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Full Length Research Paper

Biocontrol of *Phytophthora parasitica* and *Fusarium oxysporum* by *Trichoderma* spp. in *Hibiscus sabdariffa* plants under field and greenhouse conditions

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The efficiency of the biological control agent *Trichoderma* spp was tested against *Phytophthora parasitica* and *Fusarium oxysporum* in Roselle plants under field and greenhouse conditions. Under field conditions, three *Trichoderma* doses were applied to the base of the plant, and azoxystrobin and benomyl were applied at 30 and 60 days after planting. Also, 30 day-old plants were inoculated with 7 g of corncob/*Trichoderma* under greenhouse conditions, and inoculated with *F. oxysporum* and *P. parasitica* 3 or 4 days later. Field results showed that *Trichoderma longibrachiatum*-treated plants had 15% less incidence of black shank, and in one of the experiments, calyx dry weight was higher. Under greenhouse conditions, plants inoculated with *Trichoderma gamsii* and *T. longibrachiatum* had between 20 and 30% less incidence of *F. oxysporum* and *P. parasitica*, respectively. Both *Trichoderma* species increased fresh and dry plant weight, and *T. gamsii* also increased plant height. In general, *T. gamsii* and *Trichoderma virens* increased height, fresh and dry weight of Roselle plants infected with *F. oxysporum*.

Key words: *Trichoderma virens*, *Trichoderma longibrachiatum*, *Trichoderma asperellum*, *Trichoderma gamsii*, Roselle.

INTRODUCTION

In Mexico, the Roselle crop (*Hibiscus sabdariffa* L.) is important for its potential uses. Its economic value is due to the calyxes, which are used in making soft drinks, jellies, jams and cosmetics (Mungole and Chaturvedi, 2011). However, diseases have been a limiting factor to the production of Roselle. One of the most important diseases is black shank, caused by *Phytophthora parasitica* var. *nicotianae* and *Fusarium oxysporum* (Amusa et al., 2005; Agbenin and Ogunlana, 2006), which can have an incidence of up to

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40%. The first manifestation of the disease is a necrosis on the neck of the plant, with the roots and foliage affected afterward. As a result of the rot, foliar chlorosis and premature leaf-fall occur (Escalante-Estrada et al., 2001). Although chemical control remains the main strategy to combat these pathogens (Pérez, 2004) some isolates of *F. oxysporum* and *P. parasitica* exhibit resistance to fungicides (Dekker, 1976; Ferrin and Wadswordth, 1992). Currently, there is a search for natural alternatives of plant pathogen control that do not affect human health or the environment (Cohen and Caffey, 1986). One of these alternatives is the use of fungi antagonists, such as *Trichoderma* spp. These antagonists have been commonly used as biocontrol agents (Fernández-Larrea, 2001) due to their versatility, adaptability and ease of handling, in addition to their different control mechanisms, such as mycoparasitism, antibiosis, competition for space and nutrients, and induction of resistance (Mukherjee et al., 2013). Their antagonistic spectrum is broad and can affect the growth of several plant pathogens, such as *Fusarium solani, Macrophomina phaseolina, Pythium aphanidermatum* and *Alternaria solani* (Saran-Sundar et al., 2013). However, there has been no research on the effect of *Trichoderma* against black shank in Roselle plants.

The aim of this study was to evaluate the biological efficiency of *Trichoderma* spp. against the black shank incidence in Roselle plants caused by *P. parasitica* and *F. oxysporum* under field and greenhouse conditions.

MATERIALS AND METHODS

Locations and sampling sites

Field experiments were established in two plots (Plots 1 and 2) in Xalpatlahuac, Guerrero. The greenhouse experiment was conducted at the Superior Agricultural College of the State of Guerrero (CSAEGRO) located in Cocula, Guerrero, Mexico. *P. parasitica* and *F. oxysporum* were isolated from Roselle plants (*H. sabdariffa* L.) with typical symptoms of black shank. The Booth (1971) and Leslie and Summerell (2006) keys were used to identify *F. oxysporum,* while *P. parasitica* was identified using the Erwin and Ribeiro (1996) keys. The evaluated *Trichoderma* strains were T6 (*Trichoderma virens*), T9 (*Trichoderma asperellum*), T13 (*Trichoderma gamsii*), and T19 (*Trichoderma longibrachiatum*), isolated from Roselle plots and identified morphologically and molecularly.

Preparation of inocula

F. oxysporum was cultured on Potato dextrose agar (PDA) and kept at 25°C for 8 days. Once the inoculum increased, to prevent a possible loss of pathogen virulence, 50 ml of liquid PDA were placed in a flask (250 ml) with small pieces of root of Roselle seedlings, which had been previously autoclaved for 15 min at 121°C. Afterward, five slices (1 cm of diameter) with mycelial growth were added to the flasks, and stirred for 4 days in order to induce the production of conidia. *P. parasitica* was propagated as follows: Six discs with active mycelial growth (1.5 cm of diameter) were placed in Petri dishes with 20 ml of sterile distilled water and soil, and incubated under a 12/12 h dark/light cycle at 25°C during 3 days to stimulate sporangia formation. The release of the zoospores was achieved after placing the sporangia at 4°C for 30 min. A hemocytometer was used to adjust the zoospore concentration to 1×10^4 ml $^{-1}$ (Escalante-Estrada et al., 2001).

Trichoderma spp. was increased using 2 kg capacity bags filled with 400 g of maize cob, and the strains were propagated under the conditions proposed by Michel-Aceves et al. (2008). This material was used to inoculate greenhouse and field plants

Experimental design and data analysis

Field experiments were established under a randomized complete block design with five replicates. Six treatments were used: T9, T6, T13, and T19 strains; chemical controls (TQ: benomyl and azoxystrobin); and an uninoculated control. The experimental unit consisted of a plot of 7 rows, 5 m long each, with 80 cm between rows and 80 cm between plants. The greenhouse experiment was established under a completely randomized design. Six treatments (T9, T6, T13, T19, *F. oxysporum* or *P. parasitica* and an uninoculated control) were evaluated with 20 replicates (calculated using Minitab 15 software, 2007), with a total of 120 experimental units (one plant per pot) for each pathogen (*P. parasitica* and *F. oxysporum*). Data were analyzed using analysis of variance (ANOVA). Incidence data were transformed using the arcsine function, and when needed, means were compared using Tukey multiple range test ($P < 0.05$) using the Statistical Analysis System (SAS) version 9.0 statistical program.

Evaluation of *Trichoderma* spp. under field conditions

Two plots with history of presence of *P. parasitica* and *F. oxysporum* were selected, and the trial was established in autumn-winter (2012). Roselle seeds were planted in June, and the plots were immediately inoculated with 7 g of cob of each *Trichoderma* species and conidia concentration of each treatment was calculated per 10 g of cob (T6: 1×10^3, T13: 1.4×10^3; T9: 6.3×10^5, T19: 2.5×10^5). A second application was carried out a month later (August 16th and 17th) as follows: a small trench was made around the base of the plant and 7 g of cob with *Trichoderma* growth were added and benomyl was applied (0.5 kg ha^{-1}) afterward. In September, the third inoculation of each treatment with *Trichoderma* was carried out, and on the same date, the chemical treatment (azoxystrobin) was applied in the corresponding experimental units, according to the product's directions (2.5 L ha^{-1}).

Factors related to the black shank under field conditions

Soil samples were taken, and their physical and chemical properties were analyzed in the Central Laboratory at the Chapingo University. Relative humidity and temperature were recorded in a data storage unit (data logger) Extech TH10 Temperature Data Logger USB®.

Evaluation of *Trichoderma* spp. against *Phytophthora parasitica* and *Fusarium oxysporum* in greenhouse

Roselle seeds collected in the Tecoanapa, Guerrero area were used. These seeds were disinfected with 3% NaCl and planted in pots (1 L) with peat moss. After 35 days, the seedlings were inoculated with 7 g of cob + *Trichoderma mycelia* (*T. asperellum*, *T. virens*, *T. gamsii*, and *T. longibrachiatum*). Three days later, *P. parasitica* was inoculated by applying 10 ml of a suspension of

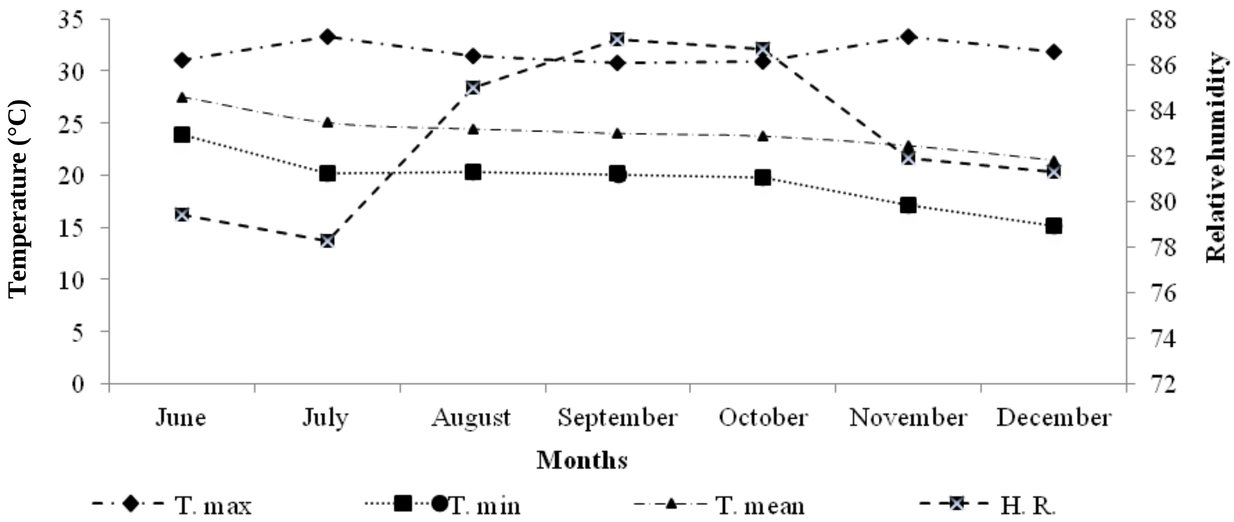

Figure 1. Average maximum, minimum temperature data and relative humidity average of each month (2012). T. Max = maximum temperature, T. Min = minimum temperature, T. mean = average temperature, H. R = relative humidity.

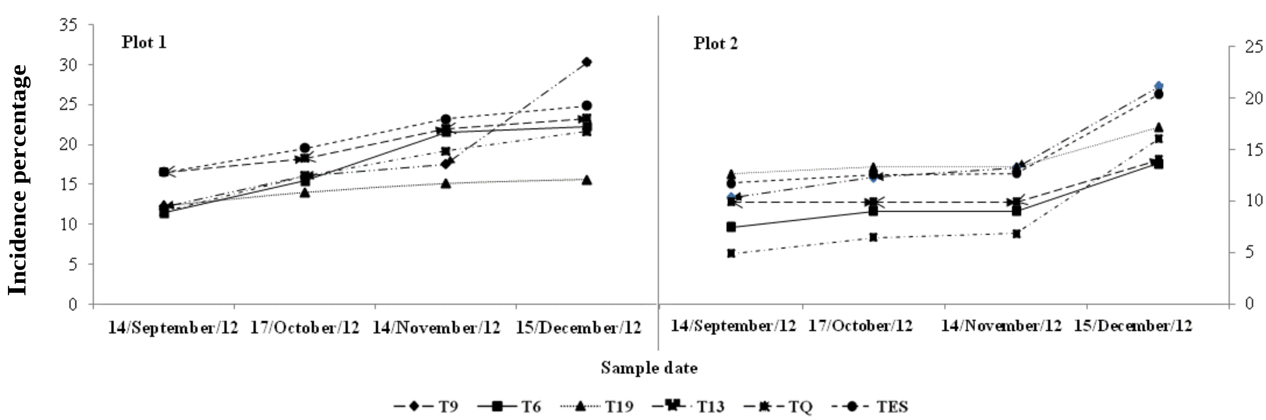

Figure 2. Incidence percentage of black shank in Roselle crops under field conditions, in two Plots (1 and 2). T9 (*T. asperellum*), T6 (*T. virens*), T13 (*T. gamsii*), T19 (*T. longibrachiatum*), TQ (chemical treatment) and TES (Control - not applied).

zoospores to the basal part of the plant at a concentration of 1 × 10^4 zoospores mL^{-1}. Before being inoculated with *F. oxysporum,* root tips were cut to increase infection. For inoculation, a conidial suspension 5 × 10^6 conidia mL^{-1} was used. Inoculated plants were left in the greenhouse at a temperature of 25 ± 2°C, and watered every 3rd day.

Response variables

In field experiments, disease incidence, plant height, stem diameter, and fresh and dry weight of the calyx (yield) at the end of the crop cycle (December 10th) were evaluated. Incidence data was collected three times: from the time diseased plants were observed, and every 20 days until harvest (December 10th). This was used to obtain the cumulative incidence. Under greenhouse conditions, the response variables were incidence (evaluated 8 days after the inoculation of each pathogen), stem diameter, plant height, root length, and fresh and dry weight of the plant. Fresh and dry weight was measured 65 days after inoculation.

RESULTS AND DISCUSSION

Factors related to black shank under field conditions

Temperature is a factor related to presence of black shank, so temperatures were collected each month from June through December. Temperatures ranged from 15 to 33°C (Figure 1). The minimum and average temperatures decreased over time, while the maximum temperature was kept constant. Another important factor was relative humidity, which varied from 78 to 87% (Figure 1). On the other hand, black shank increased over time in two locations (Figure 2). This is attributed to changes in temperature and relative humidity, in addition

Table 1. pH and fertility in evaluated plots soil.

Lot	pH	MO*	N**	P**	K**	Ca**	Mg**	Fe**	Cu**	Zn**	Mn**	B**	Arena*	Limo*	Clay*	Texture
1	4.35	1.34	77.9	12.90	52	292	45	47.48	0.46	0.21	5.87	0.51	70.2	8.0	21.8	FAA
2	4.36	0.67	144.7	7.67	84	515	201	121.02	5.10	1.54	37.41	0.42	52.2	22.0	25.8	FAA

MO, Organic matter; FAA, Franco sandy clay; *, in %; **, in $mg.Kg^{-1}$.

to the variability of rainfall, which also influenced disease incidence. Jacobi et al. (1983) reported that there is a correlation between incidence of *P. parasitica* var. *nicotianae* in tobacco and temperature, precipitation conditions (suitable conditions for pathogens) and drought (which stresses the plant).

Temperature and relative humidity are factors that impact the development of *Trichoderma*, as reported by Infante et al. (2009), because chlamydospores have more than 75% of germination under optimal conditions of humidity (> 75%) and temperature (28 - 30°C), similar events occurred in this study (Figure 1). Furthermore, Papavizas (1985) indicated that nutritional requirements in filamentous fungi are minimal and growth is favored by organic matter, humidity and optimum temperatures between 25 - 30°C. Other authors such as Widden and Scattolin (1988) and Jackson et al. (1991) mentioned that *Trichoderma* will adapt and survive in extreme conditions of temperature, pH and salinity. Soil and organic matter have a profound influence on phytopathogens growth (Singh and Sachan, 2013). Because of this relationship, soil characteristics were analyzed in this study. pH and texture were very similar in both locations (Table 1), and higher levels of nutrients were observed in Plot 2, however, Plot 1 showed higher amounts of organic matter. Kincaid et al. (1970) reported that a soil pH between 5 and 6 and high levels of potassium are correlated with the presence of black shank in tobacco caused by *P. parasitica* var. *nicotianae*. These results differ from those obtained in this work because the soil pH was 4.5. The highest incidence of black shank was found in Plot 1, where high levels of potassium were detected. Gallup et al. (2006) mentioned that black shank caused by *P. nicotianae* (*P. parasitica*) is favored for pH values higher than 6.2, and also mentioned that a soil pH between 5.5 and 6.0 will provide favorable growing conditions for tobacco, without providing favorable conditions for *P. nicotianae*.

Evaluation of *Trichoderma* spp. under field conditions

The results obtained in Plot 1 for black shank incidence showed significant differences ($P \leq 0.0001$) between treatments, ranging from 15 to 33% (Table 2). It was found that *T. asperellum* and the uninoculated control had the highest incidence of the disease (33.9 and 25%), although there were no significant differences among treatments with the same variable in Plot 2. The plants treated with *T. gamsii* and *T. virens* showed lower incidences of the disease compared to the other treatments (Table 2). However, the chemical treatment (QT) was not different from the control in the incidence of black shank. Some reports indicated that benomyl (Benlate ® 50 WP) applied to the soil decreased the incidence of *F. oxysporum*, and also increased the growth of pea (Pabón-Villalobos and Castaño-Zapata, 2012), which differs from the results observed in this work. Trillas et al. (2006) indicated that *T. asperellum* T34 requires to be combined with compost and is more effective against *Rhizoctonia solani*, decreasing up to 29% of its effect on cucumber plants. Additionally, studies on strawberry indicated that applying *Trichoderma* spp. reduced *Phytophthora cactorum* incidence in 76.6% compared with the untreated control in the 1st year of implementation. Furthermore, soil solarization and application of *Trichoderma* spp. alone or in combination, reduced rot incidence by 30.8 and 36.9%, respectively, relative to the untreated control at 2 years evaluation (Porras et al., 2007). One of the novel observations in this study was that the application of *Trichoderma* accelerated flowering in Roselle plants on 8 days, compared to control treatments and TQ.

Likewise, height, plant diameter, and fresh and dry weight of the calyx showed no significant difference among treatments ($P \leq 0.07$) in the tested plot (Tables 2 and 3). That differs from data obtained by Sivan et al. (1987), where they reported that the yield of tomato in plots treated with *Trichoderma harzianum* had an increase of 26.2%. When making comparisons between the two plots, the F-test in the ANOVA showed that height, and fresh and dry weight of the calyx differed among treatments ($P \leq 0.0001$). However, no differences in plant diameter were observed between locations ($P \leq 0.08$) (Tables 2 and 3). Our results differ from those reported by Jabnoun-Khiareddine et al. (2009). They mentioned that

Table 2. Means comparison on disease incidence, plant height, stem diameter, fresh and dry calyx in Roselle plants inoculated with *Trichoderma* spp. on plot 1.

Treatment	Incidence (%)	Height of plant (cm)	diameter stem (cm)	Calyx fresh weight (kg)	Calyx dry weight (kg)
T9	33.97^{az}	183.70^{ab}	1.44^{a}	9.32^{a}	1.260^{a}
T6	22.28^{ab}	191.84^{a}	1.54^{a}	10.78^{a}	1.331^{a}
T13	21.64^{ab}	182.70^{ab}	1.54^{a}	10.10^{a}	1.261^{a}
T19	15.58^{b}	176.42^{ab}	1.47^{a}	9.34^{a}	1.264^{a}
TQ	23.32^{ab}	175.00^{ab}	1.47^{a}	8.58^{a}	1.132^{a}
Control	25.05^{ab}	170.68^{b}	1.46^{a}	8.44^{a}	1.076^{a}

^{z}, Means within each column followed by the same letter are statistically equal (Tukey 0.05); T9, *T. asperellum*; T6, *T. virens*; T13, *T. gamsii*; T19, *T. longibrachiatum*; TQ, chemical treatment; Control (not applied).

Table 3. Means comparison on the variables incidence, height plant, diameter stem, fresh and dry calyx in Roselle plants inoculated with *Trichoderma* spp. on Plot 2.

Treatment	Incidence (%)	Height of plant (cm)	Diameter stem (cm)	Calyx fresh weight (kg)	Calyx dry weight (kg)
T9	14.24^{a}	193.40^{a}	1.34^{b}	6.64^{a}	0.872^{a}
T6	9.75^{a}	201.53^{a}	1.59^{a}	6.20^{a}	0.796^{a}
T13	8.53^{a}	203.43^{a}	1.41^{ab}	6.36^{a}	0.800^{a}
T19	14.06^{a}	200.06^{a}	1.47^{ab}	6.60^{a}	0.794^{a}
TQ	10.93^{a}	196.46^{a}	1.37^{ab}	6.10^{a}	0.770^{a}
Control	14.28^{a}	192.43^{a}	1.40^{ab}	6.10^{a}	0.749^{a}

^{z}, Means within each column followed by the same letter are statistically equal (Tukey 0.05); T9, *T. asperellum*; T6, *T. virens*; T13, *T. gamsii*; T19, *T. longibrachiatum*; TQ, chemical treatment; Control (not applied).

Table 4. Influence of *Trichoderma* spp. on incidence, growth and biomass in Roselle plants inoculated with *Phytophthora parasitica* in greenhouse at 60 days-old after inoculation.

Treatment	Incidence (%)	Height (cm)	Diameter stem (mm)	Root length (cm)	Plant fresh weight (g)	Plant dry weight (g)
P. parasítica	45^{az}	84.92^{ba}	0.52^{ba}	21.38^{a}	10.81^{b}	2.80^{c}
T9 + *P. par*	30^{c}	77.03^{bc}	0.57^{a}	21.85^{a}	15.16^{ba}	5.16^{cb}
T6 + *P. par*	40^{b}	87.50^{ba}	0.58^{a}	17.07^{a}	15.76^{ba}	5.02^{cb}
T19 + *P. par*	30^{c}	90.65^{ba}	0.51^{ba}	23.50^{a}	14.24^{ba}	6.05^{b}
T13 + *P. par*	20^{d}	56.52^{c}	0.54^{ba}	21.36^{a}	16.59^{ba}	6.18^{b}
Control	0^{e}	104.17^{a}	0.45^{b}	46.01^{a}	18.59^{a}	9.42^{a}

^{z}**,** Means within each column followed by the same letter are statistically equal (Tukey 0.05); ***P. par*****,** *P. parasitica*; **T9,** *T. asperellum*; **T6,** *T. virens*; **T13,** *T. gansii*; **T19,** *T. longibrachiatum*; Control (water).

tomato inoculated with *T. harzianum, T. virens* and *Trichoderma viride* reduced *Verticillium* spp incidence, increasing fresh root and stem weight by 50%. Additionally, Okoth et al. (2011) mentioned that *T. harzianum* combined with triple superphosphate and calcium ammonium nitrate increased seed germination of beans and corn, and also it increased stem diameter, roots and shoots growth.

Trichoderma spp., as a biological control agent of *Phytophthora parasitica* on Roselle plants in greenhouse

Roselle plants inoculated with *P. parasitica* showed typical symptoms between the 4th and 8th day after inoculation. These data are consistent with those reported by Escalante-Estrada et al. (2001) where 30 strains of *P. parasitica* were tested in Roselle plants. They also mentioned that the duration of the symptoms was variable (11 to 22 days) and that there were differences in the size of necrotic lesions caused by the evaluated strains. *P. parasitica* incidence on plants showed significant differences among biological treatments ($P \leq 0.0001$). *Trichoderma* species *T. gamsii* (20%), *T. longibrachiatum* (30%) and *T. asperellum* (30%), caused reduction of *P. parasitica* incidence in Roselle plants (Table 4), and this is attributed to the action of *Trichoderma* as a bio-control agent of several

Table 5. Influence of *Trichoderma* spp. on incidence, growth and biomass in Roselle plants inoculated with *Fusarium oxysporum* in greenhouse at 60 days-old after inoculation.

Treatment	Height (cm)	Diameter stem (mm)	Root length (cm)	Fresh weight plant (g)	Plant dry weight (g)
F. oxysporum	55.22^{cbz}	5.00^{a}	26.60^{b}	17.11^{ba}	3.32^{b}
T9 + *F. oxy*	44.08^{d}	4.45^{a}	24.40^{b}	12.73^{c}	2.87^{b}
T6 + *F. oxy*	54.98^{cb}	4.95^{a}	26.35^{b}	15.80^{bc}	2.95^{b}
T19 + *F. oxy*	47.14^{cd}	4.85^{a}	23.20^{b}	14.77^{bc}	4.06^{ba}
T13 + *F. oxy*	57.13^{b}	5.15^{a}	26.75^{b}	15.69^{bc}	3.55^{b}
Control	76.33^{a}	4.95^{a}	32.30^{a}	20.62^{a}	5.19^{a}

^{z}, Means within each column followed by the same letter are statistically equal (Tukey 0.05); F. oxy, *F. oxysporum*; T9, *T. asperellum*; T6, *T. virens*; T13, *T. gamsii*; T19, *T. longibrachiatum*; Control (water).

pathogens (Harman, 2006). Studies in other crops and other pathogens carried out by Hoyos-Carvajal et al. (2008), mentioned that *T. asperellum* (T21 and T7) reduced the incidence of *Sclerotium rolfsii* by 90% on bean seedlings, but not in the same proportion to that obtained in this work. However, these results differ from those obtained by Fernández-Herrera et al. (2007), who evaluated four commercial biological products containing *Trichoderma* spp, *Bacillus* and mycorrhizae, but did not find a reduction of the incidence of *Phytophthora capsici* on tomato plants, all of which showed the disease.

Furthermore, Roselle plants treated with *T. virens* and *T. asperellum* showed greater stem diameter (Table 4) compared to other treatments. Plants treated with *T. gamsii* and *T. longibrachiatum* showed a smaller stem diameter, which indicates that this response differs among the *Trichoderma* species; these data are consistent with those reported by Azarmi et al. (2011). There were significant differences in fresh and dry plant weight among treatments (P ≤ 0.0001). It was noted that the fresh weight increased by 30% in plants inoculated with *Trichoderma*, while in plants inoculated with *T. gamsii* and *T. longibrachiatum*, dry weight increased by 54.2%. These results were similar to those obtained by Hoyos-Cabral et al. (2008), who reported an increase of 92% of foliar dry weight, and in general in aerial structures of potato plants inoculated with two strains of *T. asperellum* (T84 and T109) against *Spongospora subterranea* f. sp. *subterranea*. Ageeb and Mohamed (2012) evaluated the same variables in maize plants inoculated with *T. harzianum* T22, and found an increase of foliar weight compared to control plants. These authors also mentioned that *Trichoderma* concentrations in the soil were positively correlated with growth variables.

Trichoderma as biological control agent against *Fusarium oxysporum* on Roselle plants in greenhouse

F. oxysporum is a cosmopolitan fungus that exists in a wide variety of pathogenic forms (Ploetz, 2006). By inoculating *F. oxysporum* in Roselle plants, chlorotic and stunted plants were observed. Similar results were obtained by Ploetz and Palmateer (2007), who also observed widespread necrosis and wilting in Roselle plants, although these results differ with those obtained by Sylvère et al. (2011), who tested two varieties of Roselle (*sabdariffa* and *altissima*) and found that *sabdariffa* is resistant to *F. oxysporum* attack, which he attributed to the amount of phenolic compounds the variety exhibited, compared with *altissima*. In this investigation, it was observed that *F. oxysporum* does not cause black shank, which differs from that obtained by Amusa et al. (2005), who mentioned that *F. oxysporum* is the main causative agent of black shank in Roselle plants, with an incidence of 26% under field conditions. There were significant differences in plant height among treatments (P ≤ 0.0001), plants treated with *T. gamsii* were 3.1% taller than control plants. The control showed differences with all treatments in all evaluated variables (Table 5), which is attributed to the clear negative effect that *F. oxysporum* has on Roselle growth, compared with uninoculated control (Agbenin and Ogunlana, 2006). Stem diameter and root length had no significant difference among treatments (P ≤ 0.14). It suggests that these variables do not determine *Trichoderma* influence on pathogen control. This contrast with that obtained by Jiménez et al. (2011) who indicated that the application of *T. harzianum* in seedlings or transplants can cause an increase in the growth of tomato plants and the development of root system, because of the production of secondary compounds that stimulate and increase the ability of roots to get nutrients. Fresh and dry plant weight showed significant differences among treatments (P ≤ 0.0001), notably in *T. virens*, *T. gam*sii and *T. longibrachiatum*-treated plants (Table 5). Badar and Qureshi (2012) indicated that *T. hamatum*, alone and/or in combination with *Rhizobium* increased the growth of sunflower plants, enhancing their root length and shoots biomass.

Conclusions

Plants treated with *T. l ongibrachiatum* showed lower (15%) black shank incidence and in one location, calyx dry weight was higher under field production.

In greenhouse, *T. gamsii* and *T. longibrachiatum* reduced *P. parasitica* incidence and increased fresh and dry plant weight. In plants infected with *F. oxysporum*, the inoculation of *T. gamsii* and *T. virens* increased their height and fresh and dry plant weight.

Conflict of Interests

The authors have not declared any conflict of interests.

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REFERENCES

Agbenin NO, Ogunlana MO (2006). Occurrence of Fusarium wilt and nematodes on red calyx Roselle (*Hibiscus sabdariffa* L.) in Northern Nigeria. J. Plant Protect. Res. 46(2):117-121.

Ageeb AS, Mohamed S (2012). Application of *Trichoderma harzianum* T22 maize as a biofertilizer supporting growth. Afr. J. Biotechnol.11(35):8672-8683.

Amusa NA, Adegbite AA, Oladapo MO (2005). Vascular wilt of Roselle (*Hibiscus sabdariffa* L. var. sabdariffa) in the humid forest region of South-western Nigeria. Plant Pathol. J. 4(2):122-125. http://dx.doi.org/10.3923/ppj.2005.122.125

Azarmi R, Hajieghrari B, Giglou A (2011). Effect of Trichoderma isolates on tomato seedling growth response and nutrient uptake. Afr. J. Biotechnol. 10(31):5850-5855.

Badar R, Qureshi SA (2012). Use of *Trichoderma hamatum* alone and in combination with Rhizobial isolates as bio-fertilizer for improving the growth and strength of sunflower. J. Basic Appl. Sci. Res. 2(6):6307-6314.

Booth C (1971). The genus Fusarium. Common wealth Institute Mycology. Kew, Surrey, United Kingdom. P. 237.

Cohen Y, Coffey MD (1986). Systemic fungicides and the control of Oomycetes. Annu. Rev. Phytopathol. 24:311-38. http://dx.doi.org/10.1146/annurev.py.24.090186.001523

Dekker J (1976). Acquired resistance to fungicides. Annu. Rev. Phytopathol.14:405-428. http://dx.doi.org/10.1146/annurev.py.14.090176.002201

Erwin DC, Ribeiro OK (1996). Phytophthora Diseases Worldwide. The American Phytopathological Society. St. Paul, Minnesota, USA. P. 562.

Escalante-Estrada YI, Osada-Kawasoe S, Escalante-Estrada JA (2001). Pathogenic Phytophthora parasitica variability in Roselle (*Hibiscus sabdariffa* L.). Mexican J. Phytopathol. 19:84-89 (Spanish).

Fernández-Herrera E, Acosta-Ramos M, Ponce-González F, Manuel Pinto V (2007). Biological management of Phytophthora capsici Leo, *Fusarium oxysporum* Schlechtend. Br. and Rhizoctonia solani Kühn in tomato (*Lycopersicon esculentum* Mill.) Mexican J. Phytopathol. 25:35-42 (Spanish).

Fernández-Larrea VO (2001). Antagonistic microorganisms for phytosanitary control. Integrated Pest Management (Costa Rica), 62:96-100 (Spanish).

Ferrin DM, Wadswordth ML (1992). Effect of metalaxyl on sporulation and growth of metalaxyl-resistant and metalaxyl-sensitive isolates of Phytophthora parasitica. Plant disease, 76:492-495. http://dx.doi.org/10.1094/PD-76-0492

Gallup CA, Sullivan MJ, Shew HD (2006). Black shank of tobacco. The Plant Health Instructor. DOI: 10.1094/PHI-I-2006-0717-01. Retrieved 11/09/12 http://www.apsnet.org/edcenter/intropp/lessons/fungi/oomycetes/Pages/BlackShank.aspx.http://dx.doi.org/10.1094/PHI-I-2006-0717-01

Harman GE (2006). Overview of mechanisms and uses of Trichoderma spp. Phytopatholy 96:190-194.http://dx.doi.org/10.1094/PHYTO-96-0190

Hoyos-Carvajal CL, Jaramillo VMS, Orduz PS (2008). Evaluation of *Trichoderma asperellum* as bioregulator Spongospora subterranea f.sp. subterranea. Rev. Fac. Nat. Agric. Medellín 61(2):4496-4502.

Infante D, Martínez B, González N, Reyes Y (2009). Mechanisms of action of Trichoderma against phytopathogenic fungi. Protect. Rev. Veg. 24 (1):14-21.

Jabnoun-Khiareddine H, Daami-Remadi M, Ayed F, El Mahjoub M (2009). Biological control of tomato Verticillium wilt by using indigenous Trichoderma spp. African J. Plant Sci. Biotechnol. 3(1):26-36.

Jackson AM, Whipps JM, Lynch JM (1991). Effects of temperature, pH and water potential on growth of four fungi with disease biocontrol potential. World J. Microbiol. Biotechnol. 7:494-501. http://dx.doi.org/10.1007/BF00303376

Jacobi WR, Manin CE, Powell NT (1983). Influence of temperature and rainfall on the development of tobacco black shank. Phytopathology, 73:139-143. http://dx.doi.org/10.1094/Phyto-73-139

Jiménez C, Sanabria Albarracin N, Altuna G, Alcano M (2011). Effect of *Trichoderma harzianum* (Rifai) on the growth of tomato plants (*Lycopersicon esculentum* L.). Rev. Fac Agron. 28(1):1-10 (Spanish).

Kincaid RR, Martin FG, Gammon NJ, Breland HL, Pritchett WL (1970). Multiple regression of tobacco black shank, root knot, and coarse root index on Soil pH, potassium, calcium, and magnesium. Phytopathology 60:1513-1516. http://dx.doi.org/10.1094/Phyto-60-1513

Leslie JF, Summerell BA (2006). The Fusarium Laboratory, Manual. Blackwell Publishing. Ames, Iowa, USA. pp. 388-389. http://dx.doi.org/10.1002/9780470278376

Michel-Aceves AC, Otero-Sanchez MA, Rojero-Martínez RD, Rodríguez-Morán NL, Ariza-Flores R, Barrios-Ayala A (2008). Mass production of Trichoderma harzianum Rifai in different organic substrates. Revista Chapingo Serie Horticulture, 14(1):185-191 (Spanish).

Mukherjee PK, Horwitz BA, Herrera-Estrella A, Schmoll M, Kenerley CM (2013). Trichoderma Research in the Genome Era. Annu. Rev. Phytopathol., 51:105-29.http://dx.doi.org/10.1146/annurev-phyto-082712-102353

Mungole A, Chaturvedi A (2011). Hibiscus sabdariffa L. a rich source of secondary metabolites. Int. J. Pharmaceutical Sci. Rev. Res. 6 (1):83-87.

Okoth SA, Otadoh JA, Ochanda JO (2011). Improved seedling emergence and growth of maize and beans by *Trichoderma harzianum*. Tropical Subtropical Agroecosystems 13:65-71 65.

Pabón-Villalobos J, Casta-o-Zapata J (2012). Management of root rot of pea (*Pisum sativum* L.) caused by *Fusarium oxysporum* Schlechtend. Fr. agron. 20(2):37-44 (Spanish).

Papavizas GC (1985). Trichoderma and Gliocladium: biology, ecology and potential for biocontrol. Annu. Rev. Phytopathol. 23:23-54. http://dx.doi.org/10.1146/annurev.py.23.090185.000323

Pérez CJ (2004). Organic farming: an alternative to sustainable development in rural Mexico. The Cotidiano, 20:95-100 (Spanish).http://dx.doi.org/10.1021/jo049945f http://dx.doi.org/10.1021/jo049243q

Ploetz RC (2006). Fusarium-induced diseases of tropical perennial crops. Phytopathology, 96 (6):648-652.http://dx.doi.org/10.1094/PHYTO-96-0648

Ploetz RC, Palmateer AJ (2007). First report of Fusarium wilt caused by *Fusarium oxysporum* on Roselle in the United States. Plant Disease, 91(5): 639.http://dx.doi.org/10.1094/PDIS-91-5-0639A

Porras M, Barrau C, Arroyo FT, Santos B, White C, Romero F (2007). Reduction of *Phytophthora cactorum* in strawberry fields by Trichoderma spp. and soil solarization. Plant Disease, 91(2):142-146. http://dx.doi.org/10.1094/PDIS-91-2-0142

Saran-Sundar M, Vinodhkumar T, Ramanathan G (2013). Evaluation of antifungal activity of metabolites from Trichoderma species against fungal phytopathogens. Int. J. Sci. Innov. Discov. 3:528-538.

SAS ® (2002). 9 OnlineDoc Copyright ©. SAS Institute Cary, NC, USA.

Singh R, Sachan NS (2013). Review on biological monitoring of soil borne fungi in vegetable crops. Hortflora Research Spectrum, 2(1):72-76.

Sylvère SR, Charles G, Atta DH, Koné D, Toueix Y, Dje Y, Branchard M (2011). Breeding of *Hibiscus sabdariffa* L.: evaluation of resistance to Fusarium oxysporum Schlecht. Emend. Snyd. and Hans in two varieties. Agric. Biol. J. N. Am. 2(1):125-133.

Trillas MI, Casanova E, Cotxarrera L, Ordovas J, Borrero C, Avilés M (2006). Composts from agricultural waste and the Trichoderma asperellum strain T-34 suppress *Rhizoctonia solani* in cucumber seedlings. Biological Control, 39:32-38. http://dx.doi.org/10.1016/j.biocontrol.2006.05.007

Widden P, Scattolin V (1988). Competitive interactions and ecological strategies of Trichoderma species colonizing spruce litter. Mycologia, 80(6):795-803. http://dx.doi.org/10.2307/3807557

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Straminipiles fungi growing on the alevins of the Nile tilapia in limnologically and trophically different water bodies

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The authors investigated the growth of straminipiles fungi on the alevins of Nile tilapia (*Oreochromis niloticus niloticus* L.) in water from eight limnologically and trophically different water bodies. Twenty-seven species were recorded. The largest number of species occurred on alevins in water from the Biala River and Fosa Pond (more biogenic), and the fewest in water from Dojlidy Pond and Lake Blizno (poor in biogenesis). The most commonly encountered species on Nile tilapia alevins were *Saprolegnia parasitica*, *Saprolegnia ferax*, *Achlya polyandra*, *Achlya oligocantha*, *Achlya prolifera*, *Leptomitus lacteus*, and *Pythium diclinum*. Amino acid, carbohydrate, and urease tests were used.

Key words: *Oreochromis niloticus*, alevins, straminipiles infections, hydrochemistry.

INTRODUCTION

The presence of wild fish production is stagnating, and the growth in overall fish production has come almost entirely from the global boom in aquaculture, especially in developing countries (Brown, 2000; van West, 2006). Over the past ten years, aquaculture production has increased, on average, by 11% per year (Delgado et al., 2003). In coming years, aquaculture will represent more than 30% of total fish production for consumption (FAO, 2012). In all hatcheries, the most important problem is aquatic fungi, especially straminipiles, growing on the fishes' eggs and young specimens (Dudka et al., 1989; Meyer, 1991; Hatai and Hoshiai, 1992; Czeczuga and Woronowicz, 1993).

The most common aquaculturally produced fish on the African continent for commercial use is the Nile tilapia (FAO-FIES, 2012), which has also been widely introduced for aquaculture on other continents (Stickney, 1986; ITIS, 2010). Thus, we decided to publish data covering the development of straminipiles fungi on the alevins of Nile tilapia from eight limnologically and trophically different water bodies.

MATERIALS AND METHODS

Short description of species

Nile tilapia, *Oreochromis niloticus niloticus* (Linnaeus, 1758) (Syn.: *L. niloticus* (Linnaeus, 1757); *Chromis niloticus* (Cuvier, 1817); *Chromis nilotica* (Cuvier, 1844); *Chromis guentheri* (Steindachner, 1864); *Tilapia nilotica, Tilapia calciati* (Gianferrari, 1924); *Tilapia nilotica nilotica* (van den Audenaerde, 1964); *Sartherodon niloticus* (Trewavas, 1978); *Oreochromis*

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niloticus (Trewavas, 1981), occur in Africa, the coastal rivers of Israel, the Nile from below the Albert Nile to the delta, and the Jebel Marra. In West Africa, its natural distribution covers the basins of the Niger, Bonue, Volta of Gambia, Senegal, and Chad, but the introduced specimens have been reported from the coastal basins. It has been widely introduced for aquaculture, with many existing strains (Lahuet, 1991). This fish occurs in a wide variety of freshwater habitats, such as rivers, lakes, sewage canals, and irrigation channels (Bailey, 1994; Skelton 2002). It feeds mainly on phytoplankton or benthic algae (ITIS, 2010). It is an oviparous species (Breder and Rosen, 1966), and they are maternal mouthbrooders (Trewavas, 1983). The extended temperature range is 8 to 42°C, and the natural temperature range is 13.5 to 33°C (ITIS, 2010).

Characteristics of water bodies

Water for the experiments was collected from eight limnologically and trophically different water bodies (two springs, two rivers, two ponds, and two lakes) located in the Northeastern region of Poland, Podlaskie District (53°07'N, 23°10'E- 53°13'N, 23°20'E):

i. Cypisek Spring, located in the South part of the Knyszynska Forest; limnocrenic type, 0.4 m width, 0.15 to 0.2 m depth, 0.6 $l\ s^{-1}$ discharge.
ii. Jaroszowka Spring, located in the North part of Bialystok; limnocrenic type, 0.65 m width, 0.12 m depth, 2.4 $l\ s^{-1}$ discharge.
iii. Biala River; 9.8 km length, 3.7 m width, 0.85 m depth; a left-bank tributary of the Suprasl River flowing through Bialystok.
iv. Suprasl River; 93.1 km length, 6.0 m width, 1.1 m depth; the largest right-bank tributary of the Middle Narew River, flowing through the Knyszynska forest.
v. Dojlidy Pond, located near Bialystok; 34.2 ha area, max depth 2.85 m. Its Southern shore borders coniferous woods, and its western part borders Bialystok.
vi. Fosa Pond, located in Branicki Park, Bialystok; 2.5 ha area, 1.75 m max depth. Swans breed and wild ducks colonies stay in this pond, as well as crucian carp.
vii. Lake Komosa; 12.1 ha area, 2.20 m max depth; surrounded densely by coniferous trees of the Knyszynska forest.
viii. Lake Blizno, located in Augustowska forest; 238.5 ha area, 28.8 m max depth.

Nineteen parameters of those water samples were determined (Table 1) according to the generally accepted methods (APHA, 2005).

Determining of straminipiles species

The investigated alevins (yolk-sac fry), covered with mycotic mycelia, were collected at the end of May from the hatchery of the Institute of Ichthyobiology and Aquaculture of the Polish Academy of Sciences in Golysz, Poland.

The following procedures were followed to determine the presence of straminipiles species on the investigated Nile tilapia alevins. Water samples (800 ml each) were placed in 1000 ml vessels, and 10 to 15 alevins were transferred to each vessel in accordance with general principles of culture (Watanabe, 2002). All the vessels were enclosed in Petri scales with the bed turned upside down to prevent possible airborne contamination by fungal spores. The vessels were stored at 15 ± 2°C, with access to daylight resembling natural conditions, and following the recommended instructions (Seymour and Fuller, 1987). Water analyses and experiments were carried out in three parallel repetitions.

The alevins in each vessel were observed every 3 to 4 days under a light microscope, and the presence of morphological structures (zoospores, antheridia, and oogonia) of aquatic fungi was recorded. Amino acid, carbohydrate, and urease tests were performed on the *Achlya, Aphanomyces, Leptolegnia, Pythium,* and *Saprolegnia* genera, according to Yuasa and Hatai (1996) and Kitancharoen and Hatai (1998). Yeast nitrogen base agar (YNB) (Difco) was used as the medium to culture the fungal isolates for the carbohydrate utilization test, and glucose yeast (GY) extract agar was used for the urease test. To prevent the growth of bacteria, ampicillin and streptomycin were applied. Basal medium was used in the amino acid assimilation test.

The medium preparation and indicator were the same as for the carbohydrate assimilation test. Bromothymol blue and phenol red were used as indicators and added to the YNB broth and GY broth, respectively. A change in the colour of the medium to pink or purple indicated a positive result, and a change to orange or yellow indicated a negative result. Those methods were described in detail in our previous paper (Czeczuga et al., 2011a). The experiments were carried out for one month. The straminipiles species were identified using the keys of Johnson et al. (2005) and Pystina (1998). The systematics of straminipiles species according to Dick (2001) were used in these experiments. The results were tested for significance using analysis of variance (ANOVA) and evaluated by Scheffé's test (Winer, 1997).

RESULTS

The chemical and physical parameters of the water used in the experiments are shown in Table 1. The most eutrophic was the water from the Biala River and Fosa Pond. Water from Lake Blizno and Dojlidy Pond had the lowest content of all forms of nitrogen, phosphates, sulphates, and chlorides. The highest levels of chemical oxygen demand (COD), chlorides, sulphates, and iron were also found in the Biala River and Fosa Pond.

Twenty-seven straminipiles species, including 22 belonging to Saprolegniales, four to Pythiales, and one to Leptomitales, were found growing on the Nile tilapia alevins (Table 2). The *Achlya*, *Pythium*, and *Saprolegnia* were the most prevalent genera. The most commonly encountered species were *Saprolegnia parasitica*, *Saprolegnia ferax*, *Achlya oligocantha*, *A. prolifera*, *Leptomitus lacteus*, and *Pythium diclinum*. The largest number of species occurred on Nile tilapia alevins in water from the Biala River and Fosa Pond (both more biogenic), and the smallest number were found in water from Dojlidy Pond and Lake Blizno (both poor in biogenesis). The results of amino acid, carbohydrate, and urease utilization are shown in Table 3. Six of the 12 amino acids tested-methionine, lysine, ornithine, phenylalanine, leucine, and glycine-could not be assimilated by the investigated straminipiles. All the stated species from the *Achlya*, *Aphanomyces*, *Leptolegnia*, and *Saprolegnia* genera assimilated glucose and starch, but they did not assimilate arabinose or salicin (except the species from the *Pythium* genus). Urease was assimilated by specimens from the *Leptolegnia, Pythium*, and *Saprolegnia* genera.

Table 1. Chemical and physical properties of water in particular water bodies (in mg L^{-1}).

Specification	Spring		River		Pond		Lake	
	Cypis	Jaroszówka	Biała	Supraśl	Dojlidy	Fosa	Blizno	Komosa
Temperature (°C)	14.5	10.2	16.8	15.2	15.8	18.0	12.6	14.9
pH	7.2	7.2	7.1	7.3	7.9	7.1	8.0	7.2
DO	16.4	11.8	8.2	18.6	9.4	6.4	16.5	14.8
BOD_5	2.6	3.6	10.8	4.2	9.7	12.8	2.8	5.1
COD (Oxidability)	9.9	4.8	16.8	11.2	15.8	20.2	4.2	9.0
CO_2	37.4	15.4	26.9	29.4	8.8	22.4	5.3	18.6
Alkalinity ($CaCO_3$ mval l^{-1})	4.6	3.9	4.3	3.6	3.2	5.8	2.5	3.8
N-NH_3	0.254	0.124	0.662	0.315	0.128	0.864	0.113	0.196
N-NO_2	0.015	0.013	0.128	0.037	0.008	0.114	0.003	0.018
N-NO_3	0.245	0.302	0.470	0.317	0.050	0.552	0.025	0.072
P-PO_4	0.920	0.974	1.820	1.020	0.242	3.598	0.140	0.706
Sulphates (SO_4)	36.8	121.0	73.2	50.4	45.7	85.1	14.1	40.6
Chlorides (Cl)	40.5	22.0	66.4	50.2	61.8	79.3	14.2	35.4
Total hardness (in Ca)	110.9	101.5	98.2	93.6	12.2	24.2	40.9	76.6
Total hardness (in Mg)	21.8	14.6	17.4	17.2	18.1	20.6	11.3	18.4
Fe	0.38	0.10	0.92	0.47	0.84	1.06	0.12	0.26
Dry residue	174.2	327.0	434.0	412.0	257.0	429.0	182.0	220.0
Dissolved solids	150.0	320.0	324.0	384.0	210.0	370.0	140.0	198.0
Suspended solids	24.2	7.0	110.0	28.0	47.0	59.0	42.0	22.0

DISCUSSION

In this study, 27 straminipiles fungal species belonging to eight genera were found to grow on the larvae of *O. niloticus* in water from eight limnologically and trophically different bodies of water in Northeastern Poland. The *Achlya*, *Pythium*, and *Saprolegnia* genera were the most prevalent.

The occurrence of zoosporic fungi has been investigated in bodies of water that are the areas of natural distribution of Nile tilapia on the African continent, mainly in the Northern part of the continent, especially in upper Egypt (Khallil et al., 1993) and lower Egypt (Ali, 2007, 2009). Zoosporic fungi were collected from accumulated rainfall water (El-Nagdy and Nasser, 2000), ponds in oases (El-Nagdy and Abdel-Hafez, 1990), the Nile River (El-Hissy et al., 1982), Nile Delta Region waters (El-Hissy and Khallil, 1989; Ali, 2007), Lake Nasser on the Aswan High Dam (El-Hissy et al., 2000), and other Egyptian lakes (Ali and Abdel-Raheem, 2003; El-Hissy et al., 2004), and fungal zoosporic species belonging to the *Saprolegnia*, *Pythium*, *Phytophthora*, and *Achlya* genera were found. Experimental transmission to two *Tilapia* species and the pathogenicity of some zoosporic fungi were investigated by El-Sharouny and Badram (1995), and 17 fungal species belonging to the *Saprolegnia, Achlya, Dictyuchus, Pythium, Allomyces*, and *Aphanomyces* genera were recorded in four organs of *Tilapia nilotica* and *Tilapia galilaela* from River Nile water. The most common were *S. ferax*, *Saprolegnia diclina, Achlya dubia, Achlya americana, Achlya racemosa, A. flagellata, Dictyuchus sterile, Pythium undulatum*, and *Aphanomyces sp.* Among the tested fungi, *S. parasitica, S. ferax*, and *A. racemosa* have been found to be the most infectious. Such species of zoosporic fungi as *Aphanomyces frigidophilus*, *Aphanomyces invadans*, *Dictyuchus pisci*, *Saprolegnia polymorpha*, *Scoliolegnia asterophora*, *P. diclinum*, and *L. lacteus* have not been recorded in bodies of water on the African continent.

