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African Journal of Agricultural Research

ARTICLES

Full Length Research Paper

Chemical composition of processed baru (*Dipteryx alata* Vog.) almonds: Lyophilization and roasting

Rodrigo Martins Fraguas*, Anderson Assaid Simão, Renato Leal Silva, Claudia Mendes dos Santos, Denise Alvaranga Rocha, Tassia Silva Tavares, Tamara Rezende Marques, Mariene Helena Duarte, Silvana Marcussi and Celeste Maria Patto de Abreu

Department of Chemistry, Biochemistry Laboratory, Federal University of Lavras - UFLA, P.O. Box 3037, Zip Code 37200.000, Lavras, MG, Brazil.

The objective of this study was to evaluate percent and mineral composition, protein digestibility, bioactive compounds (phenols and flavonoids), total soluble solids (TSS), titratable acidity, pH, profiles of fatty acids (FA) and proteins, and the organic acids of baru (*Dipteryx alata* Vog.) almonds, subjected to different treatments (lyophilization and roasting), aiming to determine which process results in a better use of the almond constituents, adding value to the fruit. High levels (g 100 g^{-1} dry matter - DM) were found for proteins: 32.04 and 36.08; lipids: 34.50 and 36.67; dietary fiber: 21.55 and 21.46; and for the following minerals (mg 100 g^{-1} DM); phosphorus: 652.35 and 703.14; and iron: 8.42 and 9.51, respectively, in lyophilized and roasted almonds. Among the FA, oleic and linoleic acids were the major ones, as well as citric acid among the organic acids, both in lyophilized and in roasted almonds. The roasting process increased the levels of lipids, proteins and of the minerals: phosphorus, calcium, magnesium, copper, zinc and iron. However, it resulted in a decrease in the levels of phenolic compounds, flavonoids, protein digestibility and in the number of absorption bands and proteins shown in the electrophoretic profile.

Key words: Chemical characterization, lyophilization, roasting, *Dipteryx alata* Vog.

INTRODUCTION

Cerrado is characterized by a great biodiversity; however, this biome is among the most threatened ecosystems in the world (Zaidan and Carreira, 2008; Santos et al., 2012). Over the past 30 years, extensive cattle ranching, monocultures and the opening of roads destroyed much of this ecosystem. According to the Brazilian Institute of Environment, only 20% of the *Cerrado* area remains

without major changes (Vera et al., 2009).

Numerous *Cerrado* species are used as food, for medicinal purposes, or for the production of handicrafts. The appreciation of native species can be encouraged through the research of their capabilities and proper management, thus contributing to profitably add value to native fruits and to the preservation of the biome

*Corresponding author. E-mail: rodrigomfraguas@hotmail.com, Tel: +55-35-3829-1272.Fax: +55-35-3829-1893.

(Aquino et al., 2007).

Baru (*Dipteryx alata* Vog.) is a fruit that belongs to the Leguminosae family, widespread in the *Cerrado* biome, which is part of the group of native species used by the regional population as a source of family income (Sano et al., 1999). It is one of the most promising species for cultivation, due to its multiple uses, such as in food, wood, medicine, industry, landscaping, and in the recovery of degraded areas (Alves et al., 2010). It has drawn the attention of researchers, due to its nutritional quality and health benefits.

The sustainable use of baru is being enhanced through various farmers' associations, regional community organizations and agroextractivist cooperatives. The use of baru almond for commercial purposes has been valued in the state of Goiás (Brazil) (Rocha and Santiago, 2008), but studies of national and international scope are still scarce.

Baru almonds stand out for their high content of proteins, insoluble fiber (Takemoto et al., 2001) and the minerals potassium, magnesium, phosphorus and zinc (Sousa et al., 2011). The oil from the seeds is composed of more than 75% unsaturated fatty acids (FA) (Vera et al., 2009), among which a substance that inhibits melanin was isolated, which demonstrates the presence of medicinal properties in this fruit (Sano et al., 1999).

The fruit of baru is used in the production of cereal bars, breads, cookies, liquors and oil extraction. In addition, there is the possibility of using barueiro in areas to be recovered, such as springs and riverbanks, because it can favor the conservation and maintenance of other associated species of flora and/or fauna (Sano et al., 1999).

The availability of highly rich sources of protein, dietary fiber, unsaturated FA and minerals, can add value to the exploration of baru, since it allows its use in different industrial products. Research has shown that processing conditions, such as roasting, improve the availability of certain nutrients, flavor, texture, besides inhibiting the action of antinutritional factors present in foods (Silva, and Fernandes, 2011).

Considering the importance of research into new products as an alternative to meet the market demand and the need to preserve native species of the Brazilian *Cerrado*, the objective of this study was to analyze the chemical composition of lyophilized and roasted baru almonds, aiming to expand the knowledge about nutritional compounds, justifying and disseminating the spread and use of this native species, besides investigating changes in its composition, resulting from processing.

MATERIALS AND METHODS

Sample collection and preparation

Baru (*D. alata* Vog.) almonds were purchased at the local market in the town of Jataí, in the south of the state of Goiás, in five replications, and transported to the laboratory. A part of the fresh almonds was lyophilized for 24 h, and the other part was subjected to a roasting process in an electric oven, at a temperature of 150°C for 30 min. They were then peeled and crushed in a cooled mill, and all the samples were placed in hermetically sealed flasks in a freezer at -18°C.

Percent composition

Moisture contents were determined in an oven at 105°C, until constant weight. The ether extract was determined using a Soxhlet continuous extractor. The crude protein was measured by the Kjeldahl method, using the conversion factor of 6.25 (N × 6.25). Ash and fixed mineral residue were obtained from a defined quantity of samples by incineration (550°C) in a muffle furnace, thus determining the percentage of residue. Total, soluble and insoluble dietary fibers were determined by the enzymatic method. The nitrogen-free extract was determined by the difference between 100 and the sum, in dry matter, of ether extract, protein, ash and total dietary fiber. These analyzes of percent composition were performed using the methodology described by the Association of Official Analytical Chemists (AOAC, 2005).

In vitro protein digestibility

The sample (with known nitrogen content) was subjected to digestion by the enzyme pepsin and then by pancreatin in their optimum pH, and the digestion was stopped by the addition of trichloroacetic acid. Then, the samples were centrifuged at 10,000 × g for 15 min, and the content of nitrogen was dosed in the supernatant. Casein was used as a standard (Akeson and Stahmann, 1964). The value obtained for casein digestibility was considered as 100%, and the digestibility values obtained for the samples were calculated based on the value obtained for casein.

Mineral composition

In order to quantify the minerals (Fe, Zn, Mn, Cu, Ca, Mg, P, K and S), the samples were subjected to a nitroperchloric digestion in digester blocks with temperature control. P and S were determined by colorimetry, K by flame photometry and Ca, Mg, Cu, Mn, Zn and Fe by atomic absorption spectrophotometry. For all analyzes, the procedures described by Malavolta et al. (1997) were used.

Phenolic compounds

The extraction of phenolic compounds was carried out with 50% methanol, under reflux for three consecutive times, at 80°C, and the extracts were collected, evaporated up to 25 ml and submitted to phenolic compound measurement, using the Folin-Denis reagent, and tannic acid as a standard (AOAC, 2005).

Total flavonoids

The contents of total flavonoids were measured using the same extracts used in the phenolic compound analyses, using the aluminum chloride colorimetric method, with catechin used as a standard (Zhishen et al., 1999).

Total soluble solids (TSS), total titratable acidity (TTA) and pH

The contents of TSS were determined according to the

methodology proposed by AOAC (2005), and the percentage of moisture which was lost in lyophilization was returned to the samples. Homogenization was performed in a polytron, the filtration of the samples in an organza fabric and readings were performed in a 121 Homis digital refractometer. The results were expressed as °Brix.

For the determination of TTA and pH, after moisture was returned, 40 ml of distilled water were added, followed by homogenization in polytron and filtration in an organza fabric. After the determination of pH in a digital pH meter, the samples were titrated with 0.1 mol L^{-1} NaOH, using phenolphthalein as an indicator, until the samples reached pH = 8.1. Calculations were made considering the weight of sample used, the volume of 0.1 mol L^{-1} NaOH spent and the number of gram equivalent of citric acid. The results were expressed in g citric acid 100 g^{-1} sample (AOAC, 2005).

Organic acids

The extraction of organic acids for chromatographic analysis was carried out with 1 g sample in 50 ml ultra pure water, under agitation, for 45 min and, subsequently, filtered through Whatman No. 40 paper. An LC 200 A Shimadzu liquid chromatograph was used, as well as a conductivity detector (CDD-6A), + polarity, using a SHIM-PACK SPR-H(G) pre-column (50 × 7.8mm) and two SHIM-PACK SPR-H columns in series (250 × 7.8 mm). The injection volume used was 20 µl. The mobile phase used was 4 mmol L^{-1} p-toluenesulfonic acid, with a flow of 0.8 ml/min and 45°C. Peaks corresponding to each acid were identified by the retention time, using the retention times of the standards as a comparison.

Fatty acid (FA) profile

Lipids were extracted according to the methodology proposed by Bligh and Dyer (1959), and esterification was performed using the methodology by Joseph and Ackman (1992).

The composition of FA was determined by gas chromatography, and the chromatograph GC-2010 (Shimadzu) was used, equipped with a flame ionization detector and a fused silica capillary column (100 m long, 0.25 mm internal diameter), containing polyethylene glycol as a liquid stationary phase. The standard used was a mixture of 37 methyl esters (SupelcoTM 37 Component FAME Mix), from C:4 to C22:6, with a purity of 99.9%.

The following operating parameters were used: "split" injection mode, split ratio 1:100; injected volume: 1 µl; detector and injector temperature: 260°C; temperature program: 4°C/min up to 140°C, remaining at this temperature for 5 min, keeping the heating ramp in 4°C/min up to 240°C, remaining at this temperature for 30 min.

In order to perform the gas chromatography, it was necessary to redissolve the samples in 0.50 ml hexane.

The identification of the peaks was performed by a comparative method with the retention times of the standard FA esters, and the results were performed by integration of the peak areas and expressed in area percentage.

Electrophoresis on polyacrylamide gel under reducing conditions

The protein profile of the defatted fractions, obtained from lyophilized and roasted samples, was observed in electrophoresis on polyacrylamide gel (Acrylamide:bis-acrylamide; 19:1) in the presence of sodium dodecyl sulfate (SDS). The extraction was carried out with 50 mg sample in 1 ml phosphate buffered saline (PBS), under agitation for 60 min, followed by centrifugation at 10,000 × g for 20 min. Different volumes (5 to 25 µl) of the supernatant were subjected to an electrophoretic run. The samples were prepared under reducing conditions (boiling at 98°C for 5 min in a β-mercaptoethanol solution); 0.5 mol L^{-1} Tris-HCl (pH 6.8); glycerol; 10% SDS (w/v); 0.1% bromophenol blue (w/v)) for use in 12% polyacrylamide gel, and the electrophoresis was performed in 0.025 mol L^{-1} Tris running buffer; 0.192 mol L^{-1} glycine; 0.1% SDS (pH 8.3) for 4 h under a current of 80 V.

The pattern of relative mass (Mr) of BIO RAD (α-lactalbumin, trypsin inhibitor, carbonic anhydrase, egg albumin, bovine albumin and phosphorylase β) underwent the same electrophoresis, enabling the calculation of the Mr_s of the protein fractions in the samples. The bands that characterize the protein profile of the samples were stained with Coomassie blue G-250, prepared in the ratio 0.2% dye to 20% acetic acid. The gel was reproduced on Scanner and, with the migration distance of the standards, a graph in logarithmic function of the Mr_s was prepared.

Statistical analysis

Data are the mean of five replicates ± standard deviation and were statistically evaluated by analysis of variance, and the means were compared using the Scott Knott test ($P < 0.05$) with the aid of the R software (R Development Core Team, 2011).

RESULTS AND DISCUSSION

The results of percent composition and protein digestibility of lyophilized and roasted baru almonds are shown in Table 1.

The lyophilized almond showed lower contents of lipids and proteins, and the contents of ash and dietary fiber were similar, in relation to the roasted almond (Table 1).

Lipid contents in lyophilized and roasted almonds were higher than those found by Vera et al. (2009) (33.28 g 100 g^{-1}) and lower than those reported by Sousa et al. (2011) (41.25 g 100 g^{-1}) and Takemoto et al. (2001) (38.40 g 100 g^{-1}); these studies were conducted with baru almonds, without any treatment. The differences between these studies may be due to several factors, such as harvest regions, maturity stage of the almonds, climate, soil, experimental conditions, among others.

The protein contents of the lyophilized (32.04 g 100 g^{-1}) and roasted (36.08 g 100 g^{-1}) almonds were higher than those observed in other studies with these almonds, whose contents ranged from 23.90 to 29.60 g 100 g^{-1} (Togashi and Sgarbieri, 1994; Takemoto et al., 2001; Vera et al., 2009); these differences are possibly related to different environmental and genetic conditions, as well as to experimental conditions (sample preparation).

The protein contents of the almonds in this study are higher than other "almonds", such as Brazil nuts (14.00 to 16.00 g 100 g^{-1}), pine nuts (13.00 g 100 g^{-1}), pecan (9.00 g 100 g^{-1}), cashew nuts (17.50 g 100 g^{-1}), hazelnuts (14.50 g 100 g^{-1}), and pistachio (20.00 g 100 g^{-1}) (Yang, 2009), emphasizing the nutritional value of baru.

When comparing, in dry matter, 100 g of baru almond flour with 100 g of black (21.30 g) and purple (22.20 g) beans, which are sources of vegetable proteins (Brazilian Food Database, 2011), it is observed that the flours from

Table 1. Percent composition and protein digestibility, in g 100 g^{-1} DM, of lyophilized and roasted baru almonds.

