg. 3.60 mg/kg Ca and 3.06 mg/kg Na. Cocoa pod husk ash had high K with low N and P. The low value of N in CPHA might be as a result of volatilization during the burning process since the carbon present in the material has been partially destroyed by burning. This is consistent with the findings of Ajayi et al. (2007) and Odedina et al. (2003) that cocoa pod ash contained N, P, K, Ca and Mg. The high pH of the organic materials especially the CPHA, is an indication that the soil used for the conduct of the experiment which is confirmed to be acidic will benefit positively from their addition, and hence, moderate the acidity of the soil. This finding is in agreement with the earlier results of Ayeni et al. (2008a, b) and Ajayi et al. (2007a, b) that CPHA increased soil pH due to its liming effects on the soil. The growth parameters of cocoa seedlings as influenced by different organic fertilizers application are presented in Table 3.

The organic fertilizer materials positively and significantly affected the growth parameters of cocoa seedlings such as plant height, stem diameter, number of leaves per plant and leaf area relative to control. Kola pod husk produced the highest plant height, number of leaves per plant and leaf area respectively relative to control and other fertilizer materials; this was closely followed in descending order by NPK 15-15-15, cocoa pod husk ash, cowpea pod husk and control (KPH > NPK 15-15-15 > CPHA >CPA> control). Generally, the values of KPH with respect to these parameters were either higher or comparable to the in-organic fertilizer (NPK 15-15-15) and other organic material.

Fresh and dry root weight of cocoa seedlings (Table 4) showed that NPK fertilizer and organic materials of plant origin were comparable in their values. However, values due to organic fertilizers of plant origin were higher compared to that of inorganic origin (NPK 15-15-15 fertilizer). This might be due to presence of other vital nutrient elements presence in the organic fertilizer mate-rials (Ca, Mg, organic carbon and other micronutrients) that are required for good seedling growth which are absent in the NPK 15-15-15 fertilizer. Similar results were obtained for both fresh and dry shoot weight of cocoa seedlings with kola pod husk having the highest shoot weight (13.13 g) relative to control (6.33 g). The mean weight differences recorded for KPH and NPK 15-15-15 were not significantly ($p = 0.05$) different from each other although the highest response was recorded with KPH. CPH and CPHA recorded similar mean values of 10.00 and 9.00 g, respectively for dry shoot weight while KPH was significantly ($p \geq 0.05$) higher relative to other materials and control.

The effects of the treatments on the chemical properties of the soil as presented in Table 5 shows that all the organic materials and most importantly the cocoa pod husk ash increased the soil pH significantly ($p \leq 0.05$) compared to NPK and control, respectively. This findings is in agreement with the result of Nottidge et al. (2007) that affirmed the role of ash as a liming material and effective source of nutrients for crops such as vegetables, maize and cocoa (Odedina et al., 2003; Ayeni et al., 2008).

The soil N contents ranged between 0.11 to 0.99 g/kg soil. NPK 15-15-15 significantly increased soil nitrogen content relative to control. The effect of the treatments on soil N status shows the soil was significantly ($p \leq 0.05$) and positively affected by all the treatments. The effect of kola pod husk on soil N was more pronounced followed by CPHA and CPH, respectively. The difference between NPK 15-15-15 and KPH in respect to soil was not significant (Table 5). The effects of the applied organic

Table 2. Chemical analysis of the organic manures used for the experiment.

Treatment	pH (H_2O)	C/N ratio	OM (%)	N (%)	P (mg/kg)	K (mg/kg)	Mg (mg/kg)	Ca (mg/kg)	Na (mg/kg)
CPH	7.02	6.00	4.02	2.63	22.93	3.89	8.25	4.98	4.19
CPHA	7.20	9.50	2.00	1.02	40.21	5.31	1.08	3.60	3.06
KPH	6.99	5.60	3.21	2.68	6.51	3.29	1.09	2.66	2.61

CPH, Cowpea pod husk; CPHA, cocoa pod husk ash; KPH, kola pod husk.

Table 3. The growth parameters of cocoa seedlings between 4 to 24 weeks after planting under different organic fertilizer application.

Treatment	Plant height (cm)	Number of leaves	Stem girth (cm)	Leaf area (cm^2)
CPH	27.81^b	9.55^b	2.24^a	47.09^b
CPHA	27.82^b	10.46^a	2.19^a	45.91^b
KPH	30.56^a	11.38^a	2.19^a	53.64^a
NPK 15-15-15	27.87^b	11.27^a	2.16^a	52.26^a
Control	19.96^c	6.86^c	1.13^b	28.96^c

CPH, Cowpea pod husk; CPHA, cocoa pod husk ash; KPH, kola pod husk.
Treatment means within each column followed by the same letter are not significantly different from each other using Duncan multiple range test at 5% level.

Table 4. The yield parameters of cocoa seedlings under different organic fertilizer application.

Treatment	Fresh root weight (g)	Dry root weight (g)	Fresh shoot weight (g)	Dry shoot weight (g)
CPH	6.30^a	5.60^a	12.86^c	10.00^b
CPHA	7.30^a	3.80^b	14.3^{ab}	9.00^b
KPH	7.53^a	3.95^b	18.66^a	13.13^a
NPK 15-15-15	6.86^a	2.85^b	15.90^b	12.83^a
Control	3.37^b	2.88^b	10.00^c	6.33^c

CPH, Cowpea pod husk; CPHA, cocoa pod husk ash; KPH, kola pod husk. Treatment means within each column followed by the same letters are not significantly different from each other using Duncan multiple range test at 5% level.

Table 5. Soil chemical analysis after the experiment under different organic fertilizer application.

Treatment	Soil pH (H_2O) 1:1	Organic carbon (g/kg)	Organic matter (%)	N (%)	P (mg/kg)	K (mg/kg)	Mg (mg/kg)	Ca (mg/kg)	Na (mg/kg)
CPH	7.05^a	2.05^a	3.59^a	0.20^b	12.66^c	1.11^a	2.21^a	4.10^a	1.13^c
CPHA	7.38^a	1.53^b	2.15^b	0.27^b	40.00^a	1.88^a	1.98^b	3.19^b	1.09^a
KPH	7.28^a	2.78^a	2.46^b	0.62^a	16.00^c	1.31^a	2.01^a	3.11^a	0.66^b
NPK 15-15-15	5.03^b	1.79^b	0.85^d	0.99^a	20.00^b	1.40^a	1.10^b	2.93^b	0.60^b
Control	5.95^b	1.00^b	0.56^d	0.11^c	12.00^d	1.20^a	0.58^c	1.42^c	0.50^b

CPH, Cowpea pod husk; CPHA, cocoa pod husk ash; KPH, kola pod husk. Treatment means within each column followed by the same letters are not significantly different from each other using Duncan multiple range test at 5% level.

material on soil P revealed that CPHA gave significantly higher mean values relative to control and NPK 15-15-15. This might not be unconnected to the higher P present in the material as revealed by the analysis of the materials (Table 2). Similarly, all the organic materials improved soil K, Mg, Ca, and Na, respectively relative to the control. CPHA recorded the highest value of 1.88 mg/kg K followed by NPK 15-15-15 (1.40 mg/kg). CPH recorded the least soil K value. There is no significant difference among all the treatments applied. The amount of Mg, Ca and Na were also positively influenced with organic fertilizers addition irrespective of sources. This result is

Table 6. The leaf chemical composition under different organic manure application.

Treatment	N (%)	P (mg/kg)	K (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Na (mg/kg)
CPH	1.98[a]	1.44[a]	5.94[b]	2.08[a]	2.11[a]	2.40[a]
CPHA	1.48[b]	1.24[b]	7.08[a]	1.08[b]	1.91[b]	1.99[b]
KPH	1.49[b]	1.31[b]	6.01[a]	0.73[c]	2.06[a]	1.99[b]
NPK 15-15-15	1.93[a]	1.20[b]	5.48[b]	2.06[a]	1.88[b]	1.08[b]
Control	1.00[c]	0.27[c]	0.81[c]	0.83[c]	0.92[c]	1.00[b]

CPH, Cowpea pod husk; CPHA, cocoa pod husk ash; KPH, kola pod husk. Treatment means within each column followed by the same letter are not significantly different from each other using Duncan multiple range test at 5% level.

consistent with the findings of Odedina et al. (2003) who reported that cocoa pod husk ash significantly increased soil OM, N, P, K, Ca and Mg, respectively. Adejobi et al. (2011a, b), in their work on the effects of organo-mineral fertilizer and cocoa pod husk ash in the soil, leaf chemical composition and growth of coffee concluded that combined application of organo-mineral fertilizer and cocoa pod husk ash increased soil N, P, K, Ca, Mg and pH. The nutrients element composition of the cocoa leaf as affected by different organic manure application is shown in Table 6. The leaf N and P composition of cocoa seedlings was either comparable or higher than the NPK 15-15-15 in leaf nutrient composition. The mean values in the fertilizer treated seedlings were significantly higher compared with that of control. Similar trend as obtained in leaf N and P compositions was recorded with Ca. CPH gave a significantly higher value relative to control.

The difference in values recorded for CPH and NPK 15-15-15 were comparable, though CPH produced a higher value, the difference was not significant. The leaf Mg contents was higher in CPH relative to control and other applied fertilizer material. The low leaf chemical composition value noticeable with the control is a clear indication that the soil is inherently low in soil fertility and basic nutrients for cocoa seedlings. Hence, application of organic fertilizer amendments is quite necessary for enhanced production.

Conclusion

The use of both chemical and organic fertilisers significantly enhanced cocoa growth parameters, fresh and dry matter yield and leaf and soil chemical com-position. However, addition of organic materials such as CPH, CPHA and KPH as nutrient sources produced a promising effects on cocoa seedlings comparable to inorganic fertilizer; hence, they are advised for the purpose of cocoa seedlings establishment.

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New technique for adventitious rooting and clonal propagation of *Piper longum* L. (pippali) through leaf cuttings

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A simple but unique protocol was developed for root production and clonal propagation of *Piper longum* L. (pippali), an important medicinal plant of India. Roots and shoots were induced in apical and basal petiolar halves of leaf using auxins. Average root number, root length and survival of rooted leaf cuttings were not significantly affected by type of auxin and leaf cuttings. Highest number of roots (13.40), root length (9.65 cm), rooting behaviour (91.69%) and survival of rooted cuttings (83.33%) were recorded in petiolar leaf cuttings treated with indole 3-butyric acid (IBA)/naphthalene acetic acid (NAA) (1000 ppm each). The petiolar leaf cuttings treated with IBA/NAA showed significantly higher percentage of shooting (83.33). The shoot number (2.0 per cutting) was also significantly highest in IBA/NAA treated leaf cuttings. *Pippali* can be regenerated via leaf-cuttings, either without hormone treatment, or for better results by using low concentrations of IBA or combination of IBA and NAA hormones. Production of planting material using leaf can substitute traditional propagules such as sucker, vine cutting, etc.

Key words: Auxins, indole 3-butyric acid (IBA), medicinal plants, naphthalene acetic acid (NAA), vegetative propagation.

INTRODUCTION

Piper longum L. (long pepper, pippali), an unisexual perennial climber with woody roots belonging to the family piperaceae is distributed throughout India. Almost all parts of it, namely roots, stems and fruits are medicinally important and used especially in the treatment of diseases of respiratory tract like bronchitis, asthma, cough, etc (Sivarajan and Balachandran, 1994). The principal pharmacological constituents are piperine and piplartine. The crude extract of *P. longum* contains 3-8% of piperine (James, 1999).

Collection of roots from wild habitats and deforestation has made this plant species a threatened taxon. As the plants are extracted from its natural habitat for use in drug formulation, the species has become very rare in the forests of Kerala (Nair, 2000). Conventionally, *P. longum* is propagated through seeds, suckers or cuttings or by layering of mature branches at the beginning of rainy season. Although, conventional propagation is beset with problems of poor seed viability, low percentage of germination and scanty or delayed rooting of vegetative cuttings. Therefore, there is a need to explore alternate propagation methods (Sarasan et al., 1993).

Vegetative propagation through leaf cutting can be a suitable way to develop plants economically and in a controlled manner. The advantage of this type of propagation is that, with this technique plants can be raised throughout the year and the mother plant is less disturbed unlike the stem cutting method. Several reports are available regarding vegetative propagation of *P. longum*. Vegetative propagation by the application of root

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Table 1. Experimental layout and allocation of treatments for rooting in leaf-cuttings of *P. longum.*

Leaf cutting	Treatment				
	T_0: Control (without auxins)	T1: IBA (1000 ppm)	T2: NAA (1000 ppm)	T3: IBA (1000 ppm) + NAA (1000 ppm)	Total
Apical halves	30	30	30	30	120
Petiolar halves	30	30	30	30	120
Total	60	60	60	60	240

promoting substances in stem cutting has been studied (Bhat et al., 1995). Soniya et al. (2002) has also reported micro propagation of *P. longum.* However, regeneration of adventitious roots and shoots from the leaf cuttings of *P. longum* at *in vivo* condition has not been attempted. As root of *P. longum* have great medicinal value this study aimed at inducing growth of root. This could be an effective and less expensive way for production of root from leaves of *P. longum*.