The straminipiles species grows on the alevins of Nile tilapia in Polish waters and on the eggs of other fish species in Europe (Czeczuga and Muszynska, 1998) and Asia (Hussein et al., 2001). It is also worth noting that *A. invadans* occurs on the larvae of Nile tilapia in water from

Table 2. Straminipiles organisms recorded on the alevins of the Nile tilapia.

Taxa	Water bodies
Straminipiles	
Peronosporomycetes	
Saprolegniales	
Achlya americana Humphrey	Bi, K
A. androgyna (W.A. Archer) John et R.L.Seym.	Bi, J, K
A. bisexualis Coker et Couch	Bi, K, S
A. debaryana Humphrey	Bi, K, S
A. klebsiana Pieters	Bi, K, S
A. oligocantha de Bary	Bi, Bl, C, J, S
A. polyandra Hildebr.	Bi, Bl, C, J
A. prolifera Nees	Bi, Bl, C, J, S
Aphanomyces frigidophilus Kitanch. et Hatai	Bi, K, S
A. invadans Willoughby et al.	F
A. laevis de Bary	Bi, K
Dictyuchus monosporus Leitgeb	Bi, C, J, S
D. pisci Khulbe et Sati	Bi
Isoachlya curvata (Minden) Cejp	Bi, K
I. monilifera (de Bary) Kauf.	Bi, C, J, K, S
Saprolegnia anisospora de Bary	Bi
S. australis R.F. Elliott	Bi
S. diclina Humphrey	Bi, C, J, K
S. ferax (Gruith) Thur.	Bi, Bl, C, J, S
S. parasitica Coker	Bi, Bl, C, J, S
S. polymorpha Willoughby	Bi, K
Scoliolegnia asterophora (de Bary) M.W. Dick	Bi, K
Leptomitales	
Leptomitus lacteus (Roth.) C. Agardh	Bi, Bl, C, J, K, S
Pythiales	
Pythium debaryanum Hesse	Bi, K
P. diclinum Tokun	Bi, Bl, C, J, K, S
P. proliferum de Bary	Bi, S
P.ultimum Trow	Bi, C, J, K
Number of species in water from Spring Cypisek (C) – 11[a]	
Number of species in water from Spring Jaroszówka (J) – 12[a]	
Number of species in water from River Biała (Bi) –26[b]	
Number of species in water from River Supraśl (S) – 13[c]	
Number of species in water from Pond Dojlidy (D) – 9[c]	
Number of species in water from Pond Fosa (F) – 27[d]	
Number of species in water from Lake Blizno (Bl) – 7[e]	
Number of species in water from Lake Komosa (K) – 15[f]	
Number of species in water from springs – 11.5[a]	
Number of species in water from rivers – 19.5[b]	
Number of species in water from ponds – 18.0[b]	
Number of species in water from lakes – 11.0[c]	

Means with the same letter are not significantly different (p>0.05).

Fosa Pond; the *Aphanomyces sp.* has been reported to occur in some Egyptian lakes (El-Hissy et al., 2004). In some species of fish, it has been associated with skin lesions and mortality of the specimens. This phenomenon

Table 3. Amino acid, carbohydrate and urease assimilation by straminipiles isolated from alevins of Nile tilapia.

Species of genus	Amino acid	Carbohydrate	Urease
Achlya	Asp, Glu, Arg, Ala	Fru, Glu, Man, Raf, Suc, Mal, Lac, Mel, Cel, Tre, Sta, Dex, Rha, Gly	-
Aphanomyces	Glu, Ala, Cys	Glu, Sta	-
Leptolegnia	Asp, Glu, Ala	Fru, Glu, Man, Mal, Mel, Cel, Tre, Sta, Dex, Gly	+
Pythium	Ala, His	Fru, Glu, Man, Gal, Raf, Suc, Mal, Lac, Mel, Cel, Tre, Sta, Dex, Rha, Gly, Sal	+
Saprolegnia	Asp, Glu, Arg, Ala, His	Fru, Glu, Man, Mal, Cel, Tre, Sta, Dex, Gly	+

Abbreviations: Amino acids: Ala- Alanine, Arg- Arginine, Asp- Asparagine, Cys- Cysteine, Glu- Glutamine, His- Histidine, Carbohydrate: Fru- Fructose, Gal- Galactose, Glu- Glucose, Man- Mannose, Mal- Maltose, Mel- Melibiose, Cel- Cellobiose, Dex- Dextrin, Gly- Glycerol, Lac- Lactose, Rha- Rhamnose, Sal- Salicin, Raf- Rafinose, Sta- Starch, Suc- Sucrose, Tre- Trehalose. "+" positive; "-" negative.

was observed in two species of fish from an excavated earthen pond at the western shore of the Suez Canal, Egypt, during the winter of 1971 (Shaheen et al., 1999). According to Lilley et al. (2009), this invasive *Aphanomyces* infection of fish, reported by Shaheen et al. (1999), was caused by the *Aphanomyces invadans* species. It has also been observed in Poland, during autumn, on the skin and muscles of *Labeo bicolor* Smith specimens in water from Fosa Pond (Czeczuga et al., 2011c).

True fungi (Olufemi et al., 1983; Salem et al., 1989) and imperfect fungi (Lightner et al., 1988) also have been isolated from African specimens of the *Oreochromis* genus. Those isolated fungal species (from adult specimens of *Tilapia*) belonged to such genera as *Aspergillus, Fusarium, Mucor, Penicillium, Rhizopus, Scopulariopsis, Curvularia,* and *Paecilomyces*. Yeasts belonging to the *Candida, Rhodotorula,* and *Torulopsis* genera also have been isolated from adult specimens of the *Oreochromis* genus (Refai et al., 2010). The most predominant diseases of farmed *Tilapia* are aspergillomycosis (Marzouk et al., 2003) and zygomycosis (Wolf and Smith, 1999). Nile tilapia in aquaculture belong to parasite- and disease-resistant species (Kotusz, 2000). However, bacterial fin inflammation (rot fin) caused by protozoans from the *Trichodina* and *Chilodon* genera have been reported (Dykova, 2006), as well as some moulds, especially true fungi and straminipiles species (Marzouk et al., 2003; Refai et al., 2010).

We found the largest number of fungus species on the larvae of Nile tilapia in water from the Biala River and Fosa Pond (the most eutrophic of the bodies of water examined), while the number of isolated species of fungi was the lowest in water from Dojlidy Pond and Lake Blizno (the least abundant in biogenesis). We previously observed this type of phenomenon while studying the growth of fungi on the fillets of two species of piranhas (Czeczuga et al., 2010) and on the eggs of Acipenseridae fish species (Czeczuga et al., 2011b). *Saprolegnia* genus, similarly to *Achlya*, is generally thought to be an opportunistic pathogen that is saprotrophic and necrotrophic (Bruno and Wood, 1999). Opportunistic fungal specimens are very virulent and able to grow on dead plant fragments and cause primary infections in fish. Infections can take place on both eggs and fish. In the eggs, the disease is manifested by abundant cotton-like mycelial growth on the cells, resulting in death. In fish, the pathogen invades epidermal tissues (often beginning at the head or fins) and spreads to the entire surface of the body.

Occasionally, the hyphae penetrate into the muscles and blood vessels of infected fishes (Hatai and Hoshiai, 1992; van West, 2006). The most eutrophic water bodies are highly favourable environment for opportunistic species. Most dead plant fragments, on which opportunistic fungal species develop, occur in eutrophic bodies of water (Czeczuga et al., 2005b, 2007).

Owing to their rapid body weight growth, high tolerance of environmental conditions, and resistance to parasites and diseases, species of the Nile tilapia play an important role as a source of animal proteins, fats, and other biologically active substances, including carotenoids, which are important to human life. Carotenoids serve as a source of vitamin A, have antioxidant actions, play a cancer-protective role, and increase the immune response in mammals. They also may be significant against coronary heart disease (Czeczuga et al., 2013). Fish are not able to synthesise carotenoids *de novo*; therefore, those compounds have to be supplied in the diet. As shown in recent studies (Boonyaratpalin and Unprasert, 1989; Czeczuga et al., 2005a), specimens of Nile tilapia are rich in this pigment. Total content of carotenoids in particular body parts ranged from 0.216 µg g^{-1} (muscle) to 0.945 µg g^{-1} (liver) wet weight and included such important carotenoids as β- carotene, lutein, zeaxanthin, canthaxanthin, and astaxanthin (Britton and Khachik 2009).

In Nile tilapia, the considerable part of the carotenoid pool in the liver is transformed by oxidation and reduction into, i.a. vitamin A, and specifically, into three types of vitamin A (A_1, A_2 alcohol; A_1, A_2 aldehyde; and anhydro vitamin A_1 and A_2). In the Nile tilapia liver, the biotransformation into vitamin A involves not only α- and β-carotene, but also xanthophylls such as lutein, zeaxanthin, tanaxanthin, canthaxanthin, and astaxanthin (Katsuyama et al., 1987). Carotenoids are also important in resistance to fish infections. The immunity of a given population is also food-dependent (MacPhee et al., 1995), and it has been established that chitin (found in fish food) stimulates fish resistance (Sakai et al., 1992). The chitinous armours of crustaceans are known to be rich in carotenoids (Czeczuga and Czeczuga-Semeniuk, 1999). It is worth noting that fewer fungi were found on the eggs of *Coregonus alba* L., which contain a large amount of carotenoids, in comparison with eggs from the same lake but with fewer carotenoids (Czeczuga and Muszynska, 1998). This could explain why the fewest fungi were found growing on the eggs of *Coregonus lavaretus* L., which contain more carotenoids, compared with eggs with lower carotenoid amounts.

According to some authors (Soin, 1968; Karnaukhov, 1973; Latscha, 1990), carotenoids serve as a source of oxygen when oxygen is deficient, thereby ensuring better health of the eggs and enhancing their resistance to mycotic infections. Nile tilapia is known to be polyphagous (Kotusz, 2000; ITIS, 2010) under natural conditions; its food consists of algae (dominated by cyanobacteria), as well as periphyton and detritus. The fish also feeds on zooplankton and other aquatic invertebrates. Algae, especially cyanobacteria, as well as crustaceans and zooplankton, are rich in carotenoids. As is known, eutrophic bodies of water are more phyto- and zooplankton-rich than are oligotrophic bodies of water.

Chemotaxis-exhibiting zoospores found in water are particularly sensitive to asparagine and glutamine (Rand and Munden, 1993). Those two amino acids occur in large quantities in animals, including fish (Smith et al., 1985). All the stated species from the *Achlya, Leptolegnia*, and *Saprolegnia* genera growing on Nile tilapia larvae assimilated asparagine and glutamine, and the *Aphanomyces* species assimilated glutamine. Species of *Pythium* genus assimilated only alanine and histidine and all carbohydrates. These findings suggest that *Pythium* species prefers the plant substrate more than animal tissue. Four species of the *Pythium* genus growing on the Nile tilapia alevins are also common in Polish bodies of water (Czeczuga and Snarska, 2001), whereas they are quite rare on the eggs of freshwater fish (Czeczuga, 1996). Species from the *Aphanomyces* genus assimilated only glucose and starch and such amino acids as alanine, glutamine, and cysteine. We observed similar phenomena in our investigations on the occurrence of fungi on *Oncorhynchus gorbuscha* (Czeczuga et al., 2011a) and *O. tshawytscha* eggs (Czeczuga et al., 2012). It has also been observed on the eggs of other *Oncorhynchus* species (Kitancharoen and Hatai, 1998).

Conclusions

Examinations of the growth of straminipiles organisms on the alevins of Nile tilapia in eight different water bodies was performed, such as springs, rivers, ponds, and lakes. The investigations showed that:

i. The water most abundant in biogenesis was from the Biala River and Fosa Pond; the least abundant was water taken from Dojlidy Pond and Lake Blizno.
ii. Twenty-seven species of straminipiles organisms were found developing and growing on the alevins of Nile tilapia, 22 of which belonged to Saprolegniales, four to Pythiales, and one (*L. lacteus*) to Leptomitales. Most of the species were representatives of the *Achlya* and *Saprolegnia* genera. Two species that cause mass mortality of fishes in aquacultures, *A. invadans* and *S. parasitica* (Hatai and Hoshiai, 1992; Lilley et al., 2003), were found.
iii. The most frequently occurring species were *A. oligocantha*, *A. prolifera*, *Isoachlya monilifera*, *S. ferax, S. parasitica*, *L. lacteus*, and *P. diclinum.*
iv. The limnological type of the body of water does not influence the number of species occurring on the alevins of *Nile tilapia*; it depends on the trophicity.
v. The greatest number of straminipiles species on Nile tilapia alevins were found in water from the Biala River (26 species) and Fosa Pond (27) (more biogenic), and the lowest were found in water from Pond Dojlidy (9) and Lake Blizno (7) (both poor in biogenesis).

This finding can be explained by the fact that pathogenic Saprolegniales species belong to opportunistic organisms that are saprotrophic and necrotrophic (Bruno and Wood, 1999). Water rich in biogenesis is more abundant in dead organic material for saprotrophic organisms; therefore, it generates better development conditions for more Straminipiles species.

Conflict of Interests

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

REFERENCES

Ali EH (2007). Biodiversity of zoosporic fungi in polluted water frainages across Niles Delta region, Lower Egypt. Acta Mycol. 42:99-111. http://dx.doi.org/10.5586/am.2007.010

Ali EH (2009). Antifungal activity of sodium chloride on Saprolegnia diclina and Aphanomyces sp. Acta Mycol. 44:125-138. http://dx.doi.org/10.5586/am.2009.011

Ali EH, Abdel- Raheem A (2003). Distribution of zoosporic fungi in the mud of major Egyptian lakes. J. Bashram. Microbiol. 43:175-184. http://dx.doi.org/10.1002/jobm.200390020 PMid:12761768

APHA (American Public Health Association) (2005). Standard methods for the Examination of Water and Wastewater, APHA, Washington, DC .
Bailey RG (1994). Guide to the fishes of the River Nile in the Republic of Sudan. J. Nat. Hist. 28:937-970. http://dx.doi.org/10.1080/00222939400770501
Breder CM, Rosen DE (1966). Modes of reproduction in fiehs. T.F.H. Publications, Neptune City, New Jersey.
Britton G, Khachik F (2009). Carotenoids in food. In: Britton G et al. (Eds.). Carotenoids, Vol. 5: Nutrition and health, Birkhäuser Verlag, Basel, Boston, Berlin, pp. 45-66.
Boonyaratpalin M, Unprasert N (1989). Effect of pigments from different sources in colour change and growth of red Oreochromis niloticus. Aquaculture 79:375-380.http://dx.doi.org/10.1016/0044-8486(89)90479-1
Brown LR (2000). Fish Farming may soon Overtake Cattle Ranching as a Food Source. Worldwatch Institute, 1776 Massachusetts Ave., NW, Washington. http://aquanic.org/news/2000/farming.htm
Bruno DW, Wood BP (1999). Saprolegnia and other Oomycetes. In: Woo PTK, Bruno DW (Eds.). Fish Diseases and Disorders. Viral, Bacterial and Fungal Infections, vol. 3. CABI Publishing, Wallingford, Oxon, UK, pp 599-659.
Czeczuga B (1996). Species of Pythium isolated from eggs of fresh-water fish. Acta Mycol. 31:151-161.
Czeczuga B, Czeczuga- Semeniuk E (1999). Comparative studies of carotenoids in four species of crayfish. Crustaceana 72:693-700. http://dx.doi.org/10.1163/156854099503735
Czeczuga B, Czeczuga- Semeniuk E, Klyszejko B, Szumiec J (2005a). Carotenoid content in the Nile tilapia (Oreochromis niloticus L.) cultured in Poland. Acta Sci. Pol., Piscaria 4 (1-2):25-32.
Czeczuga B, Czeczuga- Semeniuk E, Semeniuk A (2011a). Microfungi-like organisms developing on the eggs of pink salmon Oncorhynchus gorbuscha. Curr. Trends Microbiol. 7:21-29.
Czeczuga B, Czeczuga-Semeniuk E, Semeniuk A (2012b). Aquatic fungi developing on the eggs of chinook salmon Oncorhynchus tschawytscha and some of their biochemical characteristics. Trends Comp. Biochem. Physiol. P. 16, in press.
Czeczuga B, Czeczuga-Semeniuk E, Semeniuk A, Muszynska E (2011a). Zoosporic fungi and fungus- like organisms growing on the eggs of four species of sturgeonid fish (Acipenseriformes). Trends Comp. Biochem. Physiol. 15:83-92.
Czeczuga B, Godlewska A, Mazalska B, Muszynska E (2010). Straminipilous organisms growing on herbivorous parapitinga (Piaractus brachypomus) and carnivorous piranha (Pagocentrus nattereri) from Poland. Braz. J. Biol. 70:335-339. PMid:20549064
Czeczuga B, Muszynska E, Godlewska A, Mazalska B(2005b). Aquatic fungi growing on dead fragments of submerged plants. Limnologica 35:283-297. http://dx.doi.org/10.1016/j.limno.2005.07.002
Czeczuga B, Muszynska E (1998). Aquatic fungi growing on coregonid fish eggs. Acta Hydrobiol. 40:239-264.
Czeczuga B, Muszynska E, Godlewska A, Mazalska B (2007). Aquatic fungi and straminipilous organisms on decomposing fragments of wetland plants. Mycol. Balc. 4:31-44.
Czeczuga B, Semeniuk J, Czeczuga-Semeniuk E, Semeniuk A, Klyszejko B (2012c). Amount and qualities of carotenoids in fillets of fish species fed with natural feed in some fisheries of West African Coasts. Afr. J. Biotechnol. in press.
Czeczuga B, Semeniuk A, Muszynska E, Najecka K (2011c). Fungi and straminipilous organisms growing on some aquarium fish species in water from different water bodies. Curr. Trends Ecol. 2:63-73.
Czeczuga B, Snarska A (2001). Pythium species in 13 various types of water bodies of N.E. Poland. Acta Soc. Bot. Pol. 70:7-17. http://dx.doi.org/10.5586/asbp.2001.009
Czeczuga B, Woronowicz L (1993). Aquatic fungi developing on the eggs of certain fresh- water fish species and their environment. Acta Ichtyol. Piscat. 23:39-57.
Delgado CL, Wada N, Rosegrant MW, Meijer S, Ahmed M (2003). Outlook for Fish to 2020. Meeting Global Demand. Report by the International Food Policy Research Institute, Washington.
Dick MW (2001). Straminipilous Fungi, Systematics of the Peronosporomycetes Including Accounts of the Marine Straminipilous Protists, the Plasmodiophorids and Similar Organisms. Kluwer, Dordrecht, NL.
Dudka IA, Isaeva NM, Davydova ON (1989). Saprolegniaceae breeding fish mycoses. Mycol. Phytipathol. 23:488-498.
Dykova I (2006). Phylum Microspora. In: Woo PTK (Ed.) Fish Diseases and Disorders. Protozoan and Metazoan Infections, 2nd ed., vol. 1. CABI Publishing, Wallingford, UK, pp. 205-299. http://dx.doi.org/10.1079/9780851990156.0205
El-Hissy FT, Moubasher AH, El- Nagdy MA (1982). Seasonal fluctuation of freshwater fungi in river Nile (Egypt). Zeit. Allg. Mikrobiol. 22:521-527. http://dx.doi.org/10.1002/jobm.3630220803 PMid:7164472
El-Hissy FT, Khallil AM (1989). Studies on aquatic fungi in Delta region (Egypt). Zbl. Mikrobiol. 144:421-432.
El-Hissy FT, Ali EH, Abdel- Raheem A (2004). Diversity of zoospore of fungi recovered from the surface of water bodies in four Egyptian lakes. Ecohydrol. Hydrobiol. 4:77-84.
El-Hissy FT, El- Zagat SA, Masoud MS (2000). Monthly and vertical fluctuations of aquatic fungi at different depths in Aswan High Dam Lake, Egypt. Fung. Diver. 5:165-173.
El-Nagdy MA, Abdel- Hafez SI (1990). Occurrence of zoosporic and terrestrial fungi in some ponds of Khurga Oases, Egypt. J. Basic Microbiol. 30:233-240. http://dx.doi.org/10.1002/jobm.3620300404 PMid:2384873
El-Nagdy MA, Nasser IA (2000). Occurence of zoosporic and terrestrial fungi in accumulated rainfall water in the Riyadh region (Saudi Arabia). Fung. Divers. 5:175-183.
El-Sharouny HM, Badram RAM (1995). Experimental transmission and pathogenicity of some zoosporic fungi to Tilapia fish. Mycopathology 132:95-105. http://dx.doi.org/10.1007/BF01103781
Hatai K, Hoshiai GI (1992). Mass mortylity in cultured coho salmon (Oncorhynchus kisutch) due to Saprolegnia parasitica Coker. J. Wild. Dis. 28:532-536. http://dx.doi.org/10.7589/0090-3558-28.4.532, PMid:1474649
Hussein MMA, Hatai K, Nomura T (2001). Saprolegniasis in salmonids and their eggs in Japan. J. Wild. Dis. 37:204-207. http://dx.doi.org/10.7589/0090-3558-37.1.204 PMid:11272500
IT IS (2010). Integrated Taxonomic Information System. Report, http://www.fishbase.org/Summary/speciesSummary/php?ID=2692&genusname
Johnson TWJr, Seymour RL, Patgett DE (2005). Systematics of the Saprolegniaceae: New combination. Mycotaxon 92:11-32.
Karnaukhov WN (1973). Function of Carotenoids in Cells of Animals. Bauka, Moscow.
Katsuyama M, Komori T, Matsuno T (1987). Metabolism of three steroisomers of asthaxanthin in the fish, rainbow trout and tilapia. Comp. Biochem. Physiol. 86B:1-5.
Khallil AM, El- Hissy FT, Abdel- Raheem A (1993). Monthly variations of Oomycetes (zoosporic fungi) and aquatic Hyphomycetes at Soheg (Upper Egypt). Acta Soc. Bot. Pol. 62:67-73. http://dx.doi.org/10.5586/asbp.1993.012
Kitancharoen N, Hatai K (1998). Some biochemical characteristics of fungi isolated from salmonid eggs. Mycoscience 39:249-255.http://dx.doi.org/10.1007/BF02464005
Kotusz J (2000). Nile tilapia, Oreochromis niloticus (Linnaeus, 1757). In: Brylinka M (Ed.). Freshwater Fishes of Poland, Wyd. Nauk. PWN, Warszawa, pp. 473-475.
Latscha T (1990). Carotenoids- their Nature and Significance in Animal Feeds. F Hoffman- La Roche, Basel.
Lightner D, Redman RM, Mohney L, Sinski J, Priest D (1988). A renal mycosis of N dult hybrid red tilapia Oreochromis mosambicus and O. hornorum, cause by the imperfect fungus Paecilomyces marquandii. J. Fish. Dis. 1:437-440. http://dx.doi.org/10.1111/j.1365-2761.1988.tb00740.x
Lahuet P (1991). Tilapia, Oreochrmis spp. In: Wilson RP (Ed.) Handbook of Nutrient Requirements of Finfish. CRC Press, Boca Raton,Flöorida pp. 138-187.
Lilley JH, Hart D, Panyavachira V, Kanchanakhan S, Chinabut S, Söderhäll K, Cerenius L (2003). Molecular characterisation of the fish- pathogenic fungus Aphanomyces invadans. J. Fish Dis. 26:263-275. http://dx.doi.org/10.1046/j.1365-2761.2003.00448.x PMid:12962235
MacPhee DD, Ostland VE, Lumsden JS, Derksen J, Ferguson HW (1995). Influence of feeding on the development og bacterial gill

diesease in rainbow trout Oncorhynchus mykiss. Dis. Aqua. Org. 21:163-170.http://dx.doi.org/10.3354/dao021163

Marzouk MS, Samira SR, El- Gamal MH (2003). Mycological investigations on cultured Tilapia in Kafer El- Sheikh Governorate. Kafer El- Sheikh Vet. Med. J. 1:97-114.

Meyer FP (1991). Aquaculture disease and health management. J. Anim. Sci. 69:4201-4208.PMid:1778836

Olufemi BX, Agius C, Roberts RJ (1983). Aspergillomycosis in intensively cultured tilapia (Sarothernden sp.) from Kenya. Vet. Rec. 122:203-204.http://dx.doi.org/10.1136/vr.112.9.203

Pystina KA (1998). Genus Pythium Pringsh. Nauka, Sankt Petersburg.

Rand TG, Munden D (1992). Enzyme involvment in the invasion of brook charr Salvelinus fontinalis (Mitchill) eggs by Saprolegnia diclina (Oomycotina, Saprolegniaceae). J. Fish Dis. 15:91-94. http://dx.doi.org/10.1111/j.1365-2761.1992.tb00641.x

Refai MK, Mohamed LA, Kenawy ANM, Shimaa El- SMA. (2010). The assessment of mycotic settlement of freshwater fishes in Egypt. J. Am. Sci. 6:594-602.

Sakai M, Kamiya H, Ishii S, Atsuta S, Kobayashi M (1992). The immunostimulating effects of chitin in rainbow trout Oncorhynchus mykiss. In: Shariff I et al. (Eds.) Diseases in Asian Aquaculture. Asian Fisheries Society, Manila, pp 413-417.PMid:1633799

Salem AA, Refai MK, Eissa IAM, Marzouk MS, Bakir A, Moustafa M, Manal A (1989). Some studies on aspergillomycosis in Tilapia nilotica. Zagazik Vet. J. 17:315-328.

Seymour RL, Fuller MS (1987). Collection and isolation of water molds (Saprolegniaceae) from water and soil. In. Fuller MS, Jaworski A (Eds.) Zoosporic Fungi in Teaching and Research, Southeastern Publishing, Athens, pp. 125-127.

Shaheen AA, Elsayed E, Faisal M (1999). Isolation of Aphanomyces sp (p), associated with skin lessions and mortalities in the striped (*Mugil cephalus*) and the thin lip (*Liza ramada*) grey mulltes. Bull. Eur. Ass. Fish Pathol. 19:79-82.

Skelton HP (2002). A Complete Guide to the Freshwater Fishes of Southern Africa (Revised). Struik, Cape Town.

Smith SN, Armstrong RA, Springgate J, Barker J (1985). Infection and colonisation of trout eggs by Saprolegniaceae. Trans. Br. Mycol. Soc. 85:719-723.http://dx.doi.org/10.1016/S0007-1536(85)80268-0

Soin SG (1968). Adaptation Features of Development of Fishes. Izdatelstwo Moskowskogo Uniwersiteta, Moscow.

Stickney RR (1986). Tilapia. In: Stickney RR (Ed.) Culture Nonsalmonid Freshwater Fishes. CRC Press, Boca Raton, USA, pp. 58-72.

Trewavas E (1983). Tilappine fishes of the genera Sarotherodon, Oreochromis and Danakalia. Br. Mus. Nat. Hist., London, UK. PMid:6352061

van West P (2006). Saprolegnia parasitica, an oomycete pathogen with a fishy appetite: new challenges for an old problem. Mycologist 20:99-104.http://dx.doi.org/10.1016/j.mycol.2006.06.004

Watanabe T (2002). Pictorial Atlas of Soil and Seed Fungi: Mophologies of Cultured Fungi and Key to Species. CRC Press, Boca Raton, Florida. http://dx.doi.org/10.1201/9781420040821

Winer BJ (1997). Statistical Principles in Experimental Design. McGraw Hill, New York. PMCid:PMC1904723

Wolf JC, Smith SA (1999). Systemic zygomycosis in farmed tilapia fish. J. Comp. Pathol. 121:301-306. http://dx.doi.org/10.1053/jcpa.1999.0323 PMid:10486168

Yuasa K, Hatai K (1996). Some biochemical characteristics of the genera Saprolegnia, Achlya and Aphanomyces isolated from fishes with fungal infection. Mycoscience 39:249-255.

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Full Length Research Paper

Influence of girdling and zinc and boron application on growth, quality and leaf nutrient status of olive cv. Frontoio

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The experiment was carried out during 2008 to 2009 and 2009 to 2010 with the aim to study the effect of girdling and foliar application of zinc and boron on growth, quality and leaf nutrient status of olive cv. Frontoio. The experiment was laid out in a randomized block design with seventeen treatments. Maximum shoot extension growth (8.92 cm) was observed with the spray of 0.6% $ZnSO_4$ in combination with 0.6% boric acid. Maximum pulp:stone ratio of 2.57 and oil content of 25.62% was recorded with the foliar application of 0.6% $ZnSO_4$ in combination with 0.6% boric acid and 0.6% $ZnSO_4$ in combination with 0.4% boric acid, respectively. The highest leaf K (1.74%), Ca (1.71%) and Mg (0.15%) contents were recorded with spray of 0.5% $ZnSO_4$ in combination with 0.4% boric acid, 0.5% $ZnSO_4$ in combination with 0.5% boric acid and 0.6% $ZnSO_4$ in combination with 0.6% boric acid, respectively. From this study, it is concluded that foliar application of zinc sulphate in combination with boric acid resulted in better growth, physical characteristics and oil content of olive fruits.

Key words: Olive, girdling, zinc, boron, leaf nutrient, oil content.

INTRODUCTION

Olive (*Olea europea* L.) is an evergreen tree, which requires chilling for its fruitfulness. Olive is grown for its fruits, used for oil extraction as its oil is a rich source of polyunsaturated fatty acids (PUFA). It possesses numerous biological properties and therefore occupies a pivotal position in human nutrition. Olive is an important crop in all countries situated in the Mediterranean region. The main producers of olive include Spain, Italy, Greece, Portugal, Turkey, etc. The total world production of olives was estimated at 20.58 million tonnes (FAO, 2012). Olive cultivation in India is still in its infancy and is restricted to the states of Jammu and Kashmir, Himachal Pradesh and Uttaranchal.

In olives, fruit weight and oil content are affected by the mineral status of leaves (Jordao and Lietao, 1990). The foliar feeding of micro-nutrients in different fruit crops, including olives, has assumed a significant position to enhance their growth, productivity and nutrient status. Nutrient sprays can influence qualitative and quantitative characteristics of olive, such as fruit size, weight, and fruit oil content. Rajaie et al. (2009) while studying the effect of zinc and boron interaction on growth and mineral

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composition of lemon seedlings (*Citrus aurantifolia* L.) observed that application of zinc and boron resulted in better plant growth and was associated with the highest uptake of N, P, K, Zn, Fe, Cu and Mn under greenhouse conditions. Foliar application of zinc and boron in combination has been reported to elevate the leaf chlorophyll and N content, thus increasing shoot growth, in Persian walnut (*Juglans regia*) (Keshavarz et al., 2011). Therefore, the present experiment was undertaken to study the effect of girdling and zinc and boron application on growth, quality and leaf nutrient status of olive cv. Frontoio.

MATERIALS AND METHODS

Study site

The present investigation was conducted at the Division of Fruit Science, Faculty of Agriculture at Chatha, SKUAST-Jammu, India during 2008 to 2009 and 2009 to 2010. The experimental site was situated in the intermediate zone at latitude 32°50′ North and longitude 74°55′ East at an elevation of 1100 m above mean sea level with an annual precipitation of about 910 mm, mostly coinciding from February to June (about 80%). The mean annual maximum and minimum temperatures are 21.72 and 12.57°C, respectively.

Soil of the experimental field was clay loam with pH 6.4. The organic carbon content, available nitrogen, phosphorus and potassium contents of the soil were 0.87%, 283, 73 and 188 Kg ha^{-1}, respectively.

Experimental setup

The experiment was laid out in a randomized block design with seventeen treatments T_1: Control, T_2: Girdling, T_3: 0.4% $ZnSO_4$, T_4: 0.5% $ZnSO_4$, T_5: 0.6% $ZnSO_4$, T_6: 0.4% Boric acid, T_7: 0.5% Boric acid, T_8: 0.6% Boric acid, T_9: 0.4% $ZnSO_4$ + 0.4% Boric acid, T_{10}: 0.4% $ZnSO_4$ + 0.5% Boric acid, T_{11}: 0.4% $ZnSO_4$ + 0.6% Boric acid, T_{12}: 0.5% $ZnSO_4$ + 0.4% Boric acid, T_{13}: 0.5% $ZnSO_4$ + 0.5% Boric acid, T_{14}: 0.5% $ZnSO_4$ + 0.6% Boric acid, T_{15}: 0.6% $ZnSO_4$ + 0.4% Boric acid, T_{16}: 0.6% $ZnSO_4$ + 0.5% Boric acid, and T_{17}: 0.6% $ZnSO_4$ + 0.6% Boric acid. Each treatment was replicated thrice with single tree as one replication (Figure 1). All the bearing branches of the trees were girdled by removing 10 mm wide rings of bark, one week before full bloom. Both the nutrients were sprayed in the first week of March and repeated 30 days after the first spray, during both years of investigation.

Statistical analysis

The data were subjected to statistical analysis according to the methodology proposed by Panse and Sukhatme (2000). Critical difference (C.D) at 5% level was used for finding the significant differences, if any, among the treatment means.

Shoot extension growth

For measuring shoot extension growth, twenty uniform and healthy shoots were randomly selected all over the tree canopy in all directions. The length of each shoot was measured at the beginning and end of growing season between the points of initiation of new growth to the extremity of the shoot tip and expressed in centimeters.

Chlorophyll content

Leaf chlorophyll content was estimated by the procedure reported by Arnon (1949) and the total chlorophyll content was calculated using the following equation:

$$\text{Total chlorophyll (mg}^{-1}\text{ g}^{-1}) = 20.2\,(A_{645}) + 8.02\,(A_{663}) \times \frac{V}{1000 \times W}$$

Where, A_{645} = Absorbance at 645 nm, A_{663} = Absorbance at 663 nm, V = volume of aliquot; W = weight of the leaf tissue (g).

For the measurement of physical parameters of fruits, one hundred healthy fruits were randomly selected from each treatment.

Fruit size

Fruit size of fifteen randomly selected fruits from each replication was measured in centimeters by using a digital vernier caliper and was expressed as an average of length and diameter.

Fruit weight

Fruit weight was recorded on a top pan electrical balance with an accuracy of ±0.5 g and expressed as mean fruit weight in grams.

Fruit volume

Fruit volume (cm^3) was estimated by the water displacement method using a graduated glass cylinder.

Pulp: stone ratio

For the estimation of pulp:stone ratio, fifteen fruits were randomly selected from each replication. The flesh of fruit was separated from the stone and the ratio between the weights of pulp and stone was worked out for all the treatments.

Oil content

Oil content of fruit pulp was estimated by the Soxhlet extraction method using hexane as a solvent (AOAC, 1980) and expressed in percentage on a fresh weight basis.

Macro-nutrient

For estimation of the macro-nutrient status of experimental trees, one hundred fully expanded leaves of each treatment along with petioles were sampled from the middle portion of the previous season's shoots situated all around the canopy of the tree as recommended by Chapman (1964). Cleaning, drying, grinding and storing of samples were carried out in accordance with the procedures outlined by Kenworthy (1964). For the estimation of N, 0.5 g of plant material was digested in 15 to 20 ml concentrated sulphuric acid in the presence of a digestion catalyst as described by Jackson (1973). The aliquot thus derived was used for the estimation of total N by micro Kjeldahl method (AOAC, 1980). The estimation of P, K, Ca and Mg digestion was done in a triacid

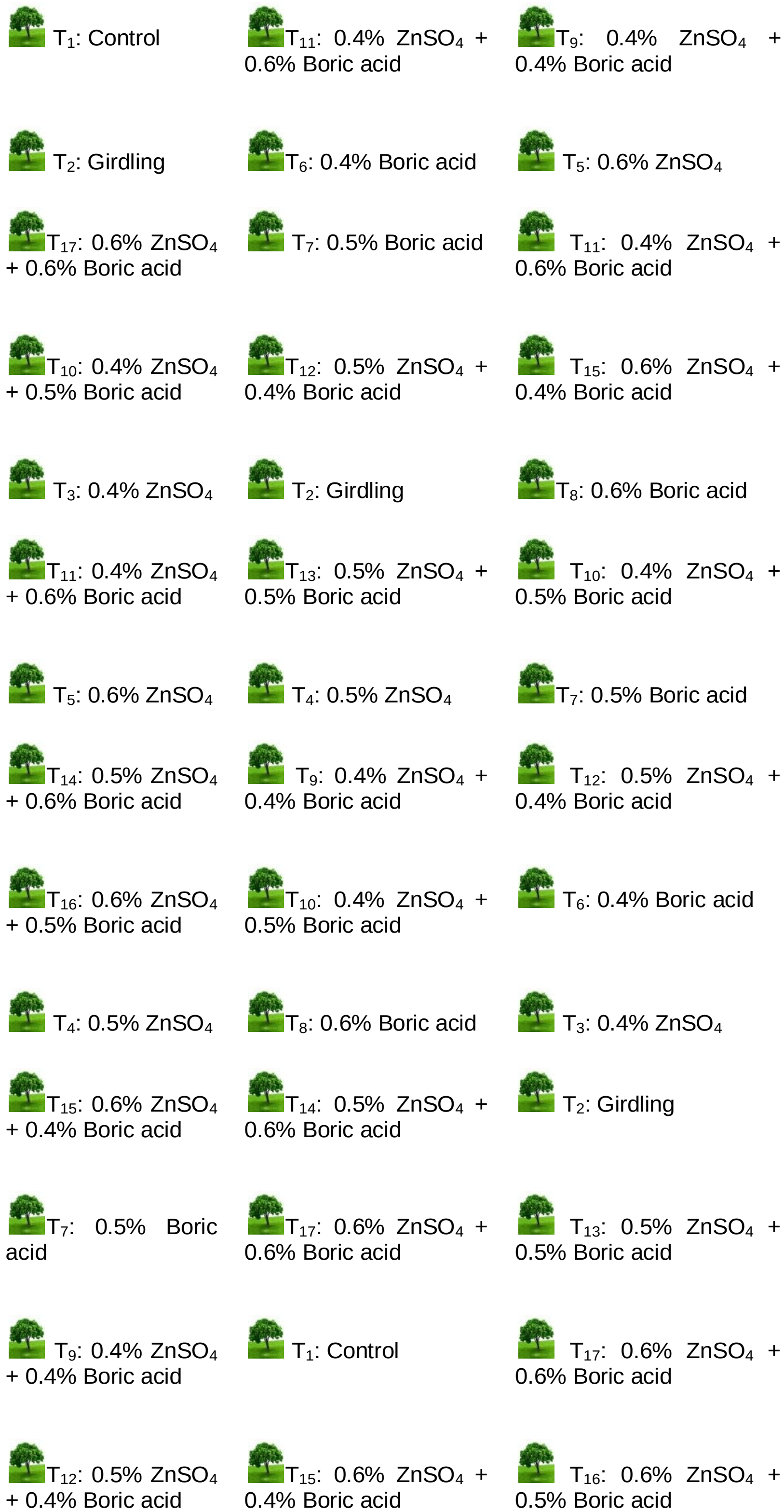

Figure 1. Layout of the experimental field.

mixture containing concentrated nitric acid, perchloric acid and sulphuric acid in the ratio of 10:4:1. All precautions, as suggested by Piper (1966), for wet digestion of leaf samples were taken. The volume of aliquot was made to 100 ml and filtered through

Table 1. Effects of girdling and zinc and boron application on shoot extension growth and chlorophyll content in olive cv. Frontoio.

Treatment	Shoot extension growth (cm)			Chlorophyll content (mg g^{-1})		
	2008-2009	2009-2010	Pooled	2008-2009	2009-2010	Pooled
T_1	6.10	6.63	6.37	1.10	1.02	1.06
T_2	5.74	6.46	6.10	1.17	1.11	1.14
T_3	6.65	6.64	6.65	1.25	1.18	1.21
T_4	6.72	6.90	6.81	1.36	1.30	1.33
T_5	7.10	6.82	6.96	1.42	1.37	1.40
T_6	7.00	7.27	7.14	1.21	1.16	1.18
T_7	7.24	7.30	7.27	1.32	1.27	1.29
T_8	7.53	7.46	7.49	1.40	1.35	1.38
T_9	7.88	7.62	7.75	1.28	1.21	1.24
T_{10}	8.03	7.65	7.84	1.44	1.31	1.38
T_{11}	8.22	7.81	8.02	1.53	1.47	1.50
T_{12}	8.46	7.89	8.18	1.35	1.30	1.32
T_{13}	9.22	8.15	8.69	1.48	1.42	1.45
T_{14}	9.15	8.37	8.76	1.51	1.45	1.48
T_{15}	9.40	8.26	8.83	1.31	1.25	1.28
T_{16}	9.41	8.38	8.90	1.38	1.32	1.35
T_{17}	9.39	8.44	8.92	1.43	1.38	1.40
C.D $_{(0.05)}$	1.11	1.35	0.86	0.11	0.13	0.08

Whatman no.1 filter paper. Total P was determined by the Vanadomolybdo phosphoric yellow colour method as described by Jackson (1973), while total K in the plant sample was estimated with the help of a Corning 410 digital Flame photometer. Calcium and Magnesium were determined on an Atomic Absorption Spectrophotometer.

RESULTS AND DISCUSSION

Effects of girdling and micro-nutrients on shoot extension growth of olive

The shoot extension growth was significantly influenced by girdling and micro-nutrient sprays of zinc and boron (Table 1). During 2008 to 2009, maximum shoot extension growth (9.41 cm) was recorded in trees sprayed with 0.6% zinc sulphate in combination with 0.5% boric acid which was statistically similar to shoot extension growth recorded in trees sprayed with 0.6% zinc sulphate in combination with 0.4% boric acid (9.40 cm), 0.6% zinc sulphate in combination with 0.6% boric acid (9.39 cm), 0.5% zinc sulphate in combination with 0.5% boric acid (9.22 cm), 0.5% zinc sulphate in combination with 0.6% boric acid (9.15 cm) and 0.5% zinc sulphate in combination with 0.4% boric acid (8.46 cm) whereas, minimum shoot extension growth (5.74 cm) was recorded in girdled trees. However, during the second year (2009 to 2010), shoot extension growth ranged from 6.46 to 8.44 cm, maximum shoot extension growth (8.44 cm) was recorded with 0.6% zinc sulphate in combination with 0.6% boric acid and least shoot extension growth (6.46 cm) was recorded in girdled trees. Pooled data showed maximum shoot extension (8.92 cm) in trees sprayed with 0.6% zinc sulphate in combination with 0.6% boric acid. The treatments *viz*., 0.6% zinc sulphate in combination with 0.5% boric acid, 0.6% zinc sulphate in combination with 0.4% boric acid, 0.5% zinc sulphate in combination with 0.6% boric acid, 0.5% zinc sulphate in combination with 0.5% boric acid and 0.5% zinc sulphate in combination with 0.4% boric acid were equally effective in registering shoot extension growth within the range of 8.18 to 8.90 cm. Minimum shoot extension growth (6.10 cm) was registered in girdled trees. These findings are in accordance with the results obtained by Singh et al. (2009). They found that interactions of zinc, at higher levels and boron either at lower or higher concentration caused significant improvement in shoot length of mango cv. Dashehari. Decrease in shoot extension growth was recorded in olive trees with girdling as compared to untreated trees and is in consonance with the findings of Ungerer and Steyn (2009) and Choi et al. (2010) who also observed decreased shoot growth in persimmon with girdling as compared to untreated trees.

Effects of girdling and micro-nutrients on leaf chlorophyll content of olive

During 2008 to 2009, the highest leaf chlorophyll content

Table 2. Effects of girdling and zinc and boron application on fruit size, fruit weight and fruit volume of olive cv. Frontoio

Treatment	Fruit size (cm)			Fruit weight (g)			Fruit volume (cm^3)		
	2008-2009	2009-2010	Pooled	2008-2009	2009-2010	Pooled	2008-2009	2009-2010	Pooled
T_1	1.27	1.29	1.28	1.13	1.18	1.15	1.31	1.33	1.32
T_2	1.34	1.37	1.35	1.21	1.26	1.24	1.39	1.41	1.40
T_3	1.31	1.33	1.32	1.16	1.22	1.19	1.35	1.37	1.36
T_4	1.34	1.36	1.35	1.20	1.24	1.22	1.38	1.40	1.39
T_5	1.35	1.39	1.37	1.22	1.27	1.24	1.40	1.41	1.41
T_6	1.36	1.38	1.37	1.21	1.28	1.24	1.40	1.42	1.41
T_7	1.43	1.40	1.42	1.27	1.30	1.28	1.44	1.46	1.45
T_8	1.45	1.41	1.43	1.29	1.33	1.31	1.44	1.47	1.46
T_9	1.44	1.44	1.44	1.27	1.36	1.32	1.46	1.49	1.48
T_{10}	1.51	1.46	1.49	1.32	1.38	1.35	1.50	1.53	1.51
T_{11}	1.55	1.43	1.49	1.34	1.41	1.38	1.49	1.54	1.52
T_{12}	1.52	1.52	1.52	1.32	1.45	1.39	1.51	1.55	1.53
T_{13}	1.60	1.54	1.57	1.39	1.50	1.45	1.59	1.63	1.61
T_{14}	1.62	1.51	1.56	1.41	1.51	1.46	1.53	1.64	1.58
T_{15}	1.56	1.54	1.55	1.35	1.48	1.42	1.55	1.59	1.57
T_{16}	1.60	1.56	1.58	1.38	1.49	1.44	1.58	1.63	1.61
T_{17}	1.62	1.57	1.59	1.39	1.51	1.45	1.59	1.62	1.60
C.D $_{(0.05)}$	0.09	0.08	0.06	0.07	0.03	0.04	0.08	0.05	0.05

(1.53 mg g^{-1} $^{-1}$) was observed in trees sprayed with 0.4% zinc sulphate in combination with 0.6% boric acid (Table 1) closely followed by trees sprayed with 0.5% zinc sulphate in combination with 0.6% boric acid, 0.5% zinc sulphate in combination with 0.5% boric acid, 0.4% zinc sulphate in combination with 0.5% boric acid and 0.6% zinc sulphate in combination with 0.6% boric acid registering values of 1.51, 1.48, 1.44 and 1.43 mg g^{-1} $^{-1}$, respectively which were similar to each other, whereas the lowest leaf chlorophyll content (1.10 mg g^{-1} $^{-1}$) was obtained in untreated trees which was similar to the chlorophyll content recorded in girdled trees (1.17 mg g^{-1} $^{-1}$). The results from the second year also exhibited similar patterns as those observed in the first year of study. The leaf chlorophyll content ranged from 1.02 to 1.47 mg g^{-1} $^{-1}$ registering the highest chlorophyll content (1.47 mg g^{-1} $^{-1}$) in trees sprayed with 0.4% zinc sulphate in combination with 0.6% boric acid and the lowest leaf chlorophyll content (1.02 mg g^{-1} $^{-1}$) in untreated trees. The pooled data showed highest leaf chlorophyll content of 1.50 mg g^{-1} $^{-1}$ in the trees sprayed with 0.4% zinc sulphate in combination with 0.6% boric acid and was at par with the values found in trees sprayed with 0.5% zinc sulphate in combination with 0.6% boric acid (1.48 mg g^{-1} $^{-1}$) and 0.5% zinc sulphate in combination with 0.5% boric acid (1.45 mg g^{-1} $^{-1}$). The lowest leaf chlorophyll content (1.06 mg g^{-1} $^{-1}$) was found in control. The findings in the present study are in line with the results obtained by Keshavarz et al. (2011) who recorded the highest chlorophyll index in Persian walnut with foliar application of 1050 mg L^{-1} zinc in combination with 174 mg L^{-1} boron. The increase in leaf chlorophyll content with foliar sprays of zinc and boron can also be attributed to the fact that zinc is part of the carbonic anhydrous enzyme, present in all the photosynthetic tissues and it is required for chlorophyll biosynthesis (Ryugo, 1988). Nitrogen and magnesium are the main constituents of chlorophyll and nitrogen and magnesium status of leaves in trees is when is improved when sprayed with zinc and boron. However, in the present investigation girdling exerted no significant improvement in total leaf chlorophyll content. The lack of response to girdling in enhancing chlorophyll may be due to elevated starch level in the leaves of girdled shoots which inhibited the photosynthetic activity as reported by Proietti and Tombesi (1990) who also obtained similar results with girdling in olive cv. Leccino.

Effects of girdling and micro-nutrients on physical parameters of olive

All the physical parameters of fruits *viz*., fruit size, fruit weight and fruit volume were significantly increased with girdling as well as with foliar application of zinc sulphate and boric acid applied singly or in combination with each other (Table 2). Foliar spray of 0.5% zinc sulphate and 0.5% boric acid was found to be the best in enhancing all the physical parameters of olive fruits. A positive effect of girdling on fruit size and weight has been reported in grapes (Ahmad et al., 2005), persimmon (Choi et al., 2010) and peach (Chanana and Gill, 2006). A positive effect of zinc on fruit weight, size and volume, as the one

Table 3. Effects of girdling and zinc and boron application on pulp:stone ratio and oil content of olive cv. Frontoio

Treatment	Pulp:stone ratio			Oil content (%)		
	2008-2009	2009-2010	Pooled	2008-2009	2009-2010	Pooled
T_1	2.21	2.28	2.24	21.23 (4.72)*	20.68 (4.66)	20.96 (4.69)
T_2	2.28	2.36	2.32	22.20 (4.82)	22.03 (4.80)	22.12 (4.81)
T_3	2.25	2.66	2.45	21.86 (4.78)	21.84 (4.78)	21.85 (4.78)
T_4	2.28	2.36	2.32	22.28 (4.82)	22.57 (4.85)	22.42 (4.84)
T_5	2.30	2.38	2.34	22.62 (4.86)	22.92 (4.89)	22.77 (4.88)
T_6	2.30	2.39	2.34	23.11 (4.91)	23.41 (4.94)	23.26 (4.93)
T_7	2.33	2.42	2.37	23.10 (4.91)	23.40 (4.94)	23.25 (4.92)
T_8	2.35	2.43	2.39	23.19 (4.92)	23.49 (4.95)	23.34 (4.93)
T_9	2.36	2.46	2.41	24.02 (5.00)	24.30 (5.03)	24.16 (5.02)
T_{10}	2.39	2.50	2.44	24.01 (5.00)	24.28 (5.03)	24.15 (5.01)
T_{11}	2.39	2.50	2.44	23.66 (4.97)	24.25 (5.02)	23.96 (4.99)
T_{12}	2.44	2.56	2.50	25.25 (5.12)	25.53 (5.15)	25.39 (5.14)
T_{13}	2.49	2.60	2.55	25.26 (5.12)	25.50 (5.15)	25.38 (5.13
T_{14}	2.43	2.57	2.50	24.73 (5.07)	25.01 (5.10)	24.87 (5.09)
T_{15}	2.48	2.59	2.53	25.49 (5.15)	25.75 (5.17)	25.62 (5.16)
T_{16}	2.51	2.62	2.56	25.17 (5.12)	25.36 (5.13)	25.27 (5.13)
T_{17}	2.53	2.60	2.57	24.77 (5.08)	25.05 (5.10)	24.91 (5.09)
C.D $_{(0.05)}$	0.05	NS	0.12	0.07	0.07	0.05

*Figures in the parentheses are transformed mean, NS: Non-significant.

the one obtained in the present investigation, has also been reported by Sharma et al. (2003) in kagzi lime and Singh et al. (2009) in mango as zinc is required to obtain good fruit size being the part of the carbonic anhydrous enzyme, present in the photosynthetic tissues and also required for chlorophyll biosynthesis (Ryugo, 1988). Similarly, boron application also resulted in an increase of fruit weight, size and volume.