Variable	Lyophilized baru	Roasted baru
Ether extract	34.50 ± 0.23[b]	36.67 ± 0.82[a]
Crude protein	32.04 ± 0.77[b]	36.08 ± 0.47[a]
In vitro digestibility	20.45 ± 0.29[a]	16.23 ± 0.13[b]
Ash	2.73 ± 0.13[a]	2.65 ± 0.16[a]
Insoluble fiber	20.74 ± 2.33[a]	20.41 ± 0.68[a]
Soluble fiber	0.81 ± 0.13[a]	1.05 ± 0.11[a]
Total fiber	21.55 ± 2.10[a]	21.46 ± 0.18[a]
[1]NFE	9.18 ± 0.90[a]	3.14 ± 0.27[b]

Data are the mean of five replicates ± standard deviation. Same letters in rows do not differ by the Scott-Knott test (P < 0.05). [1]NFE: Nitrogen-free extract. Moisture contents of baru almonds, in g 100 g^{-1}: Lyophilized = 2.35 ± 0.10; Roasted = 6.86 ± 0.09.

these almonds had higher protein levels. Since some population groups still have a diet with limited access to animal protein, the consumption of alternative plant sources, rich in protein and high in nutritional value, may be preventive or palliative measures in the treatment of nutritional deficiencies.

Due to the high contents of proteins found in this study, an evaluation of the *in vitro* protein digestibility of the samples was performed, and the values 20.45 and 16.23 g 100 g^{-1} were obtained for the lyophilized and roasted sample, respectively, in relation to the standard protein (casein). This result shows that the roasting process reduces the digestibility of the proteins present in baru almond. Protein digestibility is a very important nutritional parameter, because it evaluates the use of a protein source, thus providing a measure of the susceptibility of the protein to proteolysis. This characteristic may be influenced by heat treatment, presence of polyphenols, trypsin inhibitors and lectins, among others.

The ash contents of baru almonds were similar to those reported in the literature, which range from 2.70 to 3.18 g 100 g^{-1} (Togashi and Sgarbieri, 1994; Takemoto et al., 2001; Sousa et al., 2011).

The studied samples showed similar levels of total dietary fiber in both treatments, and insoluble fiber was, on average, 19 times higher than soluble fiber. The levels of total dietary fiber in the samples are superior to those mentioned in the literature, in studies with baru almonds, whose contents ranged from 9.21 to 19.00 g 100 g^{-1} (Togashi and Sgarbieri, 1995; Takemoto et al., 2001; Sousa et al., 2011). Baru almonds are rich in fiber, which are important in both the prevention and treatment of various diseases, such as diabetes, obesity, among others, and their consumption favors a diet of better nutritional quality.

The nitrogen-free extract or glycidic fraction is made up mainly of sugars. Thus, the highest content was found in the lyophilized almond (9.18 g 100 g^{-1} DM), which shows that roasting reduces sugar levels in almonds. This

reduction may have occurred due to their degradation and reactions with other compounds during roasting. Pyrolysis of carbohydrates (thermal dehydration) is an example of a reaction that occurs during roasting.

Table 2 shows the mineral contents of lyophilized and roasted baru almonds. The roasted seeds showed higher levels of phosphorus, calcium, magnesium, copper, zinc and iron, in relation to lyophilized baru seeds, and similar to other minerals. The contents of phosphorus, potassium, magnesium, copper, manganese, zinc and iron of the two samples were higher than those recorded by Takemoto et al. (2001), in a study with baru almonds, who found the following contents for these minerals, in mg 100 g^{-1} DM: phosphorus (358.00); potassium (827.00); magnesium (178.00); copper (1.45); manganese (4.90); zinc (4.10) and iron (4.24); however, a lower calcium content (140.00) was observed.

When compared to the contents observed by Vera et al. (2009), in a study with baru almonds from eleven regions in the state of Goiás, the values obtained in the present study were higher, in relation to the minerals (in mg 100 g^{-1} DM) potassium (920.00); magnesium (130.00); copper (1.67); manganese (5.72) and zinc (2.36); and lower in relation to phosphorus (730.00); calcium (300.00); sulfur (410.00) and iron (19,81). The differences between the contents of minerals may be associated with factors, such as differential soil composition, degree of ripeness of the seeds, season of the year when harvest was performed, among others.

Considering the recommended daily allowance (RDA), according to the Dietary Reference Intakes (DRI, 2001) of minerals for 19 to 50 year-old adults (phosphorus: 700 mg; calcium: 800 mg; magnesium: 260 mg; copper: 9 mg; manganese: 23 mg; zinc: 11 mg and iron: 8 mg), roasted baru almonds in the amount of 100 g day^{-1} would supply the need for the minerals phosphorus, magnesium and iron, while lyophilized seeds would supply the need for iron.

The iron levels observed in this study are superior to

Table 2. Mineral composition, in mg 100 g^{-1} DM, of lyophilized and roasted baru almonds.

Mineral	Lyophilized baru	Roasted baru
Phosphorus	652.35 ± 21.79[b]	703.14 ± 21.75[a]
Potassium	1,248.20 ± 65.37[a]	1,252.31 ± 121.65[a]
Calcium	71.91 ± 0.00[b]	102.65 ± 0.00[a]
Magnesium	231.14 ± 7.26[b]	277.15 ± 0.00[a]
Sulfur	354.42 ± 7.26[a]	343.87 ± 7.26[a]
Copper	1.82 ± 0.04[b]	2.30 ± 0.01[a]
Manganese	6.11 ± 0.05[a]	6.13 ± 0.08[a]
Zinc	6.31 ± 0.14[b]	7.50 ± 0.08[a]
Iron	8.42 ± 0.05[b]	9.51 ± 0.04[a]

Data are the mean of five replicates ± standard deviation. Same letters in rows do not differ by the Scott-Knott test (P < 0.05). Moisture contents of baru almonds, in g 100 g^{-1}: Lyophilized = 2.35 ± 0.10; Roasted = 6.86 ± 0.09.

Table 3. Phenolic compounds, flavonoids, TSS, TTA, pH and organic acids of lyophilized and roasted baru almonds.

Variable	Lyophilized baru	Roasted baru
Phenolic compounds (mg 100 g^{-1} DM)	326.35 ± 14.44[a]	228.24 ± 14.72[b]
Flavonoids (mg 100 g^{-1} DM)	9.63 ± 1.73[a]	1.61 ± 0.09[b]
TSS (ºBrix)	3.26 ± 0.05[a]	2.47 ± 0.06[b]
TTA (g citric acid 100 g^{-1} sample)	5.85 ± 0.01[a]	5.85 ± 0.02[a]
pH	6.59 ± 0.05[a]	6.50 ± 0.07[a]
Maleic (µg g^{-1} DM)	149.70 ± 7.11[b]	188.67 ± 10.01[a]
Citric (µg g^{-1} DM)	381.43 ± 17.10[b]	500.70 ± 21.14[a]
Quinic (µg g^{-1} DM)	65.75 ± 2.31[a]	0.00[b]
Succinic (µg g^{-1} DM)	0.00[b]	1.45 ± 0.08[a]
Lactic (µg g^{-1} DM)	0.44 ± 0.01[a]	0.00[b]

Data are the mean of five replicates ± standard deviation. Same letters in rows do not differ by the Scott-Knott test (P < 0.05). Moisture contents of baru almonds, in g 100 g^{-1}: Lyophilized = 2.35 ± 0.10; Roasted = 6.86 ± 0.09.

various foods popularly referred to as sources of iron and described in Brazilian Food Database (2011), such as cooked beets (2.13 mg), braised collard greens (2.70 mg), braised spinach (4.48 mg), raw lentils (7.91 mg) and raw beans (black - 7.64 mg), highlighting the potential use of baru almonds as a food supplement.

The contents of phenolic compounds, flavonoids, TSS, TTA, pH and organic acids in lyophilized and roasted baru almonds are described in Table 3.

The roasting process decreased the contents of phenolic compounds (30%) and flavonoids (83.28%) in the samples, in relation to the lyophilized sample. Such reduction may have occurred due to factors associated with processing, since heating can lead to the degradation of this class of compounds.

Several epidemiological studies show that phenolic compounds have multiple biological effects, such as antioxidant, anti-allergic, anti-inflammatory, anti-bacterial, antithrombotic, cardioprotective and vasodilatory (Rao, 2003; Balasundram et al., 2006; Silvério et al., 2013). Several natural antioxidants have been isolated from different plant materials, such as oilseeds, cereals, legumes, fruits, leaves, roots and herbs (Ramarathnam et al., 1995). However, studies that evaluate the antioxidant activity of seeds of tropical and subtropical fruits have been rarely reported, suggesting the need for studies with these fruits, since this is a vast field to be explored.

The lyophilized sample showed a higher content of TSS (3.26° Brix) than the roasted one (2.47° Brix), which is consistent with the contents of the glycidic fraction, that were lower in roasted samples (Table 1). No significant difference between the values of TTA (5.85 and 5.85) and pH (6.50 and 6.59) was observed in lyophilized and roasted almonds, respectively (Table 3).

The determination of organic acids in lyophilized and roasted baru almonds showed citric acid as a major

Table 4. FA composition (%) of lyophilized and roasted baru almonds.

FA	Lyophilized baru	Roasted baru
C14:0	0.17 ± 0.01^a	0.17 ± 0.01^a
C15:0	0.16 ± 0.01^b	0.96 ± 0.01^a
C16:0	7.70 ± 0.02^a	7.73 ± 0.03^a
C18:0	4.93 ± 0.30^a	4.62 ± 0.02^a
C18:1n9c	49.81 ± 1.01^a	45.66 ± 2.03^b
C18:1n9t	0.08 ± 0.01^b	0.57 ± 0.01^a
C18:2n6	25.25 ± 1.03^a	22.90 ± 1.31^a
C20:0	0.94 ± 0.02^a	0.94 ± 0.01^a
C18:3n3	2.39 ± 0.03^a	1.82 ± 0.03^b
C20:3n3	2.36 ± 0.04^b	2.66 ± 0.02^a
C20:4n6	1.87 ± 0.04^a	1.80 ± 0.01^b
C20:4n6	1.80 ± 0.20^b	2.46 ± 0.04^a
C20:5n3	1.89 ± 0.20^b	2.39 ± 0.06^a
C22:5n3	0.42 ± 0.01^b	1.06 ± 0.01^a
C22:6n3	0.30 ± 0.01^b	0.33 ± 0.01^a
∑SFA	13.90 ± 1.00^a	14.42 ± 0.03^a
∑MUFA	49.89 ± 2.13^a	46.23 ± 3.02^a
∑PUFA	36.28 ± 2.45^a	35.42 ± 3.01^a
PUFA/SFA	1.37 ± 0.03^a	1.30 ± 0.01^b
ω-6 /ω-3	3.92 ± 1.00^a	3.28 ± 0.13^a

Data are the mean of five replicates ± standard deviation. Same letters in rows do not differ by the Scott-Knott test ($P < 0.05$). ∑SFA = sum of saturated FA; ∑MUFA = sum of monounsaturated FA; ∑PUFA = sum of polyunsaturated FA; PUFA/SFA = ratio between the sum of polyunsaturated and saturated acids; ω-6 /ω-3 = ratio between the sum of omega-6 and omega-3. Moisture contents of baru almonds, in g 100 g^{-1}: Lyophilized = 2.35 ± 0.10; Roasted= 6.86 ± 0.09.

component in both samples, and the roasted sample showed the highest content (500.70 µg g^{-1} DM), which was also observed for maleic acid. Quinic acid was observed at a low concentration in the lyophilized sample, and was not detected in the roasted sample. It is believed that, during the roasting process, the temperature rise has promoted deacetylation and subsequent loss of water molecules, and its degradation product was not observed in the roasted sample, due to the absence of the pattern (Table 4).

The emergence of succinic acid in the sample that underwent the roasting process can be attributed to the hydrogenation process since, even after lyophilization, the sample retained a small amount of water, as well as the low concentration of lactic acid, a fermentation product, observed in the lyophilized sample, also reflects the presence of significant amounts of water in the sample.

Fifteen FA were detected in lyophilized and roasted baru almonds, with oleic (C18:1n9c; 49.81 and 45.66%) and linoleic (C18:2; 25.25 and 22.90%) as the major ones, respectively (Table 4). It was also observed that the roasting process for some FA induced an increase in their contents and, for others, a reduction, but without statistical significance.

The total amount of FA was, on average, 13.90 and 15.00% for saturates, 49.89% and 48.12 for monounsaturates and 36.28 and 36.86% for polyunsaturates, for lyophilized and roasted baru almonds, respectively. On the other hand, the ratio polyunsaturated/saturated (PUFA/SFA) was, on average, 1.37 and 1.30, and ω-6/ω-3, 3.92 and 3.28, with a higher percentage of unsaturated FA, compared to the saturated ones.

The contents of oleic and linoleic acid, in the samples of this study, were similar to those found in other studies, such as those conducted by Takemoto et al. (2001) (oleic, 50.40%; linoleic, 28.00%) and Vera et al. (2009) (oleic, 47.15%; linoleic, 25.51%), which also describe oleic and linoleic acids as the majority in baru almonds.

Diets that present the ratio PUFA/SFA superior to 0.45 and the ratio ω-6/ω-3 inferior to 5 (Simopoulos, 2002) are considered healthy for humans, from a nutritional point of view. Considering that the FA composition of lyophilized and roasted baru almonds was within the recommendations, these seeds can be considered good dietary sources.

The electrophoretic profile on polyacrylamide gel with samples of defatted baru flour, lyophilized and roasted, prepared under reducing conditions (with the addition of

Mr (KDa)
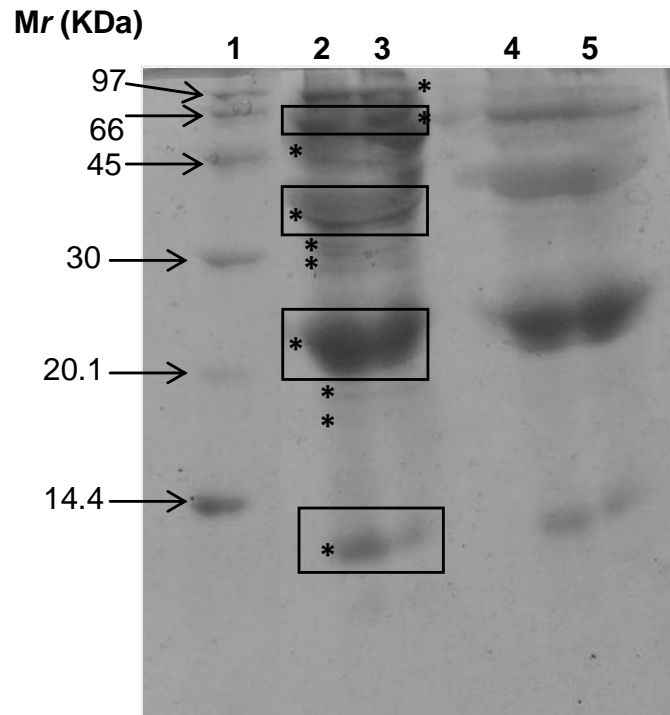
Figure 1. Electrophoresis on polyacrylamide gel in the presence of SDS (SDS-PAGE). Samples prepared under reducing conditions: **1-** Molecular weight standard (α-lactalbumin, 14.4 KDa; trypsin inhibitor, 20.1 KDa; carbonic anhydrase, 30 KDa; egg albumin, 45 KDa; bovine albumin, 66 KDa; phosphorylase β, 97 KDa; **2-** Lyophilized baru (10 μl); **3-** Lyophilized baru (20 μl); **4-** Roasted baru (10 μl); **5-** Roasted baru (20 μl). *Bands considered for the calculation of the values of relative mass in protein profiles. Boxes indicate the majority bands, highlighting a higher content of proteins with high and medium molecular weight.