MATERIALS AND METHODS

This experiment was carried out during July-September, 2009 at the institutional nursery of Regional Plant Resource Centre, Bhubaneswar, Odisha, India. Leaves were obtained from stock plants grown under shade-net house. Semi-mature and fresh leaves having 5 prominent veins were used for rooting and shooting experiment. A total of 240 leaf-cuttings (both apical and petiolar halves) were prepared out of 120 mature leaves by transverse cutting in the middle of the leaf blade. Two types of common rooting hormones viz. indole 3-butyric acid (IBA) and naphthalene acetic acid (NAA), all at four levels were used as per details given:T_0- Control (without auxins); T_1 - IBA (1000 ppm); T_2- NAA (1000 ppm); T_3- IBA (1000 ppm) + NAA (1000 ppm).

A total of 60 leaf-cuttings were treated with each type of auxin treatment in 3 replications (10 cuttings per replication) (Table 1). The rooting hormones were applied following quick dip method (Basak et al., 2000). The treated cuttings were put (at 45 deg angle) in rooting bed filled with sand:soil mixture (1:1) at the rate of 10 cuttings per block (representing one replication).

Rooting/shooting was recorded after 60 days in respect of the following parameters:

i. Percentage of rooted leaf-cuttings (rooting %)
ii. Root number per cutting
iii. Root length per cutting
iv. Percentage of shoot leaf-cuttings (shooting %)
v. Shoot number per cutting
vi. Survivability of shoot cuttings (survival %)

The data were subjected to analysis of variance followed by Student-Newman-Keuls test using GraphPad Prism (Ver,5,0). All the percentile values were converted into angular transformation for analysis.

RESULTS

Percentage of rooting

The type of auxins applied and leaf cuttings used had no relationship with number of roots developing out of plant ($p>0.05$) (Tables 2 and 3). The percentage of leaf-cuttings producing root was impacted by all four treatments (T_0- Control, T_1 - IBA (1000 ppm), T_2- NAA (1000 ppm), T_3- IBA (1000 ppm) + NAA (1000 ppm). Petiolar leaf-cuttings treated with T3 developed maximum roots (91.69%). Although there was variation in the rooting proficiency in relation to treatments, all leaf cuttings nevertheless developed roots in sand : soil mixture (1:1). But the rooting percentage varied from 70% (in apical leaf cuttings with control) to 91.69% (in petiolar leaf-cuttings with T3).

Root number per leaf-cutting

Average root number was not significantly ($p>0.05$) affected by auxin treatments in leaf-cuttings (both apical and petiolar halves) (Tables 2 and 3). The highest number of roots (13.40) was obtained in leaf cuttings treated with T3 (IBA 1000 ppm). Root number varied from 8.57 (in apical leaf cuttings with control) to 13.40 (in petiolar leaf cuttings treated with T3) (Figure 1).

Effect on length of root

The effect of auxin and leaf parts used (apical and petiolar halves) had no significant ($p>0.05$) relationship on the root length per cutting. Longest roots (9.65 cm) were obtained from leaf-cuttings treated with T3. The average length of roots ranged from 6.18 cm (in apical leaf cuttings with control) to 9.65 cm (in petiolar leaf-cuttings with T3 (Figure 1).

Shooting percentage

The variation in shooting out of leaf cutting was significantly influenced ($p<0.05$) by the type and dosage of rooting hormones (Tables 2 and 3). Petiolar leaf-cuttings treated with T3 exhibited highest percentage of shooted cuttings (83.33%). As seen in the case of rooting, shoots are also formed in leaf cutting treated with auxins irrespective of concentrations. Percentage of shoots in the treated leaf-cuttings ranged from 60% (in apical leaf cuttings with control) to 83.33% (in petiolar leaf-cuttings with T3) (Figure 1).

Table 2. Performance of adventitious rooting and multiple shoots regeneration from the leaf cutting of *Piper longum* as influenced by plant growth regulators.

Parameter/Type of leaf-cuttings	Treatment			
	T_0: (Control)	T1: (IBA 1000 ppm)	T2: (NAA 1000 ppm)	T3: (IBA 1000 ppm + NAA 1000 ppm)
Rooting (%)				
Apical leaf cutting	**70 ± 1.93**	80.24 ± 1.92	78.29 ± 2.52	87.65 ± 2.6
Petiolar leaf cutting	73.33 ± 1.92	81.47 ± 0.98	75.55 ± 4.05	**91.69 ± 2.25**
Root number				
Apical leaf cutting	**8.57 ± 0.43**	11 ± 0.12	11.03 ± 0.12	12.11 ± 0.56
Petiolar leaf cutting	8.73 ± 0.36	11.67 ± 0.52	11.21 ± 0.56	**13.4 ± 0.47**
Root length				
Apical leaf cutting	**6.18 ± 0.48**	8.79 ± 0.53	8.78 ± 0.56	9.1 ± 0.25
Petiolar leaf cutting	6.87 ± 0.43	8.6 ± 0.49	8.23 ± 0.69	**9.65 ± 0.51**
Shooting (%)				
Apical leaf cutting	**60 ± 1.92**	69.99 ± 1.93	63.33 ± 1.92	73.33 ± 1.92
Petiolar leaf cutting	63.33 ± 1.92	76.67 ± 3.85	70 ± 1.93	**83.3 ± 1.93**
shoot number				
apical leaf cutting	1.4 ± 0.06	1.4 ± 0.1	1.5 ± 0.12	1.7 ± 0.06
petiolar leaf cutting	1.6 ± 0.24	1.5 ± 0.15	1.8 ± 0.17	2 ± 0.12
Survival (%)				
Apical leaf cutting	**50.01 ± 3.86**	73.33 ± 1.92	73.33 ± 3.85	80 ± 1.92
Petiolar leaf cutting	53.33 ± 6.94	80 ± 5.17	76.66 ± 1.92	**83.33 ± 2.59**

Table 3. Statistical analysis of variance for different parameters, that is, rooting (%), shooting (%), root number and length, shoot number per leaf cutting of *P. longum.*

Parameter	Source of variation	Df	F	P
Rooting (%)	Treatment	2	0.2565	0.7765
	Type of leaf-cuttings	1	0.2466	0.6255
	No. of replications	2	0.8997	0.8997
Root Number	Treatment	2	0.04909	0.9522
	Type of leaf-cuttings	1	0.001259	0.9721
	No. of replications	2	0.1621	0.8516
Root Length	Treatment	2	0.04321	0.9578
	Type of leaf-cuttings	1	0.04364	0.8369
	No. of replications	2	0.3221	0.7287
Shooting (%)	Treatment	2	**1.155***	**0.3373**
	Type of leaf-cuttings	1	**4.575***	**0.0464**
	No. of replications	2	0.2178	0.8064
Shoot number	Treatment	2	**3.481***	**0.0527**
	Type of leaf-cuttings	1	**5.005***	**0.0382**
	No. of replications	2	0.3913	0.6818

Table 3. Contd.

Survival (%)	Treatment	2	0.4505	0.6443
	Type of leaf-cuttings	1	0.05247	0.8214
	No. of replications	2	0.2512	0.7806

*Significant at 0.05% level of probability.

Figure 1. Induction of adventitious roots and shoots in apical (upper row) and petiolar halves of leaves (lower row) of *P. longum* treated with auxin (T3, IBA/NAA at 1000 ppm each).

Shoot number per leaf-cutting

Average shoot number was significantly ($p<0.05$) affected due to various auxin treatments and varied in different leaf-cuttings (Tables 2 and 3). The highest shoot number (2.0) was obtained in leaf cuttings under the T3 treatment. Shoot number varied from 1.40 (in apical leaf cuttings with control and IBA) to 2.00 (in petiolar leaf cuttings with IBA plus NAA, that is, T3) (Figure 1).

Survival percentage in leaf cuttings

Survival percentage of rooted (and shooted) leaf-cuttings under different auxin treatments and varying doses is presented in Tables 2 and 3. Though survival percentage differed in leaf cuttings treated with different type of auxins and their combined treatments, the differential response was not significant ($p>0.05$). Petiolar leaf cuttings with T3 (IBA 1000 + NAA 1000 ppm) had the highest survival of 83.33%. Whereas the survival percentage of rooted and shooted leaf-cuttings varied from 50.01 (in apical leaf-cuttings under control) to 83.33% (petiolar leaf-cuttings with T3).

DISCUSSION

In this study, adventitious rooting and shooting were induced in leaf-cuttings of *P. longum*. Effect of growth hormones on leaf/petiolar rooting and shooting behavior was assessed under individual and combined treatment of hormones. IBA in combination with NAA was found most effective than their individual effect on rooting. These findings supported the result on adventitious rooting in stem cuttings of some mangrove species (Basak et al., 2000). In leaf cuttings, new growing points usually originate in the parenchymatous tissue closely associated with vascular tissues in the leaves. When vascular bundles of the leaf are severed but suitable growth conditions are provided new roots can be initiated but there was difficulty, in shoot regeneration. Leaf cuttings of most plants do not generate a new plant and produce only a few roots or just decay. Due to the fact

that leaf cuttings do not include an axillary bud, they can be used only for plants that are capable of forming adventitious buds (Hartmann et al., 1996). Probably, *P. longum* has the capacity to form adventitious buds in leaf-cuttings with or without been triggered by exogenous application of auxins.

In conclusion, *P. longum* can be regenerated via leaf-cuttings, either without hormone treatment, or with even better results using low concentrations of IBA or combination of IBA and NAA hormones. The non-significant differences for almost all the studied parameters in treated and control implies that *P. longum* can be propagated vegetatively at reduced cost through leaf cutting. This method, therefore, can be adopted with minimum capital to produce quality planting material.

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Full Length Research Paper

Effect of ethephon and planting density on lodged plant percentage and crop yield in maize (*Zea mays* L.)

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Increasing maize production is vital in many developing countries. One way of increasing production is through adoption of high planting densities. However, high planting densities are associated with intra-specific competition between plants for resources like nutrients, water and sunlight, as a result weak stalks develops which are susceptible to lodging. The challenge of lodging in high planting densities can be reduced by ethephon (2-chloroethylphosphonic acid). A 3 x 2 factorial experiment in randomised complete block design was carried out at New Donnington Farm, Zimbabwe in the 2011/12 rainy season to study the effects of ethephon rates and planting densities on maize. Ethephon rate had the following levels 0 l/ha (control), 0.56 l/ha and 0.84 l/ha while the density consisted of 53,333 and 80,000 plants/ha. Ethephon rate significantly ($p<0.05$) reduced the plant height of maize as compared to the control plants across different levels of planting densities. Also a significant increase ($p<0.05$) in internode diameter in the treated stands over the control plants across different levels of planting densities was recorded. Ethephon treatment significantly reduced ($p<0.05$) the percentage of lodged plants from 6.54 (control) to 4.34 and 2% for medium and high rate, respectively. Grain yield increased significantly in response to increased rates of ethephon at high planting density by 28.5 and 29.1 tons/ha for medium and high rate, respectively.

Key words: Maize, ethephon, planting, density, lodging.

INTRODUCTION

Maize is an important crop in the world and is ranked third after wheat and rice (Hoshang, 2012). In Zimbabwe and other African countries, maize is the staple crop and is grown by majority of smallholder farmers. It is estimated that more than half of the world area under maize production is in developing countries including Africa (Mejía, 2003). In Zimbabwe, despite the extensive area under production of maize in developing countries, the yields are still generally low. The poor yields can be attributed to poor agronomic practices, frequency of drought, biotic stresses among other factors.

Since maize is economically important, increasing its production is vital in African countries. One way of improving production is through adopting high planting densities. High planting densities increase the number of ears per unit area and thereby improving the grain yield (Abuzar et al., 2011). However, too high populations may cause intra-specific competition between plants for resources like nutrients, water and sunlight (Zamir, 2011). In addition, high planting densities are associated with lodging of stalks (Shekoofa and Emam, 2006). Lodging can affect photosynthesis, pollination and yield of maize crop due to poor solar radiation interception.

The challenge of lodging in high planting densities could be reduced by using ethephon (2-chloroethylphosphonic acid) (Shekoofa and Emam, 2008). Ethephon

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Table 1. The interaction between planting density and ethephon rates on final plant height (cm).

Ethephon	53, 3333 plants/ha (low density)	80, 000 plants/ha (high density)
0	277.25[a]	285.15[b]
0.56 l/ha	265.29[c]	267.6[d]
0.84 l/ha	254.15[e]	257.55[f]
p value	< 0.001	
Lsd	1.38	
cv (%)	5.3	

is an ethylene releasing compound whose effect depends on crop species, amount applied and timing of application (Khuankaew et al., 2009). It is a growth regulator which upon application, increases resistance to lodging through thickening of lower internodes, termination of apical dominance (Shekoofa and Emam, 2006). Besides reducing lodging in crop plants, ethephon has also been found to improve the water use efficiency (WUE) of plants by making water available during critical reproductive growth stages (Campos et al., 2004). Therefore, ethephon can be used to improve crop yield in dry areas which receive very low rainfall (Langan et al., 1987).