The beneficial effect of boron on these parameters might be due to its role in cell division and cell elongation. Similar findings have been reported by Dutta (2004) in mango; Bybordi and Malakouti (2006) in almond and Yadav et al. (2010) in aonla. In the present investigation, the use of zinc and boron together might have acted synergistically thereby improving the physical parameters of olive fruit. Similar results have been obtained by Banik and Sen (1997) who observed a significant increase in fruit weight with the application of zinc in combination with boron in mango whereas, Tariq et al. (2007) also obtained maximum fruit size and fruit volume with foliar spray of zinc and boron in Blood Red cultivar of sweet orange.

Effects of girdling and micro-nutrients on pulp: stone ratio of olive

In the first year of study (Table 3), pulp:stone ratio reached to a maximum of 2.53 in trees sprayed with 0.6% zinc sulphate in combination with 0.6% boric acid closely followed by trees sprayed with 0.6% zinc sulphate in combination with 0.5% boric acid, 0.5% zinc sulphate in combination with 0.5% boric acid and 0.6% zinc sulphate in combination with 0.4% boric acid registering values of 2.51, 2.49 and 2.48, respectively and were similar to each other.

The pulp: stone ratio during the second year of experimentation (2009 to 2010) ranged from 2.28 to 2.62 wherein, the maximum pulp:stone ratio (2.62) was recorded in trees sprayed with 0.6% zinc sulphate in combination with 0.5% boric acid and the minimum pulp: stone ratio (2.28) was obtained under untreated trees. The pooled data showed that maximum pulp:stone ratio (2.57) was observed in trees sprayed with 0.6% zinc sulphate in combination with 0.6% boric acid closely followed by trees sprayed with 0.6% zinc sulphate in combination with 0.5% boric acid, 0.5% zinc sulphate in combination with 0.5% boric acid, 0.6% zinc sulphate in combination with 0.4% boric acid, 0.5% zinc sulphate in combination with 0.6% boric acid, and 0.5% zinc sulphate in combination with 0.4% boric acid registering pulp:stone ratioss of 2.56, 2.55, 2.53, 2.50 and 2.50, respectively, and were similar to each other. The minimum pulp: stone ratio (2.24) was found in untreated trees.

The results are in conformity with the findings of Hamdy et al. (2007) who obtained the highest pulp:stone ratio in mango cv. Hindy Bisinara with foliar application of a mixture containing citric acid (500 ppm), boric acid (0.025%), chelated zinc (0.05%) and magnesium sulphate (0.25%).

Table 4. Effects of girdling and zinc and boron application on total leaf N, P, K, Ca, and Mg in olive cv. Frontoio

Treatment	N (%)	P (%)	K (%)	Ca (%)	Mg(%)
	Pooled	Pooled	Pooled	Pooled	Pooled
T_1	1.58(1.60)*	0.216(1.10)	1.57(1.60)	1.50(1.58)	0.135(1.06)
T_2	1.62(1.62)	0.227(1.11)	1.67(1.63)	1.62(1.62)	0.141(1.07)
T_3	1.61(1.62)	0.225(1.11)	1.67(1.63)	1.60(1.61)	0.145(1.07)
T_4	1.64(1.63)	0.226(1.11)	1.67(1.63)	1.63(1.62)	0.148(1.07)
T_5	1.62(1.62)	0.226(1.11)	1.69(1.64)	1.65(1.63)	0.149(1.07)
T_6	1.62(1.62)	0.224(1.11)	1.69(1.64)	1.66(1.63)	0.146(1.07)
T_7	1.60(1.61)	0.224(1.11)	1.70(1.64)	1.65(1.63)	0.148(1.07)
T_8	1.61(1.62)	0.219(1.10)	1.72(1.65)	1.65(1.63)	0.147(1.07)
T_9	1.61(1.62)	0.226(1.11)	1.72(1.65)	1.67(1.63)	0.146(1.07)
T_{10}	1.68(1.64)	0.224(1.11)	1.72(1.65)	1.68(1.64)	0.148(1.07)
T_{11}	1.68(1.64)	0.219(1.10)	1.73(1.65)	1.66(1.63)	0.150(1.07)
T_{12}	1.68(1.64)	0.220(1.10)	1.74(1.65)	1.71(1.64)	0.147(1.07)
T_{13}	1.61(1.61	0.223(1.10)	1.71(1.64)	1.71(1.64)	0.149(1.07)
T_{14}	1.61(1.61)	0.220(1.10)	1.70(1.64)	1.68(1.64)	0.151(1.07)
T_{15}	1.64 (1.63)	0.222(1.10)	1.71(1.64)	1.72(1.65)	0.150(1.07)
T_{16}	1.66(1.63)	0.221(1.11)	1.71(1.65)	1.70(1.64)	0.153(1.07)
T_{17}	1.63(1.62)	0.222(1.10)	1.72(1.65)	1.68(1.64)	0.154(1.07)
C.D $_{(0.05)}$	NS	NS	0.02	0.02	0.003

*Figures in the parentheses are transformed mean, NS: Non-significant.

Effects of girdling and micro-nutrients on oil content of olive

Application of 0.6% zinc sulphate in combination with 0.4% boric acid resulted in the highest oil content ((25.49%)%) which was significantly higher compared to all other treatments during the year 2008 to 2009, whereas the minimum oil content (21.23%) was extracted from fruits of untreated trees (Table 3). During the second year of investigation, application of 0.6% zinc sulphate in combination with 0.4% boric acid resulted in significantly higher oil content (25.75%) as compared to all other treatments tried in this study while, it was minimum (20.68%) in control. From the pooled data it is evident that the highest oil content (25.62%) was obtained in fruits from trees sprayed with 0.6% zinc sulphate in combination with 0.4% boric acid, which was significantly higher than all other treatments. The minimum oil content of 20.96%% was recovered from fruits of untreated trees. Overall improvement in fruit weight, size and pulp: stone ratio in the present study might be responsible for increased oil contents in olive fruit and the results obtained are in consonance with the findings of Jordao and Lietao (1990) who reported that there was a positive correlation between the fruit Zn concentration and the weight and oil content of the olive fruit.

Effects of girdling and micro-nutrients on leaf N, P, K, Ca and Mg content of olive

In the present study, leaf nitrogen and phosphorus were not affected by girdling and foliar application of zinc sulphate and boric acid applied singly or in combination. However, leaf potassium, calcium and magnesium were increased as compared to untreated trees (Table 4). Foliar application of boric acid and zinc sulphate had significant effects on the concentration of nitrogen, phosphorus and potassium in leaves and fruits of olive cultivar Zard as reported by Taheri and Talaie (2001) and the results obtained in the present study are in line with their findings.

Conclusion

From this study it may be concluded that foliar application of zinc sulphate in combination with boric acid resulted in better growth, physical characteristics and oil content of olive fruits as compared to control and single application of zinc sulphate or boric acid. Application of 0.5% zinc sulphate in combination with 0.5% boric acid proved to be the best for improving the shoot extension growth, chlorophyll content of leaves, fruit size, weight and volume, pulp:stone ratio and oil content of olive cv. Frontoio.

Conflict of Interests

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

REFERENCES

Ahmad M, Kaul RK, Kaul BL (2005). Effect of girdling, thinning and GA_3

on fruit growth, yield, quality and shelf life of grapes (*Vitis vinifera* L.) cv. Perlette. Acta Hort. 696:309-313.

AOAC (1980). Official methods of Analysis of the analytical chemists. Association of official Agricultural Chemists, 13th Edn., Washington, D.C. P1018.

Arnon DI (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24:1-15.

Banik BC, Sen SK (1997). Effect of three level of iron, boron, zinc and their interactions on growth, flowering and yield of mango cv. Fazli. Hort. J. 10(1): 23-29.

Bybordi A, Malakouti MJ (2006). Effects of foliar applications of nitrogen, boron and zinc on fruit setting and quality of almonds. Acta Hort. 726:351-357.

Chanana YR, Gill KS (2006). Impact of girdling, thinning and their combination on quality and maturity in Florda Prince peach. Indian J. Hort. 63(1):27-30.

Chapman HD (1964). Suggested foliar sampling and handling techniques for determining nutrient status of some field, horticultural and plantation crops. Indian J. Hort. 21:98-119.

Choi ST, Song WD, Park DS, Kang SM (2010). Effect of different girdling dates on tree growth, fruit characteristics and reserve accumulation in a late-maturing persimmon. Sci. Hort.126:152-155.

Dutta P (2004). Effect of foliar boron application on panicle growth, fruit retention and physico-chemical characters of mango cv. Himsagar. Indian J. Hort. 61(3):265-266.

FAO (2012). Statistical Year Book 2012. A Publication of Food and Agricultural Organization. Available at *http:// faostat3. fao.org /home /index.html#DOWNLOAD.*

Hamdy IMI, Ahmed YM, Ahmed FF (2007). Relation of fruiting in Hindy Bisinara mangoes to foliar nutrition with Mg, B and Zn and some antioxidants. In: 8th African Crop Science Society Conference, El-Minia, Egypt. 27(31):411-415.

Jackson ML (1973). Soil Chemical Analysis. Prentice Hall of India Ltd., New Delhi, P. 498.

Jordao PV, Lietao F (1990). The olive mineral composition and some parameters of quality in fifty olive cultivars in Portugal. Acta Hort. 286:461-464.

Kenworthy AL (1964). Fruit, nut and plantation crops, deciduous and evergreen. A guide for collecting foliar samples for nutrient element analysis. Memo Horticultural Department Michigan State University, Michigan, pp. 223-224.

Keshavarz K, Vahdati K, Samar M, Azadegan B, Brown PH (2011). Foliar application of zinc and boron improves walnut vegetative and reproductive growth. Hort. Tech. 21(2):181-186.

Panse VG, Sukhame PV (2000). Statistical Methods for Agricultural Workers. Publication Information Division of ICAR, New Delhi.

Piper CS (1966). Soil and Plant Analysis. Hans Publishers, Bombay, India, pp. 40-51.

Proietti P, Tombesi A (1990). Effect of girdling on photosynthetic activity in olive leaves. Acta Hort. 286:215-218.

Rajaie M, Ejraie AK, Owliaie HR, Tavakoli AR (2009). Effect of zinc and boron interaction on growth and mineral composition of lemon seedlings in a calcareous soil. International J. Plant Prod. 3(1):39-50.

Ryugo K (1988). Fruit culture: It's Science and Art. John Wiley and Sons. pp. 259-261.

Sharma AK, Singh K, Mishra SP (2003). Effect of foliar spray of zinc sulphate, 2,4,5-T and GA_3 on quality of kagzi lime (*Citrus aurantifolia* Swingle). The Orissa J. Hort. 31(2):29-32.

Singh VK, Bhriguvanshi SR, Chatterjee C (2009). Effect of micro-nutrients on growth and yield of mango (*Mangifera indica* L.) cv. Dashehari. Asian J. Hort. 4(1):112-115.

Taheri M, Talaie A (2001). The effect of chemical spray on the qualitative and quantitative characteristics of "Zard" olive fruits. Acta Hort. 564:343-348.

Tariq M, Sharif M, Shah Z, Khan R (2007). Effect of foliar application of micro-nutrients on the yield and quality of sweet orange (*Citrus sinensis* L.). Pak. J. Biol. Sci. 10(11):1823-1828.

Ungerer SF, Steyn WJ (2009). Effect of scoring and GA_3 application during full bloom on fruit set and yield in 'Triumph' Persimmon. Acta Hort. 833:207-211.

Yadav S, Shukla HS, Ram RA (2010). Studies on foliar application of NAA, GA_3, boric acid, and Ca $(NO_3)_2$ on fruit retention, growth, yield and quality of aonla (*Emblica officinalis* Gaertn,) cv. Banarasi. Hort. J. 23(2):64-67.

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Optimization of pre-treatment conditions for maximum pulp recovery with optimum quality from bael fruit (*Aegle marmelos* Correa.)

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The effect of pH, heating temperature, heating time and crude mass to water ratio on yield, total soluble solids and ascorbic acid content of pulp were studied. A central composite design was used to optimize the conditions of pulp extraction from bael fruit to maximize pulp yield, total soluble solids and maximum retention of ascorbic acid in pulp. The regression model describing the changes of pulp yield, total soluble solids (TSS) and ascorbic acid of pulp with respect to pretreatment conditions were derived with the coefficient of determination (R^2), 0.9883, 0.9814 and 0.9554, respectively. The models were found to be fit to predict the data under the different ranges of conditions. The pulp yield, TSS and ascorbic acid content of pulp obtained from bael fruit was 93.5 to 245.30%, 11-22°brix and 8.30-11.62 mg/100 g under the designed ranges, respectively. The optimized pretreatment conditions were pH; 3.3, heating time; 0.5 min, heating temperature; 70°C, and crude mass to water ratio; 1:0.8 and the pulp yield, TSS and ascorbic acid content under these conditions were 148.75%, 16.5°brix and 10.486 mg/100 g, respectively.

Key words: Yield, total soluble solids (TSS), ascorbic acid, pH, crude mass.

INTRODUCTION

The bael fruit (*Aegle marmelos* Correa. family: Rutaceae) is an important indigenous fruit of Asia and is known by different names such as Bael, Bel, Bengal Quince, Bil, Bilva, Bilpatre, Shul, Shaiphal, Vilvum etc. The ripe fruit of bael is sweet aromatic, nutritious and very palatable, being eaten by all classes of people. The excellent aroma of fruit is not destroyed even during processing therefore the fruit has untapped potential for processing. These products being highly nutritive and therapeutically important can be very easily popularized in Indian as well as international markets (Kaushik et al., 2000).

Because of Bael fruit's hard shell, mucilaginous texture and numerous seeds are not popular as fresh fruit. Although, excellent flavor, nutritive and therapeutic value of bael fruits show greater potentiality for processing into value added products (Ram and Singh, 2003). Bael fruit has been attributed with various nutritional and therapeutic properties such as in the cure of chronic

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Table 1. Experimental range and levels of the independent variables.

Variable	Range and levels		
	-1	0	+1
pH (X_1)	3	4	5
Heating time (X_2, min)	0.5	1.5	2.5
Heating temp. (X_3, °C)	70	80	90
*Crude mass : water (X_4)	0.5	1.0	1.5

*Crude mass was taken as 1 and the amount of water was varied as given in the table.

diarrhea and certain other gastrointestinal disorders. The marmelosin ($C_{13}H_{12}O_3$) content is found in the bael fruit which is known as panacea of stomach ailments (Singh and Nath, 2004; Pande et al., 1986; Roy and Singh, 1979). The edible pulp, 100 g of bael fruit contains 61.5 g water, 1.8 g protein, 0.39 g fat, 1.7 g minerals, 31.8 g carbohydrate, 55 mg carotene, 0.13 mg thiamine, 1.19 mg riboflavin, 1.1 mg niacin and 7 to 21 mg ascorbic acid (Rakesh et al., 2005).

Bael can be processed into products like preserves, refreshing beverages, powder, leather, squash, nectars, toffee, jam, syrup etc. The basic requirement for preparation of all these products is the pulp of bael fruit. The mucilaginous texture, fibres and numerous seeds make the extraction of pulp tough one. Rapid browning and development of off flavor due to enzyme activity make the extraction more complicated. The bael fruit pulp extracted by passing through the sieve without addition of water results in very sticky pulp. The pulp so obtained is unfit for handling and nearly 10% of pulp is lost during extraction, partly left with the pomace and partly sticking to the sieve (Roy and Singh, 1979; Shrestha, 2000).

This may be due to mucilage content of the pulp. Incorporation of water and application of heat coupled with acid dilute the mucilage considerably and make the pulp possible to extract easily (Roy and Singh, 1979). Kenghe and Potdar (2009) stated that water addition up to the ratio 1:1 does not impair the quality of extracted pulp even facilitate the pulp extraction. Ghosh and Gangopadhyaya (2002) extracted pulp by addition of water to pulp in proportion of 1:1 and 2:1 and centrifugation of the pulp at 4000 rpm for 10 min. Singh and Nath (2004) extracted pulp by adding water in the ratio of 1:1.25, heating at 90°C for 2 min while maintaining the pH 4.2 by 50% citric acid solution. Roy and Singh (1979) extracted pulp by adding 1:1 water, maintain the pH 4.3 by 0.5% citric acid and heating the mixture at 80°C for 1 min and passing through 20 mesh sieve. The yield by the method given by Roy and Singh was 125%.

Although, few workers have reported the development and evaluation of bael products (Verma and Gahlot, 2007; Rakesh et al., 2005; Roy and Singh, 1979), yet, there is a paucity of literature on processing technology of bael fruit pulp. This study was therefore carried out to optimize the pulp extraction method using response surface methodology.

MATERIALS AND METHODS

Fully ripe fresh bael fruits (*A. marmelos* Correa.) of *Kagazi* variety without any visual blemishes were procured from Agricultural farm of R.B.S. College, Bichpuri, Agra (India). The bael fruits were broken by hammering and the crude mass (pulp with seeds and fibres) was scooped out with the help of stainless steel spoon. The scooped crude mass was homogenized by blending manually. This crude mass was used to extract pulp.

Selection of relevant variables and experimental ranges

The extraction method involves pre-treatment parameters mainly pH, heating time, temperature and crude mass to water ratio. Therefore, the initial step was the selection of experimental ranges for the pre-treatment parameters as independent variables. The experimental ranges for the independent variables were selected as pH in the range of 3-5, heating time 0.5-2.5 min, heating temperature 70-90°C and crude mass to water ratio in range of 1:0.5-1:1.5 with respect to the reported literature and existing empirical knowledge (Roy and Singh, 1979; Kenghe and Potdar, 2009) (Table 1).

Experimental design and statistical analysis

Response surface methodology (RSM) was adopted in the experimental design. A three-level four-factor central composite face centered design was employed. The independent variables studied were pH (X_1), heating time (X_2, min), heating temperature (X_3, °C) and crude mass to water ratio (X_4), while response variables were pulp yield (%), TSS (°brix) and Ascorbic acid (mg/100 g). The 100 g of the crude mass was taken for every experiment and a total of 30 experiments were conducted. The range and experimental design matrix in coded (x) form and at the actual level of variables is given in Table 2. The response function (Y) was related to the coded variables (xi, i=1, 2, and 3) by a second degree polynomial equation (Equation 1):

$$y = b_0 + b_1 x_1 + b_2 x_2 + b_3 x_3 + b_{12} x_1 x_2 + b_{13} x_1 x_3 + b_{23} x_2 x_3 + b_{11} x_1^2 + b_{22} x_2^2 + b_{33} x_3^2 + \varepsilon \quad (1)$$

The coefficients of the polynomial model were represented by b_0 (constant), b_1, b_2, b_3 (linear effects); b_{12}, b_{13}, b_{23}, (interaction effects); b_{11}, b_{22}, b_{33} (quadratic effects); and ε (random error).

Extraction

For each experiment, 100 g of crude mass (pulp with seeds and

Table 2. The central composite face centered design employed for pulp extraction of bael fruit.

Exp. No.	Coded variables				Uncoded variables				Responses		
	X_1	X_2	X_3	X_4	pH	Time (min)	Temp (°C)	*Crude mass: Water	Pulp yield (%)	TSS (°Brix)	Ascorbic acid (mg/100 g)
1	-1	-1	-1	-1	3.00	0.50	70.00	0.50	98.1	21	11.62
2	1	-1	-1	-1	5.00	0.50	70.00	0.50	93.5	17.5	11.58
3	-1	1	-1	-1	3.00	2.50	70.00	0.50	109.8	21	10.62
4	1	1	-1	-1	5.00	2.50	70.00	0.50	101.1	19	10.12
5	-1	-1	1	-1	3.00	0.50	90.00	0.50	109.8	22	10.1
6	1	-1	1	-1	5.00	0.50	90.00	0.50	106.2	18	10
7	-1	1	1	-1	3.00	2.50	90.00	0.50	116.4	22	9.89
8	1	1	1	-1	5.00	2.50	90.00	0.50	111	20	9.97
9	-1	-1	-1	1	3.00	0.50	70.00	1.50	234.9	12	10.06
10	1	-1	-1	1	5.00	0.50	70.00	1.50	211.9	11	10.12
11	-1	1	-1	1	3.00	2.50	70.00	1.50	243.3	12	9.2
12	1	1	-1	1	5.00	2.50	70.00	1.50	223.9	11	9.32
13	-1	-1	1	1	3.00	0.50	90.00	1.50	236.9	13	9.06
14	1	-1	1	1	5.00	0.50	90.00	1.50	217.7	11	9.26
15	-1	1	1	1	3.00	2.50	90.00	1.50	245.3	13	8.3
16	1	1	1	1	5.00	2.50	90.00	1.50	221.9	12	8.6
17	-1	0	0	0	3.00	1.50	80.00	1.00	197.4	14	9.22
18	1	0	0	0	5.00	1.50	80.00	1.00	169.5	13	9.44
19	0	-1	0	0	4.00	0.50	80.00	1.00	163.3	14	10.2
20	0	1	0	0	4.00	2.50	80.00	1.00	193.6	15	9.16
21	0	0	-1	0	4.00	1.50	70.00	1.00	188.1	14	10.06
22	0	0	1	0	4.00	1.50	90.00	1.00	189.9	16	8.76
23	0	0	0	-1	4.00	1.50	80.00	0.50	100.6	21	10.78
24	0	0	0	1	4.00	1.50	80.00	1.50	230.9	12	9.06
25	0	0	0	0	4.00	1.50	80.00	1.00	193.5	16	9.23
26	0	0	0	0	4.00	1.50	80.00	1.00	196	14	9.06
27	0	0	0	0	4.00	1.50	80.00	1.00	173	15	9.21
28	0	0	0	0	4.00	1.50	80.00	1.00	197.5	16	9.39
29	0	0	0	0	4.00	1.50	80.00	1.00	183	14	9.42
30	0	0	0	0	4.00	1.50	80.00	1.00	191.2	15	9.13

*Crude mass was taken as 1 and the amount of water was varied as given in the table.

fibres) was added with water, pH was adjusted with 10.0% citric acid solution and subjected to various processing conditions as given in Table 2.

The temperature of the mixture was adjusted to the desired level (±0.5°C) by using a high precision water bath (Seco, Model 129, India). At the end of the treatment, the heated mixture was then sieved through 20 mesh screen. All the fibres and seeds were removed during sieving and the extract thus collected was considered as pulp for commercial use.

Evaluation of pulp yield, TSS

The pulp yield was calculated by following the standard method as reported by Singh et al. (2012). The TSS (°Brix) of the pulp was observed by Erma hand refractometer.

Determination of ascorbic acid

The ascorbic acid content of the pulp was determined by standard method given by Ranganna (1997). The method is based on the reduction of 2, 6-dichlorophenol indophenol by ascorbic acid. The dye which is blue in alkaline solution and red in acid solution is reduced by ascorbic acid to a colorless form.

The dye was standardized against standard ascorbic acid solution for determination of dye factor. Then the sample extract was titrated against the dye. The ascorbic acid content was determined by the following equation:

$$Ascorbic\ Acid\ (mg)/100\ g = (A * B * V * 100)/(D * W)$$

Where A is the volume in ml of standard dye used for titration, B is the weight in mg of ascorbic acid equivalent to 1 ml of indophenol

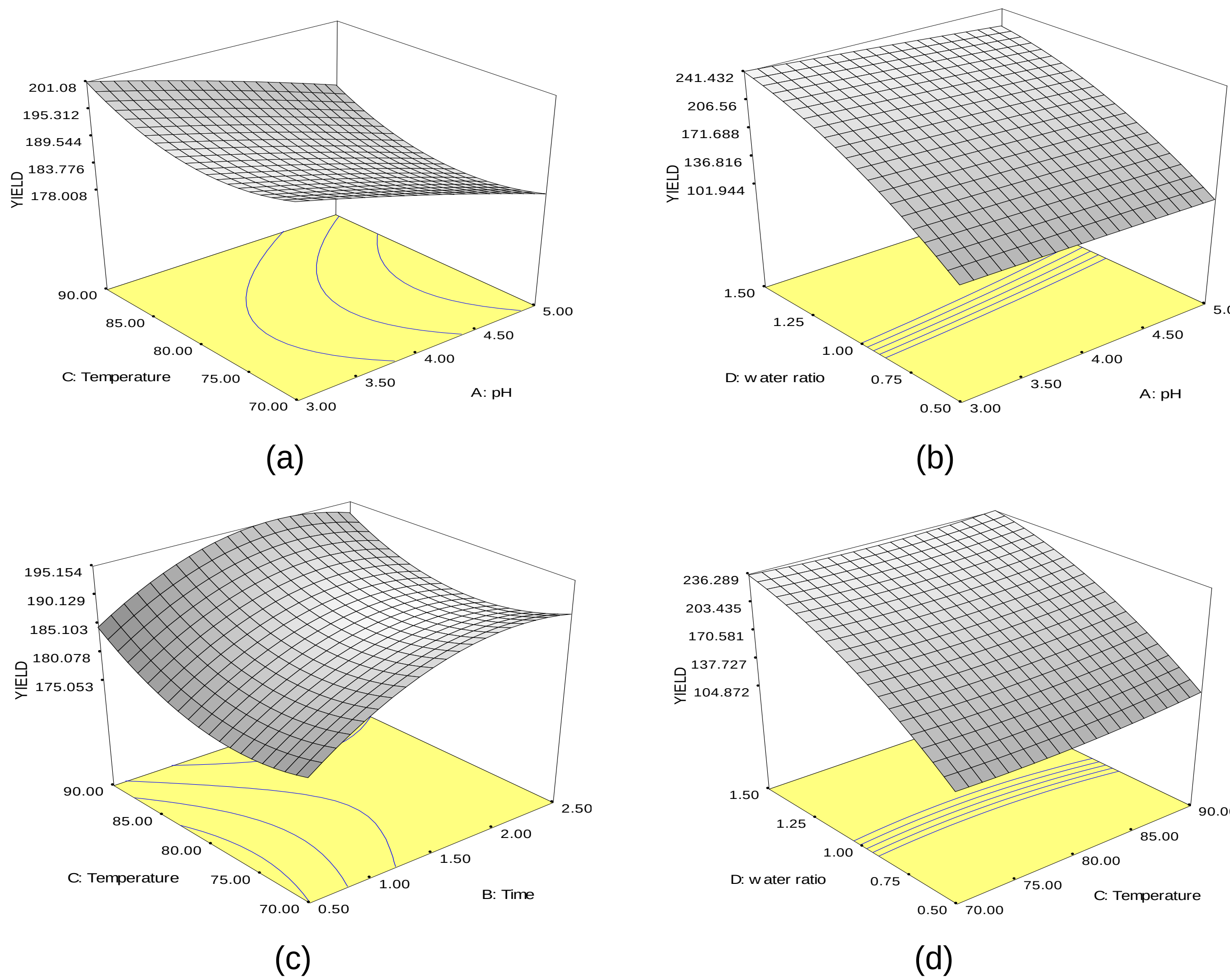

Figure 1. Response surfaces of pulp yield as a function of (a) Temperature and pH (b) water ratio and pH (c) temperature and time and (d) water ratio and temperature.

solution, that is, dye factor, V is the volume made up, D is the aliquot taken for estimation and W is the weight of the sample.

Optimization and validation of the model

Design expert version 6.0.10 (Trial version; STAT-EASE Inc., Minneapolis, MN, USA) software was used for regression and graphical analysis of the data obtained. The optimum values of the selected variables were obtained by solving the regression equation and also by analyzing the response surface contour plots. The validity and adequacy of the predictive models was done by experimental analysis at suggested optimum conditions by the design expert.

RESULTS AND DISCUSSION

The different responses under the different set of designed conditions are shown in Table 2. From the data, it is evident that the yield and quality of extracted pulp has been improved significantly from the variations in the pretreatment conditions.

The effect of processing conditions on the yield of extracted pulp

The yield of bael pulp ranged from 93.5 to 245.3% (Table 2). The minimum yield, 93.5% was observed at pH 5, time of boiling 0.5 min, temperature 70°C and crude mass to water ratio 1:0.5. The corresponding condition for maximum % yield (245.30%) was pH 3, time of boiling 2.5 min, temperature 90°C, and crude mass to water ratio 1:1.5. The response surface curves were plotted to explain the interaction of the variables and to determine the optimum level of each variable. The response surface curves for % yield are shown in Figure 1(a-d) and each figure demonstrates the effect of two factors while the other factors were fixed at middle level.

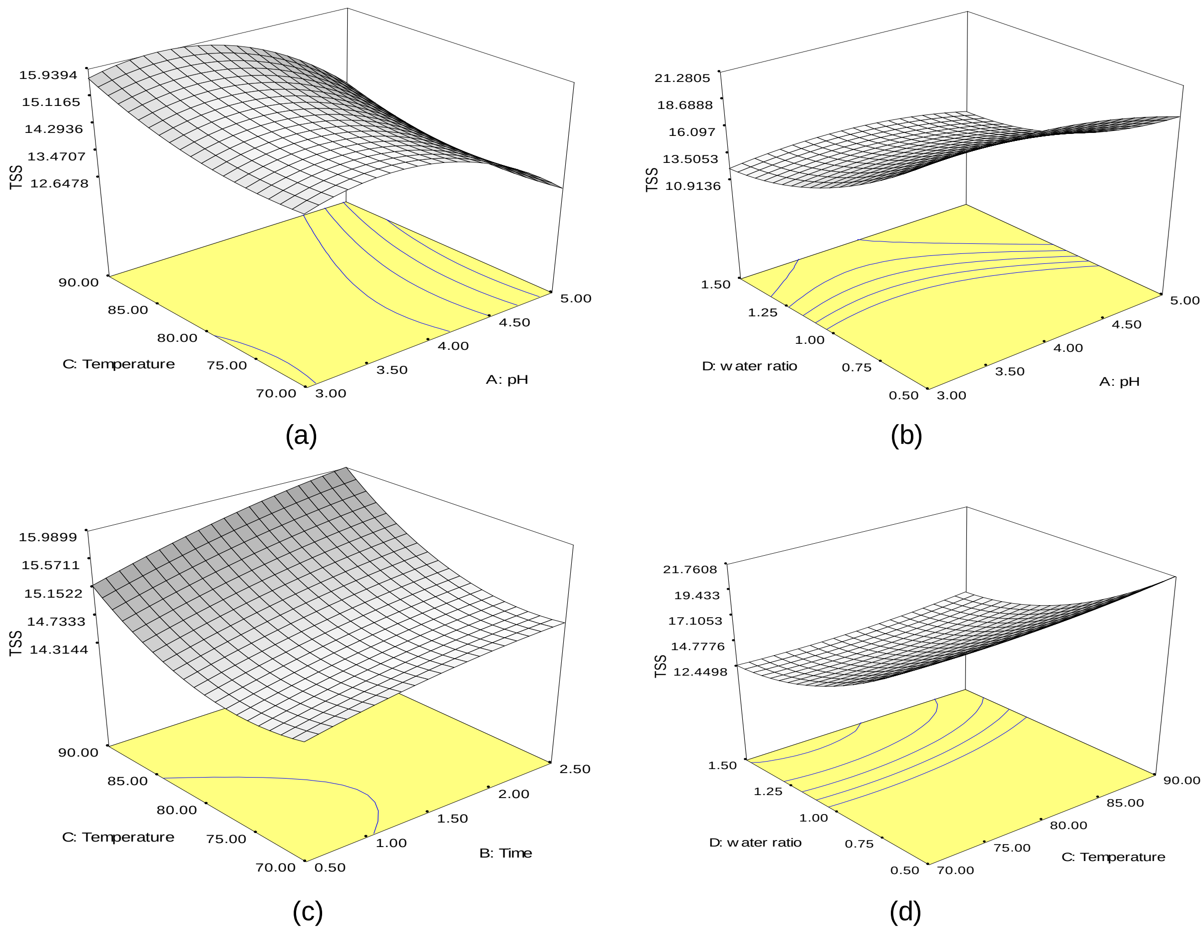

Figure 2. Response surfaces of TSS of pulp as a function of (a) Temperature and pH (b) water ratio and pH (c) temperature and time and (d) water ratio and temperature.

Figure 1a presents the interaction effect of pH (X_1) and heating temperature (X_3) on the yield of pulp. The yield increased as the pH decreased from 5 to 3 and the temperature increased from 70 to 90°C. The yield was 245.3% maximum at the heating temperature of 90°C and pH 3.0. Any decrease in heating temperature from 90°C caused the decrease in yield. High temperature helps in extraction of adhered pulp which possibly resulted to higher yield at the higher temperature. Roy and Singh (1979) and Kenghe and Potdar (2009) reported that heating at 80°C facilitated the extraction of pulp from bael fruit and increased the yield. Chopra and Singh (2001) also reported that heating at 100°C is ideal for easy extraction of pulp from wood apple fruit. Figure 1b reveals the effect of pH and crude to water ratio on the yield of pulp. It shows that the yield followed a linear behavior with increase in water ratio (1:0.5 to 1:1.5) and decrease in pH (from 5.0 to 3.0). The yield of pulp increased up to 245.3% at pH 3.0 and crude mass to water ratio 1:1.5. Any decrease in crude mass to water ratio from its maximum ratio, 1:1.5 caused decrease in pulp yield.

Figure 1c reveals the effect of heating time (X_2) and temperature (X_3) on the pulp yield. The yield followed a linear behavior with increase in heating time and temperature up to 2.0 min of heating at 90°C. With further increase in time, the yield slightly decreased, which may be due to the loss of water during heating. The maximum yield of 236.11% pulp was observed at 90°C and 1:1.5 crude mass to water ratio (Figure 1d).

The effect of processing conditions on the total soluble solids

The results showed that the TSS ranged from 11 to 22 °brix (Table 3). The minimum TSS was at pH 5, time of boiling 0.5 min, temperature 70°C, and crude mass to water ratio 1:1.5 whereas the corresponding condition for maximum TSS was pH 3, time of boiling 2.5 min, temperature 90°C, and crude mass to water ratio 1:0.5.

The interaction effect of pH (X_1) and heating temperature (X_3) on the TSS of pulp is represented in Figure 2a. TSS increased with increase in temperature

Table 3. Analysis of variance table (partial sum of squares) for response surface quadratic models for yield, TSS and Ascorbic acid of the pulp.

Source	Pulp yield		TSS		Ascorbic acid	
	Sum of squares	F-value	Sum of squares	F-value	Sum of squares	F-value
Model	74389.94	90.21	352.55	56.46	17.04	22.98
A	1015.50	17.24	17.01	38.14	6.422E-003	0.12
B	490.89	8.33	1.68	3.77	2.58	48.77
C	141.68	2.41	4.01	9.00	4.26	80.47
D	69713.78	1183.60	308.35	691.30	7.61	143.55
A^2	0.69	0.012	3.07	6.87	0.039	0.73
B^2	78.80	1.34	0.020	0.045	0.13	2.54
C^2	65.69	1.12	0.44	0.99	4.632E-003	0.087
D^2	859.62	14.59	9.47	21.24	0.57	10.70
AB	2.64	0.045	1.27	2.84	9.000E-004	0.017
AC	1.05	0.018	0.14	0.32	0.044	0.83
AD	245.71	4.17	2.64	5.92	0.096	1.81
BC	15.41	0.26	0.14	0.32	0.38	7.14
BD	0.33	5.613E-003	0.39	0.88	9.025E-003	0.17
CD	68.48	1.16	0.016	0.035	0.016	0.29
Lack of Fit	445.16	0.51	2.69	0.34	0.69	3.46
R-Square	0.9883		0.9814		0.9554	
Adj R-Square	0.9773		0.9640		0.9139	
Pred R-Square	0.9686		0.9498		0.7646	
Adeq Precision	28.847		24.940		20.129	
PRESS	2364.30		18.04		4.20	
CV	4.39		4.31		2.38	

and pH up to 90°C and 3.58 pH, respectively. The decrease in temperature from its maximum value, 90°C caused decrease in TSS. High temperature may facilitate extraction of pulp by dissolving the mucilage uniformly thereby may cause increase in total soluble solids (Roy and Singh, 1979). TSS was maximum, 21.09 °brix at pH 3.46 and crude mass to water ratio 1:0.5 (Figure 2b). With further increase in pH and water ratio, TSS decreased.

This may be due to less acid available for hydrolysis of mucilage. The mucilage is a gummy substance chemically allied to pectin and plant gums and is comprised of protein, polar glycoprotein, exopolysaccharides, polysaccharides and uranides (Narkhede et al., 2010).

Figure 2c reveals the effect of heating time (X_2) and heating temperature (X_3) on TSS of pulp. TSS followed a linear behavior and reaches its maximum value, 15.98 °brix at heating time 2.5 min and temperature 90°C. Joshi et al. (2012) suggested that heat treatment helps in complete removal of tamarind pulp adhered to the skin. The maximum TSS, 21.60 °brix of pulp was observed at 89.84°C and 1:0.5 crude mass to water ratio (Figure 2d). Increase in water causes the dilution of pulp and therefore lowers down total soluble solid. This was significant to note that crude mass to water ratio in the proportion of 1:1.5 caused the loss of pulp characteristics.

Ascorbic acid content

The ascorbic acid content of pulp ranged from 8.30 to 11.62 mg/100 g as shown in Table 1. The minimum ascorbic acid was at pH 3, time of boiling 2.5, temperature 90°C and crude mass: water ratio:: 1:1.5. The corresponding maximum retention of ascorbic acid was at pH 3, time of boiling 0.5 min, temperature 70°C, and crude mass to water ratio 1:0.5 under the various processing conditions. The loss of ascorbic acid at higher temperature which was expected is evident. The response surface curves were plotted to explain the interaction and to determine the optimum level of each variable. The response surface plots for ascorbic acid content are shown in Figure 3a-d.

Figure 3a presents the interaction effect of pH (X_1) and heating temperature (X_3) to the ascorbic acid content of the extracted pulp. The ascorbic acid content was 9.79 mg/100 g at heating temperature 70°C and pH 4.0. Any further increase in heating temperature caused gradual decrease in ascorbic acid content of the pulp. This may be due to the heat sensitivity of ascorbic acid as high

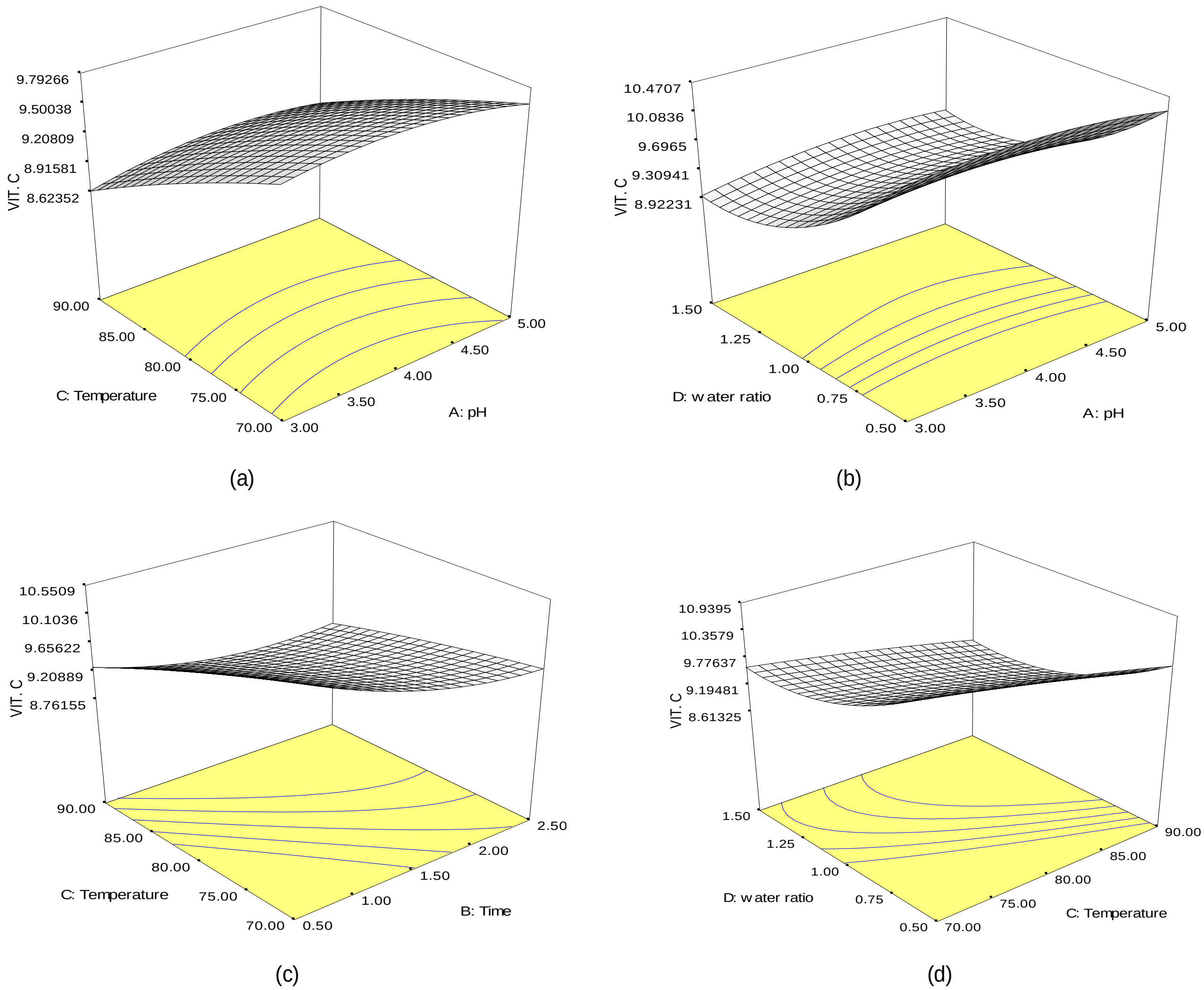

Figure 3. Response surfaces of ascorbic acid as a function of (a) Temperature and pH (b) water ratio and pH (c) temperature and time and (d) water ratio and temperature.

temperature aids the oxidation of ascorbic acid and forms dehydro ascorbic acid (Fenemma, 1985).

The ascorbic acid content of the extracted pulp was maximum 10.46 mg/100 g at crude mass to water ratio (1:0.5) and pH 4.0 (Figure 3b). With further increase in amount of water, ascorbic acid content gradually decreased. This may be due to the oxidation of ascorbic acid in water. The ascorbic acid readily oxidizes to dehydroascorbic acid, then to oxalic, and threonic acids in water (Stešková et al., 2006). The ascorbic acid content gradually decreased to minimum level 8.80 mg/100 g at maximum values of heating time 2.5 min and temperature 90°C (Figure 3c).

The ascorbic acid decreased linearly with increasing crude mass to water ratio and heating temperature. The maximum ascorbic acid of pulp 11.62 mg/100 g was observed at minimum heating temperature 70°C and minimum crude mass to water ratio 1:0.5. Minimum ascorbic acid was observed at temperature 90°C and 1:1.33 water ratio (Figure 3d).

Fitting of the model

The parameters of regression equations obtained by fitting of yield, TSS and ascorbic acid were given in Table 4. The fitness and adequacy of the model was judged by the coefficient of determination (R^2) which can be defined as the ratio of the explained variation to the total variation. The closer the R^2 value to unity, the better the empirical model fits the actual data. The coefficients of determination, R^2, for yield, TSS and ascorbic acid are 0.988, 0.981, 0.955, respectively, suggesting a good fit. The adjusted R^2 is a corrected value for R^2 after elimination of the unnecessary model terms. The adjusted R^2 was very close to their corresponding R^2 value in both models.

Table 4. Regression coefficients of predicted quadratic polynomial models for the responses

Coefficients	Pulp yield	TSS	Ascorbic acid
Intercept	186.50[a]	14.79[a]	9.35[a]
Linear			
A	-7.51[a]	-0.97[a]	0.019
B	5.22[b]	0.31[c]	-0.38[a]
C	2.81	0.47[b]	-0.49[a]
D	62.23[a]	-4.14[a]	-0.65[a]
Quadratic			
A^2	-0.51	-1.09[b]	-0.12
B^2	-5.51	-0.088	0.23
C^2	5.04	0.41	-0.042
D^2	-18.21[b]	1.91[a]	0.47[b]
Cross product			
A*B	-0.41	0.28	-7.500E-003
A*C	0.26	-0.094	0.053
A*D	-3.92[c]	0.41[b]	0.078
B*C	-0.98	0.094	0.15[b]
B*D	0.14	-0.16	-0.024
C*D	-2.07	-0.031	0.031

Statistically significant at [a] $p < 0.001$, [b] $p < 0.05$, and [c] $p < 0.10$.

High values of adjusted R^2 0.977, 0.964 and 0.914, respectively for yield, TSS and ascorbic acid also advocated significance of the models for all responses. The coefficient of variation (CV) describes the extent to which the data are dispersed. The coefficient of variation is a measure of residual variation of the data relative to the size of the mean; the small values of CV give better reproducibility.

The small CV values 4.39, 4.31 and 2.38 of the responses yield, TSS and ascorbic acid, respectively revealed that the experimental results were reliable. F-value of 90.21, 56.46 and 22.98 for yield, TSS and ascorbic acid, respectively implied that the models were significant ($P < 0.001$). The corresponding variables would be more significant if the absolute F value becomes greater and the p-value becomes smaller. The F-value for all responses was greater indicating the adequecy of the models to predict different responses at different pretreatment conditions.

Optimization and verification of process variables

The main criterion for constraints optimization was maximum pulp yield, TSS and ascorbic acid. Under the constraints, the optimum treatment conditions were found to be pH 3.28, heating time 0.5 min, boiling temperature 70°C and crude mass to water ratio 0.79. But in practice, it is difficult to maintain the recommended conditions during processing and some deviation is expected. Therefore, optimum conditions were targeted as pH 3.3, heating time 0.5 min, boiling temperature 70°C and crude mass to water ratio 0.80. Under the optimum condition (target constraint), experiments were conducted to verify the adequacy of the models. The experimental values of different responses under the optimum conditions of different variables were very close to the predicted values (Table 5), with the maximum percentage deviation of 3.67. This implied that there was a high fit degree between the observed and predicted values from the regression model.

Conclusion

The present study concluded that bael fruit pulp yield, TSS and ascorbic acid are function of pH, amount of water added and time of heating. Significant regression model describing the variation of pulp yield, TSS and ascorbic acid with respect to the independent variables (pH, heating time, heating temperature and crude mass to water ratio) were established with R^2>0.9 for all three responses. The pH and crude mass to water ratio were the most significant variable affecting the pulp yield and

Table 5. Optimization of process variables with respect to pulp yield, TSS and Ascorbic acid.

Process parameter	Optimum value (In the range)	Optimum value (Targeted)
Variables		
pH	3.28	3.3
Heating time (min)	0.5	0.5
Temperature (°C)	70	70
Crude mass: water	0.79	0.8

Responses	Predicted value	Experimental value	Deviation (%)
Pulp yield (%)	150.937	148.75	1.45
TSS (°Brix)	16.8086	16.5	1.84
Ascorbic acid (mg/100 g)	10.885	10.486	3.67

TSS whereas heating time and temperature were found significant variable affecting the ascorbic acid content of the pulp. The recommended conditions for the pretreatment conditions with respect to optimal quality were observed as pH; 3.3, heating time; 0.5 min, heating temperature; 70°C, and crude mass to water ratio; 1:0.8.

Conflict of interests

The authors have not declared any conflict of interests.

REFERENCES

Chopra CS, Singh RP (2001). Studies on extraction of pulp from wood apple fruit. Bev. Food World 28:25-27.

Fenemma OR (1985). Vitamins and minerals, Food Chemistry, Marcel Dekker Inc., New York, pp. 477-544.

Ghosh U, Gangopadhyay H (2002). Enzymatic, physicochemical and rheological behaviour of bael fruit pulp and juice. Ind. J. Chem. Tech. 9(2):123-126.

Joshi AA, Kshirsagar RB, Sawate AR (2012). Studies on standardization of enzyme concentration and process for extraction of tamarind pulp, variety Ajanta. J. Food Proc. Technol. 3(2):141. doi:10.4172/2157-7110.1000141

Kaushik RA, Yamdagni R, Dhawan SS (2000). Physico-chemical characteristics of Bael fruit at green and ripe stage of maturity. Haryana J. Hort. Sci. 29(1-2):44-45.

Kenghe RN, Potdar SN (2009). Physical properties of bael (*Aegle marmelos* Corr.) fruit and extraction of pulp. J. Maharashtra Agric. Uni. 34(2):205-207.

Narkhede SB, Vidyasagar G, Jadhav AG, Bendale AR, Patel KN (2010). Isolation and evaluation of mucilage of *Artocarpus heterophyllus* as a tablet binder. J. Chem. Pharm. Res. 2(6):161-166.

Pande NC, Singh AR, Maurya VN, Katiyar RK (1986). Studies on the biochemical changes in Bael (*Aegle marmelos* Correa.) fruit. Prog. Hort. 18(1-2):29-34.

Rakesh, Dhawan SS, Arya SS (2005). Processed products of Bael. Processed Food Ind. 8(12):25-27.

Ram D, Singh IS (2003). Physico-chemical studies on Bael (*Aegle marmelos* Correa.) fruits. Prog. Hort. 35(2):199-201.