β-mercaptoethanol and boiling) is shown in Figure 1, and the Mr values, calculated based on the migration distances of proteins of the molecular weight standard, are shown in Table 5.

Observing Figure 1 and based on the intensity of staining, it is possible to note a larger amount of proteins with a molecular weight between 97 and 20 KDa. Although protein quantitation has resulted in higher values for roasted samples, the electrophoretic profile highlights a smaller number of types of protein molecules and in a smaller amount in the roasted sample, compared to the lyophilized, since a smaller amount of bands is observed and they still have a less intense staining. It is suggested that the roasting process has resulted in fragmentation of proteins and small molecules, possibly with a Mr inferior to 4.0 KDa, as well as with free amino acids, which could not be observed on the electrophoresis gel, although they are detectable by the protein quantitation method, since it quantifies the nitrogen present in the samples.

Table 5 shows values calculated based on the migration distances of proteins in each sample evaluated

(lyophilized baru and roasted baru); however, the small changes observed in molecular weight correspond to methodological problems related to the run, for example, very high voltage, with characteristic bands that can be visualized in both profiles (Figure 1), corresponding to the same types of protein molecules.

Conclusion

D. alata Vog. (baru) almonds constitute a significant source of lipids, proteins, dietary fiber and minerals, besides having some bioactive compounds that provide health benefits. Thus, the use of this almond is suggested to enrich the diet, and it can also be used in the preparation of various food products.

The roasting process resulted in samples containing higher levels of lipids, proteins, phosphorus, calcium, magnesium, copper, zinc and iron. However, it led to a decrease in the levels of phenolic compounds, flavonoids, protein digestibility and number of types and amount of proteins from the protein profile observed on

Table 5. Migration distance and relative mass (M*r*) of the protein profile of lyophilized and roasted baru almonds.

Lyophilized baru		Roasted baru	
Migration distance (cm)	M*r* (KDa)	Migration distance (cm)	Mr (KDa)
9.31	14.72	nd	nd
6.32	19.01	nd	nd
5.37	21.17	8.84	14.23
3.86	26.34	4.97	22.38
3.57	26.75	nd	nd
3.36	28.87	3.68	27.18
2.75	32.92	nd	nd
1.75	44.42	1.77	44.09
1.19	57.32	0.90	68.94
0.50	101.66	0.45	108.99

nd: Not detected.

the electrophoresis gel.

The knowledge of these chemical constituents and of different ways of processing this almond contributes to its better use, either by the population or by the food industry, resulting in a greater use and economic value of this fruit from the Brazilian *Cerrado*.

Conflict of Interests

The authors have not declared any conflict of interests

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Full Length Research Paper

Evaluation of plasma minerals vis-a-vis physiological status and seasonal variation in goats of Shuhama Alusteng area of Kashmir valley

A. A. Dar[1]*, R. K. Jadhav[1], F. A. Rather[2], S. Bashir[2], U. Dimri[1], A. A. Khan[2], S. A. Wani[2] and M. C. Sharma[1]

[1]Division of Veterinary Medicine, Indian Veterinary Research Institute, Izatnagar Bareilley, UP-243122, India.
[2]Faculty of Veterinary Sciences and Animal Husbandry, Shuhama Alusteng, Srinagar- 190006, India.
[3]Division of Veterinary Epidemiology and Preventive Medicine, FVSc and AH, SKUAST-Kashmir, India.

Mineral disorders can have a great impact on animal performance and to draw optimum production the deficiency gap needs to be abridged. Regular assessment of livestock vis-a-vis physiological demands and/or geo-climatic variations is an important management strategy to overcome severe economic losses. A study was undertaken to evaluate the plasma mineral profile of goats in Shuhama Alusteng area of Kashmir valley, aimed to set a platform for formulation of area- and species-specific mineral supplement(s). A total of 114 blood samples from goats belonging to different physiological states were collected in four different seasons of the year. The plasma macro-minerals like Ca, P and Mg were measured using standard kits, while micro-minerals like Cu, Zn and Fe were measured using atomic absorption spectrophotometer (AAS). Young stock revealed significantly higher Ca, but lower Mg status. Also, Ca level was lower throughout the year except autumn, whereas Mg was lower in winter and spring seasons. Cu and Zn concentrations, though adequate in all the categories, were near the critical values in spring and summer. Fe was adequate but could be provided during spring. Fe concentration observed was adequate- well above the critical concentration irrespective of the physiological status or season of the year. The study also revealed a good percentage of samples deficient in one or the other mineral throughout the year, suggesting Ca, Mg, Cu and Zn supplementation during specific periods. Further, formulating a mineral supplement(s) demands larger sampling size for confirmation of these findings besides the impact study of agro-geo-climatic conditions of the area on animal mineral status.

Key words: Goats, physiological status, season, Kashmir valley, minerals.

INTRODUCTION

Agriculture and livestock production are intrinsically linked; each one being dependent on the other, with livestock sector supporting agriculture in the form of critical inputs, contributing to health and nutrition of household, supplementing incomes and finally being a dependable "bank on hooves" in times of need

(Ben Salem and Smith, 2008). This livestock-based economy contributes about 4.36% to the total Gross domestic product (GDP) of India and 24.72% to GDP-Agriculture, despite being seriously affected by various diseases. In J&K-India, 75% population is rural with agriculture as the main stay and livestock-rearing as the subsidiary one. As per 18th Livestock Census of 2007, the goat population in J&K is about 2.07 million out of the total 10.99 million livestock strength. The agriculture and allied sectors contribute about 38% to the state GDP of which 11% is contributed by livestock sector (DES, 2007). Goats supply precious animal proteins of high biological value in the form of meat, milk, plus essential minerals and fat-borne vitamins. Their increased contribution to animal production is justified by the presence of 94% of the total 674 million world population being found in the developing countries characterized by inadequate food supply and the need for increased food security for the poor (Devendra, 1999). The largest meat consumption in India is chevon/mutton with a share of 46.3% in rural areas, 49.2% in urban areas, and 47.4% nationally. The per capita consumption of meat (chicken, mutton and chevon) in India's Kashmir valley is among the highest in the country (Gandhi and Zhou, 2010). India is currently the largest producer of milk in the world, goats contributing about 3.58%.

Mineral disorders range from acute deficiency or toxicity, characterized by well marked clinical signs and pathological changes, to mild and transient conditions too difficult to diagnose and expressed as a vague unthriftiness or unsatisfactory growth and production (Vargas and McDowell, 1997). Macro-minerals Ca, P, Mg etc play a key role in skeletal development, muscle functions, and transmission of nerve impulse. Inadequacy in diet mostly causes muscular weakness, while mild to moderate deficiency causes sub-minimal productivity and increased susceptibility to other metabolic as well as infectious diseases (NRC, 2001). Trace elements (Cu, Zn, Fe etc) are required for synthesis of many proteins and activation of vast array of enzyme systems (Ceylan et al., 2008). Their concentration must usually be maintained within quite narrow limits, if the functional and structural integrity of the tissues is to be safeguarded and the growth, health and productivity of animals to be maintained at optimum levels. Their supplementation above predicted requirements during stressful conditions mitigates cellular oxidative damage and increases disease resistance (Gressley, 2009). Levels of requirements as well as thresholds of deficiency and toxicity vary with age, sex, production level, activity level, season, and pasture availability, presence of antagonists e.g. Cu and Mo etc, species and genetic strain of the animal (Guyot et al., 2009; Tashi et al., 2005). Significant species differences have been reported for Cu, I, Mo and As (Haenlein, 1991).

For maintenance of normal health and sustained efficient production of livestock, it is necessary to ensure adequate dietary intake of essential nutrients (Hosnedlava et al., 2007). Large number of livestock around the world thrives on mineral deficient diets (McDowell et al., 1993). Continued ingestion of such diets culminates in mineral imbalances in the body leading to physiological and pathological consequences (Prasad and Gowda, 2005). Mostly pastures are considered adequate in nutritive value (energy and protein) to sustain mature animals, the quality of grasses is often inadequate for growing animals whose physiological demands are higher (Pastrana et al., 1991). Mineral deficiencies that affect livestock at pasture include those of macro and micro minerals (Khan et al., 2005). Signs of mineral disorders are often non-specific and in cases of marginal deficiencies may go unnoticed. Further, the interpretation of such signs is even difficult in presence of multiple mineral deficiencies or if complicated by gastrointestinal parasitic burden (Suttle and Jones, 1989). Mineral supplementation is a least cost input for livestock improvement. Nevertheless, supplements should be used only when requirements cannot be met within the available feed, and only when local conditions dictate.

In Kashmir, goats are mainly maintained on grazing with little or no mineral supplementation. Hence, the study was undertaken to assess mineral status of goats in Shuhama Alusteng area of Kashmir valley so as to initiate the work for formulation of area specific mineral supplements and to devise the supplementation strategy for ensuring optimum production performance and prevention of health disorders.

MATERIALS AND METHODS

The study area lies between 34.23°N 74.78°E and is characterized by sub-humid temperate climate with mean annual rainfall of 744 mm and mean annual temperature of 13.4°C. In the study area, the livestock rearing is a subsidiary occupation with almost no inputs and use of technology. The animals, selected randomly, had no history of deworming/vaccination. Jugular venous blood collected in heparinized vials using 18 gauge needles from goats belonging to different physiological states viz. kids/weaners (aged 1 to 6 months), adults and in different seasons of the year as shown in Table 1, was centrifuged at 2000 rpm for 15 min to harvest plasma, stored at -20°C, and subsequently processed and analyzed for minerals.

Estimation of macro-minerals

Calcium

Plasma calcium was estimated by O-Cresolphthalein Complexone (OCPC) endpoint assay (Ca Test Kit supplied by Span Diagnostics Ltd. India). 20 µl of plasma samples were taken in labeled test tubes followed by 1000 µl of working Ca reagent. Standard was prepared in triplicate with 20 µl of Ca standard in test tubes mixed with 1000 µl of working Ca reagent. Test tube containing 1000 µl working Ca reagent was used as reagent blank. After mixing the reagents, test tubes were incubated at 37°C or room temperature

Table 1. Total number of plasma (Goat) samples collected from the study area.

Area	Season	Kids/Weaners	Dry	Pregnant	Lactating	Bucks	Total
Shuhama Alusteng	Winter	3	6	8	5	5	27
	Spring	6	4	5	13	6	34
	Summer	9	3	5	4	5	26
	Autumn	3	5	3	13	3	27
	Total	21	18	21	35	19	114

(15 to 30°C) for 5 min. Analyzer programmed (578 nm) as per assay conditions and absorbance of standards followed by plasma samples was taken against blank.

Phosphorus

Plasma inorganic phosphorus (P_i) was estimated by UV Molybdate, endpoint assay (P Test Kit supplied by Span Diagnostics Ltd. India). 10 µl of plasma samples were taken in labeled test tubes to which 1000 µl of Reagent 1 was added and mixed well. Test tubes containing 10 µl of P_i standard and 1000 µl of Reagent 1 were taken in triplicate as standard. Reagent blank was prepared by taking 1000 µl of Reagent 1 in the test tube. All test tubes containing test samples, standards and blank were mixed properly by shaking and incubated at 37°C for 5 min. Analyzer was programmed as per assay parameters (340 nm) and blanked with reagent blank. The absorbance of the standard and plasma samples was taken against blank.

Magnesium

Plasma Mg was estimated by Calmagite method (Mg Kit supplied by Crest Biosystems, India). To labeled test tubes, 0.01 ml of plasma samples were added followed by 0.5 ml of buffer reagent (L_1), and 0.5 ml of color reagent (L_2). Standard was prepared in triplicate which contained 0.5 ml of L_1 reagent, 0.5 ml of L_2 reagent and 0.01 ml of Mg standard. Test tube containing L_1 and L_2 reagents (0.5 ml each) plus 0.01 ml distilled water was used as reagent blank. The contents in test tubes were mixed well by shaking and incubated at 25°C for 5 min. Absorbance of standard and the samples was recorded against blank at 510 nm.

Estimation of micro-minerals

For estimation of trace elements the plasma samples were digested and subsequently analyzed as per the standard procedures (Kolmer et al., 1951; Sharma et al., 2003). To 3 ml of sample in digestion tubes an equal volume of concentrated HNO_3 was added and mixed well. The tubes were kept overnight at room temperature followed by low heat (70 to 80°C) digestion until the volume of the samples reduced to 1 ml. To this, 3 ml of double acid mixture (HNO_3 and $HClO_4$ in 3:1 ratio) was added and low heat digestion continued until the digested samples became watery clear and emitted white fumes. Heating was continued until the volume of the samples got reduced to ~0.5 ml. Final volume of the filtrate was made 10 ml with triple distilled de-ionized water after warming the solution. During digestion of plasma samples simultaneous digestion of reagent blank was also undertaken and final volume of 10 ml stored to have the blank. Atomic absorption spectrophotometer (AAS-Model No ECIL 4141) was used for estimation of trace minerals. At least 3 standards of known concentration were used for calibration and then the unknown test samples were analyzed. Sample analysis was done by attached computer and concentration of mineral samples was expressed in parts per million (ppm).

Statistical analysis

Collected data were analyzed for mean, standard error and analysis of variance (ANOVA) by using SPSS software (version 16).