Elsewhere, ethephon has been extensively studied as a plant growth regulator used to promote fruit ripening, abscission, flower induction, breaking of apical dominance but there is limited information to show that it has been evaluated as an anti-lodging agent in maize under high planting densities.

MATERIALS AND METHODS

A field experiment was conducted at New Donnington Farm in Zimbabwe, which lies between 30°41' E and 17° 52' S in Norton during the 2011-2012 growing season. The farm is located in Agro-ecological region 11a about 38 km from Harare along Bulawayo road. The altitude of the area is 1360 m above sea level. The mean annual rainfall is between 950 and 1000 mm per annum whilst the mean annual temperature is 20.6°C. The maximum and minimum temperatures of the area are 26 and 17°C, respectively. The study site was dominated by light, well drained sandy loam soil textures (Nyamapfene, 1991). The design used was a 3 × 2 factorial experiment in randomized complete block design with four replications and the blocking factor was the slope. The first factor was ethephon with three levels, 0, 0.56 and 0.84 l/ha and the second factor was planting density with two levels, 53,333 and 80,000 plants/ha.

Land was conventionally prepared using a tractor drawn disk harrow and a roller to obtain a fine tilth from which seedbeds were raised. A marked wire cable was used to mark planting stations and the seed was hand sown in plots of 4.5 m wide and 5.0 m long. In row spacing was 16.6 (high density) and 25 cm (low density). Inter-row spacing was 75 cm in all plots and uniformity of sowing depth was achieved by using a hand dibbler to make holes of 5 cm deep. A basal fertiliser of Compound D (7% N, 14% P_2O_5 and 7% K_2O) was applied at a rate of 300 and 450 kg per hectare under the low and high planting densities, respectively. Top dressing was done using urea (46% nitrogen), the fertilizer was split applied at the rate of 250 and 375 kg per hectare at the low and high planting densities, respectively at four and eight weeks after planting. The field was kept weed free by the use of Atrazine and Alachlor at 31/ha and 21/ha, respectively. Late and persistent weeds were controlled mechanically by the use of hoes at eight weeks after crop emergence.

Stalk borer (*Busceola fusca*) was controlled by the use of dipterex (dimethyl 2.2.2-trichloro-1-hydroxyethyl-phosphate) which was applied at four weeks and six weeks after crop emergence at a rate of 4 kg per ha. Ethephon mixed with blood buff (surfactant) was applied in the morning at 8 to 9 leaf stage using a knapsack sprayer. Each plot was surrounded by plastic walls to avoid the drift of the solution to the adjacent plots.Confider was sprayed at physiological maturity to control termites using a knapsack sprayer.

The following measurements were recorded, plant height, internode length and diameter, percentage of lodged plants and grain yield. Data was subjected to analysis of variance using GENSTAT version 14. Number of lodged plants was square root transformed prior to analysis of variance. Treatments means were separated using least significant difference test at 5% level.

RESULTS AND DISCUSSION

Ethephon rate and planting density on final plant height and internode length

There was a significant interaction ($p< 0.05$) in the final plant height between planting densities and ethephon rates. Ethephon treatment resulted in a significant ($p<0.05$) reduction of plant height across the two plant densities (Table 1). The reduction in plant height can be attributed to the observed decrease in internode lengths (Figure 1).

This finding was also in agreement with the results of Shekoofa and Emam (2006) and Khuankaew et al. (2009). However, Poovaiah and Leopold (1973) reported that stem elongation was observed after ethephon was applied to blue grass (*Poa pratensis* L) and wheat (*Triticum aestivum* L). This confirms that plant species respond differently to ethephon application.

The effect of ethephon on percentage of lodged plants and internode diameter (mm) of maize

There was significant ($p< 0.05$) reduction on the percentage of lodged plants following ethephon treatment. The minimum percentage of lodged plants were recorded where high ethephon rate was used (Figure 2). These results may be attributed to an increase in internode diameter following ethephon treatment as shown in Table 2. Shekoofa and Emam (2006) also found that ethephon increases internode diameter and is important for reducing lodging.

Effect of planting density on the percentage of lodged plants

Planting densities had a significant effect ($p< 0.05$) on

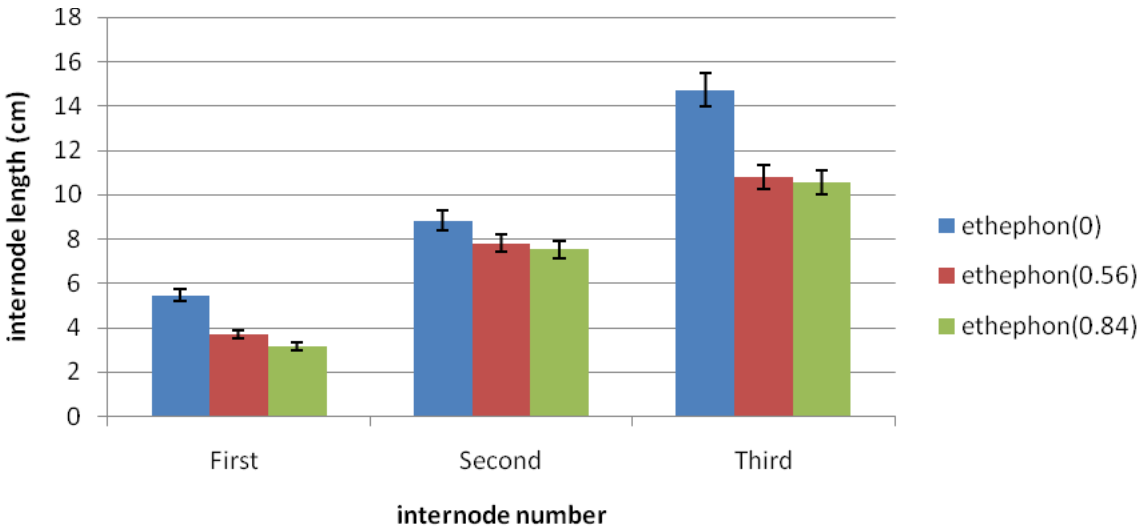

Figure 1. Effect of ethephon rates on final internode length of maize plant (cm).

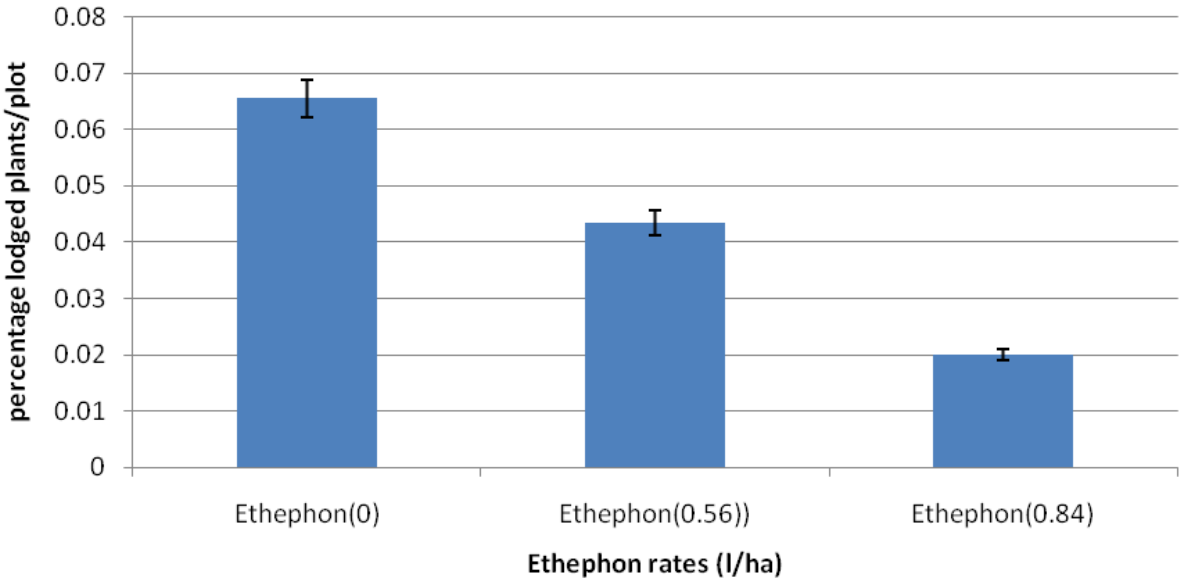

Figure 2. Effect of ethephon levels on percentage of lodged plants.

Table 2. The interaction between plant density and ethephon rates on first internode diameter of the maize plant.

Ethephon	53, 3333 plants/ha (low density)	80, 000 plants/ha (high density)
0	28.9070[a]	28.8555[b]
0.56 l/ha	32.4180[c]	31.2835[d]
0.84 l/ha	29.91[c]	29.7995[e]
p. value	< 0.001	
LSD	0.06025	
CV (%)	5.1	

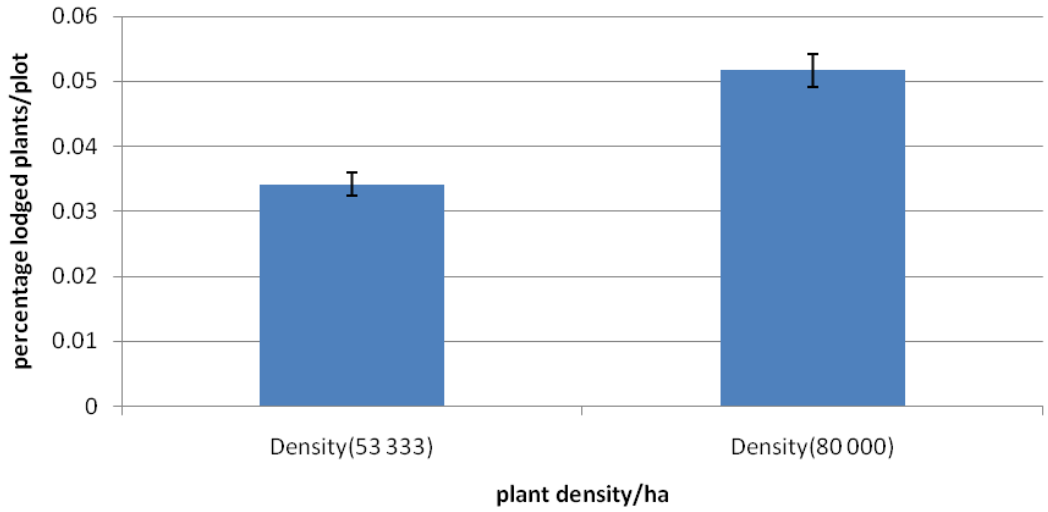

Figure 3. Effect of planting density levels on percentage of lodged plants.

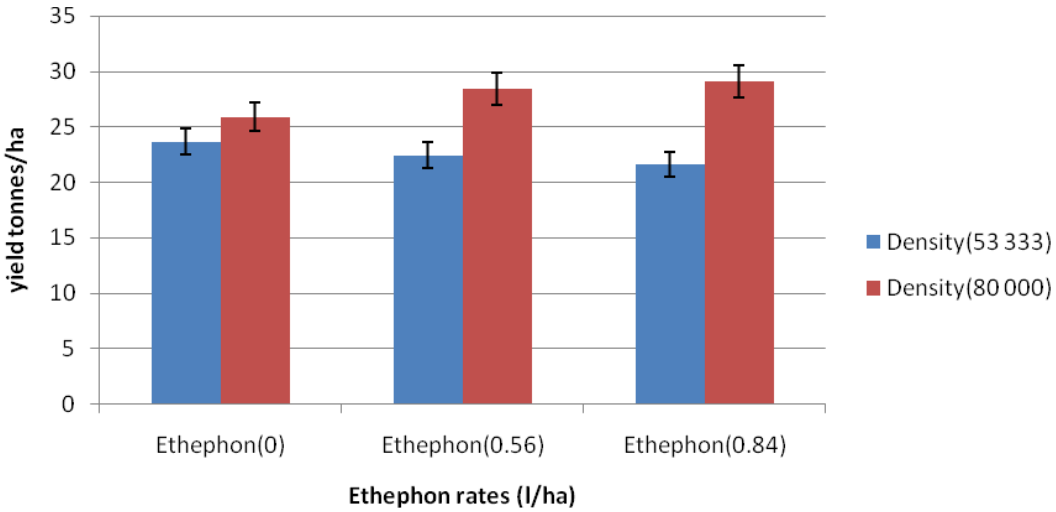

Figure 4. The interaction between different levels of planting density and ethephon rates on grain yield (t/ha).

percentage of lodged plants. There was a significant ($p<0.05$) increase in percentage of lodged plants following an increase in planting densities (Figure 3). This is in agreement with the findings by Liu et al. (2012), who reported that, an increase in planting densities result in risk of root lodging. The intraspecific competition between plants for resources like light, water and nutrients may result in etiolating of the crop thereby weakening the root system and anchoring ability.