Ranganna S (1997). Handbook of analysis of quality control of fruit and vegetable product. 2nd Ed., Tata McGraw-Hill Publ. Co., New Delhi. pp. 105-106.

Roy SK, Singh RN (1979). Studies on utilization of Bael fruit for processing-II Extraction of Bael fruit pulp. Ind. Food Packer 33(1):5-9.

Shrestha G (2000). Processing of wild Bael fruit for rural employment and income generation. Int. J. Small Scale Proc. (TTDG Food Chain). 27:15-17.

Singh A, Kumar S, Sharma HK (2012). Effect of enzymatic hydrolysis on juice yield from Bael fruit (*Aegle marmelos* Correa.) pulp. American J. Food Technol. 7:62-72. DOI 10.3923/ajft.2012.62.72.

Singh AK, Nath N (2004). Development and evaluation of whey protein enriched bael (*Aegle marmelos*) fruit beverage. J. Food Sci. Tech. 41(4):432-436.

Stešková A, Morochovičová M, Lešková E (2006). Ascorbic acid degradation during storage of fortified foods. J. Food Nutr. Res. 45(2):55-61.

Verma S, Gehlot R (2007). Standardization of processing technology for bael (*Aegle marmelos* Correa.) squash. Res. Crops 8(2):500-503.

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Sweet potatoes in Cameroon: Nutritional profile of leaves and their potential new use in local foods

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Sweet potato (*Ipomoea batatas*) leaves especially the beta-carotene fortified varieties are rich in functional macro - and micronutrients such as dietary fibres, antioxidants and other micronutrients deficient in the predominantly starchy staples of most nutritionally vulnerable Africans. In this study, the nutrient content of young leaves and succulent green stems of local and exotic varieties were evaluated using standard analytical procedures. Dry matter content was lowest and highest in young leaves of the exotic Jewel 56638 and Mbouda local varieties, respectively. Young leaf crude protein content was 15.1% in the variety Jonathan and 27.1% in the Santchou local variety. Crude fibre and ash content were higher in the young leaves and stems of the exotic varieties. Leaf total carotenoid content varied significantly across varieties. The leaves were found to soften *Gnetum africanum* vegetable sauce giving it an acceptable appearance, texture, flavour and taste. About 80% of respondents on a survey were willing to readily use sweet potato leaves to substitute for *Talinum triangulare* (waterleaf) in the preparation of *G. africanum* sauce during periods of waterleaf scarcity. These leaves can therefore provide a nutritional base in Africa (especially Cameroonian) diets for the nutritionally vulnerable in rural and urban communities.

Key words: *Gnetum africanum,* local and exotic varieties, nutrient content, sensory evaluation, sweet potato leaves.

INTRODUCTION

The growing awareness of health promoting and protecting properties of non-nutrient bioactive compounds found in vegetables has directed increased attention to vegetables as vital components of daily diets. In sub-Sahara Africa (SSA) vegetables are important dietary components and they are indispensable ingredients of

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soups or sauces that accompany the common carbohydrate staples. Plant nutrients are the main source of carbohydrates, proteins, minerals and dietary fibre as well as other non-nutrient bioactive compounds. In SSA, tubers crops provide the carbohydrates while leafy vegetables are the major sources of vitamins, dietary fibres, essential amino acids and antioxidants (Fasuyi, 2006). In spite of the recognized health advantages of leafy vegetables, the availability and consumption in tropical and sub-tropical Africa is inadequate partly due to low production, seasonality and susceptibility of these vegetables to various environmental production constraints.

Consequently, a more readily available source of vegetables is needed. Sweet potato (*Ipomoea batatas*) is a crop of great nutritional and health significance mainly due to its beta-carotene and anthocyanin properties (Taylor, 2007). In South Africa, the Agricultural Research Council and Medical Research Council are promoting the consumption of these beta-carotene fortified varieties (Laurie and Faber, 2008). In Tanzania, results of a consumer study showed that beta-carotene-rich sweet potato was more acceptable than the cream-fleshed varieties (Tomlins et al., 2007). The storage roots are often processed into a range of products including flour, chips, infant formulae, breakfast foods and snacks (Tumwegamire et al*.,* 2004). The increased use of yellow/orange flesh storage roots and green shoots of sweet potato could be one important means of alleviating vitamin A deficiency, especially among young children and women in many developing countries (Woolfe, 1992). In some parts of Nigeria, the vines of sweet potato are used as soup ingredients for their flavour, appearance, palatability, and tenderness (Tewe et al., 2003).

Sweet potato leaves are rich in micronutrients and are also consumed in several countries (Mosha and Gaga, 1999). The leaves are an excellent source of antioxidative polyphenolics (Islam et al., 2002). The leaves are consumed as tea in Japan and believed to be medicinal for the treatment of diabetes and help to reduce the risk of colon cancer (Hiroshi et al., 2000). Sweet potato leaves can be harvested almost bimonthly and the crop is generally tolerant to many diseases, pests, flooding and drought. Its leaves can therefore be served as an important source of vegetables to nutritionally deprived populations in sub-Sahara Africa especially during periods of adversity. Despite its importance, little is known in Cameroon about the nutritional content of the leaves of sweet potato. Furthermore, since the nutrient profile of a given crop is influenced by the climatic condition and mode of cultivation, it is important to elucidate the nutritional profiles of the vegetative parts of various local as well as exotic varieties grown and used in Cameroon. There is a wide range of varieties adapted to every ecological zone in Cameroon. This would stimulate interest or increase understanding and the consumption of these health promoting vines by humans.

MATERIALS AND METHODS

Sweet potato varieties

The following six exotic sweet potato varieties, Zapallo, SPK 004 Kakamega, Jewel 566638, Jewel 440331, Jonathan and Tainung obtained from the Institute of Agronomic Research for Development (IRAD) Cameroon and the four local varieties, Buea local, Kekem 2, Mbouda and Santchou obtained from local farmers in Cameroon were used in the study. These varieties were planted in an Experimental Research Plot at the University of Buea, Cameroon. Leaves and vines (shoots) from these varieties were harvested from three to four month old plants and assessed for their nutrient content.

Preparation of sweet potato shoots

Leaf and vine samples were divided into young leaves and young vines. From each sweet potato variety, a stem of about 30 cm long containing leaves was folded into two and then cut; the younger soft parts herein-after referred to as “young leaves” and the rest as “young stems”. Harvested leaves and vines were washed with distilled water to remove dust particles prior to biochemical analysis.

Proximate composition analysis

Moisture content in the samples was determined using the method of Association of Analytical Chemists (AOAC, 1990). Fresh samples were weighed then dried overnight for 24 h at 70°C in a ventilated oven. The samples were weighed again after 24 h then left in the oven at 105°C and dried for 72 h to constant weight. The samples were then cooled in the desiccators, and final weights were taken. The percentage moisture content was calculated as follows: {(fresh mass of sample – dry mass of sample)/fresh mass of sample} x 100%. The percentage dry matter was then calculated as, 100 – (% moisture content).

Ash content was determined following the procedures of AOAC (1990); 0.5 g of the finely milled sample was put in a pre-weighed 10 ml glass beaker and then placed in a muffle furnace set at 450°C for four hours to allow the sample to become ash. It was then cooled overnight in the furnace. The ash content was calculated as follows: {(Weight of beaker with ash – weight of beaker without ash)/weight of sample} x 100%.

Crude protein content was determined by the Kjeldahl method which involved digesting a given mass of the dried sample in sulphuric acid with selenium catalyst. The inorganic nitrogen converted to ammonium sulphate. Percentage nitrogen was then determined by subjecting the digest to a reaction with sodium nitroprusside-sodium salycilate reagent and the absorbance read at 650 nm. Percent nitrogen x 6.25 gave the percentage protein.

Total carbohydrate (sugars + starch) content was determined by the method of Dubois et al. (1956). Sugars were extracted with hot 95% ethanol and the residue subjected to hydrolysis with 70% perchloric acid for starch quantification. The extracts were subjected to colour development using concentrated sulphuric acid and 5% phenol and absorbance read at 490 nm. Crude fat was extracted in hexane by refluxing a given amount of dried sample for four hours in a Soxhlet apparatus at 78°C (Joslyn, 1970).

The calorific values of samples were obtained by multiplying values of crude protein, crude fat and carbohydrate by 4, 9 and 4, respectively (Akubor, 1997). Crude fibre determination was obtained according to the method of Entwistle and Hunter (1949).

Table 1. Proximate composition (%) and the energy contents of young leaves of selected exotic and local sweet potato varieties (mean ± SE).

Variety	Ash	Crude protein	Carbohydrates	Caloric value (Kcal/100 g)
Exotic				
SPK 004 kakamega	13.4 ± 0.2^{d}	19.0 ± 0.4^{b}	35.0 ± 5.0^{a}	248.2^{bc}
Zapallo	14.8 ± 0.3^{e}	22.1 ± 1.0^{c}	33.8 ± 4.9^{a}	265.5^{d}
Jewel 440331	11.9 ± 0.0^{c}	15.5 ± 0.2^{a}	48.8 ± 0.8^{e}	275.4^{d}
Jewel 566638	12.1 ± 0.5^{c}	17.8 ± 0.8^{b}	39.0 ± 0.9^{b}	244.4^{b}
Jonathan	11.9 ± 0.0^{c}	15.1 ± 0.8^{a}	38.3 ± 0.9^{b}	239.7^{b}
Tainung	13.9 ± 0.1^{e}	15.8 ± 0.0^{a}	35.5 ± 0.3^{a}	222.6^{a}
Local				
Buea local	11.5 ± 0.6^{c}	18.6 ± 0.6^{b}	39.9 ± 2.9^{b}	254.5^{c}
Kekem 2	10.4 ± 0.1^{b}	26.3 ± 0.7^{d}	41.8 ± 2.7^{c}	291.8^{e}
Mbouda	9.7 ± 0.1^{a}	26.8 ± 0.4^{d}	40.8 ± 0.3^{c}	294.4^{e}
Santchou	10.2 ± 0.0^{a}	27.1 ± 0.4^{d}	42.6 ± 1.6^{d}	304.0^{e}

Means followed by the same lower case letter in a column are not significantly different at P = 5%.

The dried plant material was defatted in hexane, and the residue was hydrolysed with trichloroacetic acid to eliminate proteins and carbohydrates and then ashed to obtain the mineral content in the sample. The residue resistant to digestion but susceptible to charring was quantified as crude fibre.

Carotenoids were extracted with cold acetone after crushing in a mortar using a pestle. The mixture was then filtered and the filtrate was transferred into a separation funnel. Petroleum ether was added to the extract and the two phases were separated. The lower portion was discarded and the upper portion was washed for a couple of times with distilled water to remove any residual acetone. The petroleum ether phase was transferred into a volumetric flask and residual water removed with sodium sulphate. The volume was then made up to 50 ml with petroleum ether and absorbance measured in a spectrophotometer at 450 nm. A carotenoid standard curve was prepared and used to quantify carotenoids in the leaf samples.

Sensory evaluation

The product used in the sensory evaluation was a popular vegetable sauce in Cameroon often composed of a mixture of the leaves of *G. africanum* popularly known as “eru' and *T. triangulare* (waterleaf) added as a softener/tenderizer usually in the ration of 1:1. About equal proportions of fresh leaves of *G. africanum* and those of the most popular and widely abundant sweet potato variety in the area “Buea Local” were steamed and then cooked in palm oil with all the required ingredients added as is traditionally prepared. In this case, sweet potato leaves were used in place of *T. triangulare* as the softener/tenderizer. The control was “eru” cooked with *T. triangulare* as is traditionally done. Using a 21-member panel comprised of randomly selected graduate and undergraduate students 10 males and 11 females from the University of Buea, each sauce was served to each consumer in a plate and each asked to assess for colour, texture and taste separately. Panellists were instructed to rinse their mouths before tasting the next sauce. For the assessment, the following verbal anchors were used; ‘preferred, ‘somewhat preferred, ‘neutral/indifferent’, ‘not preferred’ and ‘not preferred at all’. Each panellist was given a score sheet to complete after tasting the sauce. They were not told what each sauce represented.

After the blind assessment of the organoleptic attributes of the sauces, a ‘Yes/No’ question was also asked to the panellists whether they would use sweet potato leaves in place of *T. triangulare* in the preparation of *G. africanum*.

Statistical analysis

Analysis of variance (ANOVA) was carried out on the proximate composition data and differences in means were assessed using least significant difference (LSD) at 5% significance level.

RESULTS

Proximate composition of leaves

The dry matter content of the leaves ranged from 14.7 to 18.6% for the exotic Jewel 56638 and the local variety Mbouda, respectively, while the crude fat composition ranged from 1.9% in the variety Santchou to 4.6% in the Buea local. The crude fat content was generally low compared to the other nutrient components. The percentage of ash, crude protein, total carbohydrates content and caloric values varied greatly among the varieties (Table 1). Among the exotic varieties, the leaves of Zapallo had the highest protein content of 22.1% while among the local varieties Santchou had the highest protein content of 27.1%. In all the varieties studied, leaf protein content was higher than that of the stem. Starch constitutes the bulk of the total carbohydrate content of the young sweet potato stems. Besides Jewel 440331, Kekem 2, Mbouda and Santchou which had the highest carbohydrate leaf content of all varieties studied, the carbohydrate content did not vary much among the others (Table 1).

The contents of proteins, carbohydrates and dry matter

Table 2. Comparative (mean ± SD) combined proximate composition of young stems of exotic and local sweet potatoes (dry weight basis).

Nutrient type	Nutrient content (g/100 g)	
	Exotic	Local
Protein	08.8 ± 1.2[a]	11.3 ± 1.9[a]
Ash	15.9 ± 1.4[a]	07.7 ± 1.3[b]
Sugars	12.0 ± 2.6[a]	12.9 ± 2.2[a]
Starch	32.2 ± 3.6[a]	37.2 ± 3.9[b]
Total carbohydrates	44.2 ± 4.0[a]	50.1 ± 5.3[b]
Dry matter	12.9 ± 1.2[a]	18.4 ± 1.3[b]
Crude fibre	29.3 ± 1.4[a]	27.3 ± 2.5[a]
Crude fat	02.1 ± 0.4[a]	02.3 ± 0.8[a]
Caloric value (kcal/100 g)	231.5 ± 17.5[a]	265.9 ± 22.2[b]

Means followed by the same lower case letter in a row are not significantly different at P = 5%, Student t-test.

were generally higher in the local varieties than in the exotics. The local variety Mbouda had the highest leaf dry matter content of 18.6/100 g of fresh weight. In contrast, crude fibre and ash contents were generally higher in the exotic than the local varieties both in the leaves and stems (Table 2).

There were varietal differences in the crude fibre content with SPK 004 Kakamega having the highest in the leaves and stems among the exotic varieties while Mbouda had the highest values among the local varieties. The highest and lowest leaf carotenoid content of 3556.9 and 1414.6 ug/100 g was recorded for the Mbouda and Santchou local varieties, respectively (Table 3).

Sensory evaluation of sweet potato leaves in “eru” (*Gnetum africanum)* sauce

Young sweet potato leaves were found to tenderize *G. africanum* “eru” vegetable sauce similar to the traditionally used tenderizer *T. triangulare*. Roughly 85% of the panellists preferred the colour of the sweet potato-based sauce, 15% somewhat preferred it or were neutral/indifferent compared to 76 and 20%, respectively, for the *T. triangulare*-based that served as the control sauce. Based only on the colour, it was almost impossible to differentiate between the sauce prepared with leaves of the Buea local sweet potato variety and that prepared with *T. triangulare* (waterleaf). With regard to taste, about 43% preferred or somewhat preferred the sweet potato-based sauce, 33% were neutral/indifferent compared to 61 and 28%, respectively for the control (Table 4). In regard to the texture, 15% either preferred or somewhat preferred the sweet potato-based sauce and around 47% were neutral or indifferent as opposed to about 71 and 19%, respectively for the *T. triangulare*-based control sauce. Overall, 80% of the panellists indicated a readiness to use sweet potato leaves as a substitute for *T. triangulare* (waterleaf) to prepare the *G. africanum* sauce if waterleaf was unavailable as would be the case during the dry season.

DISCUSSION

The nutritional composition of sweet potato leaves and storage roots varies widely across varieties as dictated by genetic differences. The range for sweet potato leaf protein obtained in this study is consistent with the range reported by Oduro et al. (2008) (16.78 to 25.39%) with the local varieties Kekem 2, Mbouda and Santchou having the highest values of about 26% in this study. These values were compared favourably with those of cassava and other leaves that are consumed in Africa (Akindahunsi and Salawu, 2005). Hence, sweet potato leaves can be served as a good source of dietary protein comparable or in some cases superior to those of amaranth, taro, pumpkin and okra (FAO, 2006) that are widely consumed as leafy vegetables in Africa. Although, sweet potato is often considered as a food source for the poor in most parts of Africa (Adu-kwarteng et al., 2001), it remains a nutrient-rich food that can alleviate nutritional deficiencies in most parts of the continent.

Sweet potato leaves are often harvested together with the young tender apical portions of the vine; therefore nutrients contained in these portions would supplement those in the leaves. The crude fibre content was generally higher in the vines than leaves understandably so because the vine plays a supportive role in the plant and therefore requires tougher and fibrous tissues consistent with this function. The mean fibre content of the leaves was close to the values reported by Tewe (1994) but higher than those of Antia et al. (2006). This disparity is probably due to varietal differences and/or differences in climatic conditions. Overall, the leaves of sweet potato have lower fibre content than those of other tropical root crops such as cassava. The low fibre content coupled with the high moisture content of sweet potato

Table 3. Micronutrient content of young leaves and stems of selected exotic and local sweet potato varieties on fresh weight basis (FWB).

Variety	Crude fibre (g/100 g)		Total carotenoid (ug/100 g)
Exotic	**Young leaves**	**Stems**	**Leaves**
SPK 004 Kakamega	4.7 ± 0.3	4.6 ± 0.3	1508.9 ± 75.5
Zapallo	4.1 ± 0.2	3.4 ± 0.2	3154.5 ± 157.7
Jowel 440331	3.3 ± 0.1	3.5 ± 0.2	2291.9 ± 144.6
Jowel 566638	3.5 ± 0.2	4.1 ± 0.3	1650.8 ± 82.2
Jonathan	3.6 ± 0.2	4.2 ± 0.3	1483.4 ± 74.2
Tainung	2.7 ± 0.1	3.4 ± 0.2	1979.5 ± 99.0
Mean ± SD	3.6 ± 0.7	3.9 ± 0.5	2011.5 ± 639.9
Local			
Mbouda	4.5 ± 0.3	5.3 ± 0.4	3556.9 ± 177.8
Buea local	3.0 ± 0.2	5.1 ± 0.3	1851.6 ± 92.9
Kekem 2	2.4 ± 0.1	4.1 ± 0.2	1609.1 ± 80.5
Santchou	3.4 ± 0.2	5.5 ± 0.4	1414.6 ± 70.7
Mean ± SD	3.3 ± 0.9	5.0 ± 0.6	2108.1 ± 602.7

Table 4. Consumer preferences (%) for *Gnetum africanum* vegetable sauce softened with either *Talinum triangulare* (control) or leaves of the Buea local sweet potato variety.

Sensory attribute	Verbal anchor	Type of sauce/preference (%)	
		G. africanum* + *T. triangulare	***G. africanum* + Sweet potato leaves**
Colour	Preferred	76.2^{a}	85.7^{b}
	Somewhat preferred	4.8^{a}	9.5^{a}
	Neutral/Indifferent	19.0^{b}	4.8^{a}
	Not preferred	0.0^{a}	0.0^{a}
	Not preferred at all	0.0^{a}	0.0^{a}
Taste	Preferred	38.1^{a}	9.5^{b}
	Somewhat preferred	23.8^{a}	33.3^{b}
	Neutral/Indifferent	28.6^{a}	33.3^{a}
	Not preferred	9.5^{a}	19.0^{b}
	Not preferred at all	0.0^{a}	4.8^{a}
Texture	Preferred	47.6^{b}	4.8^{a}
	Somewhat preferred	23.8^{b}	9.5^{a}
	Neutral/Indifferent	19.0^{a}	47.6^{b}
	Not preferred	9.0^{a}	28.6^{b}
	Not preferred at all	0.0^{a}	9.5^{b}

Means followed by the same lower case letter in a row are not significantly different at P = 5%, Student t-test.

leaves likely makes them generally much tender than cassava leaves. The relatively tender nature and acceptable fibre content of the leaves and vines of sweet potato renders them easily digestible.

Dietary fibres facilitate digestion and are reported to prevent colon cancers (Saldanha, 1995). Thus, making sweet potato leaves an important component of many diets. Starch constituted the bulk of the total carbohydrate content in both the leaves and stems. The range (35.00 to 56.71%) is comparable to that reported by Antia et al. (2006). This high starch content makes the leaves a good source of energy in addition to its improved nutrient status and health benefits mediated by the high total carotenoid levels. We can conclude that consumption of sweet potato leaves rich in dietary fibre, vitamins and antioxidants in the form of carotenoids would be useful in neutralizing free radicals in the body and hence reduce the harmful effects of oxidative stress (Teow et al., 2007). The addition of *G. africanum* to sweet potato leaves was acceptable particularly because its appearance and the mixture and preparation did not alter the organoleptic attributes of the sauce (Laurie and Van Heerden, 2012). A possible reason for 33% of the panellists being neutral/indifferent regarding the taste of the sauce is because it was the first time of tasting a *G. africanum*-based sauce of a slightly different flavour from what they are accustomed to. It is highly likely that if the panellists had been offered the sweet potato based sauce subsequently, many of these neutral respondents would have readily accepted the taste of the sauce. This is buttressed by the fact that 80% of the respondents indicated that they would readily use sweet potato leaves as a substitute for *T. triangulare* in the preparation of the *G. africanum* if *T. triangulare* was unavailable. Replacement of *T. triangulare* with beta-carotene-rich sweet potato leaves in the preparation of *G. africanum* can be a good strategy to increase the consumption of diets rich in antioxidants. Ofori et al. (2009) indicated that sweet potato varieties with high levels of beta-carotene in their leaves and roots are potential food-based candidates for alleviating Vitamin A deficiency in Africa. Since we tested only one of the sweet potato varieties, it is possible that other varieties might be preferred even more. Further studies would be conducted to verify this possibility.

Given the acceptable taste, diverse and important nutritional components of sweet potato shoots (leaves and stems), hardiness and tolerance of the crop to high humidity detrimental to other leafy vegetables, sweet potato shoots can therefore be served as a rich and versatile vegetable to supplement the predominantly starchy dietary staples in most areas of rural sub-Sahara Africa. The consumption of beta-carotene-rich sweet potato leaves as strategy of addressing Vitamin A deficiency needs to be widely disseminated especially in rural communities where the consumption of leafy vegetables is highest because of their increased availability in remote areas.

Conclusion

The sweet potato-based sauce had an acceptable appearance, texture, flavour and taste. Most respondents of a sensory evaluation panel indicated that they will readily use sweet potato leaves in the preparation of the *G. africanum* sauce in the absence or during scarcity of *T. triangulare*. A wide dissemination of the use of beta-carotene fortified sweet potato leaves in the preparation of *G. africanum* sauce would be a good means of increasing the consumption of vitamin A and other functional micronutrients in some diets of many Africans especially those in rural areas where the consumption of vegetables is highest.

Conflict of Interests

The authors have not declared any conflict of interests.

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REFERENCES

Adu-Kwarteng E, Otoo JA, Oduro I (2001). Screening of sweet for poundability into fufu, In Proc. Of 8th ISTRC-AB Symposium. P. 138.
Akindahunsi AA, Salawu SO (2005). Phytochemical screening and nutrient/antinutrient composition of selected tropical green leafy vegetables. Afr. J. Biotech. 4:497-501.
Akubor PI (1997). Proximate composition and selected functional properties of African breadfruit and sweet potato flour blends. Plant Foods Hum. Nutr. 51:53-60. http://dx.doi.org/10.1023/A:1007948400685. PMid:9498694
Antia BS, Akpan EJ, Okon PA, Omuren IU (2006). Nutrition and Antinutritive Evaluation of Sweet Potatoes (*Ipomoea batatas*) Leaves. Pak. J. Nutr. 5(2):166-168. http://dx.doi.org/10.3923/pjn.2006.166.168
AOAC (1990). Official method of analysis of the Association of Official Analytical Chemists. (15th Ed), Washington D.C. pp. 992-995.
Dubois M, Gilles KA, Hamilton JK, Rebers PA, and Smith F (1956). Colorimetric method for determination of sugars and related substances. J. Anal Chem. 28:350-356. http://dx.doi.org/10.1021/ac60111a017
Entwistle PV, Hunter WL (1949). Report on crude fibre. J. AOAC. 32:651-656.
FAO Corporate Document Repository (2006b). Proximate composition of foods. [http:/www.fao.org/ag].
Fasuyi AO (2006). Nutritional potentials of some tropical vegetable leaf meals: chemical characterization and functional properties. Afr. J. Biotech. 5(1):49-53.
Hiroshi I, Hiroko S, Satoshi I, Tadahiro T and Akio A (2000). Nutritive evaluation of chemical composition of leaves, stalks and stems of sweet potato (*Ipomoea batatas* Lam). Food Chem, 68:350-367.
Islam SM, Yoshimoto M, Yahara S, Okuno S, Ishiguro K, Yamakawa O (2002). Identification and characterization of foliar polyphenolic composition in sweet potato (*Ipomoea batatas* L.) Genotypes. J. Agric. Food Chem. 50: 3718-3722. http://dx.doi.org/10.1021/jf020120l
Joslyn MA (1970). Methods in food analysis. 2nd Ed. A series of monographs. Academic press. New York. pp. 552-557, 709-711.
Laurie SM, Faber M (2008). Integrated community-based growth monitoring and vegetable gardens focusing on crops rich in B-carotene – project evaluation in a rural community in the Eastern Cape, South Africa. J. Food Sci. Agric. 88:2093-2101. http://dx.doi.org/10.1002/jsfa.3319
Laurie SM, Van Heerden SM (2012). Consumer acceptability of four products made from beta-carotene-rich sweet potato. Afr. J. Food Sci. 6(4):96-103. http://dx.doi.org/10.5897/AJFS12.014
Mosha TC, Gaga HE (1999). Nutritive value and effect of blaching on the trypsin and chymotrypsin inhibitor activities of selected leafy vegetables. Plant Foods Hum. Nutr. 54(3):271-283. http://dx.doi.org/10.1023/A:1008157508445; PMid:10716408

Oduro I, Ellis WO, Owusu D (2008). Nutritional Evaluation of two Leafy Vegetable; *Moringa oleifera* and *Ipomoea batatas* leaves. SRE 3(2):57-60.

Ofori G, Oduro I, Ellis WO, Dapaah KH (2009). Assessment of vitamin A content and sensory attributes of new sweet potato (*Ipomoea batatas*) geneotypes in Ghana. Afr. J. Food Sci. 3(7):184-192.

Saldanha LG (1995). Fibre in the diet of US children: Results of national surveys. Paediatrics 96:994-996.

Taylor SL (2007). Advances in Food and Nutritional Research, Revised Edition. pp. 2-3, 6-9.

Teow CC, Truong VD, Mcfeeters RF, Thompson RL, Pecota KV, Yencho GG (2007). Antioxidant activities, phenolic and B-carotene contents of sweet potato genotypes with varying flesh colours. Food Chem. 103:829-838. http://dx.doi.org/10.1016/j.foodchem.2006.09.033

Tewe OO (1994). Biochemistry and utilization of sweet potato (Ipomoea batatas) for animal feeding: implications for food security in Africa. In: Akoroda, M.O. (ed.). Root crops for food security in Africa. 5. Triennal Symposium of the International Society for Tropical Root Crops - Africa Branch. Kampala (Uganda). 22-28 Nov 1992. Ibadan (Nigeria). International Society for Tropical Roots Crops - Africa Branch (ISTRC - AB). ISBN 978-2079-00-2. pp. 324-327.

Tewe OO, Ojeniyi FE, Abu OA (2003). Sweet potato production, Utilisation, and marketing in Nigeria. Social Science Department, International Potato Centre (CIP) Lima, Peru.

Tomlins KI, Ndunguru G, Stambul K, Joshua N, Ngendello T, Rwiza E, Amour R, Ramadhani B, Kapande A, Westly A (2007). Sensory evaluation and consumer acceptability of pale-fleshed and orange-fleshed sweet potato by school children and mothers in sub-Saharan Africa. J. Sci. Food Agric. 87:2436-2446. http://dx.doi.org/10.1002/jsfa.2931

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Guidance of an agricultural robot with variable angle-of-view camera arrangement in cornfield

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Guidance performances of an agricultural robot were evaluated when the robot traveled along cornrows and performed the row to row turning maneuver in cornfield with variable angle of view (AOV) camera arrangement. The arrangement with one low-cost camera has flexible AOV by controlling Servo motors motions as human vision. In this work, a forward far AOV was used to segment clearly and quickly cornrows due to more crop information and strong contrast between cornrows and soil, and a forward near AOV with less crop information was chosen once the number of crops pixels reduced due to the spacing headland. And a lateral near AOV was adopted to guide the robot by acquiring the crop image near the end of cornrows, due to a blind spot area in front of the robot for the near AOV arrangement. Then the headland turning was performed. During headland turning, the robot obtained the cornrows information using a lateral far AOV arrangement. After the headland turning, the robot traveled in the next cornrow with the AOV same to the first far AOV camera arrangement. Guidance information was detected with an image-processing algorithm employing mathematically morphological operations, and fuzzy logic control was applied to guide the robot according to guidance information. Tests showed that the robot traveled successfully and performed the headland turning in corn field with three replications. Test data from GPS were processed and showed stable guidance behavior with a maximum guidance error 22.58 mm and good headland turning operation. The low-cost variable AOV camera arrangement has a promising field application for autonomous agricultural vehicles.

Key words: Machine vision, agricultural robot, row guidance, headland turning.

INTRODUCTION

Driving along the crop row and turning at the headland (headland turning) are the main maneuvers for autonomous agricultural vehicles. For the former, although there are many researches based on all kinds of sensors, visual navigation has been a research hotspot due to its low cost and good navigation performance (Reid et al., 2000; Mousazadeh, 2013). For example, some guidance systems based on camera were developed and worked in tractors (Easterly et al., 2010), a field sprayer (Tian et al., 1999), a weeding cultivator(Tillett et al., 2002) and a grain harvester (Benson et al., 2003). Additionally, the same method was applied for autonomous agricultural robots (Bakker et al., 2008). These applications have the common characteristics

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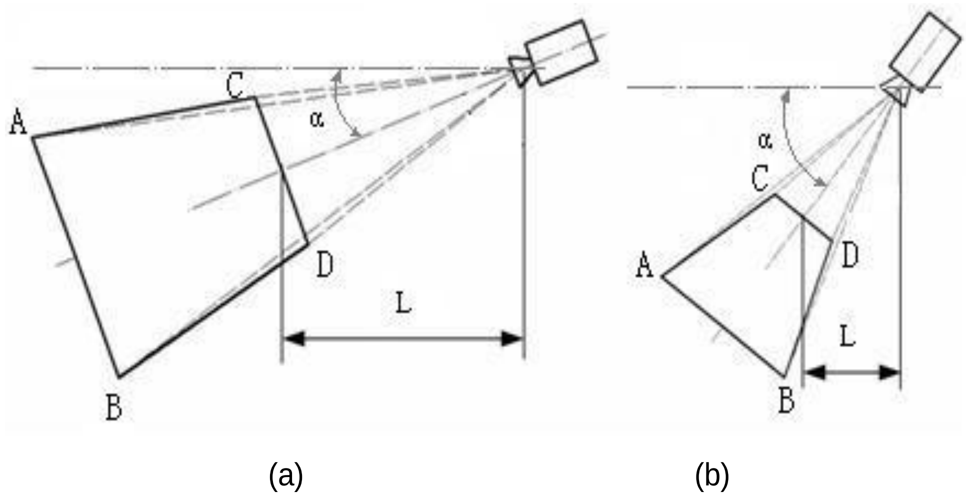


Figure 1. Vision range and blind distance of a fixed forward AOV camera. (a) Small vision angle; (b) Big vision angle.

–a fixed forward angle of view (AOV) of camera. However, the camera with a fixed AOV at a specific height that produces only a fixed vision scene cannot obtain the different scenes and more information with flexible vision angles as human vision by looking up, looking straight ahead, looking down and turning. Meanwhile, the farther forward AOV of cameras, including cameras with larger field of view indicates the longer blind distance in front of vehicle which is not appropriate to detect crop plants near the end of crop rows. On the other hand, cameras with wider field of view such as omnidirectional cameras are becoming attractive in some mobile robotic applications, but there are some questions such as geometric constraints, distortion and higher prices (Mariottini et al., 2012).

Meanwhile, more and more attention has been paid to the studies on headland turning in recent years. Zhu et al. (2007) created a suboptimal reference course for headland turning of a robot tractor and design a path-tracking controller to guide the robot tractor along the reference course by using a fiber optic gyroscope, a laser auto-tracking range finder, a potentiometer, computer simulation and field tests were performed to validate the path-tracking performance. Subramanian and Burks (2009) developed a row to row turning maneuver of an autonomous at the headlands of citrus groves on the basis of the sensors such as video camera, laser radar, inertial measurement unit and wheel encoder. But Bakker et al. (2009) identified the advantages (the smaller headland space and the less time for turning) of using all degrees of freedom of four-wheel steered vehicles for headland turning by simulating the kinematic model compared to the current manual control using only two degrees of freedom. Huang et al. (2010) presented path planning and headland turning control algorithms based on the improved pure pursuit model for autonomous agricultural machine. The simulation results showed that algorithms were simple with small headland space and high tracking accuracy.

Of course, the author and his colleagues (Xue, 2011; Xue et al., 2012) have conducted some researches on the guidance and headland turning of a robot with a variable AOV camera arrangement. The arrangement mainly consisting of a low-cost webcam and two or three low-cost Servo motors have flexible AOV as human vision. Here, according to the works done before, the author conducted further tests to validate the application of row guidance and headland turning with the variable AOV camera arrangement, when the robot traveled along the cornrows and performed the row to row turning maneuver in cornfield.

MATERIALS AND METHODS

Variable AOV camera arrangement and robot platform

A fixed forward AOV usually has such specific vision range as the ABCD area shown in Figure 1 when the camera is fixed with certain height and vision angle. Except the ABCD area, the other area belongs to blind spots and it is impossible to detect crop plants in the blind spots in front of camera with the distance L in the horizontal plane. To acquire more information, we can take the smaller vision angle α which means getting a bigger vision range, but with longer blind distance L as shown in Figure 1(a). If bigger vision angle α is taken, it indicates smaller vision range and less information but with shorter blind distance L as shown in Figure 1(b).

The variable AOV camera arrangement was developed by mounting camera on a fixture driven by Servo motors as shown in Figure 2 where Motors 1 and 2 controlled the pitch and yaw motion of camera, respectively. All angles of camera were calculated according to the camera position that camera is looking straight ahead where vision angle is set to 0˚. The pitch angle was defined as α_{up} or α_{down} when the camera is looking up and down,

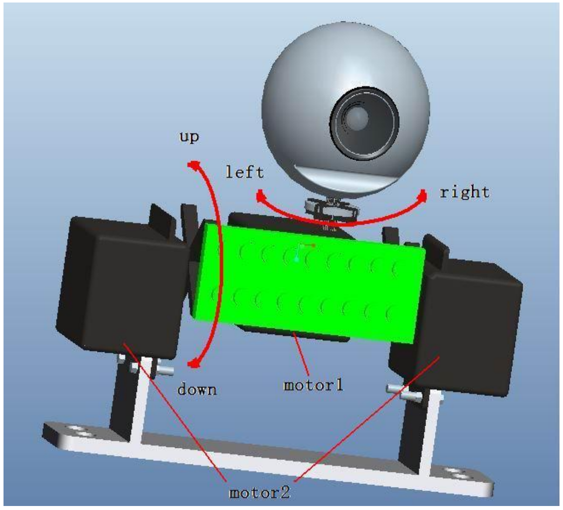


Figure 2. Arrangement of variable AOV camera.

respectively and the yaw angles were defined as β_{left} and β_{right} when the camera turns to the left and to the right, respectively. Therefore, the camera arrangement has the similar characteristics to human vision such as looking up, looking straight ahead, looking down and turning.

The developed variable AOV camera arrangement was mounted on a robot platform named "AgTracker", its detail was illustrated by Xue et al. (2012).

Row guidance methods

The variable AOV camera arrangement can be used to find guidance line by detecting crop plants flexibly. In this work, some AOVs including far AOV, near AOV and lateral AOV were used to guide the robot along cornrows at both sides of the robot. When the robot was far away from the headland, the first far AOV, with a number of crop information, was employed to segment two cornrows clearly and quickly due to the strong contrast between green cornrows and soil. Once green corn pixels reduced due to the upcoming headland space, a near AOV including less crops information was chosen. Due to a certain blind distance in front of the robot, a lateral AOV with the least crops information was implemented to guide the robot near the end of cornrows when crops pixels in the near AOV were less than the second threshold value, which implied that the end of cornrows was approaching. The second far AOV was chosen during the headland turning, and the first far AOV was used again to guide the robot in the new cornrows after the headland turning.

The measure principle of the offset and heading angle in the different AOV arrangement was detailed in the literature (Xue et al., 2012). With a comprehensive consideration of the characteristics of corn plants and corn rows, the detection algorithm of guidance row was developed to guide the robot between cornrows as shown in Figure 3. What is interested was not the whole image but a local window including two cornrows or one side cornrow when the robot platform traveled along cornrows. The local window interested can improve the real-time detection algorithm and minimize the effect of useless information and noises. In this work, different size windows were employed to different AOV camera arrangement, the details were introduced in Section 3. Subsequently, the green feature extraction was conducted to separate effectively the crop from soil image was converted to a binary image.

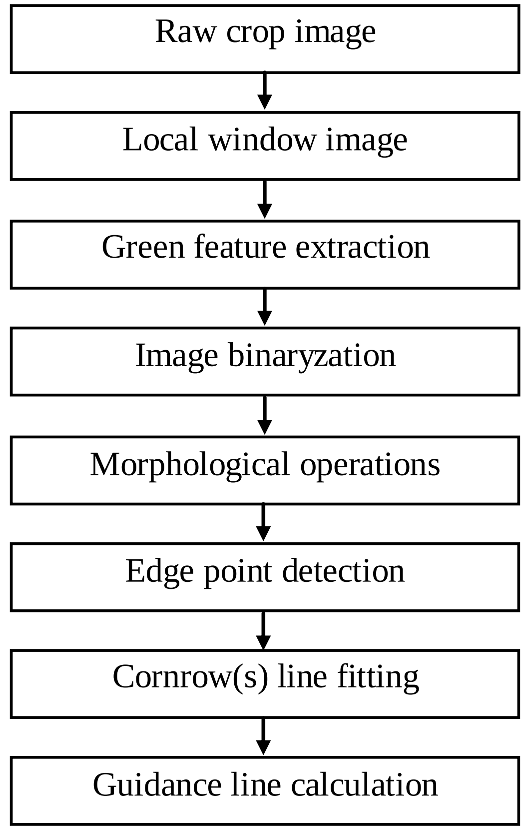


Figure 3. Detection algorithm of guidance line.

Then, mathematically morphological opening operations with line-shaped structuring element were chosen to segment and identify corn plants and corn rows. According to the new binary image, some equidistant image strips were divided, and the edge pixel point coordinates of two side cornrows were searched in each image strip for the forward AOVs. For the lateral AOV, the edge pixel point coordinates nearest to maximum pixel column were found for every corn crop. Based on the edge pixel point coordinates, the linear fitting method was used to calculate the line(s) of corn row(s). Finally, the guidance line was calculated according to the line(s) of corn row(s).

Headland turning methods

Once no crop plants appears in image, it shows that the robot is approaching the headland and it is about to perform the headland turning. Given that the robot turn left to the next row, the headland turning operation is as follows:

(1) Moving forward a distance: The robot moved forward a distance along current direction without image acquisition and processing. In the work, the distance was set to 1 m, which ensured the robot completely away from the end of cornrows.

(2) First steering operation: Without direction sensor, indoor tests and field trials in sequence were conducted to determine the speeds of both side wheels and steering time for steering to left which will be introduced below. To simplify the headland turning control, the wheel speeds and steering time were set fixed values

Table 1. Table for fuzzy control rules.

u\θ\E	-3	-2	-1	0	1	2	3
-3	3	3	2	2	1	1	0
-2	3	2	2	1	1	0	-1
-1	2	2	1	1	0	-1	-1
0	2	2	1	0	-1	-2	-2
1	1	1	0	-1	-1	-2	-2
2	1	0	-1	-1	-2	-3	-3
3	0	-1	-2	-2	-3	-3	-3

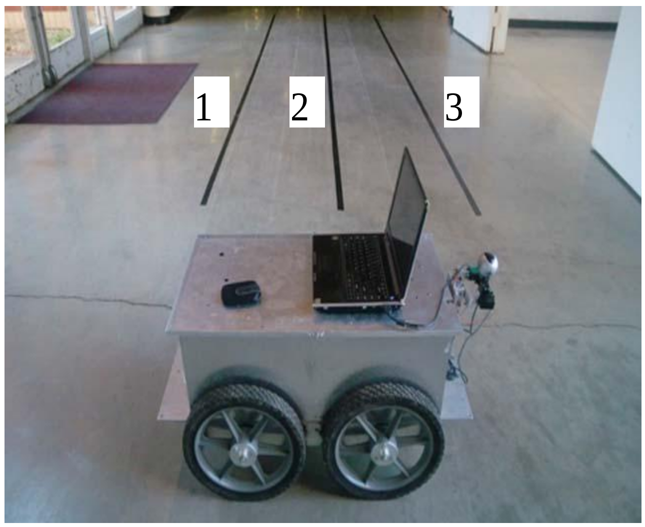


Figure 4. Indoor simulation for headland turning.

which ensured the robot turn 90° to left.

(3) First position calculation: The robot stopped and changed the camera to the second far AOV arrangement which means that the camera was turned to the direction perpendicular to longitudinal direction of the robot in the horizontal plane, thus the camera captured the image of the left cornrows and calculated the position and direction of the robot relating to the next cornrows.

(4) Backing operation: This operation must be performed, which depends on inter-rows distance in cornfield and length of the robot. According to the Step (3), the backward distance and the wheels speeds are determined. If the robot is perpendicular to the cornrows, it backs for a distance at the same speeds of both side wheels. Otherwise, it needs to change the wheels speeds to ensure the robot perpendicular to the cornrows as far as possible.

(5) Second steering operation: The operation was same to the Step (2).

(6) Second position calculation: The robot stopped again and changed the camera to the first far AOV again and then it traveled along the next cornrows according to the robot's position and heading calculated by using the far AOV arrangement.

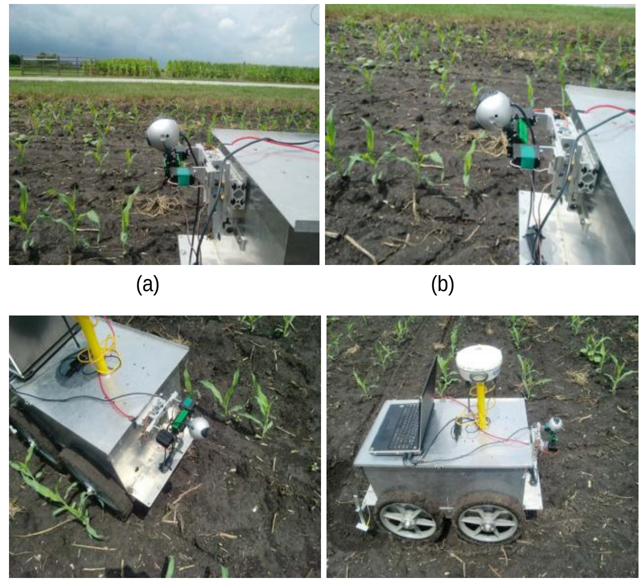
Row guidance and headland turning control

The robot was driven by Servo motors via chains and gearboxes and steered according to the different speeds of left and right wheels. After offset and heading angle of the robot are found out, the row guidance control and backing control during headland turning were implemented by adjusting pulse-width modulation (PWM) value of motors which caused change of the wheels speeds.

Fuzzy control was adopted to realize the control of row guidance with two inputs of lateral offset E and heading angle θ of the robot platform and one output U of the difference between two side PWM values as shown in Table 1. Here, the input and output fuzzy sets ere {NG, NM, NS, ZE, PS, PM, PG}, and the universes of discourse were [-30 cm, 30 cm], [-30º, 30º] and [-300, 300] for lateral offset E, heading angle θ and PWM difference U respectively, and their fuzzy domains all were {-3, -2, -1, 0, 1, 2, 3}. It should be noted that, negative sign of lateral offset, heading angle and PWM control difference means that the robot deviated from the centerline to the right side, performs clockwise deflection and steers to the right, respectively. And triangular membership function using uniform distribution, Max-Min fuzzy inference algorithm and decoupling sentencing law with the method of center of gravity were applied in the fuzzy guidance control.

During headland turning, the second far AOV camera arrangement was employed to calculate the robot's position and heading, thus it further determines how to perform the backing operation. An indoor simulation was chosen to decide the robot's position relative to the cornrows as shown in Figure 4, where three black tape lines represented three actual cornrows. After the robot accomplished first steering operation, the second far AOV was

Figure 5. Experimental corn field.



(a) (b)

(c) (d)

Figure 6. Several AOV arrangement in this work. (a) The first far AOV; (b) The near AOV; (c) The lateral AOV; (d) The second far AOV.

used to observe the third cornrow. The correspondence relationship between the actual robot position relative to the cornrows and the cornrows position in images were determined according to the different robot positions relative to the simulated cornrows.

Experimental procedures

In order to verify performances of the variable AOV guidance system, tests were conducted in cornfield of experimental farm in the University of Illinois as shown in Figure 5. Corn crops were planted in rows with about 750 mm inter-row distance and about 150 mm intra-row distance and the height of corn was about 200 mm. A webcam was selected as visual device and was connected to a laptop computer. Crop images were acquired and processed using Matlab image acquisition and processing software. After processing an image, the computer sent commands to the microcontroller in the robot platform through serial communication. For image segmentation, a large number of crop images with the different time and different weather were sampled to obtain the appropriate threshold values of images and structuring elements. The structuring element, as an essential part of open and close operations in morphological operations, was used to probe input binary images.

With a 0.2 m/s initial speed, the robot traveled along cornrows from the starting point, 30 m away from the end of cornrows to the stopping point in the next cornrows, 30 m away from the end of cornrows too. During the headland turning operation, the left and right wheel speeds were set to 0.01 and 0.2 m/s for two steering operation, the steering times of the first steering, the second steering were 8 s and backward speed was set to 0.1 m/s.

At the beginning of tests, the first far AOV (α_{down} = 25°, β_{left} = 0°) was chosen to guide the robot (Figure 6(a)). When the crop pixels fell to the first threshold, a near AOV (α_{down} = 48°, β_{left} = 0°) was adopted automatically (Figure 6(b)). Once the crops pixels became smaller than the second threshold which implies that the end of cornrows is approaching, a lateral AOV (α_{down} = 80°, β_{left} = 30°) was employed to find corn plants in left side (Figure 6(c)). Once no corn plants appeared in images, the headland turning operation was performed. During this operation, the second far AOV (α_{down} = 0°, β_{left} = 90°) was selected to calculate the robot position and direction, thus to plan the backing operation during headland turning (Figure 6(d)). Then the robot traveled to the stopping point corresponding to the starting point in the next cornrows after the headland turning.

According to row guidance method mentioned above, images from the first far AOV, the near AOV and the lateral AOV camera arrangement were processed (Figures 7, 8 and 9). It should be noted that a small rectangle window from 110 pixel row to 330 pixel row was used for the image from the far AOV, a large rectangle window from 1 pixel row to 280 pixel row for the image from the near AOV, and a large side window from 1 pixel column to 340 pixel column for the image from the lateral AOV.

The algorithm of the whole test is shown in Figure 10. Once corn pixel value Np fell to the first threshold value Thresh 1 due to the upcoming headland space, a near AOV was taken. A lateral AOV with the least crops information was implemented to guide robot near the end of cornrows when crops pixels value Np in the near AOV were less than the second threshold value Thresh 2, which implied that the end of cornrows was approaching, due to a blind distance of 1.5 m in front of the robot in this work. If the robot arrived at the end of row, headland turning operation would be performed immediately. After the headland turning, the robot traveled along the new cornrows according to the information from the far AOV arrangement.

According to the algorithm, the tests were performed with three replications from the same starting point to the same stopping point. An real-time kinematic (RTK) GPS receiver (Trimble 5800 GPS), with 1 cm accuracy was mounted to store the running trajectory data of the robot for analyzing performances of row guidance and headland turning. Once positioned, the robot was guided autonomously and was stopped at the stopping position by an operator.

From GPS data, the maximum error, average error, RMS error and standard deviation were calculated to judge row guidance accuracy. And the point after first steering operation, the point after second steering operation, the farthest point of the robot trajectory were found out to show the headland turning performances. By comparing the end points after the two steering operation and the farthest point during first steering operation in every test and

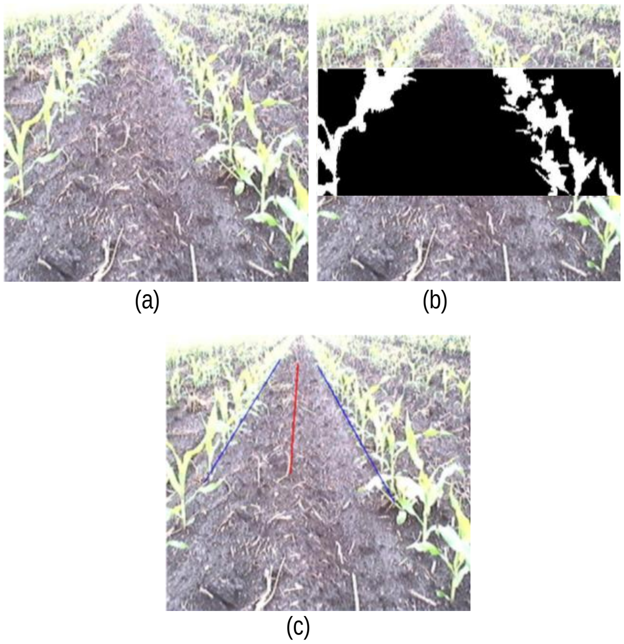


Figure 7. Far AOV results for 200 mm tall corn. (a) Raw image; (b) Binary image; (c) Processed image.