RESULTS

The overall plasma mineral concentration in goats belonging to different physiological states and in different seasons, plus the percentage of analyzed samples deficient in minerals is presented in Tables 2 and 3.

The overall Ca concentration observed was below the critical value and significantly lower ($P < 0.05$) in pregnant and lactating does, bucks and dry goats as compared to kids. The Ca concentration in spring, summer and winter was significantly lower ($P < 0.05$) than the autumn season. The percent samples observed deficient in Ca were in the order of pregnant > bucks > lactating > dry > kids/weaners, and summer > spring > winter > autumn. The P_i concentration noticed was adequate, but significantly lower ($P < 0.05$) in lactating does and dry goats as compared to bucks. In autumn and spring, the P_i concentration was significantly lower ($P < 0.05$) than winter and summer seasons. The percent samples observed P_i deficient were in the order of lactating > dry > pregnant > kids/weaners > bucks, and autumn > spring > summer > winter. The Mg concentration recorded was significantly lower ($P < 0.05$) in kids as compared to dry goats, and significantly lower ($P < 0.05$) in winter compared to the autumn season. The percent samples observed Mg deficient were in the order of lactating > kids/weaners > bucks > pregnant > dry, and spring > winter > autumn > summer.

The plasma Cu and Zn concentrations found were adequate in all the goats throughout the year except spring and summer seasons in which it was near to critical concentration. The Cu concentration observed was significantly lower ($P < 0.05$) in kids and bucks as compared to dry goats. Also, the Cu concentration recorded was significantly lower ($P < 0.05$) in spring but higher in winter season. The percent samples observed Cu deficient were in the order of bucks > kids/weaners >

Table 2. Effect of physiological status and seasonal variation on plasma minerals in goats.

Parameter	Physiological status	Seasons									
		Winter		Spring		Summer		Autumn		Overall	
		n	Mean ± S.E	n	Mean ± S.E	n	Mean ± S.E	n	Mean ± S.E	n	Mean ± S.E
Calcium (mg/dl)	Kids/weaners	3	9.49 ± 0.49^a	6	9.16 ± 0.23^a	9	9.30 ± 0.33^a	3	11.82 ± 0.58^{bB}	21	9.65 ± 0.26^B
	Dry	6	9.02 ± 0.19^b	4	8.94 ± 0.16^b	3	8.06 ± 0.64^a	5	9.19 ± 0.22^{bA}	18	8.89 ± 0.16^A
	Pregnant	8	8.76 ± 0.22	5	8.30 ± 0.35	5	8.43 ± 0.29	3	9.35 ± 0.80^A	21	8.66 ± 0.18^A
	Lactating	5	8.49 ± 0.50^a	13	8.22 ± 0.36^a	4	8.22 ± 0.55^a	13	9.90 ± 0.29^{bA}	35	8.88 ± 0.23^A
	Bucks	5	8.99 ± 0.16	6	8.85 ± 0.21	5	8.50 ± 0.27	3	9.32 ± 0.71^A	19	8.87 ± 0.15^A
	Overall	27	8.89 ± 0.14^a	34	8.59 ± 0.17^a	26	8.67 ± 0.19^a	27	9.86 ± 0.24^b	114	8.98 ± 0.10
Phosphorus (mg/dl)	Kids/weaners	3	4.35 ± 0.14^A	6	4.73 ± 0.16^{BC}	9	5.09 ± 0.59^{AB}	3	4.51 ± 0.42	21	4.80 ± 0.26^{AB}
	Dry	6	3.95 ± 0.27^{aA}	4	4.04 ± 0.19^{aAB}	3	6.77 ± 1.45^{bB}	5	4.15 ± 0.36^a	18	4.49 ± 0.35^A
	Pregnant	8	5.45 ± 0.41^{bAB}	5	4.26 ± 0.26^{abAB}	5	3.83 ± 0.14^{aA}	3	4.05 ± 0.61^a	21	4.58 ± 0.24^{AB}
	Lactating	5	5.74 ± 1.28^{bAB}	13	3.70 ± 0.26^{aA}	4	4.39 ± 0.72^{abA}	13	4.05 ± 0.18^a	35	4.20 ± 0.24^A
	Bucks	5	6.53 ± 0.31^{aB}	6	5.29 ± 0.40^{aC}	5	5.56 ± 0.51^{aAB}	3	3.36 ± 0.55^b	19	5.38 ± 0.31^B
	Overall	27	5.25 ± 0.31^b	34	4.28 ± 0.16^a	26	5.02 ± 0.33^b	27	4.04 ± 0.15^a	114	4.62 ± 0.13
Magnesium (mg/dl)	Kids/weaners	3	1.55 ± 0.13	6	1.66 ± 0.15	9	1.94 ± 0.18	3	1.97 ± 0.20	21	1.81 ± 0.10^A
	Dry	6	1.91 ± 0.18	4	2.10 ± 0.26	3	2.20 ± 0.13	5	2.34 ± 0.22	18	2.12 ± 0.11^B
	Pregnant	8	1.89 ± 0.11	5	1.96 ± 0.21	5	2.20 ± 0.22	3	2.35 ± 0.25	21	2.04 ± 0.09^{AB}
	Lactating	5	1.78 ± 0.16	13	1.88 ± 0.09	4	2.12 ± 0.35	13	2.08 ± 0.15	35	1.96 ± 0.08^{AB}
	Bucks	5	1.70 ± 0.14	6	1.81 ± 0.12	5	2.06 ± 0.16	3	1.94 ± 0.23	19	1.87 ± 0.08^{AB}
	Overall	27	1.80 ± 0.07^a	34	1.86 ± 0.06^{ab}	26	2.07 ± 0.09^{bc}	27	2.13 ± 0.09^c	114	1.96 ± 0.04
Copper (ppm)	Kids/weaners	3	1.23 ± 0.07^{bB}	6	0.63 ± 0.10^a	9	0.73 ± 0.09^a	3	0.68 ± 0.07^{aA}	21	0.77 ± 0.06^A
	Dry	6	1.18 ± 0.06^{bB}	4	0.68 ± 0.07^a	3	0.98 ± 0.21^b	5	1.11 ± 0.08^{bC}	18	1.01 ± 0.06^B
	Pregnant	8	1.08 ± 0.07^{bB}	5	0.66 ± 0.05^a	5	0.76 ± 0.14^a	3	0.80 ± 0.10^{abAB}	21	0.86 ± 0.06^{AB}
	Lactating	5	1.22 ± 0.10^{bB}	13	0.78 ± 0.05^a	4	0.83 ± 0.14^a	13	0.96 ± 0.05^{aBC}	35	0.92 ± 0.04^{AB}
	Bucks	5	0.73 ± 0.21^A	6	0.75 ± 0.06	5	0.96 ± 0.24	3	0.63 ± 0.09^A	19	0.78 ± 0.08^A
	Overall	27	1.08 ± 0.06^c	34	0.72 ± 0.03^a	26	0.83 ± 0.07^{ab}	27	0.90 ± 0.04^b	114	0.87 ± 0.03
Zinc (ppm)	Kids/weaners	3	1.67 ± 0.10^b	6	0.61 ± 0.15^a	9	0.63 ± 0.16^a	3	0.62 ± 0.10^a	21	0.77 ± 0.11
	Dry	6	1.38 ± 0.22	4	0.73 ± 0.16	3	0.92 ± 0.19	5	1.09 ± 0.28	18	1.08 ± 0.12
	Pregnant	8	1.39 ± 0.20	5	0.74 ± 0.10	5	0.86 ± 0.22	3	0.80 ± 0.21	21	1.02 ± 0.11
	Lactating	5	1.71 ± 0.14^b	13	0.60 ± 0.07^a	4	0.66 ± 0.08^a	13	0.92 ± 0.12^a	35	0.89 ± 0.08
	Bucks	5	1.43 ± 0.13^b	6	0.72 ± 0.20^a	5	0.82 ± 0.10^a	3	1.17 ± 0.11^{ab}	19	1.00 ± 0.10
	Overall	27	1.49 ± 0.09^c	34	0.66 ± 0.05^a	26	0.75 ± 0.08^{ab}	27	0.93 ± 0.08^b	114	0.94 ± 0.05

Table 2. Contd.

Iron (ppm)										
Kids/weaners	3	4.68 ± 1.01^b	6	2.26 ± 0.41^a	9	2.41 ± 0.31^a	3	3.30 ± 1.04^{abAB}	21	2.82 ± 0.31^A
Dry	6	4.01 ± 0.66	4	2.60 ± 0.74	3	2.58 ± 0.57	5	2.51 ± 0.37^A	18	3.04 ± 0.33^A
Pregnant	8	5.86 ± 1.23	5	2.85 ± 0.36	5	2.74 ± 0.49	3	5.32 ± 0.96^B	21	4.32 ± 0.58^B
Lactating	5	5.08 ± 1.27^b	13	2.57 ± 0.50^a	4	2.75 ± 0.59^a	13	3.60 ± 0.46^{abAB}	35	3.33 ± 0.34^{AB}
Bucks	5	5.67 ± 0.73^b	6	2.24 ± 0.47^a	5	2.49 ± 0.64^a	3	3.67 ± 0.60^{aAB}	19	3.43 ± 0.44^{AB}
Overall	27	5.14 ± 0.48^c	34	2.50 ± 0.23^a	26	2.56 ± 0.20^a	27	3.56 ± 0.30^b	114	3.39 ± 0.19

Means bearing different uppercase superscripts across the columns for each parameter differ significantly ($P < 0.05$). Means bearing different lower case superscripts across the rows for each parameter differ significantly ($P < 0.05$).

Table 3. Percent samples (goat plasma) deficient in minerals.

Mineral	Critical concentration*	Season	Physiological status					
			Kids/weaners	Dry	Pregnant	Lactating	Bucks	Overall
Calcium	9.0	Winter	1/3 = 33.3%	2/6 = 33.3%	4/8 = 50%	2/5 = 40%	2/5 = 40%	11/27 = 48.7%
		Spring	2/6 = 33.3%	2/4 = 50%	3/5 = 60%	7/13 = 53.8%	2/6 = 33.3%	16/34 = 47.1%
		Summer	4/9 = 44.4%	2/3 = 33.3%	3/5 = 60%	2/4 = 50%	3/5 = 60%	14/26 = 53.9%
		Autumn	0/3 = 0	1/5 = 20	1/3 = 33.3%	3/13 = 23.1%	1/3 = 33.3%	6/27 = 22.2%
		Overall	7/21 = 33.3%	7/18 = 38.9%	11/21 = 52.4%	14/35 = 40%	8/19 = 42.1%	
Phosphorus	4.0	Winter	0/3 = 0	2/6 = 33.3%	1/8 = 12.5%	1/5 = 20%	0/5 = 0	4/27 = 14.8%
		Spring	1/6 = 16.7%	2/4 = 50%	1/5 = 20%	6/13 = 46.2%	0/6 = 0	10/34 = 29.4%
		Summer	2/9 = 22.2%	0/3 = 0	2/5 = 40%	1/4 = 25%	0/5 = 0	5/26 = 19.2%
		Autumn	1/3 = 33.3%	1/5 = 20%	1/3 = 33.3%	5/13 = 38.5%	2/3 = 66.7%	10/27 = 37.0%
		Overall	4/21 = 19.1%	5/18 = 27.8%	5/21 = 23.8%	13/35 = 37.1%	2/19 = 10.5%	
Magnesium	1.5	Winter	1/3 = 33.3%	0/6 = 0	1/8 = 12.5%	1/5 = 20%	1/5 = 20%	3/27 = 11.1%
		Spring	2/6 = 33.3%	1/4 = 25%	1/5 = 20%	2/13 = 15.4%	1/6 = 16.7%	7/34 = 20.6%
		Summer	0/9 = 0	0/3 = 0	0/5 = 0	¼ = 25%	0/5 = 0	1/26 = 20.6%
		Autumn	0/3 = 0	0/5 = 0	0/3 = 0	2/13 = 15.4%	0/3 = 0	2/27 = 7.4%
		Overall	3/21 = 14.3%	1/18 = 5.6%	2/21 = 9.5%	6/35 = 17.1%	2/19 = 10.5%	
Copper	0.6	Winter	0/3 = 0	0/6 = 0	0/8 = 0	0/5 = 0	2/5 = 40%	2/27 = 7.4%
		Spring	2/6 = 33.3%	¼ = 25%	1/5 = 20%	1/13 = 7.7%	1/6 = 16.7%	6/34 = 17.7%
		Summer	2/9 = 22.2%	0/3 = 0	1/5 = 20%	¼ = 25%	1/5 = 20%	5/26 = 19.2%
		Autumn	1/3 = 33.3%	0/5 = 0	0/3 = 0	0/13 = 0	1/3 = 33.3%	2/27 = 7.4%
		Overall	5/21 = 23.8%	1/18 = 5.6%	2/21 = 9.5%	2/35 = 5.7%	5/19 = 26.3%	

the year except spring and summer seasons in which it was near to critical concentration. However, significantly higher Cu concentration in dry, lactating and pregnant goats compared to kids and bucks might be attributed to increased requirements for growth in yearling dry does, for sustained lactation in milking does, and higher progesterone level or to the increased fetal demands and utilization of maternal Cu for the development of fetal nervous system in pregnant does (Elnageeb and Adelatif, 2010). Moreover, increase in plasma Cu levels in the form of ceruloplasmin owing to increase in estrogen levels has been noticed in late pregnancy (Howell et al., 1968). The significantly lower Cu level in spring and summer compared to winter season is in agreement with the findings of Pastrana et al. (1991). The higher Zn concentration in dry goats and pregnant does might be due to increased requirements for growth in yearling dry does, increased rate of Zn accumulation in fetus and increased demands for Zn towards end of pregnancy (Elnageeb and Adelatif, 2010) in addition to higher plasma albumin levels in pregnant animals to which Zn is bound primarily (Davis, 1984). The results obtained are in agreement with the findings of Williams (1977). However, late gestation associated hemodilution may reduce serum Zn level (Masters and Fels, 1980). The significantly higher Zn concentration in winter compared to rest of the seasons is in agreement with the findings of Khan et al. (2008), but contradicts the observations made by Cermak et al. (2006) and Mtui et al. (2007). The plasma Fe concentration recorded was adequate in all physiologically varied categories and all throughout the year, hence no need of its supplementation. However, significantly higher Fe concentration observed in the pregnant stock is in agreement with the findings of Tainturier et al. (1984) who recorded relatively high Fe concentration from 3rd to 7th month of pregnancy compared to minimum concentration when lactation commenced. However, Asif et al. (1996) observed non-significant differences with respect to physiological status in plasma Fe content in cattle. The amount of Fe seems not to be dependent on the dietary Fe intake owing to its complex absorption mechanism in GIT. Fe absorption (in duodenum) is normally restricted by a mucosal block (McDowell, 1985) in which Fe in mucosal cells is released into plasma by the conversion of Fe^{3+} to Fe^{2+}. Moreover, high levels of certain divalent metals enhance the Fe requirements by competing for absorption site in the intestine (Underwood, 1981). The amount of Fe absorbed depends on Fe balance of the animal, hence Fe depleted animals absorb more Fe than the non-depleted ones. The significantly higher Fe concentration in winter compared to rest of the seasons is in agreement with the findings of Merkel et al. (1990) and Rojas et al. (1993).