Ethephon and planting density on grain yield (tons per hectare)

A significant interaction ($p< 0.05$) between planting densities and ethephon rates was observed for the grain yield (Figure 4). Addition of ethephon resulted in significant differences ($p< 0.05$) in grain yield between planting densities. Ethephon has been reported to increase maize yield under drought conditions. Since this experiment was set under rain-fed conditions, there are certain times mid dry spells were felt and as such ethephon improved the maize yield. The plant growth regulator increases the grain yield under water stress conditions by extending water availability during critical stages like grain filling (Kasele et al., 1994). The results also showed a significant increase in grain yield under high planting density when ethephon was applied. Ethephon might have reduced lodging in treatments where high planting density was used resulting in more cobs being harvested.

Ethephon had no significant effect ($p < 0.05$) on grain yield under low planting density. The plant growth regulator is not beneficial under low planting densities, as lodging is low as shown in Figure 4.

Conclusions

Ethephon reduces the plant height due to shortening of internodes. The plant growth regulator also reduces the percentage of lodged plants across all plant densities due to increase in internode diameter. Increase in planting densities tends to exacerbate lodging in maize crop. Ethephon increases grain yield under high planting densities and have no effect under low plant population.

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Seasonal variation of air, soil and leaf surface fungi of broad bean and cellulolytic ability in Upper Egypt

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Seventy-five species and 3 species varieties belonging to 21 fungal genera were collected from air, soil and leaf surface of broad bean plant on dicholran chloramphenicol malt extract agar (DCMA) and dichloran Rosebengal chloramphenicol agar (DRBC) at 28°C. The results obtained from leaf surface (phyllosphere and phylloplane), soil and atmosphere were basically similar in the two types of media and the most common fungi were: *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Cladosporium cladosporioides*, *Cladosporium sphaerospermum* and *Drechslera neergaardii*. The monthly counts of these fungi on two types of media irregularly fluctuated giving maxima value at various months. *A. flavus* was the highest fungi that produced both exo- and endo-ß-1,4-glucanases among the 9 tested isolates. Maximum production of two enzymes by *A. flavus* was 8 and 6 days after incubation at 30°C with culture medium containing glucose and cellulose as carbon sources and sodium nitrate as nitrogen source and initially adjusted to pH 6.

Key words: Airborne fungi, soil and leaf surface, broad bean, cellulolytic ability.

INTRODUCTION

Food legumes play an important and diverse role in the farming systems and in the diets of poor people around the world. They are ideal crops for simultaneously achieving three developmental goals in targeted population reducing poverty, improving human health and nutrition, and enhancing ecosystem resilience. Broad bean (*Vicia faba* L.) is one of the most important winter crops of high nutritive value in the world as well as in Egypt. *Vicia faba*, which has several common names (fava bean, faba bean, horse bean and tic bean), is a species of bean (Fabaceae) native to north Africa and southwest Asia (Elwakil et al., 2009).

Broad bean can significantly improve the quality of fish and meat. High quality dried broad beans are processed into snack foods, vermicelli, starch, and spicy bean sauce /paste. Faba bean is an excellent source of protein (20-25%), calcium (0.15%), phosphorus (0.50%), lysine (1.5%) and methionine - cystine (0.5%), from dry weight (Rabey et al., 1992).

World production of broad bean varied during the last 10 years. China came first in the production. It produced 13033750 tones while Egypt production was 155554 tones (FAO, 2009).

Numerous investigations have been carried out on fungal flora of leaf surface, soil fungi and air borne fungi of several plants cultivated in many parts of the world by several researchers (Abdel-Hafez et al., 1990, 1995, 2003; Blakeman, 1991; Khallil and Abdel-Sater, 1993; El-Kholl et al., 1994; El-Said, 1994, 2001; El-Said et al., 2006; El-Said and Saleem 2008; Gunasekera, 2004; Moharram et al., 2004, 2010; Murace and Cellitin, 2005; Saleem et al., 2010, 2013).

Cellulose, a major polysaccharide constituent of plant cell walls, is a 1,4 linked linear polymer of 8000~12000

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Gglucose units. Three major enzymes are involved in the degradation of cellulose to glucose which are endoglucanase (endo-1,4-d-glucanasem EG), cellobiohydrolase (exo-1,4-d-glucanasem CBH) and β-glucosidase (1.4-d-glucosidase, BG). EG acts in random fashion, cleaving linked bonds with in the cellulose molecule; CBH removes cellobiose units from the non reducing ends of the cellulose chain and BG degrades cellobiose and cello-oligosaccharides to glucose (Saha, 2004).

The aim of the present investigation was to study the seasonal variations of fungus flora of leaf surface, airborne fungi and soil in *Vicia faba* field cultivated in Oena governorate and cellulolytic activity of some fungal isolates and the effect of some environmental and nutritional factors on cellulase production.

MATERIALS AND METHODS

During the growing season of broad bean crop which extended from December 2011 to April 2012, a broad bean field in South Valley University in Qena city in Upper Egypt was selected to study the mycoflora of leaf surface (phyllosphere and phylloplane) and soil, as well as the airborne fungi over the broad bean field. Samples were collected fortnightly and two media types were used: dicholran chloramphenicol malt extracts agar (DCMA) Andrews and Pitt (1986) and dichloran Rosebengal chloramphenicol agar (DRBC) at 28°C King et al. (1979).

Determination of phyllosphere fungi

The dilution plate method was used as employed by El-Said et al. (2006).

Determination of phylloplane fungi

The determination of phylloplane was as employed by El-Said et al. (2006).

Determination of airborne fungi

The "exposed plate" method was used to trap fungal spores over broad bean field during growing season. Six plates of 9 cm diameter were used for each exposure (3 plates for each type of medium). The plates were exposed at 10-11 a.m., for 15 min every 15 days (Abdel-Hafez et al., 1990).

Determination of soil fungi

The dilution-plate method as described by El-Said (1994) was used for estimation of soil fungi. The plates were incubated at 28°C for 5-10 days during which the developing fungi were counted, identified purely morphologically, based on macro- and microscopic characters (Raper and Fennell, 1965; Ellis, 1971, 1976; Domsch et al., 1980; Pitt, 1985) and calculated per g dry soil.

Screening of fungal isolates for cellulase production

Nine species (the most common species) belonging to 5 genera were screened for their abilities to produce exo- and endo-β-1,4-glucanase (C_1 and C_x enzymes, respectively). Isolates were cultured on Eggins and Pugh medium (1962). Cultures were incubated at 28°C for 7 days. Using sterile cork borer, 10 mm diameter, discs were cut to inoculate 50 ml sterile liquid medium (in 250 ml Erlenmeyer flasks) of Eggins and Pugh medium (1962) for exo-glucanase production and Prasad and Verma medium (1979) for endo-glucanase. The cultures were incubated at 28°C for 7 days. The cultures were filtered and the filtrates were used to detect the activity of the enzymes as follows:

Detection of exo-β-1, 4-glucanase (C_1 enzyme)

Using a sterile cork borer, 3 cavities (10 mm diameter) were made in plates containing solid Eggins and Pugh medium (1962). A 0.1 ml of culture filtrate was dropped in each of these cavities followed by incubation at 28°C for 24 h, then the plates were flooded with chloroiodide of zinc solution and the uncolored zone gave a measure of cellulolytic power of isolates.

Detection of endo-β-1.4-glucanase (C_x enzyme)

Ten millimeters cavities were cut in plates containing solid medium of Dingle et al. (1953), filtrate obtained from 7 days old fungal cultures grown on Prasad and Verma (1979) medium was dropped in each cavity. After 24 h of incubation at 28°C, plates were flooded with chloroiodide of zinc solution and the clear zones around cavities were measured.

Factors affecting cellulase production

The effect of different ecological and nutritional factors on production of cellulase enzymes (C_1 and C_x) by *Aspergillus flavus* was shown. Since this species was found to be highly active in cellulase production so this species was used for this study. The previous isolate was grown on liquid medium (Deacon, 1985). Fifty milliliters of the medium were dispensed into each 250 ml Erlenmeyer flask and each flask was inoculated with an agar mycelial disc (10 mm diameter) of the mould obtained from 7 days old fungal cultures growing on the solid basal medium. Experiments were done to indicate the best conditions which produce a good deal of the enzyme as well as of the best expense.

Effect of temperature and time course

The inoculated flasks were incubated at 20, 30 and 40°C for 14 days and harvested at 48 h intervals. Culture fluid were filtered and centrifuged at 5000 r.p.m. for 10 min, the clear supernatants were assayed for enzyme activity.

Effect of pH values

The test isolate (*A. flavus*) was grown on the basal medium of Deacon (1985). The initial pH of the medium was adjusted with 0.1 N NaOH or 0.1 N HCL to different values ranging from 2 to 14. After inoculation with *A. flavus*, cultures were incubated at 30°C for 8 days for C_1 and C_x, respectively. At the end of incubation period the cultures were filtered, centrifuged at 5000 r.p.m. for 10 min and the clear supernatants were assayed for cellulase activity.

Effect of different carbon sources

The basal medium (Deacon, 1985) with pH 8 (the best pH for cellulase production) was supplemented with 1% of one of the following carbon sources: glucose, fructose, lactose, sucrose, cellulose, starch and carboxymethyl cellulose. The flasks were inoculated with *A. flavus* and incubated at 30°C (the best temperature of C_1 and C_x enzymes production) for 8 days (the best incubation periods for C_1 and C_x enzymes, respectively) and the cultures were filtered. After centrifugation the filtrate was used to detect the cellulase activity.

Effect of different nitrogen sources

To determine the effect of nitrogen source on cellulase production, sodium nitrate in the basal medium was replaced by the same amount of various nitrogen compounds such as: sodium nitrite, potassium nitrate, yeast extract, ammonium sulphate, ammonium nitrate and peptone in addition to sodium nitrate as a control. Cultures were incubated at 30°C for 8 days and the cultures were filtered, centrifuged and the filtrates were used for detection of cellulase activity.

Assay for cellulase activity (C_1 and C_x enzymes)

The method described by Nelson (1944) and modified by Naguib (1964) was employed. The amount of reducing sugars produced was estimated by determining the optical density (absorption spectrum) at 700 nm wave length with a spectrophotometer model (Spectronic ® Genesys ^{TM} 2PC USA). A standard curve was plotted using aqueous solution of D-glucose.

RESULTS AND DISCUSSION

The monthly total counts of phyllosphere and phylloplane surface fungi of broad bean on plates of DCMA and DRBC irregularly fluctuated giving peaks during March and March, respectively.

Thirty-four species and 3 species varieties belonging to 15 genera were collected from phyllosphere (10 genera and 20 species + 2 varieties) and phylloplane (12 genera and 21 species +1 var.) of broad bean leaves on DCMA and DRBC at 28°C (Table 1). The most common fungi of two substrates on the two types of media were: *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Cladosporium cladosporioides*, *Cladosporium sphaerospermum* and *Drechslera neergaardii*. The monthly counts of the above fungal species were widely varied and fluctuated irregularly giving maxima during different months (Figures 1 and 2). El-Said (2001) isolated *Alternaria alternata*, *Alternaria citri*. *A. flavus*, *A. fumigatus*, *A. niger*, *Chaetomium globosum*, *Cladosporium cldosporioides*, *Cochliobolus lunatus*, *Mycosphaerella tassiana*, *Setosphaeria rostrata* and *Stachybotrys chartarum* from leaf surface of banana leaves. Also, El-Said et al. (2006) found that the most common fungi isolated from 60 samples of leaf surface of broad bean on DCMA and DRBC at 28°C were: *Alternaria petroselini*, *A. citri*, *Aspergillus flavus*, *A. niger*, *C. cladosporioides* and *C. sphaerospermum*. Also, 25 species and one species variety belong to 17 genera from leaf surface of broad bean on dichloranchloram-phenicol-malt extract agar (DCMA) at 28°C and the most common species were *A. alternata*, *C. cladosporioides* and *C. sphaerospermum*. All fungal species recovered from leaf surface of broad bean on the two types of media were previously isolated but with different inciden-ces from leaf surface of several plants growing or cultiva-ted in many parts of the world (Abdel-Hafez et al., 1990, 1995; Abdel-Sater, 1993; El-Kholl et al., 1994; Murace and Cellitin, 2005; Moharram et al., 2010; Saleem et al., 2010, 2012).