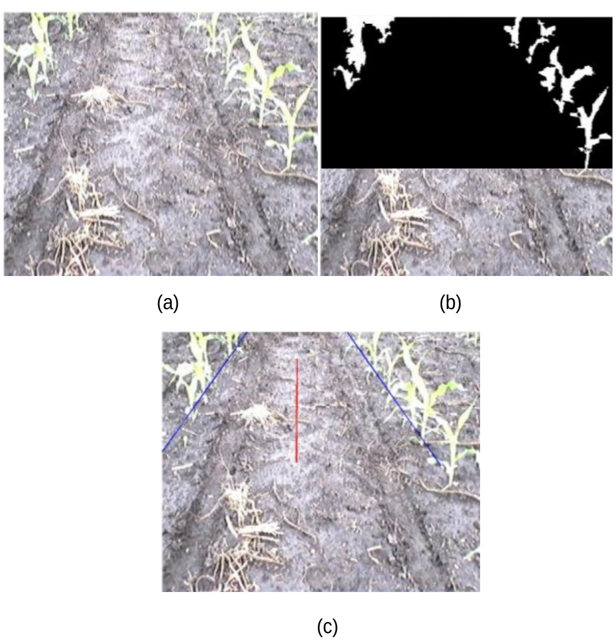


Figure 8. Near AOV results for 200 mm tall corn. (a) Raw image; (b) Binary image; (c) Processed image.

surveying the turning trajectory, the effect of the stage of moving forward a distance during headland turning was evaluated. Moreover, the backing control was evaluated according to the backward distance and the backing trajectory. And the performance of second steering operation was analyzed according to the turning trajectory and the end point.

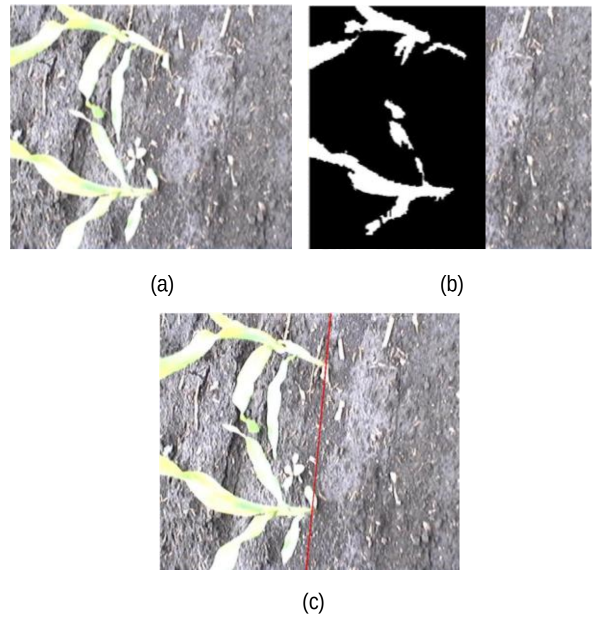


Figure 9. Lateral AOV results for 200 mm tall corn. (a) Raw image; (b) Binary image; (c) Processed image.

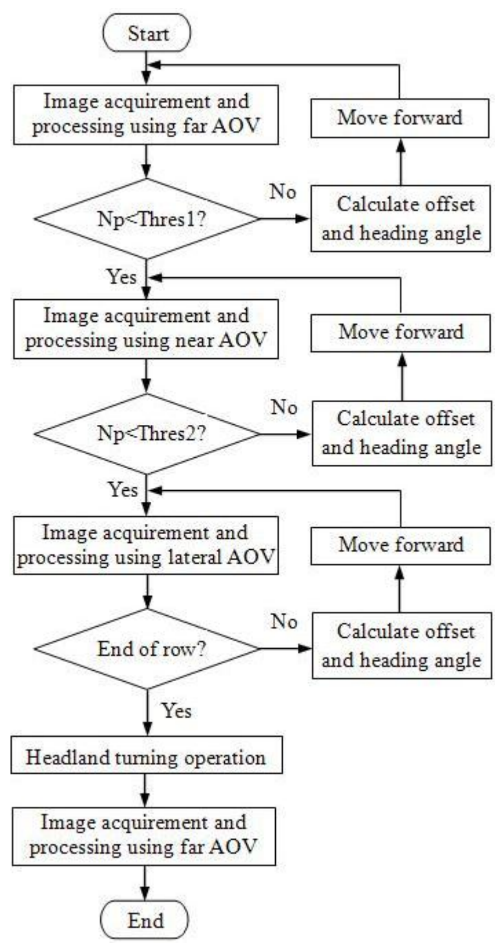


Figure 10. Algorithm of the whole test.

Table 2. Statistical results of the row guidance.

AOV stage	Maximun error (mm)	Average error (mm)	RMS error (mm)	Standard deviation (mm)
First far AOV	15.6	-0.95	6.48	6.46
Near AOV	-22.58	-8.75	10.64	6.04
Lateral AOV	-10.95	-7.14	7.37	1.86
Third far AOV	-16.48	-0.89	7.43	5.60
Total	-22.58	-3.45	7.65	6.83

Table 3. Measurement results of headland turning.

S/N	Position after first steering		Position after second steering		Position at farthest steering		Back distance
	X(m)	Y(mm)	X(m)	Y(mm)	X(m)	Y(mm)	(mm)
I	31.41	641.05	30.97	670.45	31.47	375.64	303.37
II	31.50	670.12	31.18	730.27	31.65	394.43	321.16
III	31.70	660.56	31.06	722.83	31.75	387.21	310.54

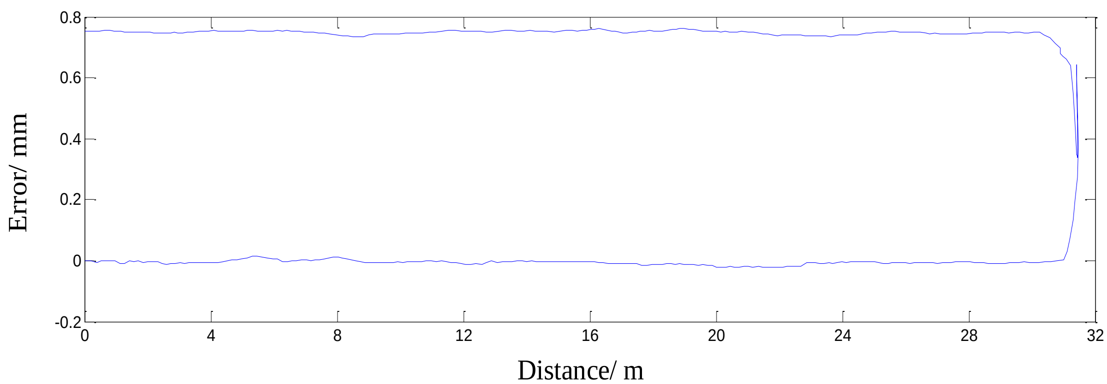


Figure 11. Trajectory of one test.

RESULTS AND DISCUSSION

Tests were performed to judge guidance accuracy of the variable AOV machine vision arrangement and headland turning performance under the test conditions mentioned above. During each test, the data from GPS were recorded automatically at 1 s intervals via serial communication cable to the laptop computer. The row guidance performances for each test were evaluated according to the maximum error, average error, RMS error and standard deviation of errors. Some points including the end points after the two steering operation and the farthest point, and the backward distance, relative to the starting point were tabled for each test.

The statistical results of the row guidance are shown in Table 2, where the errors were calculated according to the central line of the next cornrows when using the third AOV arrangement (same to the first AOV arrangement), and the measurement results of the headland turning are shown in Table 3. The "-" sign in Table 2 indicates that the error is biased to the right side of the robot at the starting point, and X and Y in Table 3 represent positions coordinates in the directions parallel to and perpendicular to cornrows, respectively. Figure 11 shows one trajectory of the three replications, when the robot traveled along cornrows from the starting point being 30 m away from the headland, implemented the headland turning to the next cornrows, and then traveled 30 m distance along the new cornrows.

From Table 2, the maximum average error and standard deviation were less than 8.78 and 6.46 mm in the total run, respectively, which indicates that the fuzzy control is striving to reduce the error to zero (Figure 11). Compared to the average errors of different AOV stage in

Table 2, there is the smallest value for the far AOV which demonstrates a more accurate detection to crop rows and a better control performance. The maximum RMS error of 10.64 mm appears in the near AOV stage, which indicates an acceptable discrete degree of error data in this stage. Although, there is the smallest amount of crop information in the lateral AOV stage, it had the smallest value of maximum error in Table 2 due to short traveling distance of 2 m.

However, the maximum error 22.58 mm which may be related to wet soil and the track trend of convergence to zero indicated that the variable AOV machine vision guidance system performed well in the corn field. In addition, the distances from the starting point of the robot, at which the camera view was adjusted from the first far AOV to the near AOV and to the lateral AOV, are basically the same for the three replication, that is, about 10 m during the first far AOV stage and about 18 m during the near AOV stage.

From Table 3, there are obvious difference in Y direction for the data of position after the two steering operation and the farthest point. At the point after first steering operation and the farthest point, the offsets in Y direction were 23.07 and 19.53 mm, respectively which indicate that the stage of moving forward a distance has effect on first steering operation, although the effect did not make the performance of headland turning worse due to good backing control mentioned below. The difference of backward distance was no more than 18.79 mm among the three tests. Moreover, from Figure 11, it shows that the robot always strived to keep perpendicular to the cornrows during the backing operation. These show good backing control by using the second far AOV camera arrangement. In addition, the maximum deviation from the center line of was 17.26 mm after the headland turning in the second test. According to this data and the trajectory in Figure 11, it shows that the developed algorithm enables the robot to close the central line of the next cornrows with high accuracy.

Conclusions

Variable AOV machine vision based guidance systems were applied to guide a robot along cornrows and implement headland turning operation. Different AOV arrangements were tested such as far AOV, near AOV and lateral AOV. Guidance lines were found in corn field by using morphological operations, and a fuzzy logic control was implemented for the row guidance. GPS data were stored to evaluate the guidance performance and headland turning operation.

According to the tests data, the variable AOV camera arrangement had acceptable accuracy with the maximum guidance error of 22.58 mm, and the headland turning operation performed well, especially using of the far AOV before backward control ensured completion of this operation. The tests show that the method mentioned is capable of guiding a robot and accomplishing headland turning operation in a cornfield with acceptable accuracy and stability. Therefore, the low-cost variable AOV machine vision has a promising field application for autonomous vehicles.

Conflict of Interests

The author has not declared any conflict of interests.

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REFERENCES

Bakker T, Bontsema J, Müller J, Asselt CJ, Henten EJV, Straten GV (2009). Automatic headland turning for four-wheel steered vehicles exploiting the full degrees of freedom. VDIBERICHT, 2060:179-186.

Bakker T, Wouters H, Asselt KV, Bontsema J, Tang L, Müller J, Straten GV (2008). A vision based row detection system for sugar beet. Comput. Electron. Agric. 60:87-95. http://dx.doi.org/10.1016/j.compag.2007.07.006

Benson ER, Reid JF, Zhang Q (2003). Machine vision-based guidance system for agricultural grain harvesters using cut-edge detection. Biosyst. Eng. 86:389-398. http://dx.doi.org/10.1016/j.biosystemseng.2003.07.002

Easterly DR, Adamchuk V,Kocher MF, Hoy RM (2010). Using a vision sensor system for performance testing of satellite-based tractor auto-guidance. Comput. Electron. Agric. 72:107-118. http://dx.doi.org/10.1016/j.compag.2010.03.004

Huang P, Luo X, Zhang Z (2010). Headland turning control method simulation of autonomous agricultural machine based on improved pure pursuit model. IFIP Adv. Inf. Commun. Technol. 317:176-184. http://dx.doi.org/10.1007/978-3-642-12220-0_27

Mariottini GL, Scheggi S, Morbidi F, Prattichizzo D (2012). An accurate and robust visual-compass algorithm for robot-mounted omnidirectional cameras. Robot. Auton. Syst. 60:1179-1190. http://dx.doi.org/10.1016/j.robot.2012.05.010

Mousazadeh H (2013). A technical review on navigation systems of agricultural autonomous off-road vehicles. J. Terramech. 50:211-232. http://dx.doi.org/10.1016/j.jterra.2013.03.004

Reid JF, Zhang Q, Noguchi N, Dickson M (2000). Agricultural automatic guidance research in North America. Comput. Electron. Agric. 25:155-167.http://dx.doi.org/10.1016/S0168-1699(99)00061-7

Subramanian V, Burks TF (2009). Headland turning maneuver of an autonomous vehicle navigating a citrus grove using machine vision and sweeping laser radar. ACTA Hortic. 824:321-328.

Tian T, Reid JF, Hummel JW (1999). An image processing based automatic steering power system. Transactions of the ASAE. 42:893-900.

Tillett ND, Hague T, Miles SJ (2002). Inter-row vision guidance for mechanical weed control in sugar beet. Comput. Electron. Agric. 33:163-177.http://dx.doi.org/10.1016/S0168-1699(02)00005-4

Xue J (2011). Headland turning of autonomous robot in corn field. Lecture Notes in Electr. Eng. 132:495-505.

Xue J, Zhang L, Grift TE (2012). Variable field-of-view machine vision based row guidance of an agricultural robot. Comput. Electron. Agric. 84:85-91.http://dx.doi.org/10.1016/j.compag.2012.02.009

Zhu Z, Chen J, Yoshida T, Torisu R, Song Z, Mao E (2007). Path tracking control of autonomous agricultural mobile robots. J. Zhejiang Univ. Sci. A 8:1596-1603. http://dx.doi.org/10.1631/jzus.2007.A1596

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Full Length Research Paper

Ergonomic study of farm women during wheat harvesting by improved sickle

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A study was conducted in Chandangaon village of Chhindwara district of Madhya Pradesh in 2009 to 2010 to determine the physical fitness, time and activity profile and physiological stress of farm women during wheat harvesting activity. A technically prepared serrated sickle was tested on farm women to improve work efficiency and to reduce the drudgery of women. The results revealed that 19.5% of working efficiency is increased by using serrated sickle as an average, one farm woman harvested 50 bundles of wheat by using serrated sickle while only 39 bundles of wheat was harvested through local sickle and using serrated sickle average working heart rate of women was found to be 110 beats/min. Similarly, energy expenditure was found to be 12 kJ/s and about 19% saving in cardiac cost of workers per unit of output in comparison to the traditional practice of local sickle.

Key words: Heart rate, ergonomic evaluation, drudgery reduction, cardiac cost of worker.

INTRODUCTION

Agriculture is one of the important labour intensive activities, where maximum percentage of women work force in rural areas is dependent for their livelihood. She performs almost each and every field activity right from sowing to harvesting. During these activities the risk of developing musculo-skeletal problems is mainly due to the inconvenient work postures (Chauhan and Saha, 1999). Wheat harvesting is a major problem for farmers/farm women. Harvesting of crops has to be done carefully as the matured grains easily detach from the ear heads/pods and, therefore, cannot be harvested by fast working tools or machines. Majority of the farm women performed this activity by local sickle with bending and squatting posture for longer time.

Though, this method is very demanding of labour and full of drudgery. It is clear that poor posture and tool design can increase the discomfort of both the healthy and less fit individuals. During harvesting activity from morning till evening, women usually adapts squatting posture and they continue to work in this posture for long duration without adapting any other posture due to which they reported severe pain in lower back, knees and cervical region (Jyotsna et al., 2005). In order to ensure health, safety, well being and thereby improving the

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Plate 1. Comparison with method of harvesting showed that Serrated sickle was easy in operation, no squatting and bending position relaxed the women against back pain with increased work force.

quality of work life and achieving higher productivity, it is essential that working equipment must be designed ergonomically and should be user friendly. By promoting such small tools, the work and work environment can be improved, physiological workload can be reduced in the agriculture and the efficiency and work output can be improved significantly.

Hence a study was conducted in Chhindwara district to reduce the drudgery and increase working efficiency of farm women during wheat harvesting by introducing serrated sickle through the heart rate method. Heart rate is one of the most accurate means of studying the energy expenditure while performing any activity.

MATERIALS AND METHODS

The study was carried out with 20 farmwomen among normal health, without any major illness involved in wheat harvesting activity, each falling between the age group of 25 to 45 years. The field experiment was conducted in the month of April to May, 2009-2010 for wheat harvesting and bundling activity. A uniform time of 6 h was given for wheat harvesting activity by theoretically equipped serrated sickle and traditionally made local sickle and 1.5 h in bundling activity per day headed for farm women (Plate 1).

During the experiment, the anthropometric rod and weighing balance were used to measure the physical characteristics like height and weight. Stop watch was used for recording the time. The heart rate was recorded by using the heart rate monitor. Based on the heart rate records the following parameters were calculated:

i. Average heart rate during rest and work,
ii. The energy expenditure was estimated from the heart rate (Varghese et al., 1994). Energy expenditure (kJ/s) = 0.159 x Average heart rate (beats/min) – 8.72,
iii. ∆HR (beats/min) = Average working heart rate – average heart rate during rest,
iv. Output (m^2/h) = area covered x duration / average time,
v. Cardiac cost of worker per unit of output (beats/ m^2 area covered) = ∆HR x duration / output.

The results were statistically analyzed using test of significance (t-test at 5% level of probability) and simple regression (r) by the method proposed by Shnedecor and Cochran (1967).

RESULTS AND DISCUSSION

To evaluate the harvesting through ergonomic point of view, 20 respondents in the age group of 25 to 45 years were selected at random and average age of the respondents engaged in wheat harvesting operation was counted as 32.50 years measuring body height of 156.50 cm and weight as 46.50 kg, respectively (Table 1).

Physiological stress of the wheat harvesters was determined on the basis of various parameters like average heart rate during work and rest, energy expenditure and physiological cost of work while performing the activity. Table 2 depicts that 19.50% of working efficiency increased by using serrated sickle as one farm woman harvested and bundled an average of 50 bundles each by using serrated sickle while only 39 bundles each of wheat was harvested and bundled through local sickle in given time frame of 6 h for wheat harvesting activity and 1.5 h for bundling activity. Physiological stress revealed that output recorded by serrated sickle was 87 m^2/h as compared to Local sickle by which 70 m^2/h area harvested. Mishra et al. (2013) also stated that improved sickle resulted in higher field capacity than simple sickle because of less pushing force required operating the sickle, which resulted in higher cutting speed and also found increased output with better harvesting efficiency and reduced drudgery by using

Table 1. Physical characteristics of selected respondents (N = 20).

Physical characteristic	Mean ± S.D.
Age (yrs)	32.50 ± 5.47
Height (cm)	156.50 ± 1.64
Weight (kg)	46.50 ± 2.63

Table 2. Evaluation of performance data of different parameters of the farm women while harvesting (N = 20).

Particular	Mean values ± S.D	
Type of implement used	**Local sickle**	**Serrated sickle**
Time (hrs.)	6	6
Numbers of bundle harvested	39 ± 2.50	50 ± 3.00
Average working heart rate (beats/min)	101 ± 4.99	110 ± 5.57
Average heart rate during rest (beats/min)	80 ± 3.55	86.50 ± 3.80
∆HR (beats/min)	21 ± 2.87	23.50 ± 3.52
Area covered/output (m^2/h)	70 ± 3.48	87 ± 3.92
Energy expenditure (kJ/s)	9.90 ± 0.57	12 ± 2.29
Cardiac cost (beats/m^2)	18 ± 2.81	14.50 ± 2.40
Reduction in drudgery (%)	-	19.00
Increase in efficiency (%)		19.50

Table 3. Correlation coefficient computed between different variables and energy expenditure (N = 20).

Particular	Energy expenditure (kJ/s)	
	Local sickle	**Serrated sickle**
Age (yrs)	-0.49*	-0.34
Height (cm)	-0.13	-0.19
Weight (kg)	0.22	-0.15
Time (min)	0.10	-0.13
Average working heart rate (beats/min)	0.94**	0.96**
∆HR (beats/min)	0.25	0.52*
Output (m^2/h)	0.07	0.17
Cardiac cost (beats/m^2)	0.15	0.46*

*Significant at P = 0.05, ** Significant at P = 0.01.

serrated sickle.

During harvesting with local sickle, the average ∆HR was 21 beats/min and energy expenditure 9.9 kJ/s while by serrated sickle, it was recorded as 23.50 beats/min and 12 kJ/s. The cardiac cost of worker was 18 beats/m^2 by local sickle while 14.50 beats/m^2 by serrated sickle. So the serrated sickle saves 19% cardiac cost of worker per unit of output and increases efficiency 19.50%. Dilbaghi et al. (2008) also found maximum output with improved sickle and reduction in total cardiac cost of worker with improved sickle over conventional sickle. The findings of the study are in conformity with Gite and Agarwal (2000) who revealed that drudgery reduction due to use of improved sickle was about 16.50% as compared to local sickle. They also revealed that improved sickle requires less effort for cutting and reduce the drudgery in harvesting. Singh (2012) also compared improved and local sickle for paddy harvesting and stated that potential demand of improved sickle is more than 2.27 million in the country. This clearly indicated the potentiality of improved sickle in the country.

Table 3 reveals that various physical measurements of respondents exhibited non significant negative correlation with energy expenditure similarly age of respondent showed significantly (P = 0.05) negative relationship (r = 0.49) with energy expenditure by harvesting with local

Table 4. Correlation coefficient computed amongst the variables of serrated sickle (N = 20).

Particular	Age (years)	Wt (kg)	Time (min)	Av. WHR (beat/min)	∆HR (beats/min)	Cardiac cost (beats/kg)	Output (m^2/h)
Age (years)	1.00						
Weight (kg)	0.38	1.00					
Time (min)	0.40	0.23	1.00				
Av. WHR (beats/min)	-0.26	-0.17	-0.11	1.00			
∆HR (beats/min)	0.19	0.59**	-0.17	0.45*	1.00		
Cardiac cost (beats/ m^2)	0.24	0.65**	0.39	0.52*	0.97**	1.00	
Output (m^2/h)	-0.40	-0.29	0.92**	0.23	0.19	-0.29	1.00

*Significant at P = 0.05, ** Significant at P = 0.01.

sickle and revealed that ageing effect working efficiency, while using serrated sickle physical parameters is not directly responsible to effect working efficiency as is shown non significantly negative correlation with energy expenditure. Energy expenditure was increasing at right angles as increase of average working heart rate during the spin of harvesting in both cases as it exhibited significantly (P = 0.01) positive correlation with local sickle (r = 0.94) and serrated sickle (r = 0.96). Heart rate difference between working and resting period of respondent [∆HR (r = 0.52)] and cardiac cost (r = 0.46) also showed positive correlation (P = 0.05) with energy expenditure and revealed that more energy was exhausted as rising of heart rate and cardiac cost also found more at same point in energy expenditure while using serrated sickle for wheat harvesting. Crouter et al. (2006) also stated that HR and physical activity (PA) can predict EE in individual subjects vary depending on age, height, weight and fitness of the subjects.

Correlation coefficient computed amongst variables of serrated sickle is demonstrated in Table 4. Perusal analysis of data recorded given the impression that the average weight of respondent showed significantly (P = 0.01) positive relationship with ∆HR (r = 0.59) and cardiac cost (r = 0.65) and exhibited the working capacity affected through fluctuation of weight of worker. Output of work (numbers of bundled harvested) was depended upon the duration of work as it confirmed positive correlation (P = 0.01) with time (r = 0.92). Cardiac cost also showed significant (P = 0.01) positive association with average heart rate difference between working and resting period (r = 0.97) during wheat harvesting with serrated sickle. Singh et al. (2010) have also reported positive relationship of Cardiac cost with average heart rate during maize shelling with tubular maize sheller.

Conclusion

Serrated sickle is women friendly tool because the assessment of technology increases the efficiency and reduces drudgery and it avoids bending and squatting posture. Serrated sickle provides safety to the workers due to its better construction and reduces musculo-skeletal disorders. It is necessary to maintain proper posture during performing these types of activities. Serrated Sickle saves about 19% cardiac cost of worker per unit of output over traditional practice. It does not require sharpening of cutting edge frequently.

This is a kind of women empowerment. It lessens the exertion and fatigue. Women feel comfortable; they earn money by reducing the labour. Their social life increases and they feel empowered in society. Hence, periodic training programmes should be organized to emphasize on educating workers regarding recognition of musculo-skeletal disorders and importance of rest pauses and maintaining proper posture while performing agricultural activities.

Conflict of Interest

The authors have not declared any conflict of interests.

REFERENCES

Chauhan MK, Saha DN (1999). Acceptable limits of physiological workload for physically active Indian women. Advance Training Course in Ergonomics, SNDT Women's University, Mumbai.

Crouter SE, Churilla JR, Bassett DR (2006). Estimating energy expenditure using accelerometers. Eur. J. Appl. Physiol. 98:601-612. http://dx.doi.org/10.1007/s00421-006-0307-5 PMid:17058102

Dilbaghi M, Gandhi S, Bimla (2008). Ergonomic evaluation of improved sickles in wheat harvesting. J. Agric. Eng. New Delhi 1:7-11.

Gite LP, Agarwal N (2000). Ergonomical comparison of local and improved sickles for wheat harvesting by women workers. Agric. Eng. Today 24(3):7-12.

Jyotsna KR, Singh K, Mehta M (2005). Ergonomic Evaluation of the Rural Women While Performing Wheat Harvesting Activity. J. Hum. Ecol. 18(4):309-311.

Mishra R, Singh YP, Mishra YD, Singh S, Singh H (2013). Dissemination of Improved Sickles for Female Agriculture Workers for Crop Harvesting. Technofame 2(1):118-123.

Shnedecor GW, Cochran WG (1967). Statistical methods, VI Edn. Oxford and IBH, New Delhi, P. 593.

Singh A, Gutam US, Pannase S, Singh A (2010). Ergonomic Evaluation of Farm Women during Maize Shelling. IRJEE 10(3):41-44.

Singh SP (2012). Physiological workload of farm women while evaluating sickles for paddy harvesting. Agric. Eng. Int. CIGR J. 14(1):82-88.

Varghese MA, Saha PN, Atreya N (1994). A rapid appraisal of occupational workload from modified scale of perceived exertion. Ergonomics 37(3):485-491. http://dx.doi.org/10.1080/00140139408963665 PMid:8143693

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Full Length Research Paper

Epidemiological study on foot and mouth disease in cattle: Seroprevalence and risk factor assessment in Kellem Wollega Zone, West Ethiopia

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A cross-sectional study was conducted in three districts of Kellem Wollega Zone of Oromia Regional State, West Ethiopia between November, 2011 to March, 2012 with the objectives of determining the seroprevalence of foot and mouth disease (FMD) in cattle and assessing the potential risk factors associated with the disease. Antibodies against non-structural protein of FMD virus using 3ABC ELISA was measured as an indicator of exposure to the virus. From the total 384 sera tested, the overall seroprevalence of FMD in Kellem Wollega Zone was found to be 21.4% (95% CI: 17.23 - 25.47). The highest seroprevalence was observed at Sayo 31.53% (9%%CI: 22.82 to 40.24) followed by Lalo Kile district 19.01% (12.51 - 25.51) and Dale Sadi district 15.26% (9.06 - 21.46). The seroprevalence of FMD in different age groups was 24.22% (95% CI: 18.62, 29.83), 16.51% (95% CI: 9.48, 23.53) and 18.75% (95% CI: 7.55, 29.94) in group of cattle aged greater than 4 years, between 2 to 4 years and less than 2 years, respectively. Higher seroprevalence observed in female 27.17% (95%CI: 20.89 - 33.45) than male 15.34% (10.17, 20.51). Seropositivity was significantly varied with sex and district of cattle ($P < 0.05$). The odds of being seropositive for female cattle was observed to be 2.05 times of the male cattle (OR; 2.05, 95% CI: 1.22 - 3.43) and Cattle from Sayo district had significantly higher seroprevalence than dale sadi cattle (OR; 2.58, 95% CI: 1.37 - 4.87). In conclusion, the result of this study showed that FMD is an important cattle disease in the study area necessitating further investigation and characterization of the circulating virus serotype to apply effective control and prevention measures.

Key words: Seroprevalence, foot and mouth disease (FMD), Kellem Wollega, 3ABC ELISA.

INTRODUCTION

Foot and mouth disease (FMD) is a highly contagious viral disease of cloven-footed animals and is one of the most important economic diseases of livestock by fever and vesicular eruptions in the mouth, on the feet and teats (Bronsvoort et al., 2004). FMD is caused by a virus of the genus *Aphthovirus*, in the family Picornaviridae, of

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which there are seven immunologically distinct serotypes: O, A, C, SAT-1, SAT-2, SAT-3, and Asia-1 (OIE, 2004). Studies have shown that five (O, A, C, SAT-1 and SAT-2) of the seven FMD serotypes were identified in Ethiopia. Serotype O was more prevalent followed by types A, SAT-2, SAT-1 and C, respectively. Serotype C was not identified after 1983; however, a serotype C specific antibody in cattle was reported (Sahle, 2004; Gelaye et al., 2005; Legess, 2008; Rufael et al., 2008).

The disease has a high morbidity although mortality is rare in adult animals. The impact posed by the disease is enormous. It affects animal's performance directly through reduction of milk yield. Death of young animals and fertility impairment due to increased abortion rate are also the grave consequences of the disease. The recovered animals remain in poor physical condition over long periods of time leading to economic losses for livestock industries (Sangare, 2002). Pastoralists are severely affected by the direct impact of the disease since their livelihood is directly linked to livestock production (Bayissa, 2009). Moreover, the disease is becoming the major constraint hampering export of livestock and livestock products. For example, the Egyptian trade bans of 2005/2006 due to FMD costs Ethiopia more than US$14 million (Leforban, 2005).

FMD is endemic to most of sub-Saharan Africa, except in a few countries in southern Africa, where the disease is controlled by the separation of infected wildlife from susceptible livestock as well as by vaccination (Sahle, 2004). In Ethiopia, FMD is endemic widely distributed and frequently noted in different farming system and agro-ecological zones of the country. During the period from 1999 -2008 on average over 77.7 numbers of FMD outbreaks were reported every year to Ministry of Agriculture and Rural Development (Gelaye et al., 2005). Recent seroprevalence studies of FMD in different districts and localities in the central, south west, northwest and in south pastoral areas of Ethiopia revealed seroprevalence of the FMD in the range of 2.5 - 21% (Bogale, 2005; Gelaye et al., 2005; Rufael et al., 2008). Regardless of its huge economic importance, FMD occurrence and risk factors associated with the disease was not substantially investigated throughout Ethiopia to indicate the real magnitude and burden of the disease in the country. Besides, there is no citable information regarding the occurrence of the disease in the Kellem Wollega Zone.

Therefore, the objectives of the present study were to estimate the seroprevalence of FMD and to assess the risk factors associated with the disease in the study area.

MATERIALS AND METHODS

Study area

The study was conducted in Kellem Wollega Zone, in three districts namely Sayo, Dale Sadi and Lalo Kile districts. Kellem Wollega located in Western Ethiopia, is found at 652 km distance from Addis Ababa. Geographically, the area fall between 08° N 25' 56" to 08° N 58'05" latitude and 034° E 33'41 longitudes with an altitude of 1701 to 1827 m above sea level. However, the elevation decline progressively west ward along the Sudan border. The zone has an average annual rainfall of 700 to 1500 mm per year. The annual minimum and maximum air temperature is 18 to 38°C, respectively. It borders with the Sudan in the west, Beneshangul Gumuz Regional State in North West and Gambella Regional State in South West of the country. The quarter of the zone is estimated to be covered with forest. The farming system of the area is mixed crop livestock farming. The livestock population of the zone is 703,877 head of cattle, 123,521 head of sheep and goat, 65,225 head of horse, mule, donkey and 572,204 head of poultry. The total human population dwelling in the area is 29,448 (CSA, 2009).

Study population

The study population was indigenous breed of cattle that are kept under traditional grazing management system in the study area. Twelve Peasant associations (PA) were selected randomly from the three districts. Approximately, about 30 animals were sampled from each PA from randomly selected herd owners.

Sample size determination

The sample size was estimated by assuming an expected prevalence of 50% to get the maximum number required to determine the prevalence. The precision was decided to be 5% at 95% confidence level. For sample size estimation the formula described by Thrusfield (1995) was used.

$$n = \frac{1.96^2 \times P_{exp}(1-P_{exp})}{d^2}$$

Where, n = required sample size; d^2 = desired absolute precision; P_{exp} = expected prevalence.

$$n = \frac{1.96^2 \times 0.5\,(1 - 0.5)}{(0.05)^2} = 384$$

Accordingly, a total 384 serum samples from cattle were collected to determine the prevalence of FMD in the study area.

Study design

A cross-sectional study was under taken on FMD in local cattle from November, 2011 to March, 2012. During the laboratory work, a total of 384 sera samples were tested using 3ABC ELISA for the detection of FMD antibodies.

Sample collection and submission

Blood samples of 10 ml were aseptically collected using plain vacutainer tubes from local cattle through jugular vein puncture. The owner(s) restrained the animals properly. The samples were labeled right after collection. Then after, the sample was allowed to clot for 4 to 5 h and then centrifuged.

Table 1. Seroprevalence of foot and mouth disease (FMD) according to district.

District	Sample	Positive	Seroprevalence (%) (95% CI)
Sayo	111	34	31.53 (22.82, 40.24)
Dale Sadi	131	20	15.26 (9.06, 21.46)
Lalo Kile	142	28	19.01 (12.51, 25.51)

Table 2. Seroprevalence of foot and mouth disease (FMD) in cattle of different sex groups.

Sex	Sample	Positive	Seroprevalence (%) (95% CI)
Female	195	53	27.17 (20.89, 33.45)
Male	189	29	15.34 (10.17, 20.51)

The serum was collected ascetically from the centrifuged blood and transferred into a single sterile cryovials and labeled with the sample code. The serum transported using ice box (at <5°C) containing icepacks to National Animal Health Diagnostic and Investigation Center (NAHDIC), Sebeta. The sera sample then stored at -20°C until laboratory investigation was undertaken.

Laboratory test

The PrioCHECK® FMDV NS 3ABC-Ab ELISA kit was designed to detect FMD virus specific antibodies in bovine serum sample. The procedure was based on a solid phase blocking ELISA. The test plates are coated with 3ABC specific monoclonal antibody (mAb), followed by incubation with antigen (3ABC protein). Consequently, test plates of the kit contain FMD virus NS antigen captured by the coated mAb. The test performed by dispensing the test samples to the wells of a test plate. After incubation, the plate was washed, the conjugate added and the chromogen (TMB) substrate was dispensed. After incubation, at room temperature (23 ± 3°C) the color development was stopped. Color development measured optically at a wave length of 450 nm shows the presence of antibodies directed against FMD virus.

Data analysis

Data were stored in Microsoft (MS) Excel and analysis was done using STATA version 11.0 for windows (Stata Corp, Texas). The total prevalence was calculated by dividing the number of 3ABC ELISA positive animals by the total number of animals tested. Logistic regression was used to analyze whether potential risk factors (sex, age, and districts) have significantly associated with disease occurrence. The degree of association between potential risk factors and seroprevalence was computed using odds ratio. FMD Seropositivity was considered as dependent variable and risk factors that would likely predict the outcome were considered as independent variables. In all of the analysis, confidence level at 95% and P < 0.05 was set to see the significance of association of the dependent and independent variables.

RESULTS

The overall seroprevalence of FMD in Kellem Wollega Zone was found to be 21.4% (95% CI: 17.23, 25.47). Tables 1, 2 and 3 show the seroprevalence FMD according to the origin, sex and age of cattle. The prevalence of FMD among the different origin of cattle indicated that the highest seroprevalence was observed in Sayo district (31.53%) followed by Lalo Kile district (19.01%) and Dale Sadi district (5.20%). Comparison of the seroprevalence of FMD between sex groups revealed a highest prevalence of 27.17% (53/195) in female cattle than in male ones (15.34% (29/189). The highest seroprevalence was observed in cattle aged greater than 4 years 24.22% (56/227) followed by in group of animals aged less than 2 years 18.75% (9/48) and between 2 to 4 years 16.51% (17/109).

Table 4 shows associations of the potential risk factors associated with FMD seropositivity. Accordingly, seropositivity was significantly varied with sex and district of cattle (P < 0.05). The odd of being seropositive for female cattle was observed to be 2.05 times of the male cattle (OR; 2.05, 95%CI: 1.22 - 3.43). Cattle from Sayo district had significantly higher seroprevalence than Dale Sadi cattle (OR; 2.58, 95% CI: 1.37 - 4.87).

DISCUSSION

The present study revealed that FMD is a significant disease in Kellem Wellega Zone with a prevalence of 21.4% (n = 82). The finding of this study was in agreement with the previous report in the Borana pastoral land where over all seropositivity of 21% was reported(Rufael et al., 2008). However, the seroprevalence was slightly lower than the previous findings in Ethiopia (Sahle, 2004) in which a seropositivity of 26.5% was reported. On the contrary, the seropositivity found in current study was higher than the report in Afar Regional State where seropositivity of 5.6% (Jembere, 2008) and in South Omo Zone where seropositivity of 8.18% was reported (Molla et al., 2010). The relative increase in the prevalence of the FMD in comparison to other findings might be due to extensive livestock

Table 3. Seroprevalence of foot and mouth disease (FMD) in cattle of different age groups.

Age	Sample	Positive	Seroprevalence (%) (95% CI)
Less than 2 years	48	9	18.75 (7.55, 29.94)
Between 2 - 4 years	109	17	16.51 (9.48, 23.53)
Above 4 years	227	56	24.22 (18.62, 29.83)

Table 4. Associations of potential risk factors associated with seropositivity of foot and mouth disease (FMD).

Variable	Sample	Positive	Prevalence	OR	95% CI	P-value
Sex						
Male	189	29	15.34	1		
Female	195	53	27.17	2.05	1.22-3.43	0.006
Age						
Between 2 - 4 years	109	17	16.51	1		
Less than 2 years	48	9	18.75	1.09	0.44-2.71	0.848
Above 4 years	227	56	24.22	1.67	0.91-3.06	0.095
District						
Dale Sadi	131	20	15.26	1		
Lalo Kile	142	28	19.01	1.36	0.71-2.59	0.353
Sayo	111	34	31.53	2.58	1.37-4.87	0.003

movement and high rate of contact at market place, grazing and watering points which are potential risk factors for the transmission of the disease in the study area.

The highest district level seroprevalence was recorded in Sayo and Lalo Kile districts, while the lowest observed in Dale Sadi district. The highest seroprevalence in Sayo and Lalo Kile districts probably reflects the higher contact pattern of domestic animals with wild animals and also due to the fact that Kellem Wollega Zone is bordered with Sudan, where possibly cattle movement across boundary occurs illegally supporting the notion that FMD peaked in cattle associated with cattle movement leading to contact of cattle in different origin, which is the predominant factor for the transmission of the disease (Rufael et al., 2008).

Animal factors like sex and age are believed to play significant role in the occurrence of a disease. Although there was no difference in the seropositivity of FMD among the different age categories statistically significant, the present study revealed the highest prevalence in cattle of age above four years (24.22%) supporting that the fact of increase in age increase the chance of exposure to the disease occurrence (Thrusfield, 1995). This is in agreement with the report of Gelaye et al. (2009). Those animals aged >4 years may have acquired the infection from multiple serotypes and/or infections. The study showed an association between sex and prevalence of FMD in cattle with the higher prevalence of the disease in female (27.17%) than male cattle (15.34%) showed the highest prevalence. The finding was in agreement with the report of Hailu et al. (2010) in which they report a higher prevalence of FMD in female (16.63%) cattle than that of male (1.37%) cattle. However, it was in agreement with the findings of other researchers in which they report a higher prevalence in male than female animals (Remond et al., 2002; Sarker et al., 2011).

Conclusion

The result of this study showed that FMD is an important cattle disease in the study area. It found that high prevalence of FMD with district and sex being the major risk factors for the occurrence of the disease in Kellem Wollega Zone, Western Ethiopia necessitating further investigation and characterization of the circulating virus serotype in the area to apply effective control and prevention measures.

Conflict of Interests

The authors have not declared any conflict of interests.

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REFERENCES

Bayissa B (2009). Foot and mouth disease seroprevalence and implications in Borana pastoral and agro pastoral systems, southern Ethiopia. MSc Thesis, Addis Ababa University, Faculty of Veterinary Medicine, Debre-Zeit, Ethiopia.

Bogale A (2005).Review of FMD: An in-depth discourse of Global, sub-Saharan and Ethiopian status (unpublished review), Addis Ababa University, Faculty of Veterinary Medicine, Research and graduate studies, Debre-Zeit, Ethiopia, pp. 1-46.

Bronsvoort BMC, Radford AD, Tanya VN, Nfon C, Kitching RP, Morgan KL (2004). Molecular epidemiology of footand- mouth disease viruses in the Adamawa provinceof Cameroon. J. Cl. Microbiol. 42:2186–2196. http://dx.doi.org/10.1128/JCM.42.5.2186-2196.2004

Central Statistical Agency (CSA)(2009).The Federal Democratic Republic of Ethiopia, Central Statistical Authority, Ethiopia.

Gelaye E, Ayelet G, Abera T, Asmare K (2009). Seroprevalence of foot and mouth disease in Bench Maji zone, south-western Ethiopia. J. Vet. Med. Anim. Health. 1:005-010.

Gelaye E, Beyene B, Ayelet G (2005). Foot and mouth disease virus serotype identified in Ethiopia. Ethiopia Vet. J. 9:75–80.

Hailu M, Mengistie T, Negussie H, Alemu S, Asaminew T (2010). Incidence of foot and mouth disease and its effect on milk yield in dairy cattle at andassa dairy farm, Northwest Ethiopia. Agri. Biol. J. 1: 969-973.

Jembere S (2008). Participatory epidemiology and seroprevalence of Foot and Mouth Disease in Afar pastoral region, Ethiopia. MSc thesis, FVM, AAU, Debre-Ziet, Ethiopia.

Leforban Y (2005). Report of a mission on foot and mouth disease in Ethiopia. Proposals for a strategic plan for a control program oriented to the export, 10–22 April 2005, pp. 12–42.

Legess Y (2008). Investigation of foot and mouth disease outbreaks and assessment of risk factors in Oromia, Amhara, and Southern Nations, Nationalities and Peoples (SNNP) regional states of Ethiopia. MSc thesis, Addis Ababa University, Faculty of Veterinary Medicine, Debre-zeit, Ethiopia.

Molla B, Ayelet G, Asfaw Y, Jibril Y, Ganga G, Gelaye E (2010). Epidemiological study on foot and mouth disease in cattle: seroprevalence and risk factor assessment in South Omo zone, South-western Ethiopia.

OIE (2004).Foot and mouth disease. In: Manual of Standards for Diagnostic Tests and Vaccines. 5th Edition, Office international des Epizooties (OIE), Paris, France, pp. 111–128.

Remond M, Kaiser C, Leberton F (2002). Diagnosis and scraning of FMD virus.Imm.micrbiol. Infect. Dis. 25(5):309-321.

Rufael T, Catley A, Bogale A, Sahle M, Shiferaw, Y (2008). Foot and Mouth Disease in Borana Pastoral system, Southern Ethiopia. Trop. Anim. Health Prod. 40:29–38. http://dx.doi.org/10.1007/s11250-007-9049-6

Sahle M (2004). An epidemiological study on the genetic relationships of foot and mouth disease viruses in east Africa. PhD Thesis, University of Pretoria, South Africa, Pretoria, pp. 84-107.

Sangare O (2002). Molecular epidemiology of foot and mouth disease virus in West Africa., PhD Thesis, University of Pretoria, Pretoria, South Africa, pp: 10-37.

Sarker S, Talukder S, Haque MH, Islam HM, Gupta SD (2011). Epidemiological study on foot and mouth disease in cattle: prevalence and risk factor assessment in Rajshahi, Bangladesh. J. Anim. Sci. (1):71-73.

Thrusfield M (1995). Veterinary Epidemiology, 2nd ed, Black well science.

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Impact of climate change on grasses cultivation potential of three altitudinal strata-agricultural lands of Mexico

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This study estimated the impact of climate change in the period 2040-2069 on six grasses potential areas in three altitudinal zones of México: 0 to1200 m (lowlands), 1200 to 2200 m (midlands) and >2200 m (highlands). Topography, soil and climate variables were used to depict potential areas. Climate data for 1961-1990 (reference climatology) and 2040-2069 were obtained from the WorldClim Earth System Grid Portal and were worked in 2.5 arc minutes raster images in the Idrisi Selva System. For the 2040-2069 climatology, three GCMs were considered: MPIM-ECHAM5, MIROC3.2 (medres) and UKMO_HADCM3, under A2 emissions scenario. The results showed that most of the potential areas with optimal conditions for grasses will remain in lowlands, however the surface with these conditions will tend to decrease for *Cenchrus ciliaris, Andropogon gayanus and Brachiaria mutica* at a rate of 35-49%, 2-63% and 15-30%, respectively, which will affect mostly to *C. ciliaris,* since it will tend to migrate to midlands. Optimal conditions surface for *C. gayana* and *C. dactylon* will not change in lowlands, but will increase in midlands 63-103% and 74-90%, respectively. For *H. rufa*, the optimum conditions surface will rise 5-17% in lowlands and 391-449% in midlands. In highlands, potential areas for grasses were estimated majorly as suboptimal, however with climate change *C. ciliaris, C. gayana* and *C. dactylon* will increase their optimal conditions surface in highlands. For *A. gayanus, B. mutica* and *H. rufa* no optimal conditions surface was determined in highlands neither in the reference climatology nor in the future climatologies.

Key words: Climate change and forage grasses; Climate change and altitudinal zones; Tropical and subtropical grasses.

INTRODUCTION

According to the Intergovernmental Panel on Climate Change (IPCC), CO_2 concentrations in the atmosphere, in preindustrial times were of 600 gigatons (Gt); concentrations currently are of 800 Gt and the expected increment in CO_2 atmosphere would be closer to 1,000 Gt by 2050 (IPCC, 2007). The increment in atmospheric concentrations of CO_2, as well as other greenhouse effect gases (GHGs), due to intensification of anthropogenic activities (Hegerl et al., 2007), is associated to the change of temperature patterns and precipitation, which will cause important effects on development and on global agricultural productivity (Attipalli et al., 2010; Deryng et al., 2011; Hsiang et al., 2011), as the soil water availability is manifested according to complex interactions between these two factors (Wang , 2005).

Numerous studies indicate that plants present a positive response to increased CO_2; which is manifested through the increment in photosynthesis, biomass and production of crops (Kimball et al., 2002; Tubiello et al., 2007). The capture of atmospheric CO_2 by photosynthesis, is crucial for the production of food, fiber and fuel for humanity (Friend et al., 2009). It has been estimated that when increasing at twice the average of CO_2 content, economic performance is increased about 10% in C_4 specie (Hatfield et al., 2011; Izaurralde et al., 2011) and up to 20% in high radiation conditions (Ghannoum et al., 2000); however, little is known about the interactive effects of environmental variables, nutrients, water availability and high CO_2 increment during the growth of C_4 plants (Leakey et al., 2009).

Moreover, as a result of increment in greenhouse gases on the atmosphere, an increment in temperature is produced, which is unambiguous (Trenberth et al., 2007); and therefore drying of many regions through increased evaporation is induced (Wang, 2005; Woodhouse et al., 2010), while the maturity crop process is accelerated, reducing leaf area duration and thus the total water requirement of crop maturity (Hatfield et al., 2011; Ojeda et al., 2011).These changes in climatic patterns, will cause profound effects on terrestrial plant growth and productivity in the near future (Attipalli et al., 2010), and, defining the geographical potential distribution on yield losses of crops, is transcendental as well as to develop mitigation strategies (Deryng et al., 2011; Justin et al., 2012).

Moreover, numerous studies indicate that agriculture must meet the dual challenge of feeding a growing population and a high demand for diets rich in meat and calories, while minimizing environmental impacts (Verena et al., 2012). In Mexico, there are studies about climate change and its impact on crop production, but few have analyzed in detail the effects of this phenomenon on forage species in particular. The country is characterized by an ample altitudinal variation (0 to 5747 m), which impose diverse c limatic conditions for agriculture. With the presence of climate change it is visualized that a re-accommodation of crops will take place as temperatures continue increasing and migration to higher altitudes is expected. Nowadays is necessary to depict how the climate conditions will be in the mean future in order to planning agriculture. This is why the objective of this study was to determine the impact of climate change on potential areas of six tropical grasses in Mexico as a function of altitudinal strata.

MATERIALS AND METHODS

The study was conducted in agricultural areas of Mexico, which were studied under the following altitudinal strata: lowlands (< 1200 masl), midzones (1200 to 2200 masl) and highlands(> 2200 masl) .

Species studied

Six forage species were included: *Andropogon gavanus* Kunth, *Brachiaria mutica* (Forssk.) Stapf, *Cenchrus ciliaris* L., *Chloris gayana* Kunth, *Cynodon dactylon* (L.) Pers., and *Hyparrhenia rufa* Nees; species of great interest for their productive potential in Mexico.

Databases and information systems

Monthly and annual data of precipitation, maximum temperature, minimum temperature and average temperature were used, from the 1961 to 1990 period (reference climatology) and from 2040 to 2069 period, to determine potential areas of the grasses under study. These climate data were obtained from the data portal of Earth System Grid (ESG) in WorldClim and were managed by raster images with a resolution of 2.5 minutes of arc, at the Idrisi Selva System (Eastman, 2012). For the 2040 to 2069 period, GCMs MPIM- ECHAM5, MIROC3.2 (medres) and UKMO_HADCM3 were considered under A2 greenhouse gas emissions scenario (IPCC, 2007). These three models have showed good fitting to Mexico environmental conditions and have been employed frequently to simulate future climatic conditions (Conde et al., 2006). To determine potential areas, other diagnostic variables were also included, such as agricultural land use, soil slope, soil texture and soil depth; which were obtained from the Environmental Information System (SIAN) of the Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias in Mexico (Díaz et al., 2012), except for agricultural land use and texture, which were extracted from the image of the soil usage series III of Instituto Nacional de Estadística, Geografía e Informática in Mexico (INEGI, 2009).