The findings of the present study suggest that goats of all categories in the study area should be supplemented with Ca throughout the year except autumn, Mg during winter and spring, and Cu and Zn during spring and summer seasons. Also, the dosage should be recommended as per the physiological needs of an animal. Moreover, the soil-plant-animal system of the area should be evaluated with respect to minerals which would confirm these findings and allow the formulation of area specific mineral supplement(s). This study could well serve as a mere indicator and set a platform for much detailed and wider scale investigations in future where in larger sample size and other livestock species throughout the valley could be studied.

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Conflict of Interest

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

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Full Length Research Paper

Effect of electromagnetic radiations on brooding, honey production and foraging behavior of European honeybees (*Apis mellifera* L.)

Pramod Mall* and Yogesh Kumar

Department of Entomology, College of Agriculture, G. B. Pant University of Agriculture and Technology, Pantnagar, 263 145 (U. S. Nagar, Uttarakhand), India.

The present investigation was carried out at HREC, Dhakrani, Dehradun during August-December, 2010. For the experiment three treatments, i) Colonies below the tower, ii) Colonies equipped with cell phone and iii) Colonies without cell phone were taken into consideration. To quantify the effect of electromagnetic radiation (EMR), all the treatments having different radiation level were maintained. It was observed that maximum brood area was found in control colonies (560.36 cm^2) followed by the colonies kept near the tower 537.85 cm^2 and lowest brood area (534.81 cm^2) was observed in the colonies equipped with cell phone. The average honey production was found to be highest (14.43 kg/hives) in the colonies placed near the tower followed by cell phone equipped colonies (13.76 kg/hive), while control colonies produced 12.80 kg/hive honey in first harvesting. There was no remarkable change in the nectar and pollen gathering behaviour of foragers and sufficient pollen and nectar stored in the colonies during the course of study. Therefore, in the light of above findings conclusion can be drawn that there is no apparent effect of EMR on brooding, honey production and foraging behaviour of *Apis mellifera* colonies.

Key words: Electromagnetic radiations, GSM 900 cell phone, behaviour, brood, *Apis mellifera*.

INTRODUCTION

Honeybees have become essential component for the success of high tech agriculture. The economical role of honeybees in worldwide pollination has been statistically valued to be around 153 billion Euros in the year 2005 (Gallai et al., 2009). Bee losses have been recorded for more than a century (Hart, 1893; Aikin, 1897; Beuhne, 1910; Wilson and Menapace, 1979). Scientists suspect many factors to be responsible for the impairing and death of the bees viz., varroa mite, pesticides, viruses, unscientific farming practices, monoculture, un-hygiene condition in the hive and dramatic change in climatic factors are the most widely cited possibilities. Radio frequency, electromagnetic radiation (EMR) has been reported to produce a number of inimical biological effects on biomolecules cells ultimately impairing the entire biological structure and functions of whole

organisms (Bawin et al., 1975, 1978; Blackman et al., 1980, 1989; Dutta et al., 1984; Goodman et al., 1995; Kwee and Raskmark, 1998; Lin-Liu and Adey, 1982; Penafiel et al., 1997; Velizarov et al., 1999; Xenos and Margas, 2003). Honeybees possess magnetite crystals in their fat body cells and they present magnetic remanence (Gould et al., 1978; Keim et al., 2002). These magnetite structures are active parts of the magneto-reception system in honeybees (Hsu and Li, 1994; Hsu et al., 2007). Honeybees can be trained to respond to very small changes in the constant local geomagnetic field intensity (Walker and Bitterman, 1989a). They can also communicate through chemical and acoustical means (Winston, 1991; Tautz, 2008). A pervasive media report asserted that cellular phones were a possible cause of honey bee colony collapse disorder in April, 2007 (Good Morning America, 2007; Lean and Shawcross, 2007). An investigation of this report revealed that the media misinterpreted the findings of a study conducted by Kimmel et al. (2007) at Koblenz-Landau University in Germany. The study made no reference to Colony Collapse Disorder (CCD) and did not look at the effects of cellular phone radiation on honeybees.

In the US, disappearance of bees was observed several years back and was associated with the rising network of electromagnetic pollution. When honeybees cannot find their way back to the hive as a result of consistent electromagnetic background noise that seems to disrupt intercellular communication within individual bees, this is known as CCD. As per the literature available, CCD has spread to Germany, Switzerland, Spain, Portugal, Italy, Greece,

Scotland, Wales and North-west England. In England, the bee population depleted 54% between 1985 and 2005 as compared to an average of 20% across Europe. Recently, a sharp decline has also been noticed in commercial bee population in Kerala posing a serious threat to beekeepers, hitting apiculture. The State has the highest density of mobile towers. Similar cases have been observed in Bihar, Punjab, Nepal and other parts of India and have been attributed to increasing electromagnetic pollution in the environment (Girish, 2010).

The behavioural pattern of bees alters when they are in close proximity to mobile phones and towers. The vanished bees are never found, but thought to die alone far from home. Bee keepers told that several hives have been abruptly abandoned. If towers and mobile phones increase, the honeybees might be wiped out within 10 years. According to Aday (1975), the radiation of 900 MHz is highly bioactive, causing significant alternation in the physiological function of living organisms.

As far as research on electromagnetic pollution in concerned, no systematic studies have been conducted on potential effect of EMR from mobile phones on honeybee behaviour in India. Here, we present results from corresponding original experiments we have carried out with honeybee populations exposed to active mobile phone radiation. The goal of these experiments was to identify potential effects of mobile phone communications on honeybee behaviour.

MATERIALS AND METHODS

The present investigation on effect of EMRs of mobile tower and cell phone on behaviour of *Apis mellifera* L. was carried out at HREC, Dhakrani, Dehradun. The observations were recorded from last week of August to first week of December, 2010. Total of 15 colonies having the bee strength of 7 to 9 frames were selected for the experiment. For the above experiment, total three treatments each having 5 colonies were considered as replication *viz.* i) Colonies just below the tower, ii) Colonies placed at 2 km far from tower equipped with cell phone and iii) Control colonies maintained 2 km away from tower and without cell phone were designed. Out of 15 colonies, 5 colonies were shifted just near the tower and 5 colonies were provided with cell phone instrument of GSM 900 MHz frequency, cell phone were placed at the bottom of the hive (Plates 1 and 2). Rest of the 5 colonies were left in the apiary and treated as control. The frequent calls continuously for 10 min were made to ring the mobile instruments at 3 h interval every day. For measuring radiation, Radio frequency meter (RF meter) was used. With the help of RF meter, Electric field (E) m V/m^2, Magnetic field (M) μ A/m^2 and Power density (P) m W/m^2 was measured. The observation on brood area was recorded in cm^2 at weekly intervals in all the 15 colonies. The observation on honey production was recorded on 15th December, 2010 at the time of honey harvesting. The procured data was subjected to two-factorial randomized block design (RBD).

RESULTS AND DISCUSSION

Brood area

Data recorded (Table 1) at weekly interval clearly implies that brood area was found maximum (636.00 cm^2) in the colonies maintained with cell phone on 10th September, 2010, whereas in control colonies the brood area was 600.00 cm^2 which is not much lower as compared to maximum brood. As far as the brood areas in respect of date of observation is concerned, the maximum mean brood area 595.26 cm^2 was found on 10th September, 2010 and minimum brood area 495.73 cm^2 was observed on 15th October, 2010, while brood areas pertaining to various treatments, the maximum 560.36 cm^2 was noticed in control colonies followed by the colonies kept near the tower where average brood area was 537.85 cm^2. The lowest brood area 534.81 cm^2 was observed in the colonies equipped with cell phone (Figure 1). However, according to the statistical analysis, none of the treatment was found to be significant. On the contrary, Sharma and Kumar (2010) reported the significant decline in colonies strength and in the egg laying rate of the queen due to EMRs.

Honey production

The experiment was started in dearth period in the end of

Plates 1a and b. Honeybees colonies with cell phone of GSM 900 MHz frequency.

August, 2010 and continued till the beginning of December. The honey stored in frames was extracted on 15th December, 2010 (Table 2). The average honey production of colonies near the tower was 14.43 kg/hives, while honey production in treatment with cell phone was 13.76 kg/hive. The control colonies produced minimum honey which was 12.80 kg/hive, while Sharma and Kumar (2010) reported in the same paper that there was neither honey nor pollen, brood and bees sustained in the colonies resulting in complete loss of the colony.

Plates 2a and b. Honeybees colonies kept near the electromagnetic radiation mobile tower.

Behavioral observation

It was observed that there was no change in the nectar and pollen gathering foragers and therefore sufficient pollen and nectar store were recorded which was suffice to rear the brood in the colonies. Bees were critically observed during ringing of cell phone and it was noticed that bees working in the frames are not paying attention towards the instrument. The colonies beneath the tower (Table 3 and Figure 2) having maximum average electric field (E) 345.40 (m V/m), while in case of cell phone equipped colonies the average range was 57.70 (V/m) and in control colonies it was very low 07.90 (m V/m) as compared to tower colonies. Whereas average magnetic field (M) observed near the tower colonies was 670.63 (µ A/m) and in mobile colonies it was measured 100.78 (m A/m). Power density (P) was also measured and it was found that honeybees colonies near the tower encountering highest average power density 260.08 (µ W/m^2) and lowest 0.10 (µ W/m^2) in control colonies.

Remarkably, one of the Queens in the hive near the tower was accidentally killed during the shifting of the hive and the workers started to reform the new Queen in the hive. The queen was successfully produced by the worker and mating was also successfully performed as healthy colony. This again clearly corroborate that there was no apparent effect of EMR on behaviour and reproduction of Queen or drone. Similar type of result observed by Mixson et al. (2009) in the final series of experiments that no effect of GSM radiation exposure was found on aggression of honey bees. They also concluded that GSM cellular phone radiation emissions do not inhibit the foraging behaviours or navigational ability of honeybees, and are thus unlikely to affect

Table 1. Effect of electromagnetic radiation of cell phone on behaviour of *Apis mellifera.*

Date of observation	Mean brood area of colony (cm^2) at weekly intervals			
	Tower	**Mobile**	**Control**	**Mean**
26/08/2010	516.20	562.40	583.60	554.06
03/09/2010	556.80	587.80	587.80	577.46
10/09/2010	561.20	636.60	600.00	599.26
17/09/2010	592.00	592.80	551.80	578.86
24/09/2010	597.00	486.80	523.40	535.73
01/10/2010	542.80	486.20	514.00	514.33
08/10/2010	455.40	477.40	580.60	504.46
15/10/2010	467.20	473.20	548.80	495.73
22/10/2010	476.40	478.00	542.00	534.13
29/10/2010	580.40	576.40	559.00	537.26
05/11/ 2010	505.80	583.20	595.40	561.46
12/11/ 2010	522.80	548.80	540.60	537.40
19/11/ 2010	571.60	546.20	558.60	558.80
26/11/ 2010	533.40	471.20	559.20	521.26
03/12/ 2010	588.80	515.20	560.60	554.86
Gm	537.85	534.81	560.36	544.34
Sem =	sem1 = 14.434	sem2 = 32.276	sem3 = 55.903	
Cd at 5% =	cd1 = 40.28689[ns]	cd2 at 90.084[ns]	cd3 at 156.030[ns]	
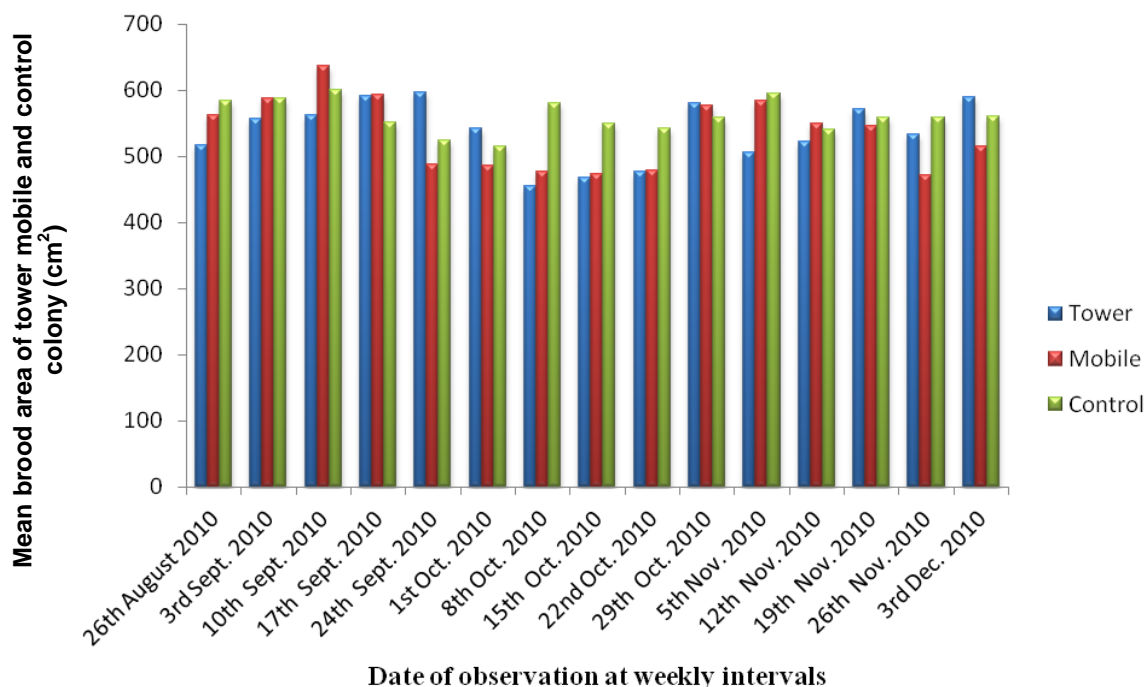
Figure 1. Effect of electromagnetic radiations on mean brood area of tower, mobile and control colonies of *A. mellifera* L.

colony health. Similarly, Kimmel et al. (2007) reported overall, 482 (63%) bees of the CG (non-exposed), 203 (56.4%) bees of the EG2 (radiation shielded down) and 365 (54.1%) bees of the EG1 (fully electromagnetic exposure) returned to their hive. These differences between the groups were not significant. Moreover, many

Table 2. Average honey production at first harvesting on 15 December, 2010.