Forty species and 1 variety representing 14 genera were collected from the air above broad bean field on plates of DCMA (14 genera and 29 species) and DRBC (10 and 32+1var.) at 28°C (Table 2). The monthly counts of fungi on DCMA and DRBC in the atmosphere over broad bean field irregularly fluctuated and widely varied between 50-12 and 100-200 colonies/6 plates in 2 expo-sures of 10 min each giving peaks during February and February, respectively (Figure 3). The most common fun-gi on the two types of media were: *A. citri*, *Alternaria petroselini*, *Alternaria pluriseptata*, *A. flavus*, *A. melleus*, *A. niger*, *C. sphaerospermum*, *Curvularia richardiae*, *D. neergaardii* and *P. chrysogenum*. The monthly counts of these fungi were widely varied and irregularly fluctuated giving maxima during various months (Figure 3). Some species were prevalent on one type of media such as*: Emericella nidulans* and *Mucor circinelloides* on DRBC. Abdel-Hafez et al. (1990) observed that the most preva-lent fungi in the air over lentil field were: *A. alternata*, *A. flavus*, *A. niger*, *Cladosporium herbarum*, *Cochliobolus spicifer*, *Curvularia pallescens*, *Fusarium moniliforme*, *F. oxysporum*, *Myrothecium verrucaria*, *Penicillium chrysogenum* and *Stachybotrys chartarum.* Abdel-Hafez et al. (1995) isolated fifty species and 3 varieties representing 26 genera were collected from the air above sugarcane field and the most common fungi were: *A. alternata, A. flavus*, *A. niger*, *A. terreus*, *P. chrysogenum* and *P. oxalicum.* Patel (2008) carried out aeromyco-logical studies on tomato (*Lycopersicon esculentum* Mill. and *Solanum melongana* L.) fields at Nashik (M. S.) during two seasons. Spores of *Cladosporium*, *Alternaria*, *Curvularia*, *Helminthosporium*, *Aspergillus*, *Penicillium*, *Fusarium* and *Periconia* were found in maximum percentage in the total air-spora. More spore catch was found in the month of January, March and August, September. During the period of investigations, 66 types of fungal spores were observed. The maximum numbers of spores were found in the second season. Low temperature, high relative humidity and moderate and alternate spell of rain show effect on release and dissemination of air borne fungal spores. Chavan (2012) studied the occurrence of Ascomycetes fungal spores over a paddy field and observed that the spores belonging to groups Deuteromcotina contributed 66.61%, Basidiomycotina 8.89%, Ascomycotina 24.56% and other types 0.35% of the total airspora. The most prevalent genera were *Curvularia*, *Fusarium*, *Helminthosporium*, *Phoma*, *Nigrospora*, *Alternaria* and *Cladosporium*. Several of the above species were also frequently isolated from the air of some Egyptian localities (Abdel-Hafez, 1989; Abdel-Sater, 1990; Abdel-Hafez et al., 1990, 1993, 1995; Patel, 2008; Chavan, 2012).

Thirty-one species and 1 variety belonging to 12 genera were collected from soil of broad bean field on plates of DCMA (9 genera and 24 species + 1 var.) and DRBC (11 and 24 + 1 var.) at 28°C (Table 3). The monthly counts of fungi on DCMA and DRBC in soil of broad bean field irregularlyfluctuatedandgivingpeaksduringdifferentmonths

Table 1. Total counts of phyllosphere (per g fresh leaves) and phylloplane (120 leaf segments) fungi, number of cases of isolation (NCI), occurrence remarks (OR) and relative importance value (RIV) of the fungal genera and species on dicholran chloramphenicol malt extract agar (DCMA) and dichloran Rosebengal chloramphenicol agar (DRBC) at 28°C.

Genera and species	Phyllosphere				Phylloplane					
	DCMA		DRBC		DCMA			DRBC		
	TC	NCI&OR	TC	NCI&OR	TC	NCI&OR	RIV	TC	NCI&OR	RIV
Alternaria	333.4	2L	666.7	2L	27	4M	55.1	16	5M	60.5
A. alternata (Fries) Keissler	-	-	-	-	3	1R	11.7	1	1R	10.7
A. chlamydospora Mouehaeca	-	-	-	-	2	1R	11.1	3	1R	12
A. cinerariae Hori & Enjaji	-	-	-	-	3	1R	11.7	-	-	-
A. citri Ellis &Pierce	166.7	1R	-	-	-	-	-	-	-	-
A. petroselini (Neergaard) Simmons Comb. nou.	166.7	1R	166.7	1R	11	3M	36.1	8	2L	25.2
A. pluriseptata (Karst. & Hart) Jorsted	-	-	500	1R	8	3M	34.5	4	3M	32.6
Aspergillus	7166.7	10H	12999.8	10H	81	9H	135.3	88	9H	147.5
A. deflectus Fennell & Raper	166.7	1R	2833.3	1R	-	-	-	2	1R	11.3
A. flavus Link	999.9	3M	2833.3	1R	21	6H	71.7	23	9H	105
A. fumigatus Fresenius	2166.6	3M	2833.3	4M	-	-	-	14	3M	39.2
A. japonicus Saito	-	-	333.3	1R	2	1R	11.1	4	1R	12.6
A. melleus Yukawa	-	-	833.3	1R	-	-	-	1	1R	10.7
A. niger Van. Tieghem	3333.5	8H	3000	6H	57	9H	121.8	43	9H	118.1
A. parasiticus Speare	-	-	-	-	1	1R	10.6	-	-	-
A. sulphureus (Fres.) Thom & Church	-	-	333.3	1R	-	-	-	1	1R	10.7
A. terreus var. *aureus* Thom & Raper	500	1R	-	-	-	-	-	-	-	-
Cladosporium	39666.6	6H	36500	8H	24	3M	43.4	18	2L	31.8
C. cladosporioides (Fres.)de Vries	37833.3	6H	11166.7	3M	7	2L	23.9	10	2L	26.5
C. sphaerospermum Penzig	1833.3	3M	25333.3	5M	17	3M	39.5	8	2L	25.2

Table 1. Contd.

Genera and species	Phyllosphere				Phylloplane					
	DCMA		DRBC		DCMA			DRBC		
	TC	NCI&OR	TC	NCI&OR	TC	NCI&OR	RIV	TC	NCI&OR	RIV
Curvularia richardiae Alcorn	166.7	1R	166.7	1R	3	2L	21.7	-	-	-
Drechslera	333.4	2L	500.1	3M	16	4M	48.9	5	3M	33.3
D. indica (Rui, Wadhwani & Twari) Mouchacca	166.7	1R	166.7	1R	1	1R	10.6	1	1R	10.7
D. neergaardii Danguash	166.7	1R	333.4	2L	15	4M	48.4	4	3M	32.1
Emericella nidulans var. *dentata* Sandhu & Sandhu	-	-	-	-	9	1R	0.2	4	1R	12.6
Fusarium	166.7	1R	333.3	1R	3	2L	21.7	4	1R	12.6

Table 1. Contd.

F. culmorum W.G. Smith (Sacc)	-	-	-	-	-	-	-	4	1R	12.6
F. merismoides Corda	-	-	-	-	3	2L	21.7	-	-	-
F. oxysporum Schlecht	166.7	1R	333.3	1R	-	-	-	-	-	-
Melanopsmma pomiformis (Pres.ex. Fr.) Sacc	-	-	333.3	1R	-	-	-	-	-	-
Mucor circinelloides Van Tiegh	166.7	1R	-	-	2	2L	21.1	2	2L	21.3
Myrothecium roridium Tode ex Fries	-	-	-	-	1	1R	10.6	-	-	-
Paecilomyces variottii Bain	333.3	1R	-	-	-	-	-	-	-	-
Penicillium	12166.8	4M	4833.3	2L	10	2L	25.6	13	2L	28.5
P. aurantiogriseum Dierckx	833.3	1R	-	-	-	-	-	-	-	-
P. camembertii Thom	9333.4	2L	1166.7	1R	-	-	-	12	1R	17.8

Table 1. Contd.

Genera and species	Phyllosphere				Phylloplane					
	DCMA		DRBC		DCMA			DRBC		
	TC	NCI&OR	TC	NCI&OR	TC	NCI&OR	RIV	TC	NCI&OR	RIV
P. chrysogenum Thom	833.3	1R	3666.6	2L	5	1R	12.8	1	1R	10.7
P. chorylophilum Dierckx	166.7	1R	-	-	-	-	-	-	-	-
P. duclauxii Delacroix	666.7	1R	-	-	5	1R	12.8	-	-	-
P. funiculosum Thom	333.4	2L	-	-	-	-	-	-	-	-
Rhizopus oryzae Went & Prisen Geerligs	-	-	-	-	1	1R	10.6	2	1R	11.3
Stachybotrys atra var. *microspora* Mathur Sankhla	1 66.7	1R	-	-	-	-	-	-	-	-
Sterile mycelium	-	-	166.7	1R	-	-	-	-	-	-
Trichothecium roseum (Pres.) Link ex Gray	-	-	-	-	2	2L	21.1	1	1R	10.7
Total counts	60667		56499.9		179			153		
Number of genera 15	10		8		12			10		
Number of species 34+3var.	20+2var.		18		21+1var.			21+1var.		

*OR = Occurrence remarks: H = high occurrence from 6-10 cases, M = moderate occurrence from 3-5cases, L = low occurrence 2 cases and R = rare occurrence 1 case.

(Figure 4). The most common fungal genera on the two types of media were: *Alternaria* (5 species), *Aspergillus* (7), *Cladosporium* (4), *Emericella* (1) and *Fusarium* (6). The most prevalent species on the two types of media were: *A. flavus*, *A. fumigatus*, *A. niger*, *C. cladosporioides*, *C. sphaerospermum* and *Emericella nidulans* var. *dentata* (Table 3). Abdel-Hafez (1994) found that the most common species in the Egyptian soils on glucose-, cellulose- and 50% sucrose-Cazpek's agar were: *A. flavus*, *A. fumigatus*, *A. niger*, *A. sydowii*, *A. terreus*, *E. nidulans* var. *dentata*, *E. nidulans* var. *lata*, *Penicillium chrysogenum*, *P. puberulum* and *Rhizopus stolonifer.* On the other hand, the most frequently encountered species in Bahreen soils were: *A. alternata*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. sydowii*, *A. terreus*, *E. nidulans*, *E. nidulans var. dentata*, *F. oxysporum*, *Nectria haematococca*,

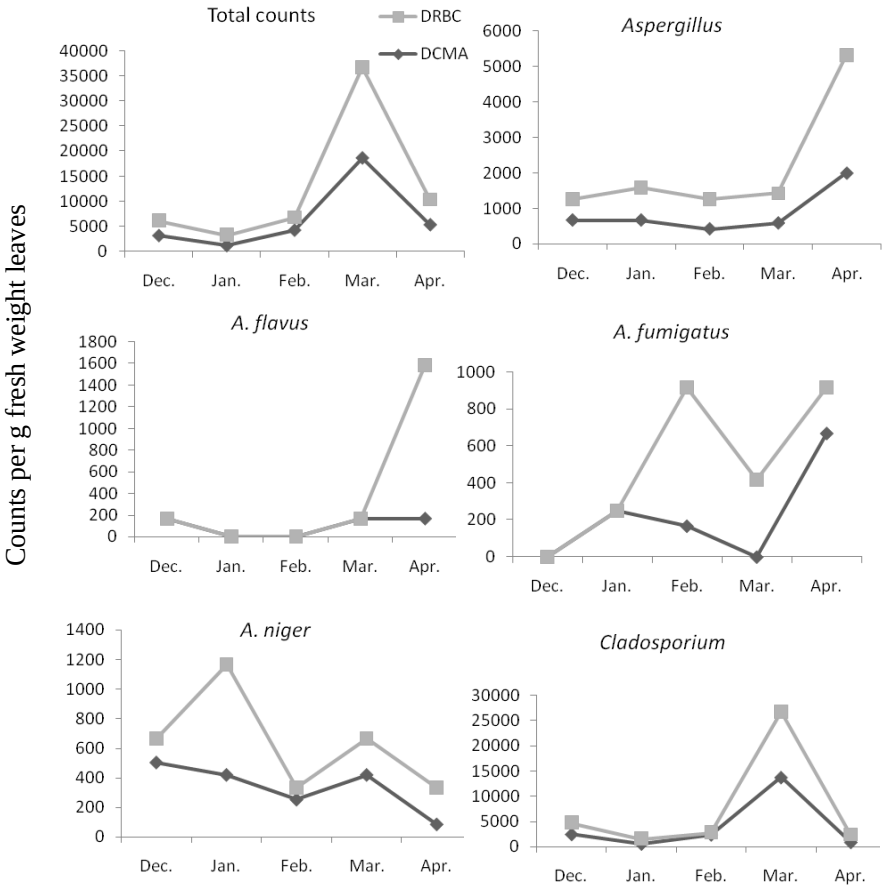

Figure 1. Monthly counts (per g fresh leaves) of common phyllosphere fungi of *Vicia faba* on DCMA and DRBC at 28°C.