Statistical analysis

Kolmogorov-Smirnov test was used to check normality on the data series of precipitation and temperature (1961 to 1990 climatology) in the three altitudinal zones. The test was run through SPSS Statistics 19 software (IBM Corp, 2010).

Since the test of normality reported in all cases that the temperature and precipitation data were not normally distributed, an analysis of variance was realized with the nonparametric statistical of Kruskal and Wallis test, which is also known as *H* test and uses sample data ranges from three or more independent populations (Kruskal and Wallis, 1952). This test was used to identify significant differences between the temperature data and precipitation data from the three altitudinal strata. The statistic is described by the expression:

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Table 1. Agro-ecological intervals for the diagnosis of potential areas for six species of tropical grasses.

Species	Mean-annual temperature(°C)			Mean-annual precipitation (mm)			Soil texture		
	Op	Sp	Mg	Op	Sp	Mg	Op	Sp	Mg
Andropogon gayanus	22-28	28-32 15-22	0-15 >32	750-1400	400-750 1400-3000	0-400 >3000	Cr	Md	Fn
Brachiaria mutica	21-30	30-46 15-21	0-15 >46	1200-2000	500-1200 2000-4100	0-500 >4100	Cr	Md	Fn
Cenchrus ciliaris	18-27	5-18 27-42	0-5 >42	400-1000	100-400 1000-3500	0-100 >3500	Md	Fn	Cr
Chloris gayana	18-37	5-18 37-50	0-5 >50	500-1500	400-500 1500-2000	0-400 >2000	Md	Fn	Cr
Cynodon dactylon	18-35	6-18 35-40	0-6 >40	625-1750	400-625 1750-4300	0-400 >4300	Md	Cr- Fn	
Hyparrhenia rufa	23-28	11-23 28-32	0-11 >32	600-1400	500-600 1400-1600	0-500 >1600	Md	Cr	

Op,optimal; **Sp**,Suboptimal and supraoptimal; **Mg**=marginal; **Cr**=Coarse; **Md**=Medium, **Fn**=Fine.

Table 2. Results of Kolmogorov-Smirnov's normality test for temperature and precipitation data from three altitudinal strata.

Variable	gl	Kolmogorov-Smirnov statistic	Significance probability
Lowlands precipitation	53824	0.119	0.0001
Midlands precipitation	35501	0.158	0.0001
Highlands precipitation	9258	0.066	0.0001
Lowlands temperature	53824	0.061	0.0001
Midlands temperature	35501	0.046	0.0001
Highlands temperature	9258	0.018	0.0001

$$H = \frac{12}{N(N+1)} \Sigma_{i=1}^{j} \frac{Ri^2}{ni} - 3(N+1)$$

Where: J = the number of samples, n_i = the number of observations in the ith sample, N = Σ_{ni}, the number of observations in all samples combined, R_i = the sum of the ranks in the ith sample.

In order to perform the statistical analysis, temperature and precipitation were derived from each cell of the raster images of these variables for the three altitudinal strata studied. This information was extracted transforming the raster images to vector of points, which were exported using Idrisi Selva system to ascii files and these were opened and manipulated in Microsoft Excel system.

Diagnostic potential areas

Potential areas were determined using a multi-criterion analysis in Idrisi Selva system and considering a qualitative scheme of three categories: areas with optimal agroecological conditions (Op), areas with suboptimal agroecological conditions (Sp) and areas with marginal agroecological conditions (Mg). They were assumed to have optimal agroecological conditions, the areas in which all environmental factors of analysis were at an optimum level for forage species; suboptimal were classified as those areas in which at least one variable of diagnosis was found in non-optimal conditions (sub- optimal or supra- optimal) for growing; finally marginal areas were taken as the ones in which at least one of the diagnostic variables maintained development restricting values for forage species studied. The information to establish this categorization was obtained from literature review reported by Duran (2011). Table 1 describes the intervals of three variables used to diagnose potential areas. The slope of the ground for all species was graded by assigning an optimal condition for slopes from 0 to 8%, suboptimal condition for slopes from 8 to 20% and marginal condition for slopes greater than 20%. Soil depth was considered in two categories, according to information available in raster image used: deep soil (optimum condition) and shallow soils (suboptimal conditions). The analysis of potential areas was made considering as surface diagnosis only agricultural use areas.

RESULTS AND DISCUSSION

Statistical analysis

As shown in Table 2, and according to the results of theKolmogorov-Smirnov test ($P < 0.0001$), temperature and precipitation data do not have a normal distribution in any of the altitudinal strata studied. Table 3 describes the basic statistics of temperature and precipitation by altitudinal stratum. According to the results of the Kruskal and Wallis test, both the temperature and precipitation varied significantly ($P<0.001$) among the three altitude

Table 3. Basic statistics of temperature and precipitation on three altitudinal strata.

Variable	n	Average	Standard deviation
Lowlands precipitation	53825	868	692.9
Midlands precipitation	35502	609	382.4
Highlands precipitation	9259	774.5	284.3
Lowlands temperature	53825	22.8	2.74
Midlands temperature	35502	17.7	2.22
Highlands temperature	9259	14.3	2.09

Table 4. Annual average values of mean temperature and accumulated precipitation for two climate scenarios in three altitudinal strata of the agricultural surface in Mexico.

Climatic scenarios	Models	Variables	Altitudinal strata		
			Lowlands	Midlands	Highlands
1961-1990		Temperature (°C)	22.8	17.7	14.3
		Precipitation (mm)	868.0	609.0	774.0
2040-2069	MPIM-ECHAM5	Temperature	25.4	21.0	17.0
		Precipitation	874.0	622.0	756.0
	MIROC3.2 (medres)	Temperature	25.7	20.5	17.4
		Precipitation	754.0	552.0	681.0
	UKMO_HadCM3	Temperature	25.5	20.4	16.7
		Precipitation	819.0	633.0	815.0

strata, so that the three topographical regions can be considered climatically different.

Climate changes in the agricultural areas of Mexico

The thermal variation ranging from 14.3°C at high altitudes to 22.8°C in low areas, combined with the variation of precipitation, produces great environmental diversity in agricultural areas of Mexico. The projections of temperature from the three GCM used, indicate a thermal increase in the range of 2.6 to 2.9, 2.7 to 3.3 and 2.4 to 3.1°C in low, mid and high lands, respectively (Table 4), passing through 1961 to 1990 period to 2040 to 2069 period, which translates into a decadal heating rate of 0.32 to 0.37, 0.34 to 0.42 and 0.30 to 0.39°C. This increase coincides with that recorded by Sivakumar et al. (2005), who estimated for Africa a warming of 0.2 to 0.5°C per decade in this century.

The thermal variation projected for the region of studyis important, as some areas will vary its temperature regime; such as areas of intermediate height that will go from a tempered condition (12-18°C of mean annual temperature, García, 1988), to a semiwarm condition (18-22°C, García, 1988, Medina et al., 1998), which will have positive effects on the surface with optimal conditions for growing tropical and subtropical species (Ruiz et al., 2011). However, the temperature increment is considered harmful for crops, since it may be shortening the crop production cycle of the actual varieties, and therefore reducing the final yield; especially if no adaptation measures are considered (Gornall et al., 2010; Ruiz et al., 2011). In the lowlands, which keep a warm temperature, near the maximum physiological thresholds of crops, the temperature increment projected in the present study may be detrimental due to the increase of heat stress and loss of water by evaporation (Gornall et al. 2010).

Regarding precipitation, the projections of the three GCMs do not maintain a coincidence as high as in the case of temperature, because while MIROC3.2 (medres) and UKMO_HADCM3 models indicate a decrease in precipitation of 13 and 6% in lowlands, MPIM-ECHAM5 model projects a slight increase of 0.7% in annual rainfall (Table 4). In addition, for midlands an increment of 2 and 4% in annual precipitation is projected, with MPIM-ECHAM5 and UKMO_HADCM3 models, respectively, and a 9.5% decrease with the MIROC3.2 (medres). In the highlands a decrease of 2 and 12% of precipitation is projected with MPIM-ECHAM5 and MIROC3.2 (medres) models, while an increase of 5% is visualized with UKMO_HADCM3 model. This lack of consistency in the modeling of future precipitation by different GCMs has been previously reported (IPCC, 2007) and it is

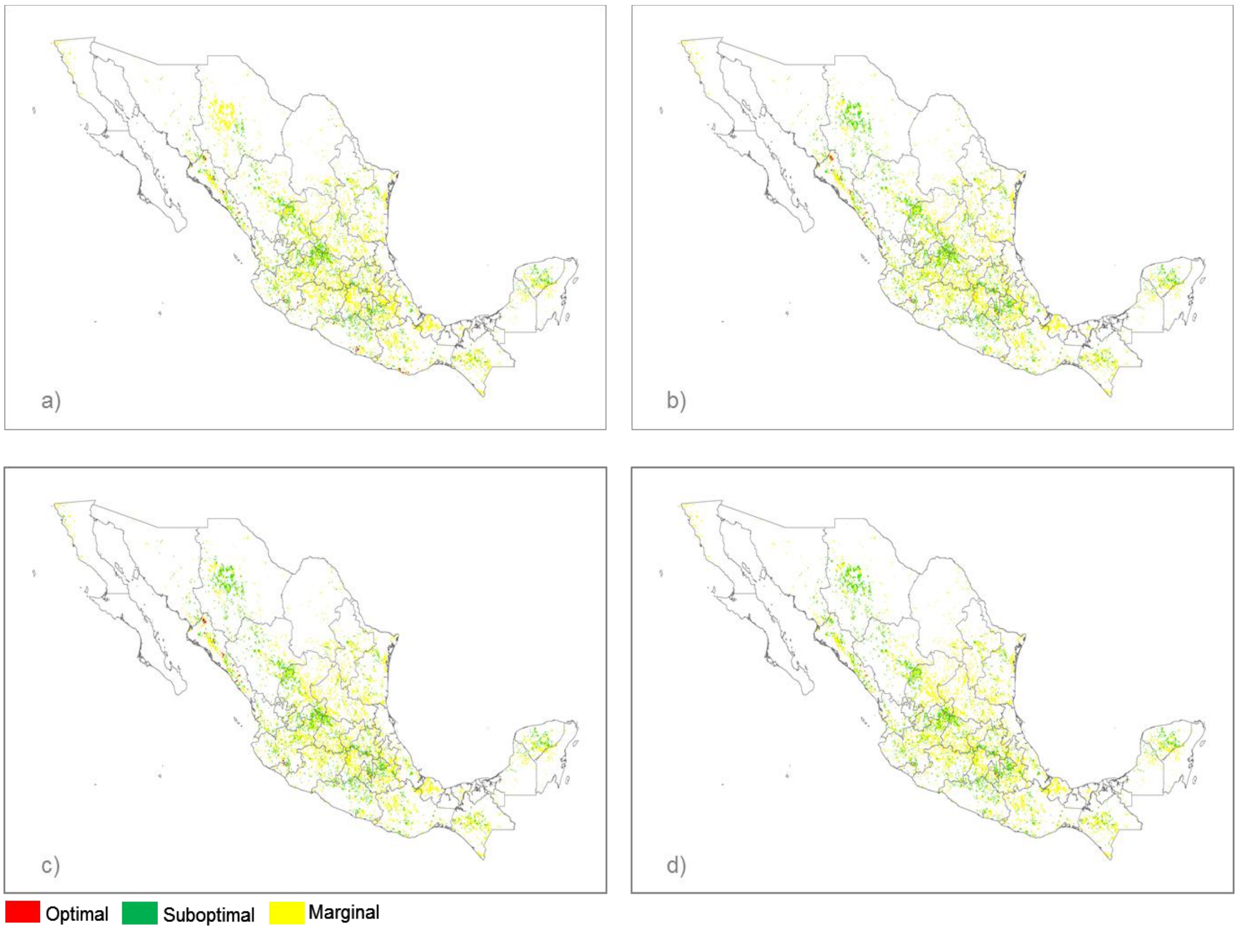

Figure 1. Potential areas for *A. gayanus*, under climatologies: a) 1961-1990, and 2040-2069, with three GCM: b) UKMO-HadCM3, c) MPIM-ECHAM5, d) MIROC3.2 (medres) and SRES A2.

emphasized in desert and semi-desert areas (Johnson and Sharma, 2009), condition that prevails in the Mexican territory. This is an important aspect, considering that rainfall is a relevant variable for hydroclimatological assessments such as crops productivity (Kumar et al., 2004; Sivakumar et al., 2005). Even little changes in rainfall may impact productivity (Lobell and Burke, 2008).

Potential areas for grasses

As expected for tropical grasses, optimal conditions for their cultivation were found mostly in lowlands during the reference period 1961 to 1990 (Figures 1 to 6), showing that climatic conditions of 0-1200 masl areas match better to the agroclimatic requirements from these grasses (FAO, 2000; Durán, 2011). For *C. ciliaris* and *C. gayana,* favorable environmental conditions were detected in the three altitudinal strata, as a result of their more ample environmental ranges (FAO, 2000).

In the maps of these figures, the effect of climate change over the grasses potential areas may also be seen. The sense of predictions of potential areas from GCMs coincided in four specie; they agree in predicting that optimal surface will decrease in lowlands and will increase in midlands and eventually in highlands for *C. ciliaris, A. gayanus* and *B. mutica*.

They also converge in establishing that *H. rufa* will increase its optimal surface in all altitudinal strata, showing that this species tolerates hotter environments and nowadays agricultural lands of México does not offer environments as hot as this species requires (Durán, 2011).

Decreasing potential areas in lowlands for grasses is in correspondence to the statement from Wan et al. (2005) and Bertrand et al. (2008a) who referred to possible negative effects on the prairies because of the high temperatures, especially in hot-dry regions, where plant physiological processes are directly affected by rising temperature.

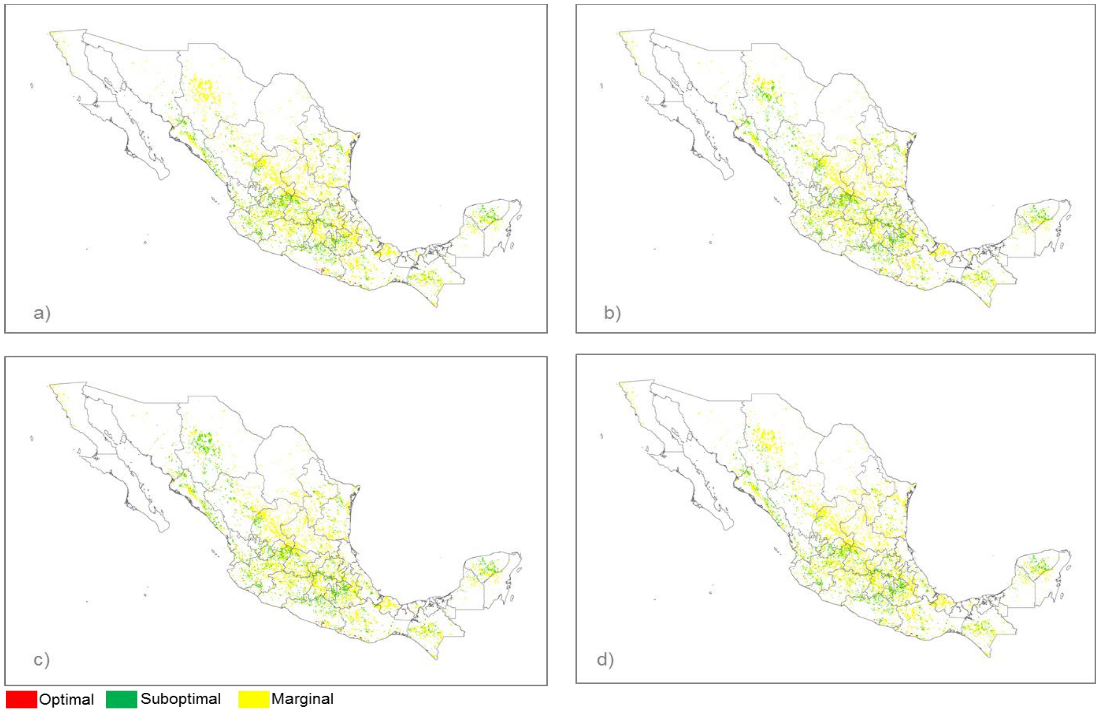

Figure 2. Potential areas for *B. mutica*, under climatologies: a) 1961-1990, and 2040-2069, with three GCM: b) UKMO-HadCM3, c) MPIM-ECHAM5, d) MIROC3.2 (medres) and SRES A2.

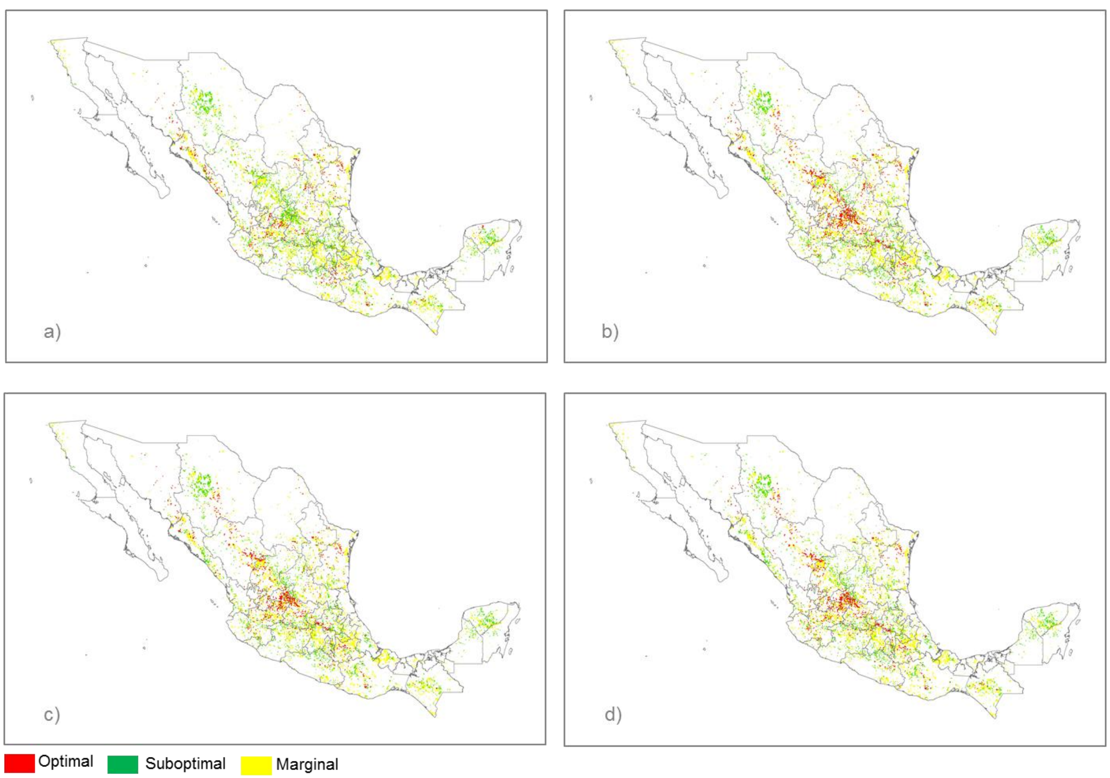

Figure 3. Potential areas for *C. ciliaris*, under climatologies: a) 1961-1990; and 2040-2069 with three GCM, b) (UKMO-HadCM3); c) MPIM-ECHAM5; d) MIROC3.2 (medres) and SRES A2.

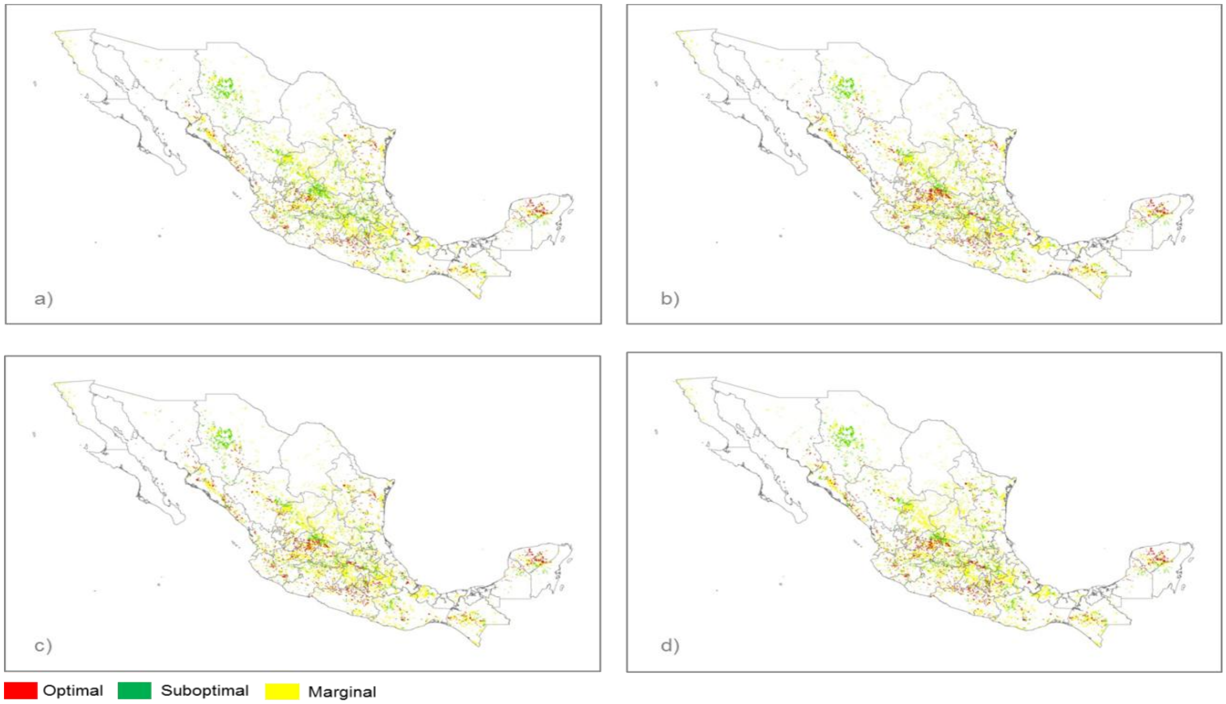

Figure 4. Potential areas for *C. gayana*, under climatologies: a) 1961-1990, and 2040-2069 with three GCM, b) UKMO-HadCM3, c) MPIM-ECHAM5, d) MIROC3.2 (medres) and SRES A2.

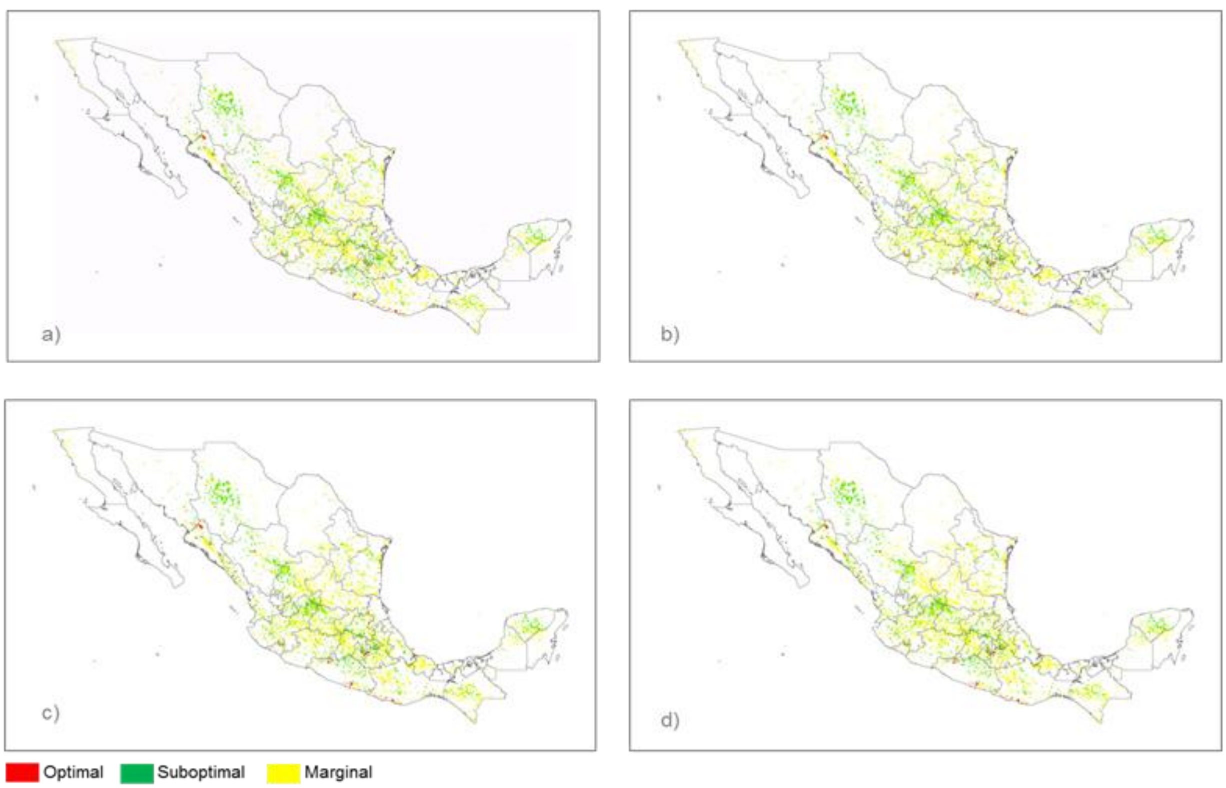

Figure 5. Potential areas for C. dactylon, under climatologies: a) 1961-1990, and 2040-2060 with three GCM, b) UKMO-HadCM3, c) MPIM-ECHAM5, d) MIROC3.2 (medres) and SRES A2.

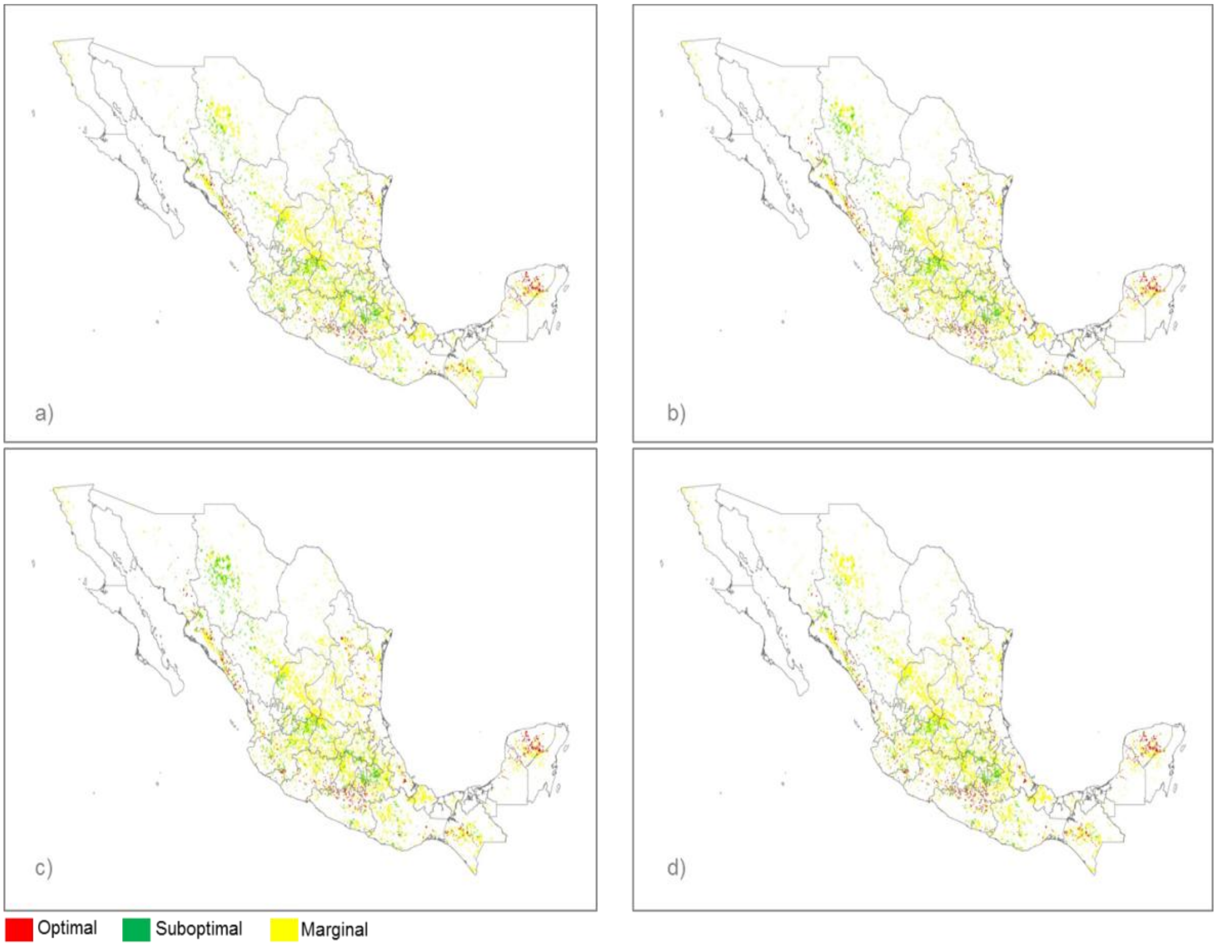

Figure 6. Potential areas for *H. rufa*, under climatologies: a) 1961-1990, and 2040-2069, with three GCM: b) UKMO-HadCM3, c) MPIM-ECHAM5, d) MIROC3.2 (medres) and SRES A2.

For *C. gayana* and *C. dactylon* climate predictions from MPI_ECHAM5 and UKMO_HADCM3 models yielded potential areas quite similar, with optimal surface in lowlands and midlands, superior to that from the reference climatology map (Figures 4 and 5). However, potential areas elaborated with the MIROC3.2 (medres) climatology (2040-2069) resulted sensitively different for lowlands, with an optimal surface 7% less than that from the reference climatology map. These opposite results are mainly due to the differences in the precipitation simulations from the GCMs, since MIROC3.2 (medres) estimated lower annual rainfall volumes in the three altitudinal strata (Table 4). This fact is evidencing that crops potential areas depiction is sensitive to the GCMs climate simulation variations even among GCMs that are considered similar in predicting climate change for México (Conde et al., 2006).

Differences among GCMs also were observed at determining suboptimal surfaces for grasses. However, based on three cases of complete coincidence (3 GCMs) and four coincidences between UKMO_HADCM3 and MIROC3.2 (medres) models, and four coincidences between UKMO_HADCM3 and MPI_ECHAM5 models, it may be stated that there is a tendency for suboptimal surface to increase in lowlands and to decrease in midlands and highlands, which is related to the tendencies observed for optimal surfaces in the three altitudinal strata (Table 5).

Some interesting cases derived from dynamic potential areas, promoted by climate change, involve the appearance of optimal areas in midlands and highlands of the northern and center regions of the country. This is the case for *C. ciliaris, C. gayana* and *H. rufa* (Figures 3, 4 and 6). These regions had already been reported with changes in crops patterns due to climate change (Ramírez et al., 2011; Santillán et al., 2011).

According to the results, it may be concluded that climate change will cause that tropical grasses potential areas move towards midlands and highlands in the future. Thus, a redistribution of crops lands probably will

Table 5. Potential areas for six grasses in three altitudinal strata and two climatic scenarios in México.

Grass	Altitude zone	Scenario (1961-1990)		Scenario 2040-2069					
				MPIM-ECHAM5		MIROC3.2 (medres)		UKMO_HadCM3	
		Optimal and suboptimal potential areas (ha)							
		Optimal	Suboptimal	Optimal	Suboptimal	Optimal	Suboptimal	Optimal	Suboptimal
Andropogon gayanus	Lowlands	164,122	1,894,659	147,280	1,935,292	59,965	1,964,792	161,064	1,909,548
	Midlands	4,061	2,643,665	22,311	2,970,164	22,295	2,800,376	22,311	3,207,595
	Highlands	0	597,224	0	1,153,472	0	1,142,503	0	1,182,214
Brachiaria mutica	Lowlands	69,631	1,943,637	57,334	1,974,806	49,105	1,861,253	59,004	1,961,888
	Midlands	0	1,726,758	0	2,296,665	0	1,619,046	2,010	2,218,312
	Highlands	0	409,153	0	907,769	0	816,231	0	996,192
Cenchrus ciliaris	Lowlands	786,747	1,915,465	463,710	2,238,502	403,483	2,298,729	510,597	2,191,615
	Midlands	759,409	3,558,706	1,755,678	2,562,438	1,721,815	2,596,300	1,949,280	2,368,835
	Highlands	6,005	1,248,036	378,823	875,218	410,878	843,163	414,789	839,252
Chloris gayana	Lowlands	1,500,584	1,062,650	1,515,397	1,078,930	1,425,710	1,118,464	1,504,117	1,082,884
	Midlands	756,726	3,023,112	1,459,904	2,154,841	1,237,426	2,214,712	1,534,320	2,325,593
	Highlands	12,149	1,202,442	238,395	932,544	264,651	861,163	294,308	922,195
Cynodon dactylon	Lowlands	301,827	1,765,121	307,464	1,783,276	280,727	1,746,071	311,476	1,761,177
	Midlands	48,632	3,133,221	92,691	2,924,213	84,795	2,741,770	88,809	3,176,774
	Highlands	0	1,313,922	16,225	1,252,024	38,490	1,172,591	22,297	1,295,578
Hyparrhenia rufa	Lowlands	1,165,892	756,257	1,346,185	584,590	1,229,833	597,349	1,360,760	544,855
	Midlands	48,672	1,860,988	239,178	2,059,540	267,279	1,339,382	239,097	1,992,730
	Highlands	0	1,034,744	0	1,018,467	0	878,712	0	1,124,049

take place in these altitudinal zones, during the present century.Since in the present, the midlands include most of the agricultural surface, this redistribution may not to have a high impact; however when atmospheric warming and climate change push the crops to migrate to highlands, it will impose a complicated situation since nowadays only 18% of agricultural surface is located within highlands.

CONCLUSIONS

The temperature projections of the three GCM used, consistently indicate temperature increase in the range of 2.6 to 2.9, 2.7 to 3.3 and 2.4 to 3.1°C in low, mid and high lands, respectively, going from the 1961 to 1990 period to the 2040 to 2069 period. Precipitation projections were not that consistent among the models, since some indicate decreased rainfall and others an increment, but in all cases these changes are located in the range from 0.7 to 13%. Two of the three models reported a decrement in annual precipitation for the period 2040 to 2069 in lowlands, an increment of 2 to 4% in midlands, and a drop precipitation of 2 to 12% in highlands. The MIROC3.2 (medres) model simulated consistently lower annual precipitation amounts in the three altitudinal strata.

The projected climate changes will affect the amount and altitudinal distribution of the surface with optimal and suboptimal agroclimatic conditions for the growth of the six grass species studied. The potential cultivation surface with optimal agroclimatic conditions currently focuses more on lowlands (0 to 1200 m) for all grasses, while the potential surface with suboptimal conditions basically is grouped in intermediate altitude lands (1200 to 2200 m). With expected climatic changes, most of the optimal surface will remain in lowlands, but will tend to decrease in these areas for *C. ciliaris, A. gayanus, and B. mutica* at a rate of 35 to 49 %, 2 to 63% and 15 to 30%, respectively. These impacts will be reflected most on the optimal surface for *C. ciliaris,* which will tend to migrate mainly to midlands. The surface with optimal conditions for *C. gayana* in lowlands will tend to stay, but in midlands it will increase between 63 and 103%. A similar case is that of *C. dactylon* which its optimum surface condition will tend to stay in the same amounts in lowlands, but will increase between 74 and 90% in midlands. Finally for *H. rufa*, the surface with optimum conditions will increase from 5 to 17% in lowlands and from 391 to 449% in midlands.

In highlands, potential areas for grasses were detected basically as suboptimal. However, with climate change *C.ciliaris, C. gayana* and *C. dactylon* will increase their optimal surface dramatically. A different situation was detected for *A. gayanus, B. mutica* and *H. rufa* which resulted with non-optimal surface neither in the reference climatology nor in the climate change climatologies.

Conflict of Interests

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

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REFERENCES

Attipalli RR, Girish KR, Agepati SR (2010). The impact of global elevated CO2 concentration on photosynthesis and plant productivity. Curr. Sci. 99(1):46-57.

Bertrand A, Prevost D, Bigras FJ, Lalande R, Tremblay GF, Castonguay Y, Belanger G (2008a). Alfalfa response to elevated CO_2 varies with the symbiotic rhizobial strain. Plant Soil 301:173–187. http://dx.doi.org/10.1007/s11104-007-9436-9

Conde C, Ferrer R, Orozco S (2006). Climate change and climate variability impacts on rainfed agricultural activities and possible adaptation measures. A Mexican case study. Atmósfera 19(3):181-194.

Deryng D, Sacks W, Barford C, Ramankutty N (2011). Simulating the effects of climate and agricultural management practices on global crop yield. Glob. Biogeochem. Cycles GB2006 25:18.

Díaz PG, Guajardo PRA, Medina GG, Sánchez CI, Soria RJ, Vázquez AMP, Quijano CJA, Legorreta PF, Ariel RCJ (2012). Potencial productivo de especies agrícolas de importancia socioeconómica en México. 1a. Ed. INIFAP. Xalapa, Ver., México P. 140.

Durán PN (2011). Cambio climático y potencial productivo de especies forrajeras en la región Centro-Occidente de México. Tesis doctoral. Universidad de Guadalajara-CUCBA. Zapopan, Jalisco, México P. 180.

Eastman JR (2012). Idrisi Selva Manual, Manual Version 17. Clark Labs, Clark University. Worcester, Mass., USA. P. 322.

FAO. Ecocrop (2000). Version Online. URL: http://www.ecocrop.fao.org.

Friend AD, Geider RJ, Behrenfeld MJ, Still CJ (2009). Photosynthesis in global-scale models. In Photosynthesis in silico (eds Laiska, A., Nedbal, L. and Govindjee), Springer, The Netherlands pp. 465–497. http://dx.doi.org/10.1007/978-1-4020-9237-4_20

García E (1988). Modificaciones al sistema de clasificación climática de Köppen (para adaptarlo a las condiciones de la República Mexicana). 4a. Ed. Offset Larios. México, D.F. P. 219.

Ghannoum O, von Caemmerer S, Ziska LH, Conroy JP (2000). The growth response of C4 plants to rising atmospheric CO_2 partial pressure: A reassessment. Plant Cell Environ. 23:931–942. http://dx.doi.org/10.1046/j.1365-3040.2000.00609.x

Gornall J, Betts R, Burke E, Clark R, Camp J, Willet K, Wiltshire A (2010). Implications of climate change for agricultural productivity in the early twenty-first century. Phil. Trans. R. Soc. B. 365:2973-2989. http://dx.doi.org/10.1098/rstb.2010.0158

Hatfield JL, Boote KJ, Fay P, Hahn P, Izaurralde L, Kimball RC, Mader BA, Morgan T, Ort J, Polley D, Thomson WA, Wolfe D (2008). Agriculture. In The effects of climate change on agriculture, land resources, water resources, and biodiversity in the United States. U.S. Climate Change Science Program and the Subcommittee on Global Change Res., Washington, DC.

Hatfield JL, Boote KJ, Kimball BA, Ziska LH, Izaurralde RC, Ort D, Thomson AM, Wolfe D (2011). Climate impacts on agricultura: Implications for crop production. Agron. J. 103:351-370. http://dx.doi.org/10.2134/agronj2010.0303

Hegerl GC, Zwiers FW, Braconnot P, Gillet NP, Luo Y, Marengo JA, Nicholls N, Penner JE, Stott PA (2007). Understanding and Attributing Climate Change. Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate 83 Change., S. e. al., Ed., Cambridge University Press.

Hsiang SM, Kile CM, Cane AM (2011). Civil conflicts are associated with the global Climate. Nature. 476:438–441. http://dx.doi.org/10.1038/nature10311

IBM Corp. (2010). IBM SPSS Statistics for Windows, version 19.0. Armonk, NY: IBM Corp.

Instituto Nacional de Estadística Geografía e Informática (INEGI). 2009. Guía para interpretación cartográfica: Uso de suelo-vegetación

Serie III. D. F., México. P. 71.
Intergovernmental Panel on Climate Change (IPCC). 2007. Climate change 2007: mitigation of climate change. Contribution of working group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Metz B, Davidson O, Bosch P, Dave R, Meyer L (eds.). Cambridge University Press, UK. and USA, P. 851.
Izaurralde RC, Thomson AM, Morgan JA, Fay PA, Polley HW, Hatfield JL (2011). Climate impacts on agriculture: implications for forage and rangeland production. Agron. J. 103:371-381. http://dx.doi.org/10.2134/agronj2010.0304
Johnson F, Sharma A (2009). Measurement of GCM skill in predicting variables relevant for hydroclimatological assessments. J. Climate, 22:4373–4382. http://dx.doi.org/10.1175/2009JCLI2681.1
Justin EB, Desai RA, Dirmeyer PA, Foley AJ (2012). Effects of land cover change on moisture availability and potential crop yield in the world's breadbaskets. Environ. Res. Lett. 7:1-9.
Kimball BA, Kobayashi K, Bindi M (2002). Responses of agricultural crops to free-air CO_2 enrichment. Adv. Agron. 77:293-368. http://dx.doi.org/10.1016/S0065-2113(02)77017-X
Kimball BA (2007). Global change and water resources. In: Lascano RJ, Sojka RE (ed.). Irrigation of agricultural crops. 2nd edition. ASA, CSSA and SSSA. Madison, WI. Agron. Monogr. 30:627-654. http://dx.doi.org/10.1111/j.13652486.2007.01430.xhttp://dx.doi.org/10.1111/j.1365-2486.2007.01486.x
Kruskal WH, Wallis AW (1952). Use of ranks in one-criterion variance analysis. Journal of the American Statistical Association 47(260):583-621. http://dx.doi.org/10.1080/01621459.1952.10483441
Kumar KK, Kumar KR, Ashrit RG, Deshpande NR, Hansen JW (2004). Climate impacts on Indian agriculture. Int. J. Climatol. 24:1375-1393.http://dx.doi.org/10.1002/joc.1081
Leakey ADB, Ainsworth EA, Bernacchi CJ, Rogers A, Long SP, Ort DR (2009). Elevated CO_2 effects on plant carbon, nitrogen and water relations: six important lessons from FACE. J. Exp. Bot. 60:2859–2876.http://dx.doi.org/10.1093/jxb/erp096 PMid:19401412
Lobell DB, Burke MB (2008). Why are agricultural impacts of climate change uncertain? The importance of temperature relative to precipitation. Environ. Res. Lett. 3:1-8. http://dx.doi.org/10.1088/1748-9326/3/3/034007
Medina GG, Ruiz CJA, Martínez PAR (1998). Los climas de México: una estratificación ambiental basada en el componente climático. Libro téc 1. SAGAR-INIFAP, P. 104.
Ojeda BW, Sifuentes IE, Í-iguez CM, Montero MMJ (2011). Impacto del cambio climático en el desarrollo y requerimientos hídricos de los cultivos. Agrociencia. 45:1-11.
Ramírez LMR, Ruiz CJA, Medina GG, Jacobo CJL, Parra QRA, Ávila MMR y Jesús Pilar Amado ÁJP (2011). Perspectivas del sistema de producción de manzano en Chihuahua, ante el cambio climático. Rev. Mex. de Cs Agríc. Pub. Esp. (2):265-279.
Ruiz CJA, Medina GG, Ramírez DJL, Flores LHE, Ramírez OG, Manríquez OJD, Zarazúa VP, González EDR, Díaz PG, De la Mora OC (2011). Cambio climático y sus implicaciones en cinco zonas productoras de maíz en México. Rev. Mex. de Cs Agríc. Pub. Esp. (2):309-323.
Santillán ELE, Blanco MF, Magallanes QR, García HJL, Cerano PJ, Delgadillo RO, Valdez CRD (2011). Tendencias de temperatura extremas en Zacatecas, México. Rev. Mex. De Cs Agríc. Pub. Esp. (2):207:219.
Sivakumar MVK, Das HP, Brunini O (2005). Impacts of present and future climate variability and change on agriculture and forestry in the arid and semi-arid tropics. In: increasing climate variability and change. Salinger JM, Sivakumar VK, Motha RP (eds.). Springer, USA, pp. 31-72.
Trenberth KE, Jones PD, Ambenje P, Bojariu R, Easterling DR, Kleint-Tank A, Parker D, Rahimzadeh F, Renwick JA, Rusticucci M, Soden B, Zhai P (2007). Observations: Surface and Atmospheric Climate Change. Climate Change 2007: The Physical Science Basis. Contribution of WG I to the Fourth Assessment Report of the IPCC., S. e. al., Ed., Cambridge University Press.
Tubiello FN, Soussana JF, Howden SM (2007). Crop and pasture response to climate change. Proc. Natl. Acad. Sci. USA. 104:19686-19690. http://dx.doi.org/10.1073/pnas.0701728104, PMid:18077401 PMCid:PMC2148358
Verena S, Navin R, Jonathan AF (2012). Comparing the yields of organic and conventional agriculture. Nature. 00(000): pp. 1-6.
Wan S, Hui D, Wallace L, Luo Y (2005). Direct and indirect effects of experimental warming on ecosystem carbon processes in a tallgrass prairie. Global Biogeochem. Cycles 19:2014 10.1029/2004GB002315. http://dx.doi.org/10.1029/2004GB002315
Wang G (2005). Agricultural drought in a future climate: results from 15 global change models participating in the IPCC 4th assessment. Clim. Dyn. 25:739–753. http://dx.doi.org/10.1007/s00382-005-0057-9
Woodhouse CA, Meko DM, MacDonald GM, Stahle DW, Cook ERA (2010). 1,200-year perspective of 21st century drought in southwestern NorthAmerica. Proc.Natl Acad. Sci. USA 107:21283–21288. http://dx.doi.org/10.1073/pnas.0911197107

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Full Length Research Paper

Effect of ultraviolet (UV) protectant added emamectin benzoate on codling moth (*Cydia pomonella* L.).

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The efficacy of emamectin benzoate with ultraviolet (UV) protectant at different doses was compared with the emamectin benzoate for the control of codling moth on the apple trees at Eğirdir/Isparta and Çivril/Denizli during 2012. The types of treatments were control (water), the highest concentration of emamectin benzoate (0.20 g L^{-1}) and different doses (0.12, 0.15, 0.17 and 0.20 g L^{-1}) of emamectin benzoate with UV protectant. Emamectin benzoate with UV protectant preparation showed 56.71, 79.69, 84.12 and 95.07% effects at 0.12, 0.15, 0.17 and 0.20 gL^{-1}, respectively in Eğirdir/Isparta as compared to 40.50% emamectin benzoate application showed effect at 0.20 gL^{-1}. In control plots, 15.17% damaged fruits were determined. Fruit damage in control plots was statistically significant from the treatment plots. In Çivril/Denizli, effects of emamectin benzoate with UV protectant preparation at 0.12, 0.15, 0.17 and 0.20 gL^{-1} concentration were observed as 69.45, 92.78, 95.34 and 96.44%, respectively. Effect of emamectin benzoate at 0.20 gL^{-1}dose was determined as 65.97%. In control plots, 37.81% damaged fruits were determined and fruit damage in control plots was statistically significant from the treatment plots. The results of this study showed emamectin benzoate with UV protectant preparation was more effective at lower dosage than the highest concentration of emamectin benzoate preparation.

Key words: Ultraviolet (UV) protectant, emamectin benzoate, codling moth, dosage.

INTRODUCTION

The cultivated apple, *Malus domestica* Borkh., belongs to the Rosaceae (Rose) Family, is a small deciduous tree that originated in Anatolia, Caucasia, Turkistan, and Europe and now grown in very different ecological conditions (Özongun et al., 2004). Turkey is the third-leading producer after China and the United States (Anonymous, 2013) and more than 500 cultivars of apple are known in Turkey (Özbek, 1978). A large portion of apples is produced in Isparta, Karaman, Niğde, Denizli and Antalya providence that shared about 60% of the total production (TUIK, 2012).

The codling moth, *Cydia pomonella* L., is a member of the Lepidoptera family Tortricidae and key pest of pome fruit in almost worldwide. It is a major pest in the apple growing areas of Turkey and causes over 40 to 60% crop losses under insufficient control methods (TAGEM, 2011). It also attacks pears, walnuts, and other tree fruits. The larvae are fruit feeders and cause an injury known as a "sting" or a deep entry into the fruit. Growers do generally not tolerate more than one damaged fruit in 100 (<1%). In our climate conditions, codling moth occurs in 2 generations a year, but in some years and in some

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places 3rd generation could be observed (TAGEM, 2011). There are several methods that can be used to control codling moth. Since 1984, forecasting and warning system based insecticide treatment has been applied 2 to 4 times yearly (Hepdurgun vd., 2001). However, undesired side effects of the pesticides on environment and human have forced the researchers to seek the alternative control methods. The natural enemies can reduce codling moth population however it's not very effective because of short life cycle spending outside of the fruit. The use of granulosis virus and pheromone-based mating disruption techniques also are effective but only applied with chemical treatments (Weddle et al., 2009). The most vulnerable time for the codling moth is between when it hatches, and when it enters the fruit (Geier, 1963). Once it's in the fruit, it is safe from the outside world. In this very short time period, historically, organophosphates and pyrethroids has been played important role to control codling moth (Miletic et al., 2011). The control efficacy of insecticides is threatened by the development of resistance which has recently been documented from different classes of insecticides in codling moth populations (Charmillot et al., 2005). According to Charmillot et al. (2005), emamectin benzoate is the only insecticide which has not been observed development of resistance.

Emamectin benzoate is a new insecticide of Syngenta Crop Protection derived from naturally occurring avermectin family isolated from the soil microorganism *Streptomyces avermitilis* Kim and Goodfellow (Ioriatti et al., 2009). Emamectin benzoate has improved thermal stability and water solubility which resulted better and broader insecticidal activity than avermectin (Jansson and Dybas, 1996; Fanigliulo and Sacchetti, 2008). It penetrates the leaf tissues (translaminar activity) and accumulates within the leaf so it has long residual activity in ingestion system of Lepidoptera (Ioriatti et al., 2009). Besides having strong effects against Lepidoptera, it is safe on useful organisms and may be considered a valuable tool for the control of codling moth as a component of integrated pest management.