Treatment	Control colonies (kg/hive)
Tower	14.43
Mobile	13.76
Control	12.80

Table 3. Average EMR frequency on *Apis mellifera* colonies during the observation period.

EMR Measurement	Tower	Mobile	Control
Electric field (E)	345.40 (m V/m)	57.70 (V/m)	07.90 (m V/m)
Magnetic field (M)	670.63 (μ A/m)	100.78 (m A/m)	18.72 (μ A/m)
Power density (P)	260.08 (μ W/m^2)	10.60 (μ W/m^2)	0.10 (μ W/m^2)

recent studies have also demonstrated that various tissues, cellular activities, memory, and learning in humans, rats, and mice are not affected when subjected to GSM or GSM-like microwave radiation (Cobb et al., 2004; Dasdag et al., 2003, 2004, 2008; Dubreuil et al., 2002, 2003; Forgacs et al., 2006; Joubert et al., 2007; Kumlin et al., 2007; Sienkiewicz et al., 2000; Smith et al., 2007; Thorlin et al., 2006; Tillmann et al., 2007). Nevertheless, these findings should not downplay the potential health hazards involved with the use of cellular phones.

Negative results are never appealing; however, no uniform consensus on the effects of microwave radiation on biological processes has been demonstrated in the existing literature. Contrary to the general belief, Mixson et al. (2009) reported that the 900 and 1800 MHz frequencies utilized by GSM technology are not a likely cause of, or a contributing factor in, colony collapse disorder. Other possible causes of CCD and factors contributing to honeybee population declines including biological pathogens (Cox-Foster et al., 2007), agrochemicals, climate change, and genetically modified crops must continue to be investigated. Moreover, the copious disagreement among published findings pertaining to the effects of cellular phone radiation on humans and other animals necessitates that researchers continue to investigate the biophysical interactions between microwave radiation and biological systems for the welfare of entire human community on this planet.

Though many reports published in different journal, books and newspaper revealed the insignificant effect of EMR on the humans and animals. Schneider and Lewis (2004) observed the induction of honeybee worker piping by the electromagnetic fields of mobile phones might have dramatic consequences in terms of colony losses due to unexpected swarming. The active mobile phone handsets in beehives noticeably induce the rate of worker piping. However, no evidence for piping of the laying queen was observed, whereas in earlier report Walker and Bitterman (1989b) observed magnetic corollary at even 26 nanoTesla (nT) for changes in the foraging behaviour of bees. On the other hand Dimitris et al. (2004) reported Pulsed radio frequency, EMR from common GSM mobile phones, with a carrier frequency at 900 MHz, "modulated" by human voice, decreases the reproductive capacity of *Drosophila melanogaster* by 50 to 60%, whereas the corresponding "non-modulated" field decreases the reproductive capacity by 15 to 20%. A national survey performed in the United States (Bee Alert Technology, 2007), reported that implication regarding a direct correlation between erratic honeybee behaviour and mobile phone-generated electromagnetic fields would substantiate one more explanation for the "disappearance" of bee colonies around the world. This phenomenon accounts for 43% of all bee losses, apart from overwintering (39%), mite disease, (15%) and pesticides (3%). Recently, Sahib (2009) suggested that cell phones and cell phone towers near beehives interfere with honeybee navigation. He found that when a mobile phone was kept near a beehive it resulted in collapse of the colony in 5 to 10 days, with the worker bees failing to return home, leaving the hives with just queens, eggs and hive-bound immature bees. Daniel (2010) described the potential effects of electromagnetic waves originating from mobile phones on honeybee behaviour. Active mobile phone handsets have a dramatic impact on the behaviour of the bees, by inducing the worker piping signal.

Conclusion

Several findings reported that sharp declines and potential health hazards in honeybee populations due to cellular phones and GSM radiation could considerably weaken the infrastructure of food webs across the globe.

Some countries have sought to limit the proliferation of mobile towers with strict rules. But in India no such rules have been formulated or implemented. Given the proliferation of mobile phone towers and their vital role in communications, solutions to the problem will not be as simple as eliminating the towers. As per the present investigation, all the treated colonies either with cell phone or tower radiation had perform well as normal colony and none of the colonies were perished during the experiment. Besides the fulfilling the requirement of growing offspring all the colonies had sufficient store of pollen and nectar which was harvested in the December, 2010 after accomplishment of the experiment. Indeed, the EMRs may harm the health of living creature in long run however; the immediate and direct impact is yet need intensive research to draw a firm conclusion.

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Conflict of Interest

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

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Full Length Research Paper

Oats forage management during winter and nitrogen application to corn in succession

Deise Dalazen Castagnara[1]*, Lucas Guilherme Bulegon[2], Paulo Sérgio Rabello de Oliveira[2], Tiago Zoz[3], Marcela Abbado Neres[2], Bruno Borges Deminicis[4] and Fábio Steiner[3]

[1]Federal University of Pampa, Uruguaiana – Rio Grande do Sul, Brazil.
[2]State University of West Paraná, Brazil.
[3]State University Paulista Júlio Mesquita Filho, Brazil.
[4]Federal University of Espiríto Santo, Brazil.

Tests were conducted aimed at studying the combination of different uses of soil cultivated with white oats cv. IPR 126 during winter with different of nitrogen (N) fertilization managements in corn in succession. Oats management regimes included grazing with 10 or 20 cm of residue heights (G10 or G20); mechanically cut (for haymaking) with 10 or 20 cm of residue heights (C10 or C20); without grazing or cutting with tillage (ST); and without grazing or cutting with conventional (SC) soil preparation. Nitrogen management schemes included 100:0:0; 0:25:75; 0:50:50; 0:75:25; 0:100:0; and 0:50:50 (during pre-sowing and V_4 and V_8 growth stages). The experiment was conducted on Oxisol as a randomized block design in a layout of plots (soil regimes) with split plots (management of N) with three replications. The quantity and characteristics [concentrations of carbon (C) and N] of the straw deposited by oats as well as yield components and grain yield of corn were investigated. The ST and SC soil regimes provided greater deposition of straw with less concentration of N but greater concentration of C, with, consequently, a greater C:N ratio. Addition of C to soil was greater in soil regimes where the oats were not grazed or cut. The management of N did not affect the characteristics of the corn cultivation, and the foliar-N content and productivity were lower in soil regimes ST and SC. The corn had limited productivity when established in succession to white oats with straw higher than 4000 kg ha^{-1} and a C:N ratio more than 34. The use of white oats for grazing or cutting on 10- and 20-cm residue heights did not affect negatively the productivity of corn introduced in succession. Independent of the quantity and C:N ratio of straw in coverage, N management did not alter the foliar-N content, the characteristics of the plants, or the yield components and grain yield of corn.

Key words: *Avena sativa*, immobilization, mineralization, C:N ratio, *Zea mays*.

INTRODUCTION

Crop-livestock integration is a technique that allows the production of forages and grain production to be switched in the same area throughout the year. To obtain income by producers, forage production in times of shortage

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	mg dm^{-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.

and the deposition of straw occur simultaneously through thecultivation of forage species in areas normally kept fallow in dry-season grain crops. In this type of crop-livestock integration, the benefits added to the soil by the tillage system are enhanced by the introduction of forage species (Loss et al., 2011), which generally accumulate more carbon than agricultural crops, ensuring soil cover (Embrapa, 2009).

Among the forages used, oats is attractive because it can be used as a cover plant in addition to its applications in forages (Floss et al., 2007). However, the amount of oats straw deposited on the surface of the soil must be in agreement the successor crop. Excessive quantities of straw preceding the sowing of corn are not desired because during decomposition it can compromise the availability of N due to its high carbon:nitrogen ratio (C:N) (Silva et al., 2006) and the occurrence of microbial immobilization of N (Amado et al., 2003).

Cultivation of corn is one of the most demanding of fertilizers, especially N (Cancellier et al., 2011). Greater quantity requirements and other factors influence the productivity and burdens of production costs (Melo et al., 2011).

Times and methods of N fertilizer application on corn are widely studied in agriculture, especially in systems exclusive for direct seeding. However, in crop-livestock integration systems, N application studies are still scarce (Sandini et al., 2011). Its handling is more complex due to the great dependence on climatic conditions (Cantarella and Duarte, 2004), and techniques that maximize the absorption of N by plants and minimize their losses to the environment can contribute to improving the sustainability of production systems.

The above work has been prepared on the assumption that the maintenance of adequate amounts of crop residue on the soil surface associated with split applications of N can reduce N losses in the system through immobilization and synchronize N availability to plants by mineralization. In this context, this study aimed to investigate combining different regimes of soil cultivated with white oats during the winter period with different splitting of N fertilization in oats/corn succession in a crop-livestock integration system.

MATERIALS AND METHODS

This work was conducted during the period from May 2009 to March 2010 at the "Antonio Carlos dos Santos Pessoa Teacher" experimental farm (latitude 24°33'22'' S and longitude 54°03'24'' W, at an altitude of approximately 400 m) at the State University of West Paraná - Campus Marechal Cândido Rondon, on an Oxisol. The area was being managed under no tillage, following the succession of soybean/corn/oats crops in the three latest agricultural yields. At the time the study was implemented, the soil had the physicochemical properties described in Table 1.

The climate of the region, according to the Köppen classification, is a Cfa-type shrubland, with well-distributed rainfall throughout the year (IAPAR, 2006). Climatic data of the experimental period were obtained from an automatic climatological station at the State University of West of Paraná, about 100 m from the experimental area and are presented in Figure 1.

The experimental design was a randomized block in a track with split plots with three replications. On the plots were allocated six soil regimes: G10, grazing with a 10-cm height of residue; G20, grazing with a 20-cm height of residue; C10, mechanically cut (for haymaking) with residue of 10 cm; C20, cut with a residue of 20 cm; ST, without grazing or cuts with tillage for sowing of the summer crop; and SC, without grazing or cuts with conventional preparation of soil for sowing of the summer crop; these were also in the sub-plots of the N fertilization management regimes (Table 2). Plots had dimensions of 15 × 30 m, and each plot was subdivided into six sub-plots with dimensions of 5 × 15 m.

The white oats (*Avena sativa* cv. IPR 126) sowing occurred on May 24 using a precision seed drill attached to a tractor at a seed density of 70 kg ha^{-1} distributed in rows spaced 0.17 m apart without a starter fertilizer. Three cuts or grazings were made, the first 55 days after the emergence of oats and the others at 30-day intervals.

For the three grazings, Holstein cows in lactation were used, which weighed approximately 550 ± 28.5 kg. The cows were distributed in the plots (paddocks) where they grazed for two days to obtain the desired heights of residue (10 and 20 cm). To obtain the desired heights, manning variables were used according to the put-and-take technique (Mott and Lucas, 1952). For treatments that were cut, a mechanical harvester coupled to a tractor set to the desired cutting height (10 and 20 cm) was used, always on the final day of grazing.

In succession of oats, corn was sown. At the time, the plots intended for soil regimes G10, G20, C10, C20, and ST were desiccated using the herbicide glyphosate (1800 g ha^{-1} active ingredient) with a volume of 250 L ha^{-1}, while the plots designated for soil regime SC were prepared mechanically via grating and leveling.

To determine the quantity of straw, sampling occurred seven days before the sowing of corn with the aid of a metallic square with known area (0.25 m²). The square was randomly placed twice in each sub-plot, and all straw on the soil surface contained inside was collected. Following collection, the material was passed through a sieve with a mesh of 3 mm for the withdrawal of excess soil. It was then subjected to kiln-drying with forced ventilation of air at a temperature of 55°C for 72 h and later weighed to determine the dry mass. After weighing, the quantities of straw deposited per hectare and the material was crushed in a Willey grinder to
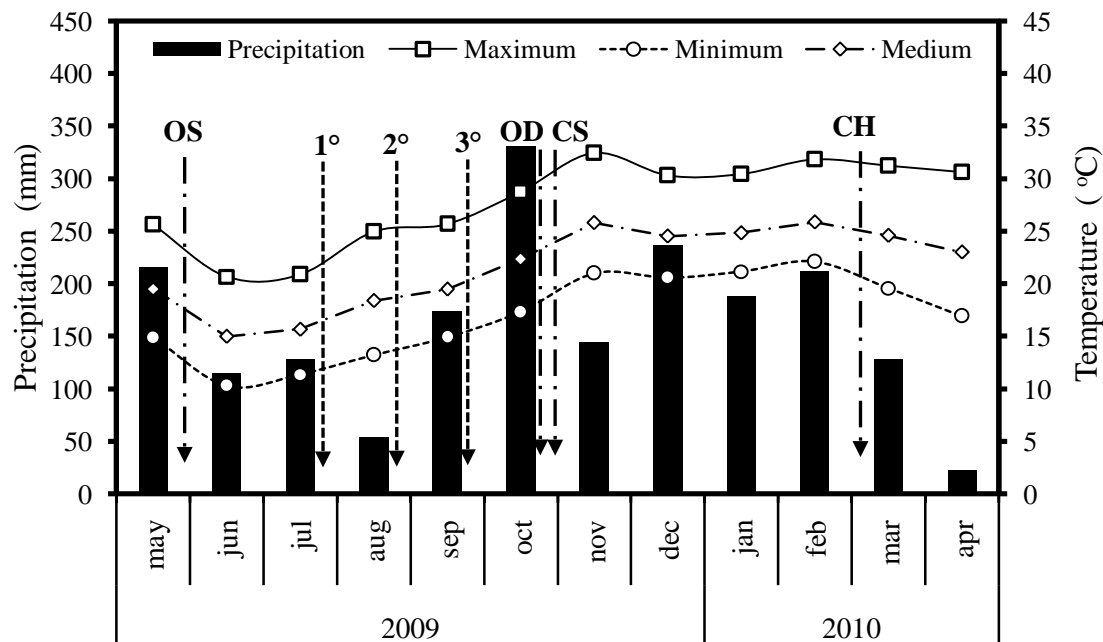
Figure 1. Monthly averages of maximum, minimum, average and cumulative rainfall during the months of the trial period. OS: Oats sowing; 1º, 2º, 3º: first, second and third grazing or cutting oats, respectively; OD: Oat desiccation; CS: Corn sowing; CH: Corn harvest. Source: Automatic Climatological station of Experimental Stations of Unioeste, Marechal Candido Rondon-PR, 2009-2010.