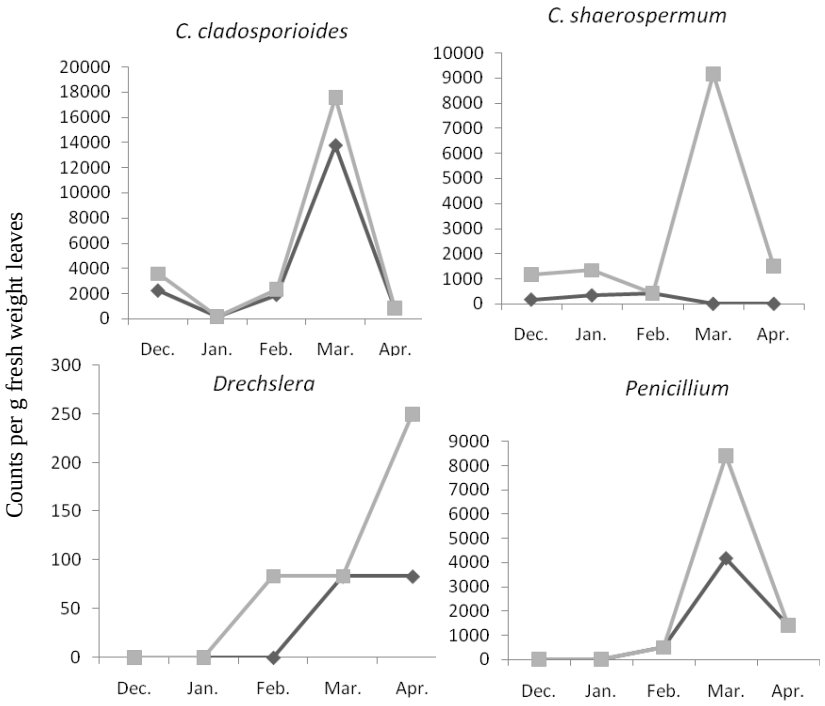

Figure 1. Contd.

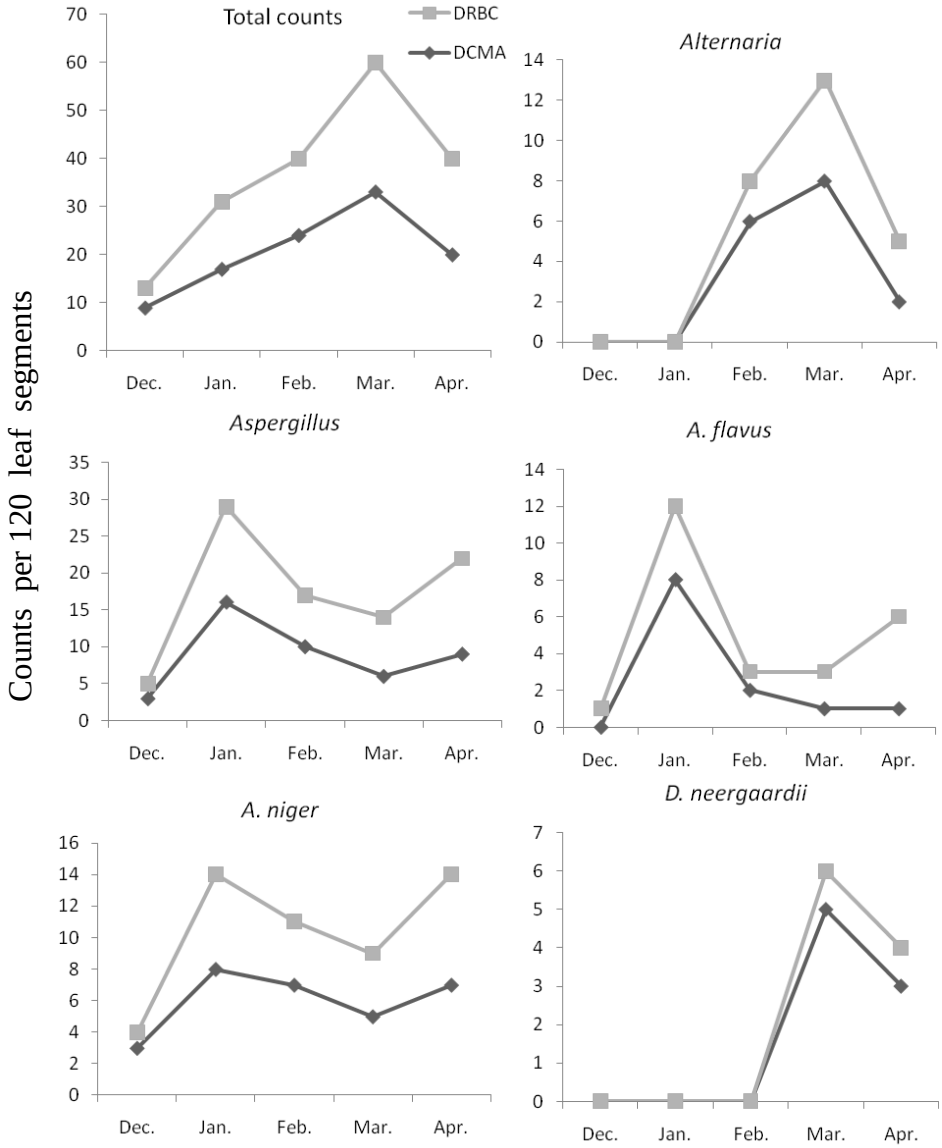

Figure 2. Monthly counts (per 120 leaf segments) of common phylloplane fungi of *Vicia faba* on DCMA and DRBC at 28°C.

Table 2. Total counts (TC, calculated per 30 plates in 10 exposures of 15min), number of cases of isolation (NCI, out of 10) and occurrence remarks (OR) of various fungal genera and species recovered from air of *Vicia faba* field on dicholran chloramphenicol malt extract agar (DCMA) and dichloran Rosebengal chloramphenicol agar (DRBC) at 28°C.

Genera and species	DCMA		DRBC	
	TC	NCI&OR	TC	NCI&OR
Alternaria	221	9H	147	8H
A. alternata (Fries) Keissler	1	1R	-	-
A. chlamydospora Mouehaeca	7	1R	18	1R
A. citri Ellis &Pierce	8	1R	13	3M
A. petroselini (Neergaard) Simmons Comb. nou.	157	7H	56	4M
A. pluriseptata (Karst. & Hart) Jorsted	25	2L	55	4M
A. rahpani Grosves Skolko	-	-	4	1R

Table 2. Contd.

Genera and species	DCMA		DRBC	
	TC	NCI&OR	TC	NCI&OR
A. tenuissima (Kunze:Pers.) Wilshire	6	1R	-	-
A. triticina Prasada & Prabhu	17	1R	1	1R
Aspergillus	108	10H	83	8H
A. flavus Link	9	5M	6	1R
A. fumigatus Fresenius	-	-	14	1R
A. japonicus Saito	-	-	7	1R
A. melleus Yukawa	3	2L	11	3M
A. niger Van. Tieghem	90	10H	40	6H
A. parasiticus Speare	3	2L	1	1R
A. sulphureus (Fres.) Thom & Church	1	1R	3	1R
*A.*sydowii (Bain. & Start.)Thom &Church	1	1R	3	1R
Cladosporium	155	4M	143	5M
C. cladosporioides (Fres.)de Vries	-	-	74	4M
C. sphaerospermum Penzig	155	4M	69	3M
Cochliobolus lunatus Nelson & Haasis	1	1R	-	-
Curvularia richardiae Alcorn	8	3M	4	1R
Drechslera	60	7H	22	6H
D. indica (Rui, Wadhwani & Twari) Mouchacca	2	2R	1	1R
D. neergaardii Danguash	58	7H	21	6H
Emericella nidulans var. *dentata* Sandhu &Sandhu	-	-	13	3M
Epicoccum purpurascens Ehrenb. ex Schlecht	3	2L	-	-
Fusarium	17	3M	121	5M
F. chlamydosporum Wollen weber & Reinking	-	-	15	1R
F. equiseti (Corda) Sacc	-	-	16	1R
F. lateritium Nees' Syst.	-	-	2	1R
F. merismoides Corda	-	-	22	1R
F. oxysporum Schlecht	7	2L	2	1R
F. poae (Peck) Wollenweber	10	2L	9	2L
F. scripi Lambotte & Fautr	-	-	55	2L
Gibberella fujikuroi (Sawada) Ito	2	1R	1	1R
Mucor	25	1R	25	3M
M. circinelloides Van Tiegh	-	-	25	3M
M. hiemalis Wehmer	25	1R	-	-
Penicillium	130	6H	95	4M
P. camembertii Thom	1	1R	1	1R
P. chrysogenum Thom	104	5M	77	3M
P. chorylophilum Dierckx	25	1R	2	1R
P. funiculosum Thom	-	-	15	1R
Stachybotrys parvispora Hughes, Mycol Pap.	6	2L	-	-
Sterile mycelia	8	2L	2	1R
Ulocladium	4	2L	-	-
U. alternariae (Cooke) Simmons	1	1R	-	-
U. chlamydosporum Mouchacca	3	2L	-	-
Total counts	748		654	
Number of genera 14	14		10	
Number of species 40+1 var.	29		32+1var.	

*OR = Occurrence remarks: H = high occurrence from 6-10 cases, M = moderate occurrence from 3-5 cases, L = low occurrence 2 cases and R = rare occurrence 1 case.

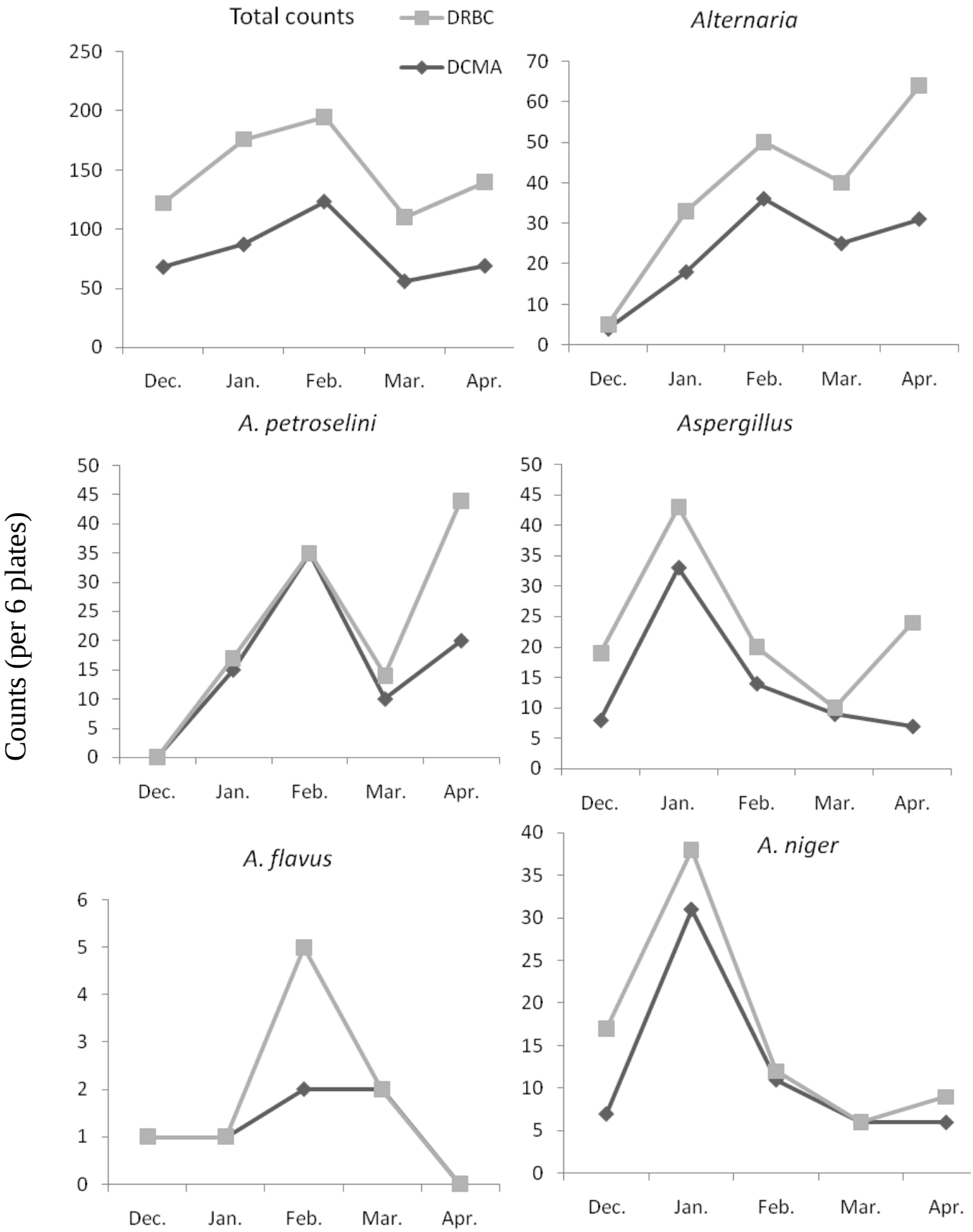

Figure 3. Monthly counts (per 6 plates) of common airborne fungi on DCMA and DRBC at 28°C.