The active ingredient (MAB1a) of emamectin benzoate is susceptible to ultraviolet (UV) light playing a major role in photodegradation (Crouch and Feely, 2005; Mushtaq et al., 1998). The reduction of toxicity is correlated with the concentration and the UV light exposure time which may be the reason of reduction in control efficacy of emamectin benzoate in the field (Zhu et al., 2011). Formulation and field application of high concentration of emamectin benzoate are not recommended because of resistance development and economic cost. Instead, there are several reports suggesting mixing the chemicals with UV protectants might be a practical and economic way to improve persistence and efficiency of pesticides against target pests under field conditions (Tan and Song, 2006; Zhu et al., 2011). Other solutions, such as microcapsule formulation (Wei et al., 2010) and other adjuvants (Tu and Randall, 2001; Topuz et al., 2011) might be useful.

The aim of the present study was to evaluate and compare the efficacy of different rates of emamectin benzoate with or without UV protectant on codling moth, *Cydia pomonella* (L.) under field conditions.

MATERIALS AND METHODS

Study areas

The study was conducted between April to October, 2012 in 3 ha apple orchard with scarlet spur variety grafted on 7 years old MM106 named apple in Eğirdir/Isparta and in 3 ha apple orchard with starkrimson variety in Çivril/Denizli in Mediterranean region of Turkey.

Chemicals

Emamectin benzoate containing PROCLAIM 05 SG® (water soluble, granule (SG) 5% emamectin benzoate) and Emamectin benzoate with UV protectant was provided by syngenta crop protection (Turkey). UV protectant formulation is not commercially available yet, for this reason, information about UV protectant is kept confidential.

Emamectin benzoate with UV protectant was tested in 0.12, 0.15, 0.17 and 0.20 g L^{-1} concentrate formulation, recommended by syngenta crop protection. Based on this, the maximum concentration (0.20 g L^{-1}) was chosen for emamectin benzoate application to compare treatments. The control plots were treated with water.

Field efficacy tests

Date and number of pesticide applications were implemented based on information for forecasting and early warning system received from Republic of Turkey, Ministry of Food, Agriculture and Livestock in Isparta and Denizli Province. Pesticides were applied three times on May 29, June 16 and July 07 in Eğirdir/Isparta and four times on May 08, May 30, July 14 and August 01 in Çivril/Denizli. Due to weather conditions, forecasting and early warning system alerted for codling moth infestation to apply one extra pesticide in Çivril/Denizli.

Treatments were performed by the hanging type of atomizer tractor with 1500 L storage capacities, check the arm connecting PTO (power take-off). During the applications, 8 to 12 L of pesticide were applied for each tree. Trials were conducted according to the experimental design of randomized block design in four replications, that is, three apple tree rows with one block each. Counts were made in the middle trees. During pesticide applications, nylon curtains were used between the blocks to protect dose interfere with each other. Fallen apples at the bottom of the each sampling trees were counted and recorded prior to each application of spray until the harvest. In harvest time, all fruits were counted as damaged or undamaged fruit.

Results of the field efficacy trials were subjected to an analysis of variance (ANOVA), and means were separated by Tukey's test (P = 0.05) (Statistica© 7.1 for Windows©; Statsoft Inc., Tulsa, OK). Data were arcsine transformed before analysis, and Levene's test was used to verify homogeneity of variances.

Table 1. Percentage rates (%) of damaged and undamaged fruits with codling moth larvae recorded from Eğirdir trials in 2012 (mean±SE).

Treatments	Doses ($g\ L^{-1}$)	No. of damaged fruit	No. of undamaged fruit	Total no. of fruit	Average fruit damage (%)[a]	Average effects of plots [a, b]
UV Protectant Emamectin benzoate	0.12 $g\ L^{-1}$	27.25±5.03	391.75±27.67	419.00±32.37	6.37±0.72[c]	56.71[c]
UV Protectant Emamectin benzoate	0.15$g\ L^{-1}$	7.25±1.03	269.75±66.15	277.00±67.05	2.90±0.43[b]	79.6[b]
UV Protectant Emamectin benzoate	0.17 $g\ L^{-1}$	8.00±1.29	350.25±68.26	358.25±68.61	2.43±0.50[b]	84.12[b]
UV Protectant Emamectin benzoate	0.20 $g\ L^{-1}$	2.00±0.40	273.75±45.44	275.75±45.57	0.75±0.15[a]	95.07[a]
Emamectin benzoate	0.20 $g\ L^{-1}$	26.25±5.80	290.00±65.01	316.25±70.47	8.66±0.88[c]	40.50[d]
Control (H_2O)	-	45.75±6.07	252.00±13.32	297.75±18.76	15.17±1.28[d]	-

[a] Data marked with same letters in columns indicate not have significant ($p \leq 0.05$) differences in numbers (TUKEY test). [b] Mortality values were corrected according to Abbott's formula.

Statistical analysis

Results of the field efficacy trials were subjected to an analysis of variance (ANOVA), and means were separated by Tukey's HSD (Honestly Significant Difference) ($p \leq 0.05$) (SPSS Version 17.0). Data were arcsine transformed before analysis and percentages of damaged fruits depending on the pesticide doses were calculated according to Abbott's formula in control and treatment blocks;

$$P.E. = \frac{(D.F.C.P. - D.F.T.P.)}{D.F.C.P.} \times 100$$

P.E. : Percentage effects
D.F.C.P. : Damaged fruits at control plots
D.F.T.P. : Damaged fruits at treated plots

RESULTS

Trials conducted in apple orchards, 7776 pieces of apple fruit in Eğirdir and 25950 pieces of apple fruit in Çivril were counted before and during the harvest and damaged or undamaged fruits were recorded separately. The percentage of damaged and undamaged fruit rates from Eğirdir trials are shown in Table 1. Total of 7776 pieces of fruits were counted and 466 damaged and 7310 undamaged fruits were recorded in Eğirdir. The emamectin benzoate with UV protectant preparation showed 6.37%, 2.90%, 2.43% and 0.75% infestation with codling moth larvae at 0.12, 0.15, 0.17 and 0.20 $g\ L^{-1}$, respectively. Emamectin benzoate (0.20 $g\ L^{-1}$) showed 8.66% damaged fruits. In control plots, 15.17% damaged fruits were determined (Table 1).

Emamectin benzoate with UV protectant at 0.20 $g\ L^{-1}$ was found to be most effective and statistically significant from the other treatments. The percentage of fruit damage recorded at 0.17 and 0. 15 $g\ L^{-1}$ concentration was moderate and they were formed statistically in same group. The lowest concentration (12.5 $g\ L^{-1}$) of emamectin benzoate with UV protectant did not differ significantly from the highest concentration of emamectin benzoate at 0.20 $g\ L^{-1}$ and they were found to be the least effective.

Results of the field efficacy evaluation from Çivril trials are reported in Table 2. Total of 25950 pieces of fruit were collected and 3213 of them were counted as damaged. Fruit damages were recorded as 11.38, 2.72, 1.76 and 1.31% at 0.12, 0.15, 0.17 and 0.20 $g\ L^{-1}$ dilution rates of the emamectin benzoate with UV protectant, respectively. The emamectin benzoate at 0.20 $g\ L^{-1}$ and control plots showed 12.66% and 37. 81% damaged fruits, respectively (Table 2). The percentage of fruit damage recorded in the plots treated with the lowest concentration (0.12 $g\ L^{-1}$) of emamectin benzoate with UV protectant did not differ significantly from the highest concentration of emamectin benzoate (0.20 $g\ L^{-1}$). They were found to be less effective. Other doses were very effective and statically placed in same group. They were significantly different from that in the control plots treated with water.

Both in Eğirdir and Çivril, percentages of damaged fruits calculated according to Abbott's formula at different treatment doses are shown in Table 1 and 2. UV protectant added emamectin benzoate at 0.20 $g\ L^{-1}$ showed the highest impact (95.07% in Eğirdir and 96.44% in Çivril) on codling moth infestation. This result

Table 2. Percentage rates (%) of damaged and undamaged fruits with codling moth larvae from Çivril trials in 2012 (mean±SE).

Treatments	Doses (gL^{-1})	No. of damaged fruit	No. of undamaged fruit	Total no. of fruit	Average fruit damage(%)[a]	Average effects of plots [a, b]
UV Protectant Emamectin benzoate	0.12 g L^{-1}	132.50±18.31	1032.75±128.46	1165.25±145.12	11.38±0.73[b]	69.45[b]
UV Protectant Emamectin benzoate	0.15 g L^{-1}	16.75±5.51	706.50±210.29	732.25±214.58	2.73±0.60[c]	92.78[a]
UV Protectant Emamectin benzoate	0.17 g L^{-1}	18.75±5.13	1064.25±212.92	1083.00±217.09	1.76±0.33[c]	95.34[a]
UV Protectant Emamectin benzoate	0.20 g L^{-1}	16.00±4.20	1155.75±219.15	1171.75±223.07	1.31±0.14[c]	96.44[a]
Emamectin benzoate	0.20 g L^{-1}	128.00±21.72	887.50±144.26	1015.50±164.17	12.66±0.71[b]	65.97[b]
Control (H_2O)	-	491.25±57.98	837.50±157.81	1328.75±210.51	37.81±2.59[a]	-

[a] Data marked with same letters in columns indicate not have significant ($p \leq 0.05$) differences in numbers (TUKEY test).[b] Mortality values were corrected according to Abbott's formula.

was followed by other dilutions of UV protectant added emamectin benzoate at 0.17, 0.15, and 0.12 g L^{-1}, respectively. The highest concentration of emamectin benzoate at 0.20 g L^{-1} showed the least impact with 40.50% in Eğirdir and 65.97% in Çivril.

DISCUSSION

Well-timed insecticide applications are often the most effective method for managing codling moth. Several lower-risk insecticides, such as insect growth regulators and other new products, have recently been labeled for use against codling moth. In Serbia, Chlorantraniliprole and the combination of chlorpyrifos and cypermetrin application exhibited high performance on codling moth larvae. Insect growth regulator novaluron and pyriproxifen showed high efficacy on first generation but less efficiency on second generation. The lowest efficiency was recorded with Azinphos-methyl on both generations (Miletic et al., 2011). The use of azinphos-methyl and phosmet has been restricted on apples and there have been several reports of codling moth resistance. However, azinphos-methyl and phosmet are the most commonly used insecticides for codling moth. Other broad-spectrum insecticides, labeled for codling moth, such as synthetic pyrethroids and carbamates, are dangerous to beneficial insects and/or are labeled for limited use in the orchards (Anonymous, 2012).

Emamectin benzoate is highly effective against lepidopteran pests and widely used in the world. Two years of testing in Tursi-Policoro (Matera), southern Italy, Emamectin benzoate has shown a high control of *Helicoverpa armigera* (Lepidoptera: Noctuidae) when compared with the standards Indoxacarb and Spinosad (Fanigliulo and Sacchetti, 2008). Dose response bioassays showed that emamectin benzoate had a high level of intrinsic toxicity to early stage larvae of *C. pomonella*. Field trials confirmed the efficacy of emamectin benzoate on codling moth when applied at 7 day intervals (Ioriatti et al., 2009).

The active ingredient (MAB1a) of emamectin benzoate was substantially degraded by UV light. MAB1a benzoate was found to be stable for 6 weeks at 25°C in buffer solutions (pH 5 to 8) in dark. The natural organic matters such as humic acids present in natural water may serve as photosensitizers and increase the photodegradation rate of emamectin benzoate (Mushtaq et al., 1998). The degree of degradation was correlated with chemical concentration. However, the highest concentration (1000 mg a.i. per liter) of the emamectin benzoate solution exposed to the long period of UV exposure (up to 120 h) showed in more than half of the chemical being photodegraded (Zhu et al., 2011).

The photodegradation of emamectin benzoate could reduce the control efficacy against insect pests. UV light exposure might be the major reason for the reduced control efficacy of emamectin benzoate to *Chilo suppressalis* (Lepidoptera: Crambidae) in the field (Zhu et al., 2011). Adding UV protectants, especially kojic acid (Zhu et al., 2011), microcapsule formulation (Wei et al., 2010) and other adjuvants (Guan et al., 2005) could be useful for prolonging the residual activity of emamectin benzoate against target pests under field conditions.

Based on the results obtained in this study, emamectin benzoate with UV protectant formulation was more effective in the field because of being more persistent on the surface of the plant. The lowest concentration doses of emamectin benzoate with UV protectant (0.12 g L^{-1}) have same effects with standard formulation (0.20 g L^{-1}) of emamectin benzoate without UV protectant. Addition of UV protectant to standard formulation (0.20 g L^{-1}) of emamectin benzoate showed the highest mortality effects on codling moth in the both fields.

It can be concluded that, emamectin benzoate may be considered a valuable tool for the control of codling moth within an IPM program. But it is extremely photolabile in the presence of UV light in the field. Addition of UV protectant, as seen in this study, could enhance the performance of emamectin benzoate. Using lower concentration of insecticide with this kind of technology could increase the efficiency of pesticides in agriculture and also reduce the undesired side effects of the insecticides on the environment.

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Conflict of Interests

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

REFERENCES

Anonymous (2012) USDA Bulletin, 2012. Codling Moth Management in Apple. http://www.rma.usda.gov/handbooks/ (Date accessed: July 2012).

Anonymous (2013). "Production of Apple by countries". UN Food & Agriculture Organization. http://www.fao.org/publications/sofa/2013/en/ (Date accessed: December 2013).

Charmillot PJ, Pasquier D, Briand F (2005). Resistance of the codlingmoth Cydia pomonella to insecticides. Tests by topical application on diapausing larvae collected in autumn 2003. Rev. Suisse. Vitic. Arbor. Hort. 37:123–127.

Crouch LS, Feely WF (1995). Fate of [14C] emamectin benzoate in head lettuce. J. Agric. Food. Chem. 43:3075–3087. http://dx.doi.org/10.1021/jf00060a016

Fanigliulo A, Sacchetti M (2008). Emamectin benzoate: new insecticide against Helicoverpa armigera. Commun. Agric. Appl. Biol. Sci. 73(3):651-653. PMid:19226807

Geier PW (1963). The life history of the codling moth, *Cydia pomonella* (L.), (Lepidoptera: Tortricidae) in the Australian Capital Territory. Aust. J. Zool. 11:323–367. http://dx.doi.org/10.1071/ZO9630323

Guan S, Zhon GH, Huang WX, Liu JX, Li XG, Hu MY (2005). Advances on research and application of ultraviolet radiation protectant of pesticide. J. Yangtze. Univ. (Nat Sci. Edit) 25:10-14.

Hepdurgun B, Zümreoglu A, Demir ST, Ibis MA (2001). Early studies on mating disruption technique of codling moth, *Cydia pomonella*, in the Aegean Region, Turkey. Pheromones for Insect Control in Orchards and Vineyards. IOBC wprs Bulletin 24(2):43-46.

Topuz H, Durmuşoğlu E, Balci H (2011). Adjuvants used in plant protection. Turk. Bull. Entom. 1(3):197-208.

Ioriatti C, Anfora G, Angeli G, Civolani S, Schmidt S, Pasqualini E. (2009). Toxicity of emamectin benzoate to *Cydia pomonella* (L.) and *Cydia molesta* (Busck) (Lepidoptera: Tortricidae): laboratory and field tests. Pest Manag. Sci. 65(3):306-12. http://dx.doi.org/10.1002/ps.1689, PMid:19097022

Jansson RK, Dybas RA (1996). Avermectins: Biochemical mode of action, biological activity, and agricultural importance in I. Ishaaya [ed.], "Insecticides with novel modes of action: Mechanism and application". Springer-Verlag, New York.

Miletic N, Tamas N, Graora D (2011). The control of codling moth (*Cydia pomonella* L.) in apple trees. Zemdirbyste Agriculture, 98(2):213-218.

Mushtaq M, Chukwudebe AC, Wrzesinski C, Allen LRS, Luffer-Atlas D, Arison BH (1998). Photodegradation of emamectin benzoate in aqueous solution. J. Agric. Food Chem. 46: 1181-1191.http://dx.doi.org/10.1021/jf970561i

Özbek S (1978). Special Fruit. Çukurova University. Faculty of Agriculture Publications, Adana, Turkey. 128:488.

Özongun Ş, Dolunay E M, Öztürk G, Karakuş A, Kankaya A, Küden A (2004). Elma Adaptasyon Denemesi 1. Tarım ve Köyişleri Bakanlığı, Tarımsal Araştırmalar Genel Müdürlüğü, Eğirdir Bahçe Kültürleri Araştırma Enstitüsü Müdürlüğü, Isparta, P. 54.

TAGEM (2011). T.C. Ministry of Food, Agriculture and Livestock, General Directorate of Agricultural Research and Policy, General Directorate of Research Plant Health, Apple Technical Instruction Booklet. pp. 21-24.

Tan W, Song B (2006). Research on effect of four natural ultraviolet light absorbers on photostabilization of Azadirachtin-A. Agric.Sci. China 5(11):855-858.

Tu M, Randall JM (2001). "Adjuvants, 8.1-8.24". In "Weed Control Methods Handbook: Tools and Techniques for Use in Natural Areas (Eds: M. Tu, C. Hurd & J. M. Randall). The Nature Conservancy, The Global Invasive Species Team (Web page: http://www.invasive.org/gist/handbook.html), (Date accessed: July 2012).

TUIK, 2012. Turkish Statistical Institute Website. (Date accessed: 01.12.2013). www.tuik.gov.tr

Weddle P W, Welter SC (2009). Thomson D. History of IPM in California pears – 50 years of pesticide use and the transition to biologically intensive IPM. Pest Manage. Sci. 65:1287–1292. http://dx.doi.org/10.1002/ps.1865, PMid:19856383

Wei L, Lu FS, Guo WT, Li H (2010). Preparation and characterization of emamectin benzoate Microcapsules. Chin. J. Appl. Chem. 27:1381-1385.

Zhu J, Yueping H, Gao M, Zhou W, Hu J, Shen J, Zhu Y C (2011). Photodegradation of Emamectin Benzoate and Its Influence on Efficacy against the Rice Stem Borer, Chilo suppressalis. Crop Prot. 30:1356-1362. http://dx.doi.org/10.1016/j.cropro.2011.06.006

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Full Length Research Paper

Alleviating harmful effects of chilling stress on rice seedling via application of spermidine as seed priming factor

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Chilling stress is a major limiting factor for rice production in many parts of the world. The study was carried out in the Seed Research Laboratory of the Department of Crop Science, Ferdowsi University of Mashhad in summer 2011. Rice (cv. Khazar) seeds were soaked in 50, 150 and 300 mg L^{-1} spermidine aerated solutions for 48 h and then dried back to the original moisture content and were sown in three temperatures (28°C as normal, 12 and 8°C as chilling stress). Chilling stress (8°C) reduced the root (39%) and shoot (52%) growth in untreated seeds, while the reduction of 11% root growth and 20% shoot growth was observed when the seeds were primed with 300 mg L^{-1} spermidine solution. The electrolyte leakage (EL) of the seedling leaves significantly increased in low temperatures as it was 67% in 8°C in compared with normal condition. Chilling stress significantly increased superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) activity in rice seedlings leaves though this increase was not significant for glutathione reductase (GR). Seed priming with spermidine had a positive effect on seedlings leaves antioxidant activity in every temperature conditions. As in 8°C, the sharp increase (73%) in SOD activity was occurred at 300 mg L^{-1} spermidine solution and it was 23 and 46% for CAT and APX, respectively. In general, seed priming with spermidine alleviated the chilling effect, probably as a result of activating antioxidants production processes and membrane stabilizing in cellular structures.

Key words: Antioxidant, chilling injury, polyamine, priming, seedlings.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important cereals in the world feeding approximately 4 billion people, while chilling stress is a major limiting factor for its production in many parts of the world. The range of air temperature around 15 to 20°C lead to chilling damage in rice though the level of damage is related to period of chilling stress, growth stage and genotypes (Pouramir et al., 2013). In northern regions of Iran, rice is sown from early April to middle of May, when mean temperature is around 15°C which leads to rotten rice seedlings, causing heavy seed loss and a delayed in growth period (Sharifi, 2010).

Chilling stress in germination and vegetative growth growth stages causes poor germination and emergence,

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delayed in seedling growth, leaf discoloration, reduced height and tiller numbers and consequently, lose yield in rice (Takeoka et al., 1992).

A lot of research on several plants demonstrated that seed invigoration treatments have effectively improved germination and early seedling growth particularly at suboptimal conditions (Bradford, 1986; Afzal et al., 2002; Farooq et al., 2005).

Seed priming is one of the seed invigoration treatments that allows seeds to absorb water to a point where germination processes start but radicle emergence does not occur (Bradford, 1986). Shorter germination time, emergence over all seedbed environmental and broad temperature range of germination, leading to uniformity, better crop establishment and therefore improved harvest quality and yield, particularly under abnormal and stress situation in the field are the typical responses to seed priming (Farooq et al., 2007).

Polyamines including spermidine, putrescine and spermine have been recognized as a group of plant growth regulators with the great effects on plant growth and development. Plant growth rate is positively related to amount of polyamine levels in a wide range of environmental conditions (Watson and Malmberg, 1998).

Polyamines play different roles in the plant including binding with different macromolecules and stabilize their structures. In addition, they are able to play act as regulators in various fundamental cellular processes such as cell division, differentiation and proliferation, cell death, DNA and protein synthesis and gene expression (Igarashi and Kashiwagi, 2000; Childs et al., 2003). Iqbal and Ashraf (2005) demonstrated that wheat (*Triticum aestivum* L.) seed priming with spermidine, spermine and putrescine increased photosynthetic capacity and also wheat growth under saline conditions. Xu et al. (2011) revealed that tobacco (*Nicotiana tabacum* L.) seed priming with putrescine improved germination percentages, germination index, seedling length and dry weight in comparison with controls which caused chilling tolerance. Farooq et al. (2007) showed that sunflower (*Helianthus annuus* L.) seeds priming with polyamines (spermidine and putrescine) increased germination percentages, root and shoot length, early seedling growth and decreased emergence time. However, Lin and Kao (1995) revealed that with increasing salinity levels, the spermine content did not change in root and shoot tissues of the rice but spermidine and putrescine levels were increased though they were not able to ameliorate of the growth inhibition of seedlings imposed by NaCl. Pretreatment of rice seeds with putrescine caused an increase in its level in shoots but could not relieve the inhibition effect of NaCl on seedling growth. Several study have shown that exogenous application of spermidine as an important polyamine can play an important role in recovering the plasma membrane damage and electrolyte leakage (EL) in rice genotypes and cucumber in response to environmental stresses like chilling, salinity and drought (Kubis, 2008; Roy et al., 2005). Xu et al. (2011) showed that the amount of seedling leaf relative water content (RWC) improved under chilling stress via seed priming with putrescine probably by osmotic adjustment or might alter in cell wall stretchiness. Cao et al. (2008) reported that seed soaking with putrescine improves chilling tolerance of maize seeds.

The objectives of this research were firstly, to investigate the physiological and biochemical changes in rice seedlings during chilling stress and secondary, the possibility of induce chilling tolerance in early seedling growth of rice by seed priming with spermidine.

MATERIALS AND METHODS

Seed

Rice seeds were obtained in October, 2010 from the Rice Research Institute, Rasht, Iran. All seeds were harvested in the same season based on seed uniformity and without any spots of diseases. They were transferred to Seed Research Laboratory of the Department of Crop Science, Ferdowsi University of Mashhad and were placed in air-tight container and kept at 4°C for further use. Germination test using between papers on 4 replicates of 25 seeds revealed the germination of 94%.

Seed priming

Seeds were soaked 48 h in three spermidine solutions (50, 150, 300 mg L^{-1}), distilled water and unprimed seed as control. Distilled water treatment was used to eliminate the possible effect of water on seed priming. Then seeds were washed six times by distilled water and dried back to the original seed moisture content at 28 ± 2°C.

Emergence in sand

Seeds were sown in 1 cm depth of moist sand in trays (45 × 25 × 10 cm). Trays were put in controlled conditions in a growth chamber at 28°C and 15/9 h light/dark for 19 days for normal temperature conditions. In order to induce chilling stress conditions, another two sets were put in the same above conditions for 15 days , before moving at 8 and 12°C for 4 days, respectively.

The experiment was carried out in a completely randomized design with 4 replicates of 25 seeds. Emergence was counted daily for 15 days.

Time taken to 50% emergence (E50) was calculated using the following equation (Equation 1) of Coolbear et al. (1984) modified by Farooq et al. (2005):

$$E50 = ti + \frac{\left[\left(\frac{N}{2}\right) - ni\right](tj - ti)}{nj - ni} \quad (1)$$

Where N is the final number of emerged seeds, and ni and nj the cumulative number of seeds emerged by adjacent counts at times ti and tj when ni < N/2 < nj.

Mean emergence time (MET) was calculated according to the equation (Equation 2) of Khajeh-Hosseini et al. (2009):

$$\text{MET} = \frac{\sum nt}{\sum n} \quad (2)$$

Where n is the number of seeds newly emerged at time t; t = number of days from sowing.

Then on the final day, seedlings from each replicate were carefully removed from the seed bed and washed before measuring the shoot and root length.

Biochemical analysis

Samples (second uppermost leaf from main stem) were taken from control and chilling stressed plants on 19th day after sowing.

Membrane permeability

In order to estimate the amount of leaf membrane damage, the amount of EL was determined (Blum and Ebercon, 1981). Six leaves samples were washed and then soaked in tubes containing 40 ml distilled water for 12 h. Afterward, the first electrolyte conductivity (C1) of the solutions was determined by a conductivity meter (4510, Jenway, manufactured in Camlab House, Norman Way Industrial Estate, Over, Cambridge CB24 5WE, United Kingdom). After all, samples were sealed in boiling water for 20 mi and then, the second electrolyte conductivity (C2) of the solutions was determined after equilibration at 25°C. Membrane permeability (EL) was defined as follows (Equation 3):

$$\text{EL } (\%) = (C_1/C_2) \times 100 \quad (3)$$

Relative water content (RWC)

To evaluate the leaf RWC, 0.5 g fresh weight of leaf samples (fw) was soaked 24 h in tubes including 40 ml of distilled water. Then leaves were quickly weighed (tw) and were kept in oven at 70°C for 48 h to calculate dry weight (dw). RWC was calculated by the following equation (Equation 4):

$$\text{RWC } (\%) = \frac{fw - dw}{tw - dw} \times 100 \quad (4)$$

Enzyme assays

Leaf fresh samples (0.1 g) was powdered in liquid nitrogen and homogenized in 1 ml of 0.1 M potassium phosphate buffer of pH 7.8 containing 1 mM ethylene diamine tetra acetic acid (EDTA) by a homogenizer into microtubes. Insoluble materials was removed by refrigerated centrifuge (Beckman Coulter headquarters in Brea, California, USA) at 12000 g for 20 min at 4°C and the supernatant used as the source of enzyme extraction. To determine the activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR), 100 µl of supernatant were taken and all steps of antioxidants determination carried out at 4°C. SOD activity was assayed by monitoring the inhibition of the photochemical reduction of nitroblue tetrazolium, according to Yu and Rengel (1999). CAT activity was assayed by measuring the initial rate of hydrogen peroxide disappearance according to Velikova et al. (2000). APX and GR activities were determined according to Yamaguchi et al. (1995) and Lee and Lee (2000), respectively.

RESULTS

Temperature and priming had no significant effect on final emergence percentages (E%), E50 and MET (Table 1) since counting of emerged seeds was finished in Day 15, while chilling stress treatment was induced in Day 16. Although, seed priming with spermidine solutions had higher E% and lower E50 and MET than unprimed seeds in all temperatures (Table 1).

Although chilling stress considerably decreased the root (39%) length, seed priming with spermidine improved the root length in both chilling stress conditions and normal temperature. Under optimum temperature conditions seedlings raised from primed seeds with 50 mg L^{-1} spermidine solution had the highest root length (90 mm) though in 12 and 8°C stress conditions the highest root length (68 and 66 mm) was obtain from primed seeds with 300 mg L^{-1} spermidine solution (Table 1).

Chilling stress significantly reduced shoot length (52%) compare with control (Table 1). Although, seed priming improved the shoot growth in all temperatures particularly at 8°C where shoot length increased (67%) from 61 mm in untreated seeds to 102 mm in primed seeds with 300 mg L^{1} spermidine solution (Table 1).

Chilling stress reduced the growth of both root and shoot. But its effect on shoot was stronger than the root hence, the root shoot length ratio increased (28%). Seed priming decreased the root shoot length ratio in all temperatures particularly at 8°C (Table 1).

The RWC of the seedling leaves decreased in both untreated and treated seeds in chilling stress conditions. Seed priming slightly increased RWC in all temperature though these differences were not significant (Table 2).

The EL of the seedling leaves significantly increased in low temperatures. This increase was 67% in 8°C compared with normal condition. However, rice seed priming by spermidine solutions decreased the leakages in every temperature conditions (Table 2). For instance in 8°C, seed priming with spermidine 300 mg L^{-1} decreased (26%) the leakages from 25.2 in untreated seeds to 18.6 in treated seeds.

Chilling stress significantly increased SOD, CAT and APX activity in rice seedlings leaves though this increase was not significant for GR (Table 2). The highest increase in antioxidant activity due to chilling stress with 54% belongs to SOD and this amount was 38 and 25% for CAT and APX, respectively. Seed priming with spermidine had a positive effect on seedlings leaves antioxidant activity in every temperature conditions (Table 2). As in 8°C, the sharp increase (73%) in SOD activity occurred at 300 mg L^{-1} spermidine solution and it was 23 and 46% for CAT and APX, respectively.

DISCUSSION

Rice seed priming with spermidine improved emergence and speed of emergence though the differences were not significant. Chilling stresses (8°C) reduced the root (39%) and shoot (52%) growth in untreated seeds, while the reduction of 11% root growth and 20% shoot growth was observed when the seeds were primed with spermidine

Table 1. The effects of chilling stress and seed priming of rice with spermidine on final emergence percentages (E%), time taken to fifty percent emergence (E50), mean emergence time (MET), root length (RL), shoot length (SL) and ratio of root/shoot length (RL/SL) of the seedlings.

Treatment		E (%)	E50 (days)	MET (days)	RL (mm)	SL (mm)	RL/SL
T1	P1	78	7.81	5.12	74	127	0.58
	P2	79	7.77	5.16	79	136	0.58
	P3	83	7.53	4.83	90	152	0.59
	P4	85	7.24	4.45	89	150	0.59
	P5	85	6.98	4.51	81	149	0.54
T2	P1	80	7.67	4.98	51	91	0.56
	P2	79	7.74	5.10	50	92	0.54
	P3	83	7.11	4.62	65	102	0.64
	P4	84	6.93	4.58	67	110	0.61
	P5	87	6.85	4.39	68	112	0.61
T3	P1	79	7.79	5.01	45	61	0.74
	P2	78	7.58	4.81	47	69	0.68
	P3	82	7.41	4.75	50	76	0.65
	P4	84	7.17	4.55	62	98	0.63
	P5	87	7.12	4.62	66	102	0.65
LSD		9.79	1.05	0.94	10.56	15.68	0.02

P1, Untreated seeds (control); P2, distilled water; P3, spermidine 50 mg L^{-1}; P4, spermidine 150 mg L^{-1}; P5, spermidine 300 mg L^{-1}; T1, temperature of the whole period was 28°C; T2, 15 days at 28°C and then 4 days at 12°C; T3, 15 days at 28°C and then 4 days at 8°C.

Table 2. The effects of chilling stress and seed priming with spermidine on relative water contents (RWC), electrolyte leakages (EL) and enzyme assays of the seedling leaves of the rice.

Treatment		RWC (%)	EL (%)	SOD (unit.g^{-1} fw)	CAT (µmol.g^{-1} fw min^{-1})	APX (unit.g^{-1}fw)	GR (µmol. g^{-1} fw)
T1	P1	85	15.1	11.0	90	18.5	13.04
	P2	86	13.8	11.1	89	17.8	12.41
	P3	87	12.4	12.2	98	19.3	14.11
	P4	87	10.2	14.3	110	23.2	14.5
	P5	89	10.6	14.2	112	26.8	15.88
T2	P1	82	16.7	16.6	111	21.2	14.04
	P2	81	17.1	16.1	110	22.4	15.42
	P3	83	15.9	20.2	120	25.9	17.15
	P4	85	13.2	25.6	125	29.2	16.05
	P5	86	13.5	24.5	135	30.1	17.33
T3	P1	78	25.2	16.9	124	23.2	16.21
	P2	78	25.5	18.1	126	23.1	15.47
	P3	79	22.7	24.2	136	28.9	18.03
	P4	83	19.2	28.3	138	30.6	18.61
	P5	82	18.6	29.2	153	33.8	19.42
LSD		12.06	4.07	5.24	16.36	4.61	8.71

P1, Untreated seeds (control); P2, distilled water; P3, spermidine 50 mg L^{-1}; P4, spermidine 150 mg L^{-1}; P5, spermidine 300 mg L^{-1}; T1, temperature of the whole period was 28°C; T2, 15 days at 28° C and then 4 days at 12°C; T3, 15 days at 28°C and then 4 days at 8°C.

300 mg L^{-1} (Table 1). Therefore, chilling reduced the shoot growth stronger than the root growth in early seedling growth of rice as reflected in a higher root shoot length ratio, while spermidine protected the seedlings against chilling. Decreasing shoot length due to chilling was reduced from 52 to 20% as a result of seed priming

with spermidine whereas it was reduced from 39 to 11% for root length. Therefore, protecting effect of spermidine on shoot length was stronger than that of the root length in chilling stress conditions (8°C). Chilling stress can affect root and shoot growth through inhibiting both cell division and expansion (Pahlavanian and Silk, 1988; Tardieu and Granier, 2000). The activity of the A-type cyclin dependent kinase (CDKA), a major regulator of cell cycle progression, is associated with the decrease in leaf growth rate of some species under stress conditions. Rymen et al. (2007) claimed that temperature directly affects enzyme kinetics in many biochemical reactions and thereby the growth rate of plant organs. They reported that leaf growth inhibition by low night temperature is tightly linked to the reduction of cell production which is a consequence of prolonged cell cycle duration and not of a reduced cell number in the leaf meristem.

West et al. (2004) indicated that decrease in cell production and a smaller mature cell length was the reason of growth reduction of the *Arabidopsis* stressed roots but average cell cycle duration was not affected. Thus, the reduced cell production was a consequence of smaller number of dividing cells (meristem size reduction). There are some evidences demonstrating the role of exogenous application of polyamines in cold acclimation (Groppa and Benavides, 2008). Polyamines regulate plant growth and development via several physiological processes. These compounds are thought to possess sharp effects on plant growth and development (Watson and Malmberg, 1998). Therefore, in the current study, improving root and shoot length caused by seed priming in chilling stress might be as a result of increased cell division within the apical meristem, resulting in enhanced plant growth. Polyamines play regulatory roles in various important cellular processes including cell division, differentiation and proliferation, cell death, DNA and protein synthesis and gene expression alongside the stabilizing macromolecular structures (Igarashi and Kashiwagi, 2000; Childs et al., 2003; Kusano et al., 2008). Xu et al. (2011) also indicated that germination, seedling length and dry weight of chilling-sensitive tobacco varieties enhanced by putrescine treatments under chilling conditions.

Chilling stress causes damage in plant by production of reactive oxygen species (ROS), however, plant have antioxidant enzymes playing a vital role in improving chilling tolerance (Bolkhina et al., 2003). Studies showed that polyamines are able to improve plants antioxidant system against ROS and therefore decrease the rate of cells injuries and increase plant root and shoot growth under chilling stress conditions (Bolkhina et al., 2003; Xu et al., 2011).

Temperature and priming had no significant effect on RWC though spermidine slightly improved the leaves RWC probably by osmotic adjustment or changing cell wall stretchiness.

More ELs (67%) was observed in the leaves of the rice seedlings under chilling stress (8°C), while it was 27% in seedling leaves raised from seeds primed with 300 mg L^{-1} spermidine solution. Therefore, priming relieved 40% of EL that can be related to priming positive effect on cell membrane and organs. Cell membranes are the major targets of environmental stresses. Enhanced EL was considered to be a symptom of membrane damage induced by chilling stress and deterioration (Pessarakli, 1999). Chilling stress results in several malfunctions at cellular levels including damage to membranes hence increasing their permeability (Simon, 1974; Pessarakli, 1999). Exogenous application of spermidine as an important polyamine is an effective way to recover the plasma membrane damage and to mitigate the EL in response to environmental stresses such as drought, salinity and chilling (He et al., 2002; Roy et al., 2005; Kubiś, 2008). Saeidnejad et al. (2012) showed that maize seedling EL was recovered by seed priming with spermidine in chilling conditions.

In the current study, antioxidants system effectively responded to the stress conditions and seed priming with spermidine enhanced the responses. Increase in antioxidants activity in chilling stress condition is a proper response to production of activates oxygen species (ROS) induced by chilling (Wang and Li, 2006). Therefore, the activities of antioxidative enzymes are related to chilling stress tolerance though the generation of ROS is a common event in growth and developmental processes. ROS can interrupt plant normal metabolism by oxidative damage of different cellular components, including DNA, proteins, lipids, and pigments which results in reduced photosynthetic capacity and growth inhibition (Pessarakli, 1999; Bolkhina et al., 2003; Xu et al., 2011). Antioxidants in the plants protect them against activate oxygen species. Several studies demonstrated that antioxidants such as SOD, CAT, APX and GR can play an important role in plant chilling tolerance by scavenging the activate oxygen species (Afzal et al., 2002; Blokhina et al. 2003; Wang and Li 2006). Shen et al. (1999) demonstrated that SOD and APX activity in chilling-tolerant cucumber (*Cucumis sativus* L.) cultivars was higher than chilling sensitive ones. In a study on the maize, it was showed that CAT plays an important role in plant protection against chilling stress (Prasad, 1997). Lee and Lee (2000) indicated that higher APX activity in plant leaves under chilling stress is a more efficient scavenging system which may result in stronger protection against ROS during chilling. Similarly, Saruyama and Tanida (1995) demonstrated that increase in the activity of APX and CAT leads to chilling tolerance in rice cultivars.

Polyamines accumulation in plant cell can reduce chilling injury. As putrescine accumulation reduced the chilling injury in tomato (*Solanum lycopersicum* L.) (Kim et al., 2002), chickpea (*Cicer arietinum* L.) (Nayyar, 2005) and maize (Cao et al., 2008) under chilling stress. Many studies demonstrated that genetic modification of the polyamine biosynthetic pathway is a useful tool to recognize the function of polyamines in plant responses to abiotic stresses in both crops and model plants (Igarashi and Kashiwagi, 2000; Childs et al., 2003; Kusano et al., 2008). It was shown that antioxidant activities in tobacco plant under chilling conditions can be enhanced by seed priming with polyamine (Xu et al., 2011).

Conclusion

In this study, chilling had a negative effect on rice seedling physiological and biochemical properties that leads to reduction of the root and shoot length and also increasing in the antioxidants activities. Seed priming with spermidine was able to decrease the negative effect of chilling stress due to activating antioxidants production processes and membrane stabilizing in cellular structures. We believe that our results provide additional support to the role of spermidine as a polyamine in mitigating chilling effect on rice early growth stage. Application of other polyamines to enhance chilling tolerance of rice seedling could be the subject of future studies.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Afzal I, Basra SMA, Ahmad N, Cheema MA, Warriach EA, Khaliq A (2002). Effect of priming and growth regulator treatment on emergence and seedling growth of hybrid maize (*Zea mays* L.). Int. J. Agric. Biol. 4:302–306.

Blokhina O, Virolainen E, Fagerstedt KV (2003). Antioxidants, oxidative damage and oxygen deprivation stress: A Rev. Annu. Bot. 91:179–194. http://dx.doi.org/10.1093/aob/mcf118

Blum A, Ebercon A (1981). Cell membrane stability as a measure of drought and heat tolerance in wheat. Crop Sci. 21:43–47. http://dx.doi.org/10.2135/cropsci1981.0011183X002100010013x

Bradford KJ (1986). Manipulation of seed water relations via osmotic priming to improve germination under stress conditions. Hortscience 21:1105–1112.

Cao D, Hu J, Gao C, Guan Y, Zhang S, Xiao J (2008). Chilling tolerance of maize (*Zea mays* L.) can be improved by seed soaking in putrescine. Seed Sci. Technol. 36:191-197.

Childs A, Mehta D, Gerner E (2003). Polyamine-dependent gene expression. Cell Mol. Life Sci. 60:1394-1406. http://dx.doi.org/10.1007/s00018-003-2332-4, PMid:12943227

Coolbear P, Francis A, Grierson D (1984). The effect of low temperature pre-sowing treatment under the germination performance and membrane integrity of artificially aged tomato seeds. J. Exp. Bot. 35:1609–1617. http://dx.doi.org/10.1093/jxb/35.11.1609

Farooq M, Basra SM, Khan MB (2007). Seed priming improves growth of nursery seedlings and yield of transplanted rice. Arch. Agron. Soil Sci. 53: 315-326. http://dx.doi.org/10.1080/03650340701226166

Farooq M, Basra SMA, Hafeez K, Ahmad N (2005). Thermal hardening: a new seed vigor enhancement tool in rice. J. Integr. Plant. Biol. 47:187–193. http://dx.doi.org/10.1111/j.1744-7909.2005.00031.x

Granier C, Inzé D, Tardieu F (2000). Spatial distribution of cell division rate can be deduced from that of p34cdc2 kinase activity in maize leaves grown at contrasting temperatures and soil water conditions. Plant. Physiol. 124:1393-1402. http://dx.doi.org/10.1104/pp.124.3.1393, PMid:11080314 PMCid:PMC59236

Groppa M, Benavides M (2008). Polyamines and abiotic stress: Recent advances. Amino. Acids. 34:35-45. http://dx.doi.org/10.1007/s00726-007-0501-8

He L, Nada K, Tachibana S (2002). Effects of spermidine pretreatment through the roots on growth and photosynthesis of chilled cucumber plants (*Cucumis sativus* L.). J. Japan. Soc. Hort. Sci. 71: 490-498. http://dx.doi.org/10.2503/jjshs.71.490

Igarashi K, Kashiwagi K (2000). Polyamines: mysterious modulators of cellular functions. Biochem. Bioph. Res. Co. 271: 559-564. http://dx.doi.org/10.1006/bbrc.2000.2601

Iqbal M, Ashraf M (2005). Changes in growth, photosynthetic capacity and ionic relations in spring wheat (*Triticum aestivum* L.) due to pre-sowing seed treatment with polyamines. Plant. Growth. Regul. 46:19-30. http://dx.doi.org/10.1007/s10725-005-5901-8

Khajeh-Hosseini M, Lomholt A, Matthews S (2009). Mean germination time in the laboratory estimates the relative vigour and field performance of commercial seed lots of maize (*Zea mays* L.). Seed. Sci. Technol. 37:446-456.

Kim TE, Kim SK, Han TJ, Lee JS, Chang SC (2002). ABA and polyamines act independently in primary leaves of cold-stressed tomato (*Lycopersicon esculentum*). Physiol. Plantarum. 115:370-376. http://dx.doi.org/10.1034/j.1399-3054.2002.1150306.x

Kubiś J (2008). Exogenous spermidine differentially alters activities of some scavenging system enzymes H_2O_2 and superoxide radical levels in water-stressed cucumber leaves. J. Plant. Physiol. 165:397-406. http://dx.doi.org/10.1016/j.jplph.2007.02.005

Kusano T, Berberich T, Tateda C, Takahashi Y (2008). Polyamines: essential factors for growth and survival. Planta 228:367-381. http://dx.doi.org/10.1007/s00425-008-0772-7

Lee DH, Lee CB (2000). Chilling stress-induced changes of antioxidant enzymes in the leaves of cucumber: In gel enzyme activity assays. Plant Sci. 159: 75-85. http://dx.doi.org/10.1016/S0168-9452(00)00326-5

Lin CC, Kao CH (1995). Levels of endogenous polyamines and NaCl-inhibited growth of rice seedlings. Plant. Growth Regul. 17:15-20.

Nayyar H (2005). Putrescine increases floral retention, pod set and seed yield in cold stressed chickpea. J. Agron. Crop. Sci. 191:340-345. http://dx.doi.org/10.1111/j.1439-037X.2005.00158.x

Pahlavanian AM, Silk WK (1988). Effect of temperature on spatial and temporal aspects of growth in the primary maize root. Plant. Physiol. 87:529-532. http://dx.doi.org/10.1104/pp.87.2.529

Pessarakli M (1999). Effect of Low Temperatures on the Structure of Plant Cells. In: Hudak J, Salaj J. Hand Book of Plant and Crop Stress. New York: Marcel-Dekker Inc; P. 231-270. http://dx.doi.org/10.1201/9780824746728

Pouramir F, Khajeh-Hosseini M, Esfahani M (2013). Methods for rice genotypes cold tolerance evaluation at germination stage. Int. J. Agric. Crop Sci.18:2111-2116.

Prasad TK (1997). Role of catalase in inducing chilling tolerance in pre-emergence maize seedlings. Plant Physiol. 114:1369–1376.

Roy P, Niyogi K, Sengupta DN, Ghosh B (2005). Spermidine treatment to rice seedlings recovers salinity stress-induced damage of plasma membrane and PM-bound H+-ATPase in salt-tolerant and salt-sensitive rice cultivars. Plant Sci.168: 583-591. http://dx.doi.org/10.1016/j.plantsci.2004.08.014

Rymen B, Fiorani F, Kartal F, Vandepoele K, Inzé D, Beemster GT (2007). Cold nights impair leaf growth and cell cycle progression in maize through transcriptional changes of cell cycle genes. Plant Physiol. 143: 1429-1438. http://dx.doi.org/10.1104/pp.106.093948

Saeidnejad AH, Pouramir F, Naghizadeh M (2012). Improving chilling tolerance of maize seedlings under cold conditions by spermine

application. Notulae. Sci. Biol. 4: 110-117.
Saruyama H, Tanida M (1995). Effect of chilling on activated oxygen-scavenging enzymes in low temperature-sensitive and -tolerant cultivars of rice (*Oryza sativa* L.). Plant Sci. 109:105–113. http://dx.doi.org/10.1016/0168-9452(95)04156-O
Sharifi P (2010). Evaluation on sixty-eight rice germplasms in cold tolerance at germination. Rice Sci. 17:77-81. http://dx.doi.org/10.1016/S1672-6308(08)60107-9
Shen W, Nada K, Tachibana S (1999). Effect of cold treatment on enzymic and nonenzymic antioxidant activities in leaves of chilling-tolerant and chilling-sensitive cucumber cultivars. J. Japan. Soc. Hort. Sci. 68:967–973. http://dx.doi.org/10.2503/jjshs.68.967
Simon E (1974). Phospholipids and plant membrane permeability. New. Phytol. 73:377-420. http://dx.doi.org/10.1111/j.1469-8137.1974.tb02118.x
Takeoka Y, Al Mamun A, Wada T, Kaufman PB (1992). Reproductive Adaptations of Rice to Environmental Stress. Japan: Japan Scientific Societies Press.
Tardieu F, Granier C (2000). Quantitative analysis of cell division in leaves: methods, developmental patterns and effects of environmental conditions. Plant Mol. Biol. 43:555-567. http://dx.doi.org/10.1023/A:1006438321386
Velikova V, Yordanov I, Edreva A (2000). Oxidative stress and some antioxidant system in acid rain treated bean plants: Protective role of exogenous polyamines. Plant. Sci. 151:59-66. http://dx.doi.org/10.1016/S0168-9452(99)00197-1
Wang LJ, Li SH (2006). Salicylic acid-induced heat or cold tolerance in relation to Ca2+ homeostasis and antioxidant systems in young grape plants. Plant. Sci. 170:685–694. http://dx.doi.org/10.1016/j.plantsci.2005.09.005
Watson MB, Emory KK, Piatak RM, Malmberg RL (1998). Arginine decarboxylase (polyamine synthesis) mutants of *Arabidopsis thaliana* exhibit altered root growth. Plant J. 13:231-239. http://dx.doi.org/10.1046/j.1365-313X.1998.00027.x
West G, Inzé D, Beemster GT (2004). Cell cycle modulation in the response of the primary root of Arabidopsis to salt stress. Plant. Physiol. 135: 1050-1058. http://dx.doi.org/10.1104/pp.104.040022, PMid:15181207 PMCid:PMC514139
Xu S, Hu J, Li Y, Ma W, Zheng Y, Zhu S (2011). Chilling tolerance in Nicotiana tabacum induced by seed priming with putrescine. Plant Growth Regul. 63:279-290. http://dx.doi.org/10.1007/s10725-010-9528-z
Yamaguchi K, Mori H, Nishimora M (1995). A novel isoenzyme of ascorbate peroxidase localized on glyoxysomal and leaf peroxisomal memberanes in pumpkin. Plant Cell Physiol. 36:1157-1162. PMid:8528608
Yu Q, Rengel Z (1999). Micronutrient deficiency influences plant growth and activities of superoxide dismutase in narrow-leafed lupines. Annu. Bot. 83:175-182. http://dx.doi.org/10.1006/anbo.1998.0811

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Forage production, straw, structural characteristics and nutritional value of white oat in crop-livestock integrated systems

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This study was aimed to evaluate the production of forage and residual straw, structural characteristics and nutritional value of white oat under different soil use in an integrated crop-livestock system. The experiment was conducted in the autumn–winter seasons of 2009, 2010 and 2011 using a randomized block design in a scheme of tracks. Six forms of soil were distributed in tracks and were used to cultivate oats: residue grazing with a height of 10 or 20 cm (G10 and G20); cutting for haymaking with a residue height of 10 or 20 cm (C10 and C20); without grazing or cutting of oat plants with subsequent direct sowing of the summer crop (NC - TS) and without grazing or cutting with subsequent conventional tillage of the soil for sowing of the crop summer (NC - CS). In 2009, 2010 and 2011 three, one and two evaluations were accomplished each year, respectively. We studied the production of forage and residual straw, structural characteristics and nutritional quality of the forage produced. The total forage production in 2009 and 2011 did not differ between soil uses; however, it was higher in the uses with a residue height of 10 cm in 2010. The white oat under cuts or grazing gave high forage dry matter, while straw production was reduced with cuts or grazing. Forage of superior quality and with better production distributed throughout the autumn–winter was obtained when the white oat was managed with grazing or cuttings.