Table 2. Managements of nitrogen for corn.

Managements (kg ha^{-1})	Pre sowing*	Sowing **	Coverage	
			V$_4$	V$_8$
100:0:0	100	40	0	0
0:25:75	0	40	25	75
0:50:50	0	40	50	50
0:75:25	0	40	75	25
0:100:0	0	40	100	0
50:50:0	50	40	50	0

*Application seven days before to sowing of corn; ** Provided by formulated 8:20:15 (N: P$_2$O$_5$: K$_2$O). V$_4$, V$_8$: stages of vegetative development of corn.

determine the concentrations of N and C. The N concentration was determined by sulfuric acid digestion and distillation using a Kjeldhal semi-micro system (Embrapa, 2009), while C was obtained from the determination of organic matter using a muffle furnace, as described by Silva and Queiroz (2006). To estimate the concentration of C in organic matter, organic matter content values of samples were divided by 1.72, as recommended by Peixoto et al. (2007). The C and N stocks were calculated from the residual straw and concentrations of elements.

The additions of C and N to the soil by the systems studied were estimated from the amount of dry matter of straw deposited by the oats and their concentrations. Average contribution of the root system is considered to be 30% of total C and N contained in the shoot. When calculating C addition, it was considered an average of 40% of this element in plant dry matter of oats (Costa et al., 2008).

The sowing of corn occurred on October 29, 2009, using the triple hybrid CD 384 with a row spacing of 0.70 m and density of 4.2 seeds per meter, with a population density goal of 60,000 plants ha^{-1}. A starter fertilizer was used at a rate of 200 kg ha^{-1}, formulated as 8-20-15. Fertilization occurred in the RS and SC regimes based on recommendations for corn of the Committee on Chemistry and Soil Fertility (CQFS-RS/SC, 2004). The fertilizer was a fixed dose of 40 kg ha^{-1} of N for all management schemes adopted, and applications were performed at the V$_4$ and V$_8$ phenological stages, as recommended by Ritchie et al. (2003) (Table 2). The pre-seeding application was performed seven days prior to sowing corn, and in all applications the source of N was urea (45% N).

When the emergence of female inflorescences occurred, sampling was performed to diagnose leaf N content according to Malavolta (1997). The sampled leaves were washed with deionized water, had the midrib discarded, and were dried in an oven with

Table 3. F values calculated for the amount, concentration ([ ]), stock (S), added (A) and C/N ratio of straw deposited by oat cv. IPR 126 under different soil uses.

Source of variation	DF	Straw	[ ] of N	[ ] of C	S of N	S of C	A of N	A of C	C:N ratio
Block	2	3.839^{ns}	0.726^{ns}	0.154^{ns}	3.003^{ns}	4.584^{ns}	3.003^{ns}	3.839^{ns}	0.066^{ns}
Nitrogen (N)	5	0.459^{ns}	0.768^{ns}	2.102^{ns}	0.604^{ns}	0.467^{ns}	0.604^{ns}	0.459^{ns}	0.327^{ns}
Error 1	10								
Soil uses (SU)	5	68.023^{**}	436.251^{**}	27.025^{**}	6.186^{**}	69.223^{**}	6.186^{**}	68.023^{**}	408.807^{**}
Error 2	10								
NxSU	25	1.014^{ns}	1.409^{ns}	2.041^{ns}	1.263^{ns}	1.240^{ns}	1.263^{ns}	1.014^{ns}	1.397^{ns}
Error 3	50								
CV1 (%)		13.96	9.38	1.92	14.78	13.40	14.78	13.96	10.96
CV2 (%)		20.18	6.55	3.03	15.05	22.03	15.05	20.18	9.49
CV3 (%)		14.34	7.63	2.39	14.99	14.56	14.99	14.34	12.31

$^{ns},^{**}$: Not significant, significant at 1% probability by the F test, respectively. CV1, CV2 and CV3 (%): coefficients of variation for nitrogen (N), soil use (SU), and interaction NxSU, respectively.

Table 4. Amount, concentration ([ ]), stocks (S), added (A) and C/N ratio of straw deposited of white oat cv. IPR 126 in different soil uses.

Soil uses	Straw (kgha^{-1})	[] of N (gkg^{-1})	[] of C (gkg^{-1})	S of N (kgha^{-1})	S of C (kgha^{-1})	A of N (kgha^{-1})	A of C (kgha^{-1})	C:N ratio
G10	1886^{b*}	31.58^{a}	490.17^{b}	58.77^{bc}	924.48^{b}	76.40^{bc}	980.61^{b}	15.58^{b}
G20	2162^{b}	32.02^{a}	500.81^{b}	69.24^{ab}	1084.60^{b}	90.01^{ab}	1124.27^{b}	15.74^{b}
C10	1790^{b}	32.04^{a}	489.84^{b}	57.19^{c}	875.83^{b}	74.34^{c}	930.57^{b}	15.34^{b}
C20	2269^{b}	31.31^{a}	496.13^{b}	70.97^{a}	1126.61^{b}	92.27^{a}	1179.73^{b}	15.93^{b}
ST	4050^{a}	15.49^{b}	527.39^{a}	62.18^{abc}	2136.68^{a}	80.83^{abc}	2106.21^{a}	34.58^{a}
ST	4051^{a}	15.21^{b}	531.90^{a}	61.62^{abc}	2156.47^{a}	80.11^{abc}	2106.71^{a}	35.40^{a}

Means followed by the same letter within a column are not significantly at 5%. G10, grazing with 10 cm height of the residue of; G20, grazing with a height of 20 cm of waste; C10, cut for haymaking with residue of 10 cm; C20, cut for haymaking with residue of 20 cm; ST, without grazing or cuts with tillage sowing of the summer crop; SC, without grazing or cuts with conventional preparation of soil for sowing of the summer crop

forced-air ventilation at a temperature of 55ºC for 72 h. Grinding and determination of N content occurred according to the methodology proposed by Embrapa (2009).

When harvest occurred on March 5, 2010, biometric characteristics were determined on 10 plants selected at random within the area of each plot. Data collected included stem diameter (with the aid of digital caliper measured between the first node visible from the ground surface), plant height (distance between the ground surface and the insertion of the last fully expanded leaves), and height insertion spike (distance between the ground surface and the base of the first spike).

Following biometric evaluations, manual harvest occurred (during March 2010) by collecting all the useful cobs from each plot. Of these, 10 spikes were taken at random to determine the number of kernel rows per spike and number of kernels per row (by manual counting), spike diameter (with the aid of a digital caliper), and spike length (with the aid of ruler graduated in centimeters). All harvested ears were subjected to a mechanized trail. The yield was estimated by weighing the grain obtained from the trail and corrected for kg ha^{-1} densities. The mass of 1000 grains was estimated by manually counting and weighing eight samples of 100 grains. The humidity of the samples was corrected to 13% moisture using a digital determiner.

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

RESULTS AND DISCUSSION

There was a significant effect of soil regime on the C:N ratio of oat straw. There were also significant effects in concentrations and stock of N and C in the straw and quantities of N and C added to the soil by oat straw (Table 3).

When the oats were subjected to cutting or grazing, the production of straw was lower than the soil regimes without cutting or grazing (Table 4). The result was expected due to harvesting of dry matter forage, because the regrowth capacity of the plants will be reduced as they undergo successive harvests, hampering the recovery of new leaf area and new accumulation of dry mass. Similar results were obtained by Flores et al. (2007) and Lopes et al. (2009). When working with oats and ryegrass intercrop under grazing stubble heights of 10, 20, 30, and 40 cm post grazing, these authors obtained values ranging from 1850-1860 to 5170-5400 kg ha^{-1} dry matter, from lowest to maximum height, respectively. In the same study, Flores et al. (2007) had

residual straw amounts of 6050 kg ha^{-1} dry matter in an area not subject to grazing. The presence of straw on the soil surface acts as a barrier for absorbing animal trampling (Lopes et al., 2009), and even amounts of straw near 2000 kg ha^{-1} are unable to compromise the yield of subsequent crops (Flores et al., 2007).

The soil regimes not cut or grazed led to deposition of straw with higher concentrations of C and lower N concentrations compared to the straw deposited by oats in other soil regimes (Table 4). The results obtained for the concentration of N in straw were expected, as the cutting or grazing of plants eliminates the possibility of lignification of plant structures and stimulates the regrowth and emergence of new shoots and new leaves, which, in turn, have N contents higher than that contained in the biomass of older plants. When oat plants are maintained under free growth (such as ST and SC), lignification of the cell wall (Campos et al., 2002) and reduction in crude protein (Vasconcelos et al., 2009) occur with a consequent reduction in N concentration (Henriques et al., 2007). When oats had been subjected to cutting or grazing, tillering and leaf area renewal were stimulated, and the plants remained in a vegetative stage with higher N concentration in dry matter.

In the stocks of estimated N in the straw and addition of N to the soil, higher values were obtained when oats were harvested by cutting with a height of 20-cm residue compared to the samples that received cutting or grazing with a residual height of 10 cm. The result is due to the deposited amounts of straw and differences in the concentration of this nutrient in the straw deposited. When the oats were not subjected to grazing or cutting, the N concentration was reduced by more than 50%, but higher straw deposition compensated for this reduction. In the areas where the oats were subjected to cutting or grazing, the obtained concentrations of N were similar to the straw of 10- and 20-cm heights by providing greater deposition amounts of straw; the plots cut with a residual height of 20 cm consequently provided a greater N stock (Table 4). Already for C, the largest stock and greater addition of C were obtained in soil regimes without cutting or grazing of the oats compared to other regimes (Table 4). The results observed for C are consistent with the concentration of the dry weight and amount of straw deposited.

The study of C stocks in the straw has received increased attention with the adoption of new concepts of sustainability, since the addition of plant residue in soil tillage is of utmost importance to maintaining and increasing the levels of organic matter in the soil (OMS), which has a key role in maintaining sustainable production over time (Lopes et al., 2009). Regarding the addition of C, grasses have great potential to add it to the soil, as reported by Rossi et al. (2012). The authors also noted the addition of C to the soil to inter plant with grain crops. The study of C addition to soil is important since it has a direct relationship with soil properties, enabling the soil to perform its functions and ensuring its quality (Vezzani and Mielniczuk, 2009).

In the case of the C:N ratio, the amount of straw obtained in the area where the oats had not been managed was higher than the straw deposited in areas where oats were subjected to cutting or grazing (Table 4). The values observed for the C:N ratio showed a direct relationship with age of plant development. This is because increased age of development causes an increase in the concentration of dry weight structural components, which are rich in C. There is also a concomitant decrease in cellular contents (Zanine and Macedo, 2006) and reduction in the amount of N in dry matter. Based on the classification proposed by Moreira and Siqueira (2006), the straw deposited by oats that was not subject to cutting or grazing had a high C:N ratio (>30), while the ratio of straw deposited by oats cut or grazed was considered low (<20). The addition of crop residues with high C:N ratios in the soil can cause a depletion in N due to the high demand of N by microbes causing N immobilization in soil. When the C:N ratio is low, the liberation of the element via mineralization occurs (Moreira and Siqueira, 2006).

In corn, only significant effects of soil regime on the leaf N content and grain yield were observed (Tables 5 and 6). None of the characteristics or yield components of the studied corn plants were affected by N management regimes or their interactions with soil regimes (Tables 5 and 6). This result may be related to the climatic conditions of the experiment (Figure 1), especially the period that encompassed the N applications.

The leaf N content of corn grown in areas subjected to cutting or grazing was higher than corn grown in areas where oats were not handled (Table 7). This result shows the occurrence of balance between the decomposition of straw and the processes of immobilization and mineralization of nutrients and the nutritional requirements of corn and is consistent with those observed by other authors (Hurtado et al., 2010). It is worth noting that cutting or grazing contribute to N cycling (Assmann et al., 2003) by stimulating the renewal of plant leaves, which retains N longer in the system and slows losses mainly due to leaching.

The lower leaf N content in soil regimes where there was greater deposition of straw can be attributed to immobilization of N due to the high C:N ratio present in these residues (Silva et al., 2007). In accordance with a high C:N ratio, the higher level of C in the straw added to soil by oats that is available for soil microbes enables greater immobilization of N, resulting in reduction in levels of mineral N in the soil and possibly lower concentration of nutrients in plant dry matter (Silva et al., 2007).

The productivity followed a similar pattern to that observed for leaf N. Productivity was higher in soil regimes with cutting or grazing and lower in soil regimes with greater deposition of straw (Table 7). The lowest

Table 5. F values calculated for the characteristics of corn plants grown under different soil uses (Soil Use) and managements of nitrogen (Nitrogen).

Source of variation	DF	Foliar nitrogen	Plant stand	Spikes index	Plants height	Height spikes insertion	Stem diameter
Block	2	6.012^{ns}	1.260^{ns}	0.428^{ns}	2.476^{ns}	5.110^{ns}	1.084^{ns}
Nitrogen (N)	5	1.791^{ns}	1.583^{ns}	0.200^{ns}	1.253^{ns}	0.430^{ns}	1.031^{ns}
Error 1	10						
Soil use (SU)	5	12.882^{**}	1.091^{ns}	1.668^{ns}	1.680^{ns}	0.566^{ns}	1.096^{ns}
Error 2	10						
NxSU	25	1.724^{ns}	1.551^{ns}	1.662^{ns}	0.989^{ns}	1.002^{ns}	1.016^{ns}
Error 3	50						
CV1 (%)		3.60	8.65	16.15	4.62	8.33	6.52
CV2 (%)		7.23	7.12	12.2	5.11	7.27	5.45
CV3 (%)		3.22	6.84	11.57	5.03	7.5	7.54

^{ns,**}: Not significant, significant at 1% probability by the F test, respectively. CV1, CV2 and CV3 (%): coefficients of variation for nitrogen (N), soil use (SU), and interaction NxSU, respectively.