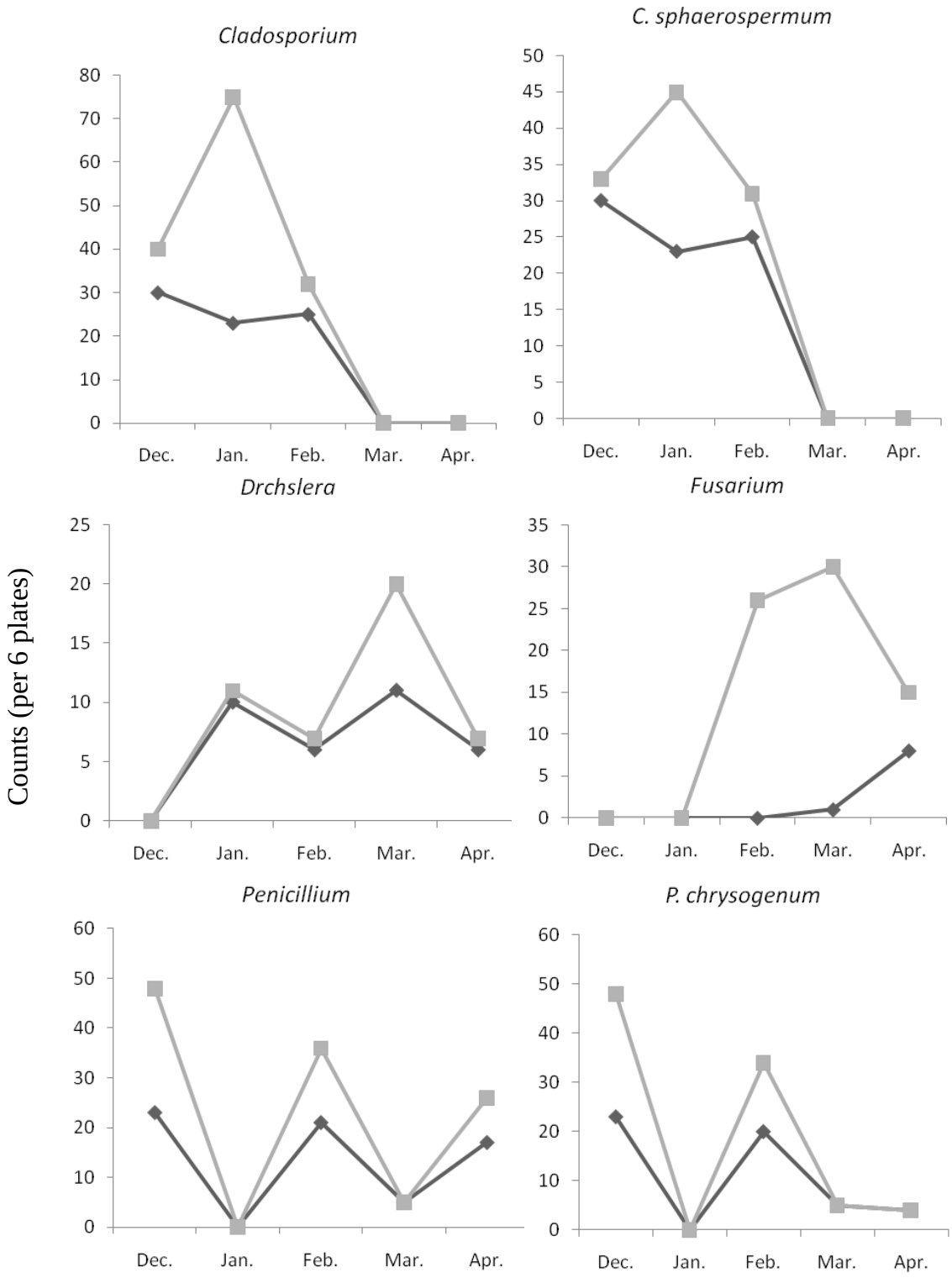

Figure 3. Contd.

P. chrysogenum and *P. corylophilum* (El- Said, 1994). El-Said and Saleem (2008) isolated sixty three species and 5 varieties belonging to 30 genera from cultivated, saline and desert soil in Western region in Libya. The most prevalent species from the three types of soils were: *A. alternata*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *E. nidulans*, *F. oxysporum*, *M. tassiana*, *Nectria haematococca* and *P. chrysogenum.* The above species were isolated

Table 3. Total counts (TC, calculated per 1 g soil), number of cases of isolation (NCI, out of 10) and occurrence remarks (OR) of various fungal genera and species recovered from soil of *Vicia faba* field on dicholran chloramphenicol malt extract agar (DCMA) and dichloran Rose bengal chloramphenicol agar (DRBC) at 28°C.

Genera and species	DCMA		DRBC	
	TC	NCI&OR	TC	NCI&OR
Alternaria	14333.4	3M	10000	2L
A. alternata (Fries) Keissler	3333.3	1R	-	-
A. chlamydospora Mouehaeca	1666.7	1R	-	-
A. petroselini (Neergaard) Simmons Comb. nou.	1666.7	1R	6666.7	2L
A. pluriseptata (Karst. & Hart) Jorsted	1000	1R	3333.3	1R
A. triticina Prasada & Prabhu	6666.6	1R	-	-
Aspergillus	71666.7	6H	205000.2	7H
A. cremeus Kwon & Fennell	1666.7	1R	-	-
A. flavus Link	23333.4	3M	50000	2L
A. fumigatus Fresenius	6666.6	2L	23333.4	3M
A. japonicus Saito	26666.7	2L	25000	1R
A. melleus Yukawa	-	-	1666.7	1R
A. niger Van. Tieghem	13333.3	2L	85000.1	5M
A. parasiticus Speare	-	-	20000	1R
Botryotrichum atrogriseum Van Beyma	-	-	21666.7	2L
Cladosporium	115000.1	8H	61666.7	6H
C. cladosporioides (Fres.)de Vries	98333.4	3M	3333.3	1R
C. cucumerinum Ellis & Arth	3333.4	2L	8333.3	2L
C. oxysporum Berk & Curt	5000	1R	6666.7	1R
C. sphaerospermum Penzig	8333.3	2L	43333.4	3M
Curvularia richardiae Alcorn	1666.7	1R	-	-
Drechslera neergaardii Danguash	6666.6	2L	5000	2L
Emericella nidulans var. *dentata* Sandhu &Sandhu	5000	1R	8333.4	3M
Fusarium	15000.1	4M	41666.8	3M
F. equiseti (Corda) Sacc	-	-	8333.3	1R
F. oxysporum Schlecht	6666.7	1R	6666.7	1R
F. poae (Peck) Wollenweber	5000	2L	11666.7	2L
F. sambucinum Fukel	1666.7	1R	11666.7	1R
F. scripi Lambotte & Fautr	-	-	1666,7	1R
F. tricinctum (Sacc) Corda.	1666.7	1R	1666.7	1R
Gibberella fujikuroi (Sawada) Ito	-	-	1000	1R
Myrothecium	65000	2L	95000	2L
M. roridum Tode ex Fries	1666.7	1R	-	-
M. verrucaria (Alb. & Sch.) Dit.	63333.3	2L	95000	2L
Penicillium	3000	2L	60000	2L
P. chrysogenum Thom	1333.3	1R	60000	2L
P. chorylophilum Dierckx	1666.7	1R	-	-
Stemophyilum botryosum Wallroth	-	-	3333.3	1R
Sterile mycelium	-	-	166.7	1R
Total counts	297333.5		512833.6	
Number of genera 12	9		11	
Number of species 31 + 1 var.	24+1var.		24+1var.	

*OR= Occurrence remarks: H = high occurrence from 6-10 cases, M = moderate occurrence from 3-5cases, L= low occurrence 2 cases and R = rare occurrence 1 case.

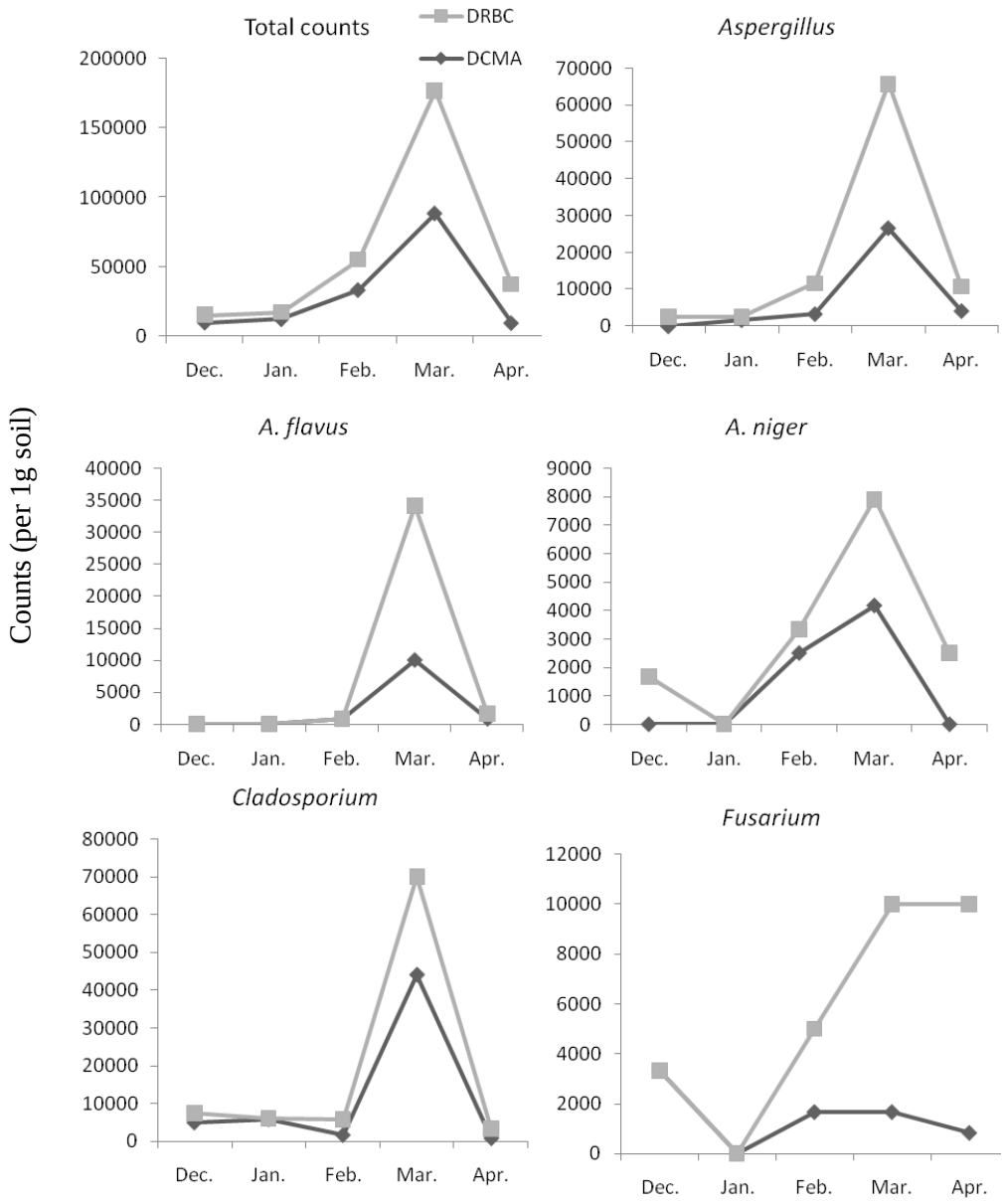

Figure 4. Monthly counts (per 1 g soil) of common soil fungi on DCMA and DRBC at 28°C.

Table 4. Degree of cellulolytic activities (calculated as diameter of clear zone in mm) of the most common fungal isolates.

Fungal isolate	Exo-ß-1,4-glucanase (C_1)	Endo-ß-1,4-glucanase (C_X)
Alternaria citri	29H	21W
A. petroselini	31H	24M
Aspergillus flavus	42H	29H
A. fumigatus	30H	25M
A. niger	26M	23W
Cladosporium cladosporioides	27M	20W
C. sphaerospermum	20W	18W
Drechslera neergaardii	26M	18W
Penicillium chrysogenum	33H	22W

*Activity remarks H = high 29 - 42 mm, M = moderate 24 - 28 mm and W = Week 20 - 23 mm.