Key words: *Avena*, proteins, nutritional analysis.

INTRODUCTION

Pastures are the major component of the diets of ruminants and are the most economical source in livestock systems (Skonieski et al., 2011). Even though southern Brazil possesses favorable soil and climatic

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Table 1. Physico-chemical characteristics of soil experimental area.

Depth	P	OM	pH	Al+H	Al^{3+}	K^{+}	Ca^{2+}	Mg^{2+}	BS	CEC	V	Sand	Silt	Clay
cm	$mgdm^{-3}$	g dm^{-3}	$CaCl_2$		--------------$cmol_c.dm^{-3}$ --------------						%		---------g kg^{-1}--------	
0-10	19.6	21.1	4.9	5.0	0.0	0.9	5.1	2.2	8.2	13.3	62.1	54.2	117.6	828.2
10-20	19.5	19.9	4.9	5.0	0.0	0.9	5.1	2.2	8.2	13.2	61.9	54.2	117.6	828.2

OM: organic matter; BS: bases sum; CEC: cation exchange capacity; V: saturation of bases.

conditions for growing many forage species (Meinerz et al., 2011), it still faces shortage of fodder in the autumn–winter.

In order to rectify the shortage of high quality forage (Aguinaga et al., 2008), hibernal forages like white oat can be grown under an integrated crop-livestock system in areas of direct seeding kept in fallow during the winter period (Balbinot Junior et al., 2011). Such system not only helps to provide vegetal cover (Cassol et al., 2011) but also contributes to the sustainability over time.

However, maximum forage production and the success of an integrated crop-livestock system (Confortin et al., 2010) depend on the interaction of various components that interfere with plant growth (Aguinaga et al., 2008; Carvalho et al., 2010). Despite their high yield potential, hibernal pastures are characterized by a short cycle of use with rapid and abrupt changes in the structure of plants and nutritional value of the forage produced (Carvalho et al., 2010).

Among the components that alter plant development, management can potentially define the growth and productivity of pastures (Skonieski et al., 2011), which also affects their nutritional value. The height management, for example, determines the dry matter produced and the level of residual straw through defoliation (Aguinaga et al., 2008).

Black oat and ryegrass, or their associations have been studied for production of forage and straw under different managements (Cassol et al., 2011; Balbinot Junior et al., 2011), but results for white oat (*Avena sativa*) are still scarce in scientific circles.

In this context, the objective of the present work was to study forage production and residual straw, the structural characteristics and nutritional value of white oats under different soil uses in an integrated crop-livestock systems.

MATERIALS AND METHODS

The study was conducted during May 2009 to September 2011 in an experimental area with coordinates: latitude 24°33' 22'' S and longitude 54° 03' 24'' W and approximate altitude of 400 m. The soil (Table 1) was classified as oxisol (LVe). The area was under a direct seeding system in succession soy/corn/oats since 2006.

The climate of the region, according to the Köppen classification is Cfa type shrub soil, with well-distributed rainfall throughout the year and summers. Climatic data of the experimental period were obtained in an automatic climatological station of the State University of West of Paraná, which is approximately 100 m from the experimental area and is presented in Figure 1. The experiment was implemented in the autumn–winter of 2009, 2010 and 2011 in a randomized block design in a track scheme with three blocks and six tracks totaling 18 experimental units of 15 × 30 m each. In the strips were distributed six forms of soil use cultivated with oats: grazing with a residue height of 10 cm (G10); grazing with a residue height of 20 cm (G20); cutting for haymaking with a residue height of 10 cm (C10); cutting for haymaking with a residue height of 20 cm (C20); without grazing or cutting with subsequent direct seeding of the summer crop (NC - TS) and without grazing or cutting with conventional tillage of the soil for sowing of the summer crop (NC - CS).

Data were studied separately for each year, and due to variability in the year-to-year number of cuts or grazing, the experimental designs adopted for the analysis of the data were also variable. For the analysis of data collected in 2009, a randomized block design with scheme tracks in time as split plots was adopted. In the strips (plots), the six forms of soil use cultivated with white oat (G10, G20, C10, C20, NC-TS and NC-CS) and subplot-sampling growing periods (1^{st}, 2^{nd} and 3^{rd}) were allocated. In 2010, due to the occurrence of drought (Figure 1) only one cut or grazing was performed, so the experimental design was randomized block schemes on tracks.

In 2011 were sampled again in the design of randomized blocks on tracks with split plot in time, where the six ways of using soil cultivated with oats were allocated to main plots (G10, G20, C10, C20, NC-TS and NC-CS) and two growing periods were allocated to subplots (1^{st} and 2^{nd}). The 2009, 2010 and 2011 sowings were made on 24 May, 10 June and 29 May, respectively. In 2009 and 2011, the oats IPR 126 were sown, while in 2010, the oats URS Guapa lineage UFRGS 998011-2 were cultivated. In the three years, oats were seeded with a precision seeder coupled to a tractor in rows spaced 0.17 m apart using 70 Kg ha^{-1} seed without the use of fertilizer.

In 2009, the 1^{st} grazing or cutting was made on July 20 (55 days after sowing), while the 2^{nd} and 3^{rd} cuttings or grazing were respectively performed on 19 August and 15 September, 2009. In 2010, the 1^{st} grazing or cutting was performed on 8 August while maintaining similar interval after sowing as that of 2009. Due to the occurrence of drought (Figure 1) and the shorter cycle of oats URS Guapa than that of IPR 126, no re-growth was possible after the 1^{st} grazing or cutting in the area in 2010. In 2011, as there was adequate and regular rainfall than in 2010, the 2^{nd} grazing or cutting was performed 32 days after the 1^{st} grazing or cutting which was made on 24 July (56 days after sowing).

Lactating Holstein cows with live weights of approximately 550 ± 28.5 kg and an average milk production of 18 ± 2.5 kg day^{-1} were used in the grazing. The cows were distributed in paddocks and grazed for about two days until the desired residue heights (10 and 20 cm) were obtained. In order to get the desired residue heights in

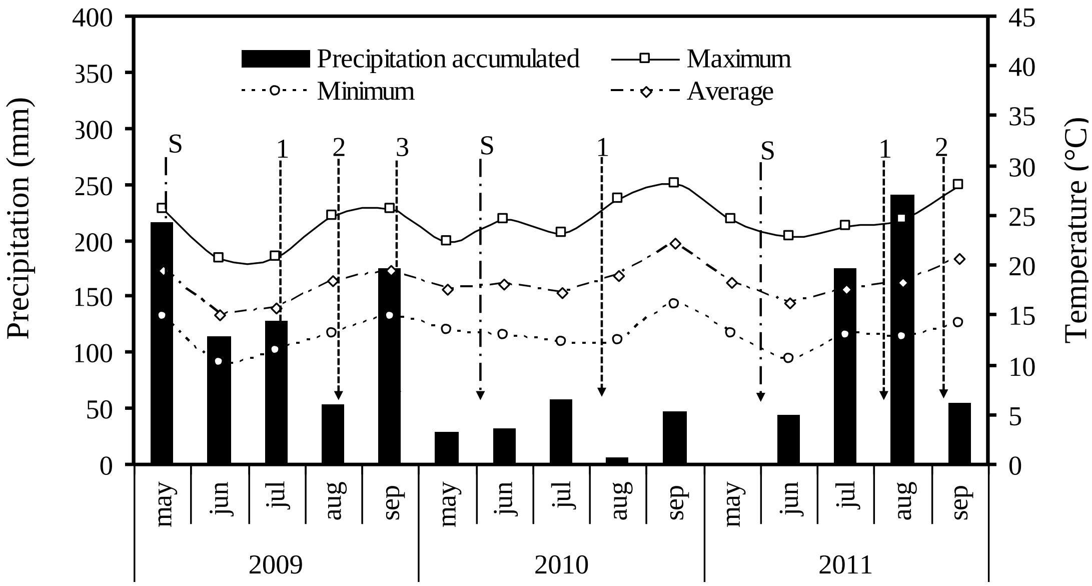

Figure 1. Monthly averages of maximum temperature, minimum and average and cumulative rainfall during the months of the experimental period in each year. S: sowing oats, 1, 2 and 3: cutting or grazing performed each year. Source: Climatological Station Auto Center of Experimental Stations Unioeste, Rondon-PR, 2009-2011.

the paddocks, manning variables were used according to the technique *put-and-take* (Mott and Lucas, 1952). For soil uses with cut, a mechanical mower coupled to the tractor regulated set at the desired cutting height (10 and 20 cm) was used and cutting was always made on the closing day of grazing. In tracks of soil uses without cutting or grazing, without were performed in oats, only its free development up to the point of desiccation.

In the years 2009 and 2010, corn and soybean were sown in succession to oats, respectively. During these sowings, the tracks submitted to the soil use for grazing or cutting and strip intended for use without grazing or cutting for direct sowing of the summer crop were desiccated using the herbicide glyphosate (1800 g of ai ha^{-1}) with a volume spray of 250 L ha^{-1}. The band destined for soil use without cutting or grazing and with subsequent conventional tillage of soil for sowing of the summer crop was mechanically prepared with the aid of a light grid. After harvesting the grain crops in each year, the experimental area remained fallow until the new sowing of oats.

Measurements of structural characteristics (plant height, number of tillers, number of leaves per tiller, final leaf length, stem diameter and leaf/stem ratio) and sampling to determine the dry matter yield of oats were performed immediately before each cutting or grazing, while sampling to determine the residual straw was mad after cutting or grazing. Plant height was measured at five random points in each plot using a ruler. The tiller density was determined by manual counting of all tillers contained in a known area (0.25 m²) in each plot. The number of leaves per tiller, final leaf length and stem diameter were measured in 10 randomly selected tillers in each plot. The stem diameter was determined with the aid of digital pachymeter. The leaf/stem ratio was determined using approximately 50 g samples collected from each plot and subjected to manual separation into leaf blades and stems + sheaths and kiln dried.

The dry matter production was estimated by sampling using a metallic square of 0.25 m² area randomly placed twice in each plot. All plants contained in the interior of the specified were cut and placed in labeled plastic bags for weighing in the laboratory in order to compose a sample containing two sub-samples for each plot. After weighing one representative sub-sample of forage produced on each plot, it was subjected to drying in an oven with forced air circulation at 55°C for 72 h. After drying the samples, they were ground using a mill with knives and a stainless steel chamber type Willey with a 1 mm sieve for subsequent determination of the nutritive value.

In soil uses with cuts or grazing, plants were sampled at predetermined heights, while in plots destined for soil uses without cutting or grazing oats, the samples were collected at a height of 15 cm from the ground level, featuring forage that would be available to be harvested, either in the form of cutting or grazing. For determining the residual straw, a metallic square of 0.25 m² in area was again randomly placed twice in each plot, and all the plant material contained inside it was collected at the level of the ground surface and kiln dried. The nutritional values of forage including crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose, lignin and cellulose were determined by chemical analyses (Silva and Queiroz, 2006). The data collected were subjected to analysis of variance, and averages were compared using Tukey's test at 5% probability.

RESULTS AND DISCUSSION

In year 2009, both forage dry matter and residual straw yields were significantly affected by the interaction effects of the factors involved (Table 2). In the 1st growth period, dry matter production was similar in all the soil uses. In the 2nd and 3rd growth periods, the highest dry matter yield were obtained when the oats in soil uses without

Table 2. Dry matter production of residual straw by white oat cv. IPR 126 under different soil uses in three growth periods in the autumn-winter 2009.

Usage soil	Dry matter production (kg ha^{-1})				Residual straw (kg ha^{-1})			
	Growth periods			Total	Growth periods			Total
	1º	2º	3º		1º	2º	3º	
G10	1374_{aA}	1017^{cB}	891^{bB}	3282^{ns}	1183^{aA}	891^{bAB}	646^{bB}	2720^{b}
G20	1451_{aA}	1511^{bA}	988^{bB}	3950	1242^{aA}	1222^{bA}	714^{bB}	3178^{b}
C10	1412^{aA}	1042^{cB}	902^{bB}	3356	1176^{aA}	959^{bAB}	679^{bB}	2814^{b}
C20	1289^{aB}	1580^{bA}	1049^{bB}	3918	1180^{aA}	1007^{bAB}	809^{bB}	2996^{b}
NC - TS	1380^{aC}	2349^{aB}	4467^{aA}	4467	1480^{aC}	2482^{aB}	4415^{aA}	8377^{a}
NC - CS	1290^{aC}	2208^{aB}	4425^{aA}	4425	1290^{aC}	2208^{aB}	4409^{aA}	7907^{a}
Mean	1366	1618	2120	3900	1259	1462	1946	
CV1 (%)	13.02				12.12			
CV2 (%)	6.29				6.63			
CV3 (%)	8.02				9.23			

^{ns}Not significant. Means followed by the same lowercase and uppercase in the column on the line do not differ statistically at a 5%. CV1, CV2 and CV3 (%): coefficient of variation factor for the period (P), soil use factor (SU) and interaction PxSU respectively. G10 and G20: grazing with residue height of 10 cm and 20 cm, C10 and C20: cutting for haymaking with residue height of 10 cm and 20 cm; NC - TS and NC-CS: no grazing or cutting with subsequent direct seeding or conventional soil tillage for sowing of the summer crop.

grazing or cuts, this fact was due by of forage was provided by grazing or cutting, not being deposited on the soil (Table 2).

The dry matter production in soil uses with a residue height of 10 cm, regardless of the use of cutting or grazing, was lower in the 2nd growth period. This could be attributed to the speed of recovery of leaf area of plants, which after grazing or cutting depends mainly on the remaining leaf area (Lemaire and Chapman, 1996). A direct relationship between the height of the forage crop and speed of recovery of leaf area was also previously observed (Rocha et al., 2004). In the 3rd growth period, dry matter production in soil uses with cutting or grazing at residual heights of 10 or 20 cm was similar (Table 2). Due to differences in sampling heights, differences in dry matter production were expected. However, due to the uneven development of the plants as a result of drought (Figure 1), these differences were not detected.

In soil use with a residue height of 10 cm, the dry matter production was higher in the 1st growth period, but decreased in the 2nd and 3rd due to speed of restoration of leaf area of plants after harvest. In grazing trial at a residual height of 20 cm, dry matter production decreased only in the 3rd period, while in cutting, there was an increase in the 2nd period with a subsequent decrease, due to the physiological age of the plants that entered the reproductive stage. No significant difference was observed in total dry matter production among the different soil uses studied (Table 2). The observed yields per period or total are considered satisfactory because they resemble the results of other studies (Bortolini et al., 2004; Cassol et al., 2011).

The production of residual straw was affected by the interaction of factors, being higher in the 2nd and 3rd growth periods in soil uses without grazing or cutting (Table 2). The observed increase in residual straw production with advancing growth period in the treatment without grazing or cutting was attributed to the accumulation of dry matter by plants.

In soil uses with grazing or cutting, there was a decrease in residual straw with the advancing growth period due to an advancement in cycle of oats and stem elongation. When the plants reach the reproductive period and lengthen their stems, the living leaves become part of the extract upper canopy in the pasture. Therefore, only the stem and leaves first issued by the tiller remain in the extract below (0 to 10 or 0 to 20). Introducing the plant with smaller leaves than other dry matter, and low participation in straw residual dry matter.

The interaction effect of the factors on the structural characteristics of oats grown in 2009 was also significant ($p<0.05$). Plant height was higher in soil uses without grazing or cuts and lower in uses with a residual height of 10 cm in the 2nd and 3rd growth periods (Table 3). In the 2nd growth period, the oats subjected to cut of oat at residue height of 20 cm had higher plant height than that subjected to grazing. This could be due to the effects of treading by the animals, which can damage plants and/or tillers and harming the re-growth in the grazing treatment. With the advance in growth period, an increase in plant height was observed in all soil use treatments due to stretching of plant internodes during the beginning of the reproductive stage (Langer, 1979). Similar results were obtained by Confortini et al. (2010) studied different

Table 3. Structural characteristics of white oat cv. IPR 126 under different soil uses in three growth periods in the autumn-winter 2009.

Usage soil	Plant height (cm)			Tiller density (tiller m^{-1})			Number of leaves per tiller		
	Growing period			Growing period			Growing period		
	1º	2º	3º	1º	2º	3º	1º	2º	3º
G10	29.17^{aB}	37.27^{dA}	36.44^{cA}	664.00^{a}A	416.67^{d}B	372.00^{c}	2.33^{aA}	2.33^{bA}	2.20^{bA}
G20	29.67^{aB}	42.27^{cA}	41.45^{bA}	662.67^{a}A	417.33^{d}B	358.00^{c}C	2.52^{aA}	2.35^{bA}	1.93^{bA}
C10	27.83^{aB}	35.70^{dA}	37.44^{cA}	653.33^{a}A	642.67^{a}A	508.00^{a}B	2.30^{aA}	2.58^{bA}	2.13^{bA}
C20	29.67^{aB}	45.40^{bA}	4355^{bA}	645.33^{a}A	557.33^{b}B	444.00^{b}C	2.51^{aA}	2.35^{bA}	1.85^{bA}
NC - TS	29.42^{aC}	66.87^{aB}	96.22^{aA}	672.67^{a}A	486.00^{c}B	286.00^{d}C	2.44^{aC}	4.03^{aB}	5.25^{aA}
NC - CS	30.58^{aC}	66.87^{aB}	96.22^{aA}	643.00^{a}A	484.00^{c}B	285.33^{d}C	2.35^{aB}	3.68^{aB}	5.33^{aA}
Mean	29.39	49.06	58.56	656.83	500.67	375.56	2.41	2.89	3.12
CV1 (%)	5.78			9.91			10.11		
CV2 (%)	4.32			4.18			7.44		
CV3 (%)	2.54			4.72			12.41		

Usage soil	Final leaf length (cm)			Stem diameter (mm)			Leaf / stem ratio		
G10	37.23^{aA}	30.57^{cB}	29.22^{bB}	2.86^{aA}	2.86^{bA}	2.97^{bA}	3.93^{aA}	3.59^{bA}	2.17^{aB}
G20	38.16^{aA}	40.82^{bA}	33.99^{aB}B	2.73^{aC}	3.83^{aA}	3.36^{bB}	4.25^{aA}	4.66^{aA}	2.37^{aB}
C10	39.66^{aA}	31.52^{cB}	30.36^{bB}	2.79^{aA}	2.84^{bA}	2.99^{bA}	3.98^{aA}	3.53^{bA}	2.10^{aB}
C20	35.36^{aA}B	40.03^{bA}	36.56^{aB}	2.97^{aB}	3.84^{aA}	3.38^{bB}	4.06^{aA}	4.74^{aA}	2.29^{aB}
NC - TS	38.46^{aB}	47.67^{aA}	34.11^{aB}B	3.01^{aB}	4.03^{aA}	4.38^{aA}	3.93^{aA}	2.17^{cB}	0.76^{bC}
NC - CS	38.18^{aB}	46.51^{aA}	33.78^{aB}B	2.84^{aB}	4.05^{aA}	4.21^{aA}	3.94^{aA}	2.32^{cB}	0.77^{bC}
Mean	37.84	39.52	33	2.87	3.57	3.55	4.01	3.5	1.74
CV1 (%)	11.06			5.99			9.72		
CV2 (%)	7.91			5.81			7.01		
CV3 (%)	5.88			6.36			11.42		

Means followed by the same lowercase and uppercase in the column on the line do not differ statistically at a 5%. . CV1, CV2 and CV3 (%): coefficient of variation factor for the period (P), soil use factor (SU) and interaction PxSU respectively. G10 and G20: grazing with residue height of 10 cm and 20 cm, C10 and C20: cutting for haymaking with residue height of 10 and 20 cm; NC - TS and NC-CS: no grazing or cutting with subsequent direct seeding or conventional soil tillage for sowing of the summer crop.

intensities by sheep grazing on oats.

Tillering of oats was inhibited with the soil uses adopted, with the exception of soil use with cut at a residue height of 10 cm; in which higher tiller density was recorded in the 2nd and 3rd growth periods (Table 3). The differences are due to the stimulation of tillering promoted by removal of the shoots of plants (cut). However, this stimulus was not enough to keep the tiller density in grazing managements, due to the death of tillers and plants by animal trampling.

A reduction in the number of tillers was observed from the 1st to the 3rd growth periods. This behavior, as well as the lowest number of tillers in plants that were not grazed or cut in the 3rd growth period, was due to the entry of plants into the reproductive phase (Langer, 1979) and the reallocation of photo-assimilates. Upon reaching the reproductive stage, the competition for resources between plants is higher resulting in senescence of the younger tillers senesce as a result of translocation of the photo-assimilates and nutrients to the older tillers to ensure development and seed production (Castagnara et al., 2010).

The number of leaves per tiller was higher in soil uses without grazing or cut in the 2nd and 3rd trial period; but no differences were observed in the 1st period. In soil uses without cutting or grazing, there was an increase in the number of leaves in the course of the growing periods. It is noteworthy that there were only whole leaves that were fully expanded.

In the 2nd growth period, the largest leaf length was observed in soil uses without cutting or grazing, with the lower value recorded for the oat grazed at a residue height of 10 cm. In the 3rd growth period, the highest and lowest length of leaves was observed in soil uses with cutting at residue heights of 20 and 10 cm, respectively.

Therefore, with a residue height of 10 cm, the remaining bundle sheath is shorter in the tiller unlike in the plants managed at a residue height of 20 cm. Thus, the new leaves formed in the apical meristem located at the base of the tiller become smaller because they travel

Table 4. Nutritive value of white oat cv. IPR 126 under different soil uses in three growth periods in the autumn-winter 2009.

Usage soil	CP (g kg^{-1})			NDF (g kg^{-1})			ADF (g kg^{-1})		
	Growing period			Growing period			Growing period		
	1º	2º	3º	1º	2º	3º	1º	2º	3º
G10	234.0^{aA}	219.2^{aA}	205.2^{aA}	494.5aB	510.1^{bB}	574.6^{bA}	288.2^{aB}	298.1^{bB}	353.9^{bA}
G20	233.5^{aA}	238.3^{aA}	201.5^{aA}	498.9^{aB}	510.1^{bB}	581.0^{bA}	301.6^{aB}	307.5^{bB}	357.9^{bA}
C10	233.0^{aA}	210.5^{aA}	197.0^{aA}	498.3^{aB}	519.4^{bB}	575.7^{bA}	292.1^{aB}	302.1^{bB}	359.0^{bA}
C20	241.0^{aA}	235.9^{aAB}	200.9^{aB}	497.7^{aB}	511.4^{bB}	582.5^{bA}	296.7^{aB}	295,4^{bB}	354.1^{bA}
NC - TS	239.5^{aA}	157.5^{bB}	75.7^{bC}	501.2^{aC}	562.3^{aB}	691.5^{aA}	291.1^{aC}	334.3^{aB}	422.7^{aA}
NC - CS	233.3^{aA}	160.9^{bB}	80.4^{bC}	498.5^{aC}	568.2^{aB}	683.5^{aA}	294.9^{aC}	331.1^{aB}	417.1^{aA}
Mean	235.7	203.7	160.1	498.2	530.2	614.8	294.1	311.4	377.5
CV1 (%)	6.46			2.14			3.38		
CV2 (%)	6.78			4.06			5.35		
CV3 (%)	9.32			2.91			3.37		
	Hemicellulose (g kg^{-1})			Lignin (g kg^{-1})			Cellulose (g kg^{-1})		
G10	206.2^{aA}	212.0^{bA}	220.7^{bA}	31.8^{aC}	41.4^{bB}	52.6^{bA}	233.6^{aA}	255.9^{bA}	258.7^{bA}
G20	203.3^{aB}	209.9^{bAB}	223.1^{bA}	31.0^{aC}	42.1^{bB}	52.4^{bA}	240.9^{aA}	250.3^{bA}	256.8^{bA}
C10	206.1^{aA}	217.3^{bA}	216.7^{bA}	32.5^{aC}	41.7^{bB}	54.2^{b}A	241.8^{aA}	253.3^{bA}	257.7^{bA}
C20	201.0^{aB}	216.1^{bAB}	228.4^{bA}	31.1^{aC}	41.5^{bB}	53.7^{bA}	242.4^{aA}	252.5^{bA}	258.9^{bA}
NC - TS	210.1^{aC}	228.0^{aB}	268.8^{aA}	29.9^{aC}	43.9^{aB}	63.5^{aA}	243.3^{aB}	290.6^{aA}	315.2^{aA}
NC - CS	203.6^{aC}	237.1^{aB}	266.4^{aA}	31.4^{aC}	45.1^{aB}	62.1^{aA}	240.4^{aB}	299.0^{aA}	311.0^{aA}
Mean	205	220.1	237.3	31.3	42.6	56.4	240.4	266.9	276.4
CV1 (%)	3.61			1.7			3.75		
CV2 (%)	4.43			2.32			7.48		
CV3 (%)	3.39			2.61			4.11		

Means followed by the same lowercase and uppercase in the column on the line do not differ statistically at a 5%. CV1, CV2 and CV3 (%): coefficient of variation factor for the period (P), soil use factor (SU) and interaction PxSU respectively. G10 and G20: grazing with residue height of 10 and 20 cm, C10 and C20: cutting for haymaking with residue height of 10 and 20 cm; NC - TS and NC-CS: no grazing or cutting with subsequent direct seeding or conventional soil tillage for sowing of the summer crop.

a shorter way until they emerge and become fully expanded. In contrast, the highest leaf length in soil use where the plants were cut at a residual height of 20 cm was due to the remaining length of the sheath and the absence of animal trampling and uniformity of cut plants. Changes in final leaf length in soil uses with grazing are due to animal trampling and uneven height of the forage crop, which becomes more pronounced following the grazing (Carvalho et al., 2010).

Stem diameter was lower in the 2nd growth period in soil uses with a residue height of 10 cm, while in the 3rd period; higher stem diameter was recorded in plants managed without cutting or grazing soil use system. These changes are due to the development of the shoots of the plants because the diameter of the stems increases in direct proportion to the strength required to support its organs (leaves).

In the leaf/stem ratio, there was a significant difference from the 2nd growth period, in which the largest leaf/stem ratio was obtained in the forage produced on soil uses with a residue height of 20 cm, followed by soil uses with a residue height of 10 cm. However, these managements did not differ in the 3rd period of growth. In soil uses without grazing or cutting, the leaf/stem ratio was much lower in the 2nd and in the 3rd period of growth (Table 3). Changes in leaf/stem ratio are due to the stem elongation of plants due to the inter-node lengthening with the arrival of the reproductive phase (Langer, 1979). Similar results for oat were obtained by Tonate et al. (2014) that compared leaf/stem ratio decreasing grown with the advance of the period, these authors attributed the fact to the natural cycle of the plant in relation to the productive age.

The nutritional value of forage produced in 2009 was affected by interaction of the factors involved (Table 4). The CP concentration in forage differed between the soil uses in the 2nd and 3rd growth periods of oats, with lower and decreasing values observed over the periods of growth in soil uses without grazing or cutting (Table 4). These results are different to those cited by Berbigier et al. (2013) studied ryegrass at different heights of cuts (5,

Table 5. Production of dry matter of forrage and of residual straw, and leaf / stem ratio of white oat cv. URS Guapa grown under different soil uses in the autumn-winter 2010.

Usage soil	Dry matter (kg ha^{-1})	Residual straw (kg ha^{-1})	Leaf / stem ratio
G10	5620^{a}	1364^{c}	0.73^{b}
G20	4708^{c}	1833^{b}	1.04^{a}
C10	5649^{a}	1389^{c}	0.74^{b}
C20	4633^{c}	1861^{b}	1.02^{a}
NC - TS	5289^{b}	6535^{a}	0.81^{b}
NC - CS	5278^{b}	6635^{a}	0.80^{b}
Mean	5196	3270	0.86
CV (%)	1.38	2.00	8.14

Means followed by the same letter in the column do not differ statistically at 5%. G10 and G20: grazing with residue height of 10 and 20 cm, C10 and C20: cutting for haymaking with residue height of 10 and 20 cm; NC - TS and NC-CS: no grazing or cutting with subsequent direct seeding or conventional soil tillage for sowing of the summer crop.

7 and 10 cm) in 3rd periods of cuts, which are drop in CP content, which did not occur in this study, only the soil uses that without grazing or cutting oats. With respect to fiber content of the forage (NDF, ADF, hemicellulose, lignin and cellulose), there was a significant difference only in the 2nd and 3rd periods of growth, and the forage produced on soil uses without grazing or cuts had higher fiber contents than the other uses. When the oat was subjected to cutting or grazing, the values of NDF and ADF were similar in the 1st and 2nd periods and increased in the 3rd period, while a progressive increase was observed in other soil uses (without grazing or cutting) (Table 4).

The hemicellulose content remained constant when the oat was managed at a residue height of 10 cm (G10 and C10), but increased with the grow period of evaluations in other soil uses (G20, C20, NC-TS and NC-CS). An increase in lignin concentrations was observed with growth periods for all soil uses studied, while the cellulose content remained constant over the period when the oat was cut or grazed and increased only in the absence of grazing or cutting.

Changes in the concentration of CP and fibrous components of forage are due to structural changes of plants as the inter-node lengthens, resulting from advancing age and the plant entering the reproductive phase (Langer, 1979). As they grow, forage plant density decreases with the decrease in the proportion of leaves and increase in the proportion of stem/stalk. In other words, there is an increase in the levels of structural compounds (cell wall), such as cellulose, hemicellulose and lignin and, in parallel, a decrease in the cellular content, with the consequent reduction in nutritional value (Van Soest, 1994).

In 2010, the highest production of dry matter was obtained for soil uses when oats were handled with a residual height of 10 cm, either for cutting or grazing, and lower dry matter occurred in soil uses with a waste of time 20 cm is for cutting or grazing (Table 5). The yields obtained are similar to those observed in other studies (Bortolini et al., 2004; Cassol et al., 2011) and are adequate despite the unfavorable climatic conditions during the growing period of the plants (Figure 1). A greater amount of residual straw was obtained with the soil uses without cuts or grazing, and lowest in the oats managed at a residue height of 10 cm (Table 5). The results were expected because the amount of residual straw is directly related to the height of harvested forage and the remaining material.

The structural characteristics studied were not altered by soil uses, except for the leaf/stem ratio, which was higher in soil uses with a residue height of 20 cm (Table 5). This result is due to the components of the harvested forage, because with the increase of the height of the forage crop, the amount of harvested leaves was higher than the number of stems in relation to other soil uses, providing a higher leaf/stem ratio.

The nutritional value of forage produced by URS Guapa oats in 2010 was similar in all soil uses studied, and the average values were 140; 644; 339; 305; 37 and 295 g/kg DM for CP, NDF, ADF, hemicellulose, lignin and cellulose, respectively. The possible lack of difference between the soil uses is linked to the performance of only one review (cutting or grazing). This was due to the weather conditions during the growing period of oats in 2010, where severe water stress, which limited crop growth and low production of dry matter occurred (Figure 1). The low rainfall limit the growth of oats and accelerate its cycle from the vegetative stage to the reproductive phase. Thus, the plants quickly gained height due to a stretching of the inter-nodes, causing a reduction in leaf/stem ratio and a decrease in the nutritional value of the forage produced. This fact is also reported by Ramos Junior et al. (2013) who studied the oats on water deficit

Table 6. Dry matter production of residual straw by white oat cv. IPR 126 under different soil uses in two growth periods in the autumn-winter 2011.

Usage soil	Dry matter production (kg ha^{-1})			Residual straw (kg ha^{-1})		
	Grown period		Total	Grown period		Total
	1º	2º		1º	2º	
G10	4135^{aA}	1853^{bB}	5989^{ns}	1005^{cA}	973^{bA}	1978^{b}
G20	3368^{cA}	1467^{cB}	4835	1239^{bA}	1115^{bA}	2354^{b}
C10	4162^{aA}	1856^{bB}	5892	982^{cA}	981^{bA}	1963^{b}
C20	3392^{cA}	1383^{cB}	4775	1229^{bA}	1131^{bA}	2360^{b}
NC - TS	3757^{bB}	5998^{aA}	5998	4285^{aB}	5998^{aA}	10283^{a}
NC - CS	3751^{bB}	6046a^{A}	6046	4218^{aB}	5980^{aA}	10198^{a}
Mean	3761	3101	3423	2160	2696	
CV1 (%)	4.10			2.32		
CV2 (%)	3.59			2.56		
CV3 (%)	3.50			3.18		

^{ns} Not significant. Means followed by the same lowercase and uppercase in the column on the line do not differ statistically at a 5%. CV1, CV2 and CV3 (%): coefficient of variation factor for the period (P), soil use factor (SU) and interaction PxSU respectively. G10 and G20: grazing with residue height of 10 cm and 20 cm, C10 and C20: cutting for haymaking with residue height of 10 cm and 20 cm; NC - TS and NC-CS: no grazing or cutting with subsequent direct seeding or conventional soil tillage for sowing of the summer crop.

reduction and its characteristics compared to other grasses as millet and sorghum.

In 2011, the interaction of the factors studied had significance on forage and residual straw production and on the structural characteristics of oats, except the tiller density. In the 1st growth period, higher forage dry matter was obtained in soil uses where the oat was managed at a residue height of 10 cm. In the 2nd period, dry matter production was higher in soil uses without grazing or cuts and lower when the plant was managed at a residue height of 20 cm (Table 6). The reduction in dry matter production for use with grazing or cutting is due to the advancement in the phenological cycle of oats, beginning in directing photo-assimilates to the formation of reproductive structures (Langer, 1979). On the other hand, the increase in forage production in the uses without grazing or cutting is due to dry matter accumulation that occurred because forage was not harvested. Total forage production over the 2nd growth periods did not differ between the soil use types. The yields observed during the periods of growth individually, or the accumulated total for the two were similar to those reported in other studies under similar conditions (Bortolini et al., 2004; Cassol et al., 2011).

The production of residual straw was higher in soil use without grazing or cuts in both evaluation periods, but in the 1st period, the soil uses with a residue height of 10 cm resulted in less straw production (Table 6). Higher biomass accumulation was observed in the 2nd period, which could be attributed to biomass accumulation by plants deposited on the soil surface, and was similar to that observed in other studies with various soil uses in the winter (Lopes et al., 2009; Balbinot Junior et al., 2011).

The structural characteristics studied in oats were affected by an interaction of factors, except the tiller density that changed only in accordance with the growth periods (Table 7). In the 1st period of growth, the plant heights were similar in all soil uses, however, plant heights increased in the 2nd period in the absence of grazing or cutting. When they are not grazed or cut, the oat pastures accelerate their development cycle, elongating internodes and showing early flowering (Rocha et al., 2004), which in turn results in higher plant height.

The tiller density was reduced from the 1st to the 2nd growth period due to the death of tillers, which occurs when the reproductive stage is reached and the reallocation of photo-assimilates to the formation of reproductive structures (Castagnara et al., 2010). In periods, the growth and number of leaves per tiller was less for soil use with a residue height of 20 cm. This resulted from the heights of sampling and the methodology used in the evaluation, because only whole and fully expanded leaves were counted.

In the 1st period of growth, a greater final leaf length was obtained in the soil uses with a residual height of 20 cm. In the 2nd period, the soil uses with a residue height of 20 cm gave higher leaf length. Variations in the final leaf length are due to the relationship between the height of sampling and length of leaves at different insertion levels in the tiller. From the base to the apex of the leaf blade, the length increases as the leaves succeed in the tiller (Lemire and Chapman, 1996) resulting in longer leaves at harvest.

Table 7. Structural characteristics of white oat cv. IPR 126 under different soil uses in two growth periods in the autumn-winter 2011.

Usage soil	Plant height (cm) Grown period 1º	Plant height (cm) Grown period 2º	Density of tillers (tillers m^2) Grown period 1º	Density of tillers (tillers m^2) Grown period 2º	Density of tillers Mean	Number of leaves per tiller Grown period 1º	Number of leaves per tiller Grown period 2º
G10	48.29^{aA}	41.43^{bB}	366.25	347.93	357.09^{a}	3.61^{aA}	3.18^{bB}
G20	47.73^{aA}	40.46^{bB}	353.46	308.29	330.87^{a}	2.16^{bA}	2.03^{cA}
C10	48.97^{aA}	40.76^{bB}	360.39	354.04	357.22^{a}	3.37^{aA}	3.06^{bB}
C20	48.50^{aA}	40.64^{bB}	358.56	305.13	331.85^{a}	2.11^{bA}	1.92^{cA}
NC - TS	48.80^{aB}	71.87^{aA}	356.58	292.58	324.58^{a}	3.53^{aB}	4.46^{aA}
NC - CS	48.73^{aB}	72.95^{aA}	349.39	290.92	320.15^{a}	3.37^{aB}	4.31^{aA}
Mean	48.5	51.35	357.44^{A}	316.48^{B}		3.02	3.16
CV1 (%)	2.42		4.41			3.62	
CV2 (%)	4.92		9			10.79	
CV3 (%)	6.65		8.99			2.97	

Usage soil	Final leaf length (cm) 1º	Final leaf length (cm) 2º	Stem diameter (mm) 1º	Stem diameter (mm) 2º	Leaf / stem ratio 1º	Leaf / stem ratio 2º
G10	39.31^{cA}	41.48^{bA}	3.58^{aA}	3.31^{bA}	1.56^{cB}	2.12^{bA}
G20	45.61^{aB}	51.08^{aA}	3.46^{aA}	3.66^{bA}	2.33^{aB}	2.80^{aA}
C10	39.71^{cA}	40.50^{bA}	3.47^{aA}	3.24^{bA}	1.55^{cB}	2.05^{bA}
C20	45.86^{aB}	52.86^{aA}	3.51^{aA}	3.62^{bA}	2.24^{aB}	2.69^{aA}
NC - TS	42.55^{bA}	39.79^{bA}	3.58^{aB}	4.55^{aA}	1.76^{bA}	1.09^{cB}
NC - CS	42.58^{bA}	40.45^{bA}	3.59^{aB}	4.54^{aA}	1.71^{bA}	1.08^{cB}
Mean	42.6	44.36	3.53	3.82	1.86	1.97
CV1 (%)	1.47		5.86		0.65	
CV2 (%)	3.36		4.6		2.1	
CV3 (%)	2.52		5.42		2.1	

Means followed by the same lowercase and uppercase in the column on the line do not differ statistically at a 5%. CV1, CV2 and CV3 (%): coefficient of variation factor for the period (P), soil use factor (SU) and interaction PxSU respectively. G10 and G20: grazing with residue height of 10 and 20 cm, C10 and C20: cutting for haymaking with residue height of 10 and 20 cm; NC - TS and NC-CS: no grazing or cutting with subsequent direct seeding or conventional soil tillage for sowing of the summer crop.

Stem diameter was influenced by soil uses only in the 2nd growth period, being higher in the uses without cutting or grazing, and yet increasing from the 1st to the 2nd growth period (Table 7). The result is due to the development and dry matter accumulation of plants, as the stem diameter increases to the strength required to support the leaves. For the leaf/stem ratio, a characteristic behavior was observed for the heights of the forage crop. Both in the 1st and 2nd periods of growth, the leaf/stem ratio was higher in soil uses with a residual height of 20 cm. For soil uses with grazing or cutting, there was an increase in leaf/stem ratio of forage obtained from the 1st to the 2nd growth period. While a reduction was observed for soil uses no grazing or cutting with subsequent direct seeding or conventional soil tillage for sowing of the summer crop (Table 7). The differences are due to the distribution of extracts of the leaves in the canopy and the heights of the forage crop, which alter the quantities of stem forage. In soil uses without grazing or cutting, the plants accelerated their cycle, and their internodes lengthened early (Rocha et al., 2004), due to the entry into the reproductive phase.

The changes in nutritional value of forage produced by oats IPR 126 in 2011 was consistent with the structural changes observed, and all studied components were altered by the interaction of the factors (Table 8). The CP concentration changed only in the 2nd period of growth being higher in the forage produced on soil uses with a residue height of 20 cm and less in the absence of grazing or cutting. Only the forage produced by oats managed at a residue height of 20 cm did not show a reduction in the concentration of CP from the 1st to the 2nd growth periods (Table 8). This fact is related due to leaf in the forage, then the remaining residue (Table 7).

Concentrations of NDF, ADF, hemicellulose, lignin and cellulose were higher in soil uses without grazing or cutting in comparison to the others only in the 2nd period of growth. However, in this evaluation, all the soil uses favored forage production with higher NDF (Table 8). The other fibrous components of forage studied (ADF, lignin,

Table 8. Nutritive value of white oat cv. IPR 126 under different soil uses in two growth periods in the autumn-winter 2011.

Usage soil	CP (g kg^{-1}) Grown period 1º	CP (g kg^{-1}) Grown period 2º	NDF (g kg^{-1}) Grown period 1º	NDF (g kg^{-1}) Grown period 2º	ADF (g kg^{-1}) Grown period 1º	ADF (g kg^{-1}) Grown period 2º
G10	177.3^{aA}	148.4^{bB}	509.0^{aB}	538.8^{bA}	307.7^{aA}	317.6^{bA}
G20	176.0^{aA}	170.6^{aA}	505.9^{aB}	541.7^{bA}	308.3^{aA}	318.2^{bA}
C10	172.6^{aA}	145.0^{bB}	506.9^{aB}	539.8^{bA}	301.9^{aA}	312.4^{bA}
C20	175.3^{aA}	172.9^{aA}	510.5^{aB}	539.9^{bA}	305.7^{aA}	322.0^{bA}
NC - TS	176.7^{aA}	106.0^{cB}	509.1^{aB}	689.7^{aA}	308.0^{aB}	417.2^{aA}
NC - CS	173.9^{aA}	100.4^{cB}	508.7^{a}B	696.2^{aA}	303.8^{aB}	416.7^{aA}
Mean	175.3	140.5	508.3	591	305.9	350.7
CV1 (%)	3.63		4.86		2.2	
CV2 (%)	5.33		3.27		6.36	
CV3 (%)	3.16		2.59		2.31	

Usage soil	Hemicellulose (g kg^{-1}) 1º	Hemicellulose (g kg^{-1}) 2º	Lignin (g kg^{-1}) 1º	Lignin (g kg^{-1}) 2º	Cellulose (g kg^{-1}) 1º	Cellulose (g kg^{-1}) 2º
G10	201.3^{aA}	221.2^{bA}	44.0^{aA}	45.2^{bA}	289.7^{aA}	295.5^{bA}
G20	197.6^{aA}	223.5^{bA}	41.5^{aA}	43.6^{bA}	282.9^{aA}	295.3^{bA}
C10	205.0^{aA}	227.4^{bA}	41.0^{aA}	43.7^{bA}	290.9^{aA}	289.9^{bA}
C20	204.8^{aA}	217.9^{bA}	45.4^{aA}	44.4^{bA}	282.9^{aA}	288.5^{bA}
NC - TS	201.1^{aB}	272.4^{aA}	42.8^{aB}	55.3^{aA}	289.6^{aB}	338.8^{aA}
NC - CS	204.9^{aB}	279.5^{aA}	44.5^{aB}	55.9^{aA}	283.6^{aB}	334.6^{aA}
Mean	202.4	240.3	43.2	48	286.6	307.1
CV1 (%)	15.15		3.83		5.02	
CV2 (%)	11.85		9.38		8.84	
CV3 (%)	7.63		5.57		4.59	

Means followed by the same lowercase and uppercase in the column on the line do not differ statistically at a 5%. CV1, CV2 and CV3 (%): coefficient of variation factor for the period (P), soil use factor (SU) and interaction PxSU respectively. G10 and G20: grazing with residue height of 10 cm and 20 cm, C10 and C20: cutting for haymaking with residue height of 10 and 20 cm; NC - TS and NC-CS: no grazing or cutting with subsequent direct seeding or conventional soil tillage for sowing of the summer crop.

hemicellulose and cellulose) were higher in the 2nd growth period in the forage managed without cutting or grazing (Table 8). The observed differences in the components of the nutritional value of the forage is due to the types and heights of management imposed on oats in different soil uses, and the advancement in the age of the plants and changes in the proportions of the components of the forage (leaves and stems). The determination of fiber components and CP in fodder crops is essential for the study of their nutritional value. In the analysis of NDF, the total concentration of cellulose, hemicellulose and lignin of the cell wall is estimated, and the concentration of these components in the forage is inversely related to the ability of DM intake by animals (Van Soest, 1994). Besides the limitation in dry matter consumption imposed by the effect of the filler on diets with a high concentration of NDF, depression consumption in ruminants can be attributed to a deficiency of CP (Van Soest, 1994).

In three years of conducting the study (2009, 2010 and 2011) and in all the evaluations performed, the forage produced had CP concentrations greater than 70 g kg^{-1}, which is a value considered as the lower limit for ruminants (Van Soest, 1994). The NDF concentration observed throughout the study remained below the maximum limit recommended for ruminant feed (550 to 600 g kg^{-1}) (Van Soest, 1994), except in the forage produced in 2010 (Table 5) and produced by oats in the soil uses no grazing or cutting with subsequent direct seeding or conventional soil tillage for sowing of the summer crop. In the last grown period in year 2009 (Table 4) and 2011 (Table 8). Thus in 2010 the soil uses no grazing or cutting with subsequent direct seeding or conventional soil tillage for sowing of the summer crop, and in the year 2009 and 2011 is 3rd grown period not produced conditions for the forage produced would not be recommended for the supply to ruminants as an exclusive food diet.

Conclusions

High dry matter production , close to 5000 kg ha - 1 , are obtained with the management of oat with cuts or grazing residual height of 10 or 20 cm , or When taken with a residual height of 15 cm at the end of the cycle. The dry matter production was drastically reduced with cuts or grazing use, not the realization of the 3rd, 2nd or cutting or grazing of livestock farming systems integration that takes place in grain crops in succession being.To obtain forage of higher quality and better production distributed over the autumn- winter oat should be managed with grazing or cutting.The use of grazing or successive cuts in oats provides forage production with higher nutritional value compared to single crop at the end of the cycle.

Conflict of Interests

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

REFERENCES

Aguinaga AAQ, Carvalho PCF, Anghinoni I, Pilau A, Aguinaga AJQ, Gianluppi GDF (2008). Morphological components and forage production of oat (*Avena strigosa,* Schreb) and annual ryegrass (*Lolium multiflorum,* Lam) pasture managed at different heights. R. Bras. Zootec. 37(2):1523-1530.

Balbinot Junior AA, Veiga M, Moraes A, Pelissari A, Mafra AL, Piccolla CD (2011). Winter pasture and cover crops and their effects on soil and summer grain crops. Pesq. agropec. bras. 46(1):1357-1363.

Berbigier C, Bicca AMO, Menezes FP, Berbigier P (2013). Influence of diferente Heights of cut in dry matter production and protein contente in gross ryegrass. Rev. FZVA. 19(1):33-42.

Bortolini PC, Sandini I, Carvalho PCF, Moraes A (2004). Winter Cereals Submitted to Cuts in Double Purpose System. R. Bras. Zootec. 33(3):45-50.

Carvalho PCF, Rocha LM, Baggio C, Macari S, Kunrath TR, Moraes A (2010). Structural and productive characteristic of oat and ryegrass mixed pastures managed in four intensities with variable stocking rate. R. Bras. Zootec. 39(5):1857-1865.

Cassol LC, Piva JT, Soares AB, Assmann AL (2011). Yield and structural composition of oat and ryegrass subjected to different periods of cutting and nitrogen fertilization. Rev. Ceres. 58(1):438-443.

Castagnara DD, Radis AC, Souza FH, Neres MA, Mesquita EE (2010). Structural and productive characteristics of the common black oats in five regrowth ages in the West of Paraná. Cultiv. o Saber. 3(4):116-129.

Confortin ACC, Rocha MG, Quadros FLF, Glienke CL, Rossi GE, Moraes AB (2010). Structural and morphogenical characteristics of black oats and Italian ryegrass on pasture submitted to two grazing intensities. R. Bras. Zootec. 39(11):2357-2365.

Langer RHM (1979). How grasses grow, London, Edward Arnold, pp. 60.

Lemaire G, Chapman D (1996). Tissue flows in grazed plant communities. In: Hodgson J, Illius AW. (eds). The ecology and management of grazing systems, Wallingford, CAB International, pp. 3-36.

Lopes ML, Carvalho PCF, Anghinoni I, Santos DT, Aguinaga AQ, Flores JPC, Moraes A (2009). Crop-livestock integration system: effect of oat and italian ryegrass sward height management on soybean yield. Ciênc. Rural. 39(3):1499-1506.

Meinerz GR, Olivo CJ, Fontaneli RS, Nornberg JL, Agnolin CA, Scheibler RB, Horst T, Fontaneli RS (2011). Nutritive value of forage of genotypes of double purpose winter cereals. R. Bras. Zootec. 40(6):1173-1180.

Mott GE, Lucas HL (1952). The design, conduct en interpretation of grazing trials on cultivated and improved pastures. In: INTERNATIONAL GRASSLAND CONGRESS, Pensilvania, Proceedings. Pensilvania: State College: pp. 1380-1395.

Ramos Junior EU, Machado RAF, Olibone D, Castoldi G, Ramos BM (2013). Cover crops growth under water déficit. Semina: Ciências Agrárias 34(1):47-56.

Rocha MG, Montagner DB, Santos DT, Freitas FK, Pilau A, Frizzo A (2004). Productive parameter of a winter pasture submitted to alternatives of utilization. R. Bras. Zootec. 33(2):1386-1395.

Silva DJ, Queiroz AC (2006). Food Analysis: chemical methods and biological. UFV, P. 235.

Skonieski FR, Viégas J, Bermudes RF, Norberg JL, Ziech MF. Rocha OAD, Meinerz GR (2011). Botanic and structural composition and nutritional value on intercropped ryegrass pastures. R. Bras. Zootec. 40(3):550-556.

Tonate F, Pedreira BC, Pedreira CGS, Pequeno DNL (2014). Black oats and anual ryegrass havested by ligth intercepitation or fixed rest interval in integrates crop-livestck systems in São Paulo State, Brasil. Ciênc. Rural. 44(1):104-110.

Van Soest PJ (1994). Nutritional ecology of the ruminant, Ithaca, Constock Publishing Associates, P. 476.

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