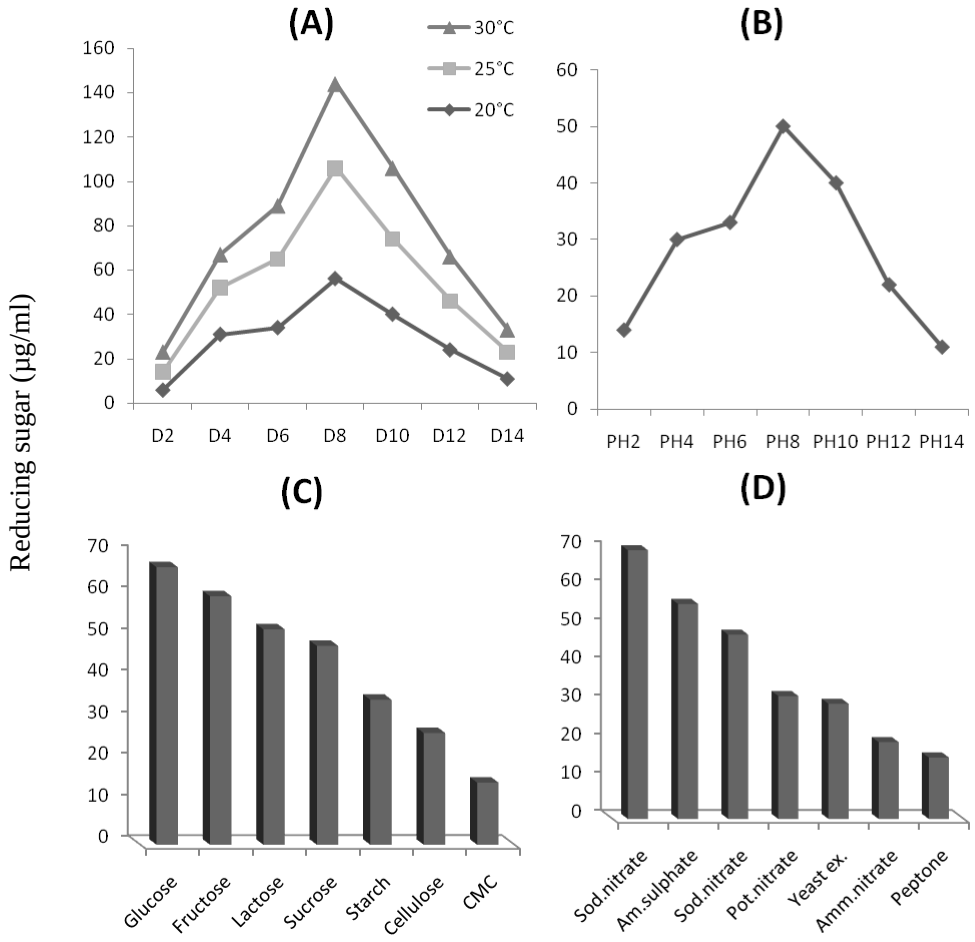

Figure 5. Effect of time course and temperature (A), pH values (B), carbon sources (C) and nitrogen sources (D) on production of exo-ß-1,4-glucnase by *A. flavus*.

with different numbers and frequencies from various soils in many places of the world by several workers (Abdel-Hafez et al., 1990; Moubasher and Mazen, 1991; Abdel-Hafez, 1994; Karl and Iain, 2004 and El-Said and Saleem, 2008).

Cellulolytic activities of some fungal isolates

Nine species (most common species) belonging to 5 genera were screened for their abilities to produce C_1 and C_x enzymes on solid media and proved to be active to utilize cellulose but with different degrees (Table 4). Five isolates (55.6% of total isolates) showed high activity in production of C_1 enzyme only and these were: *A. citri*, *A. petroselini*, *A. flavus*, *A. fumigatus* and *P. chrysogenum*. On the other hand, one fungal isolate exhibited high activity on production of C_X enzyme only and this was *A. flavus.*

Three and two isolates (33.0 and 22.2% of total isolates) were found to be of moderate production of C_1 and C_X enzymes, respectively, while one and six isolates (11.1 and 66.6% of total isolates) were of weak cellulolytic activity. Most of the above fungal isolates were reported as cellulase producers, but with variable capabilities by several workers (Abraha and Gashe, 1992; Abdel-Hafez et al., 1995; Moharram et al., 1995, 2004; Berlin et al.,2005; Rashid et al., 2009; Sohila et al., 2009; Saleem et al. 2010,2013).

A. flavus was the highest fungi in the production of endo and exo-ß-1,4glucanase in this investigation so it was chosen for further studies to achieve the most favorable environmental and nutritional conditions for C_1 and C_x enzymes production.

Maximum production of exo and endo-β-1,4-glucanase by *A. flavus* was obtained after 8 and 6 days of incubition at 30°C with culture media containing glucose and cellulose as a carbon sources and sodium nitrate as nitrogen source and the culture medium was initially adjusted to pH 6 (Figures 5 and 6). These findings are almost in agreement with those reported by El-Said et al. (2006). They found that *F. oxysporum* was the highest fungi in producing endo-β-1,4-glucanses among the 70 tested isolates obtained from 60 samples leaves of *Vicia faba*.

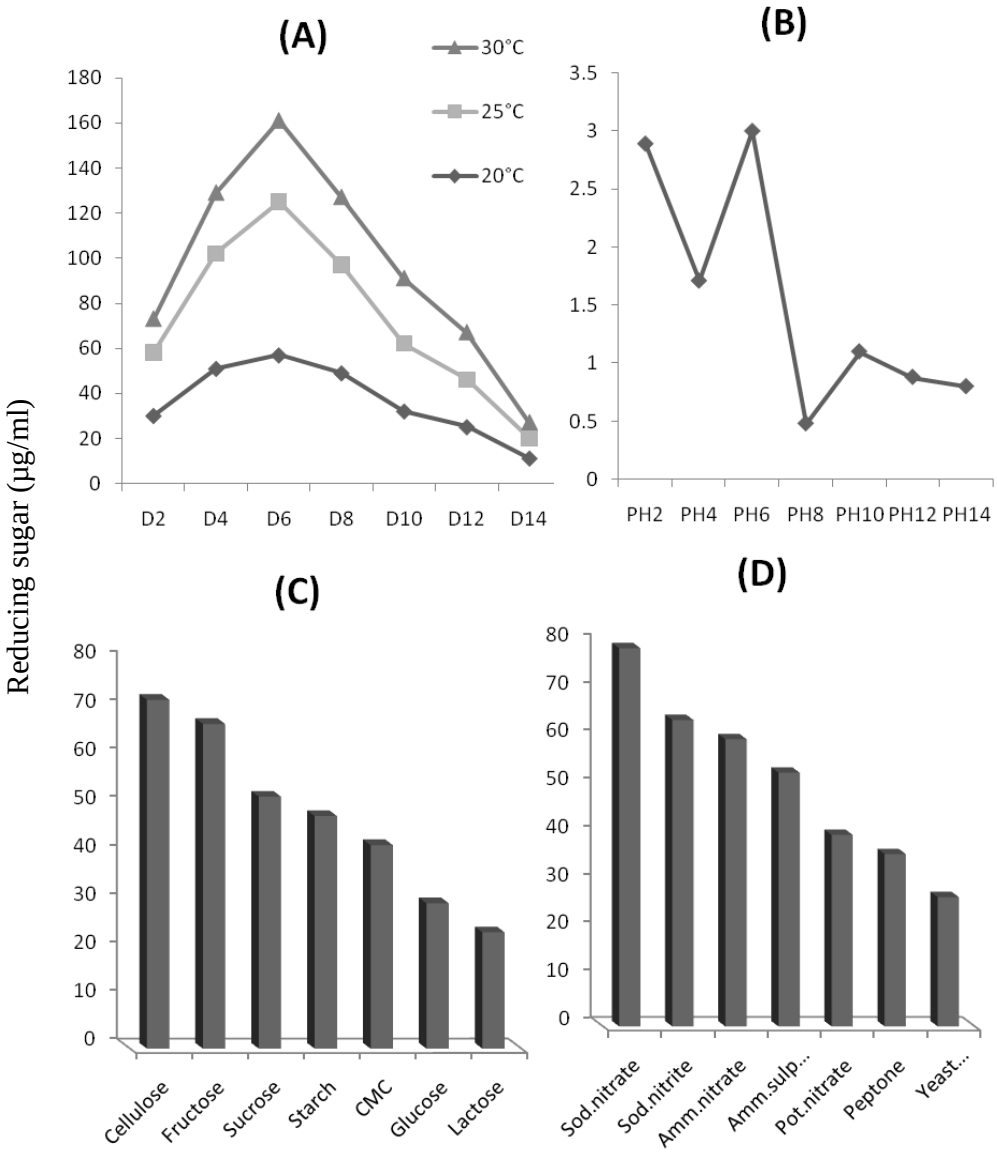

Figure 6. Effect of time course and temperature (A), pH values (B), carbon sources (C) and nitrogen sources (D) on production of endo-ß-1,4-glucnase by *A. flavus*.

Maximum production of endo-β-1,4- glucanses by *F. oxysporum* was achieved after 8 days of incubation at 30°C with culture medium containing carboxymethyl cellulose as carbon and peptone as nitrogen source and initially adjusted to pH 6. Immanuel et al. (2007) studied the effect of environmental factors on production of cellulase enzyme by *A. fumigatus* and *A. niger*. They reported that the optimum pH for cellulase production was 6 to 7 and optimum temperature was about 40°C. El Said and Saleem (2008) found that maximum production of endo-ß-1,4 glucanase by *Cheatomium globosum* was achieved 6 days after incubation at 30°C with incorporation of maltose as carbon source and NH_4NO_3 as nitrogen source in the culture medium which is initially adjusted to pH 6. Recently Saleem et al. (2013) found that maximum production of exo- and endo-ß-1,4 glucanase by *Mucor circinelloides* and *A. flavus* was achieved 6 days after incubation at 30°C with incorporation of fructose or sucrose as a sole carbon source and potassium nitrate or sodium nitrate as a sole nitrogen source, respectively in the basal medium initially adjusted to pH 6.

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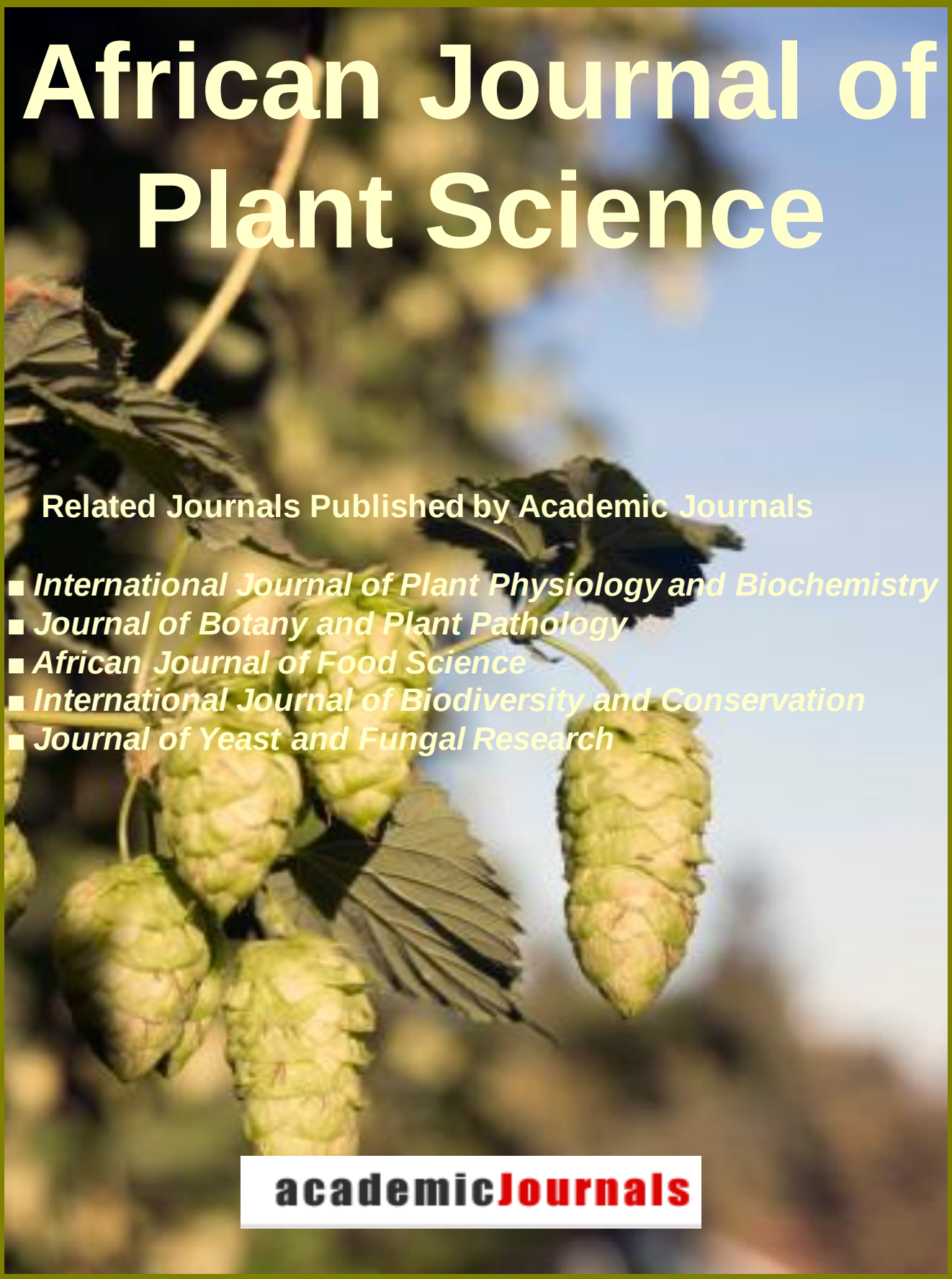

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