Table 6. F values calculated for the yield components and corn productive under different soil uses (Soil Use) and managements of nitrogen (Nitrogen).

Source variation	DF	Spike diameter	Length spikes	Kernels rows per Spike	Grains per row	Thousand grains weight	Grain yield
Block	2	0.497^{ns}	1.400^{ns}	1.985^{ns}	0.752^{ns}	0.031^{ns}	1.361^{ns}
Nitrogen(N)	5	1.129^{ns}	1.218^{ns}	0.652^{ns}	0.567^{ns}	1.205^{ns}	2.471^{ns}
Error 1	10						
Soil use (SU)	5	2.687^{ns}	0.227^{ns}	0.938^{ns}	0.771^{ns}	1.676^{ns}	56.882^{**}
Error 2	10						
N*SU	25	1.275^{ns}	1.788^{ns}	1.173^{ns}	1.728^{ns}	1.663^{ns}	1.494^{ns}
Error 3	50						
CV1 (%)		2.51	9.75	4.51	7.51	12.06	3.36
CV2 (%)		2.23	10.47	6.57	6.39	9.98	3.74
CV3 (%)		2.69	8.36	5.25	5.04	9.26	5.17

^{ns,**}: Not significant, significant at 1% probability by the F test, respectively. CV1, CV2 and CV3 (%): Coefficients of variation for nitrogen (N), soil use (SU), and interaction NxSU, respectively.

Table 7. N foliar content and productivity of corn in succession of white oat under different soil uses.

Soil uses	Foliar N (g kg^{-1})	Grain yield (kg ha^{-1})
G10	34.39^{a}	8025^{a}
G20	34.21^{a}	8070^{a}
C10	33.80^{a}	8339^{a}
C20	33.07^{a}	8271^{a}
ST	30.04^{b}	7364^{b}
SC	30.18^{b}	7057^{b}

Means followed by the same letter within a column are not significantly at 5%. G10-grazing with 10 cm height of the residue of; G20, grazing with a height of 20 cm of waste; C10, cut for haymaking with residue of 10 cm; C20, cut for haymaking with residue of 20 cm; ST, without grazing or cuts with tillage sowing of the summer crop; SC, without grazing or cuts with conventional preparation of soil for sowing of the summer crop.

yield of corn when no cutting or grazing of oats occurred was caused by low N availability to plants because the inadequate supply of N is considered a major factor limiting the yield of corn (Cancellier et al., 2011). The deposition of large amounts of straw with a high C:N ratio (Table 4) and its decomposition contributed to the occurrence of microbial immobilization of N available in the soil (Hutchison and Walworth, 2007), reducing availability of this nutrient to plants. Sandini et al. (2011) observed a reduction in the productivity of the crop when grown in rotation with winter forage that received no N fertilizer and also attributed their results to the occurrence of microbial immobilization due to high input of crop residues with high C:N. Another factor that should be emphasized is that soil preparation induced more favorable conditions for leaching of N in the soil. This occurs when the soil structure is disrupted, thereby hindering the fixation of N in the soil with the other molecules present. Still worth highlighting is that N moves and is absorbed primarily via mass flow. Thus, due to disruption of the soil that is damaged, N can be leached by water. Unstructured soil has lower water retention enabling water to quickly reach greater depths where the plants are unable to make use of the N.

The greater yield in plots subjected to grazing or cutting reveals that there was no competition between corn plants and soil microorganisms for N in the soil and applied through fertilization. The lack of statistical difference between plots that were cut or grazed also shows that potential productivity losses caused by animal trampling were offset by their waste disposal, balancing productivity in areas with the presence of animals. In a study of soil management in corn, Balbinot Jr. (2011) also found no differences in crop yield when grown in rotation with winter forage.

The process of N immobilization due to the maintenance of large amounts of straw on the soil surface is not completely negative in production systems aimed at sustainability. The maintenance of organic N helps prevent or at least minimizes the occurrence of nitrogen loss due to leaching. Its gradual accumulation of organic forms enhances the ability of N supply over time, while the benefits of the straw can mitigate the effects of drought, reducing the evaporation of water and maintaining soil moisture for a longer period (Cruz et al., 2007), contributing to the development of corn (Ferreira et al., 2009).

The lack of significance of the N fertilization managements on corn yield can be explained by timing of N application, since all others received the application in the V_4 stage (with the exception of management 100:0:0), time is defined as the potential crop yield (Ritchie et al., 2003). This result confirms that even in the 0:25:75-management scheme, applying the lower portion (25 kg ha^{-1} N) was sufficient to determine the maximum crop yield potential in the environmental conditions and management regimes studied.

In the 100:0:0-management scheme, in which all applied side-dressed N was applied in advance (as is typical), the presence of crop residues on the surface may have contributed to the retention of applied N, which was initially held but mineralized in order to meet the demand for corn, even in soil regimes with higher amounts of crop residues and the lowest C:N ratio. This management aims to increase the availability of N in the early stages of crop development and reduce the effect of N immobilization by soil microorganisms to decompose crop residues with high C:N ratios (Pöttker and Wiethölter, 2004).

These results show that all N-management schemes studied are applicable to corn using the soil regimes tested. However, according to Kluthcouski et al. (2006), only in soils with straw provided with a continuous supply of adequate organic matter content can N fertilization be anticipated in years with regular rainfall. Otherwise, in addition to rising costs with the increase in applications, there is the risk of loss of applied mineral N by leaching due to excess rains.

Conclusions

Corn has limited productivity when established in succession to oats with straw greater than 4000 kg ha^{-1} and a C:N ratio greater than 34, regardless of anticipation or of split-N fertilization. The use of oats for grazing or crop residue on heights of 10 and 20 cm does not compromise the productivity of corn introduced in succession. When corn is established with a fixed dose of 40 kg ha^{-1} N at seeding, the amount of N in pre-sowing and side-dressing at the V_4 and V_8 leaf stages does not affect the leaf N content, characteristics of the plants, or yield components and production, regardless of the quantity and C:N ratio of crop residue cover.

Conflict of Interests

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

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Full Length Research Paper

Screening of free living rhizobacteria associated with wheat rhizosphere for plant growth promoting traits

Adesh Kumar[1]*, Shambhoo Prasad[1] and Sushil Kumar Singh[2]

[1]Department of Plant Molecular Biology and Genetic Engineering, N. D. University of Agriculture and Technology, Kumarganj, Faizabad-224229 (U. P.) India.
[2]Department of Plant Pathology, N. D. University of Agriculture and Technology, Kumarganj, Faizabad-224229 (U. P.) India.

The use of plant growth promoting rhizobacteria (PGPR) for the benefits of agriculture is gaining worldwide importance and acceptance and appears to be the trend for the future. PGPR are bio-resources which may be viewed as a novel and potential tool for providing substantial benefits to the agriculture. Plant growth promoting rhizobacteria (PGPR) are known to influence plant growth by various direct or indirect mechanisms. In search of efficient PGPR strains with multiple activities, a total of 58 isolates belonging to *Pseudomonas, Azotobacter* and *Bacillus* were isolated from wheat rhizospheric soils collected from various districts of Uttar Pradesh. These rhizospheric isolates were biochemically characterized and screened for their plant growth promoting traits like production of indole acetic acid (IAA), ammonia production, siderophore production, phosphate solubilization, salt tolerance and antibiotic sensitivity test activity. The isolates of *Pseudomonas* (86.36%), and *Azotobacter* (76.13%) produced IAA, whereas only 38.09% of *Bacillus* isolates were able to produce IAA. Ammonia production was most common trait of *Pseudomonas* (90.89%), and *Azotobacter* (66.43) and *Bacillus* (76.19%). Phosphorus solubilization was detected in the isolates of *Azotobacter* (66.23%), *Pseudomonas* (45.35%), and *Bacillus* (23.80%). Siderophore production was exhibited by 9.61 to 20.17% of isolates. On the basis of multiple plant growth promoting activities eighteen isolates (nine *Azotobacter*, six *Pseudomonas* and three *Bacillus*) were evaluated for quantitative IAA production, antibacterial and salt tolerance. All the *Azotobacter* isolates were shown to produce higher range (95.60 to 175.20 μg/ml) of IAA, while *Pseudomonas* produced (44.40 to 95.60 μg/ml) IAA. The isolate Bc2 also showed potential of producing high amount of IAA. The isolate Azt5, Azt9, Ps2, Bc2 and Bc3 were found resistant even at 20 μg/ml concentration of tetracycline in the medium. Salt tolerance even at 7% NaCl concentration was observed in Azt5, Bc1 and Bc3 isolates. This study has pointed out that few isolates could exhibit PGP traits, which may promote plant growth directly and indirectly.

Key words: Plant growth promoting rhizobacteria (PGPR), wheat, indole acetic acid, ammonia, siderophore, P solubilization, salt tolerance, antibacterial activity.

INTRODUCTION

Cereals such as rice, wheat and maize, are the major grains that sustain humanity. Wheat grows in temperate climates and it is staple food for 35% of the world's population. On the other hand, it provides more calories and proteins in the diet than any other crop (Laegreid et al., 1999). Wheat is one of the major crops cultivated in India and all over the world. Climatic conditions and modern agriculture have been severally modifying and

polluting the natural environment. The increasing demand for a steady and healthy food supply by a burgeoning human population will require efficient management practices along with controlling disease that reduce crop yield. During last few decades, agricultural production has increased due to the use of high yielding varieties and enhanced consumption of chemicals, which are used both as fertilizers to provide nutrition and as protection agents to control the damage caused by phyto-pathogens. Excessive use of chemicals and change in traditional cultivation practices has resulted in the deterioration of physical, chemical and biological health of the cultivable soil. Microbial diversity in soil is considered important for maintaining the sustainability of agriculture production systems (Stark et al., 2007). The variability in the performance of Plant Growth Promoting Rhizobacteria may be due to various environmental factors that may affect their growth and exert their effects on plants. The environmental factors include climate, weather conditions, soil characteristics or the composition or activity of the indigenous microbial flora of the soil. To achieve the maximum growth promoting interaction between PGPR and crop plants, it is important to discover how the rhizobacteria exerting their effects on plant and weather, the effects are altered by various environmental factors including the presence of other microorganisms (Bent et al., 2001).

The functions of soil biota are central to decomposition processes and nutrient cycling. Soil is considered a storehouse of microbial activity, though the space occupied by living microorganisms is estimated to be less than 5% of the total space. Therefore, major microbial activity is confined to the 'hot-spot', that is, rhizosphere. The growth of many microorganisms in the rhizospheric region depends upon the root exudates released by the plants (Bais et al., 2006). Interactions between plant and microbes are intensely studied and especially those that benefit plant growth. PGPRs may benefit the host by causing plant growth promotion or biological disease control. PGPR activity has been reported in strains belonging to several genera such as *Azotobacter, Pseudomonas, Azospirillum, Acetobacter, Burkhalderia* and *Bacillus* (Kloepper et al., 1989; Glick 1995; Glick and Bashan, 1997; Rangrajan et al., 2002; Ahmad et al., 2005; Fischer et al., 2007; Joseph et al., 2007; Sachdeva et al., 2009; Agrawal et al., 2011). PGPR can exhibit a variety of characteristics responsible for influencing plant growth. The common traits include production of growth regulators (auxin, gibberellins, ethylene etc), siderophore, ammonia, Phosphorus solubilization, HCN and antibiotics(Wahyudi et al., 2011; Etesami et al., 2009; Ahmad et al., 2008; Ahmad et al., 2005; Dilfuza, 2005; Valverde et al., 2003). Plant diseases are responsible for

annual crop losses at a total value of more than 200 billion (Agrios, 2005). Resistant plants and chemicals are often used to control plant diseases. Resistance does not exist against all diseases and the breeding of resistant plants takes many years. The use of microbes to control diseases, which is a form of biological control, is an environment-friendly approach. The microbe is a natural enemy of the pathogen, and if it produces secondary metabolites, it does so only locally, on or near the plant surface, that is, the site where it should act. In contrast, the majority of molecules of agro-chemicals do not reach the plant at all (Flores et al., 2006). An effective plant growth promoting and biological control strains isolated from one region may not perform in the same way in other soil and climatic conditions (Duffy et al., 1997; Johnson et al., 1998). Isolating of native strain adapted to the environment and their study may contribute to the formulation of inoculants to be used in region crops. The different stages of life cycle of wheat consist of elongation, flowering, fruiting and ripening stages. It is found that rate of roots exudates released by the root of the wheat at flowering stage is higher as compared to other stages, hence greater microbial biota and activity is expected during this stage (Huddedar et al., 2000). Thus, the present study aims to investigate native PGPR free living bacteria, associated with rhizosphere of wheat during flowering stage to evaluate their ability to enhance the growth and yield of wheat under the ecological condition of Uttar Pradesh.

MATERIALS AND METHODS

Soil sample collection

Soil samples were collected from the rhizosphere of different wheat growing areas of Uttar Pradesh during the flowering stage. The wheat plants were uprooted from the field and rhizosphere soil was pooled and filled in sterile polythene bags.

Isolation and characterization of plant growth promoting rhizobacteria

All the strains of *Pseudomonas, Azotobacter* and *Bacillus* were isolated from the rhizosphere of wheat grown in various locations in Uttar Pradesh, India. The *Pseudomonas* strains were isolated on Kings B medium containing (Protease peptone 20 g, Glycerol 10 ml, K_2HPO_4 1.5 g $MgSO_4.7H_2O$ 1.5 g, Agar 18 g, pH 7.2) per litre of distilled water; whereas *Azotobacter* on Jensen's medium containing (Sucrose 20 g, K_2HPO_4 1 g, $MgSO_4$ 0.5 g ,NaCl 0.5, Na_2MoO_4 0.001 g, $FeSO_4$ 0.01 g, $CaCO_3$ 2 g, Agar 18 g, pH 7.0) per litre of distilled water, and *Bacillus* on the nutrient agar containing 5 g peptone, 3 g beef extract and 18 g Agar per liter of distilled water. The plates were incubated at 30°C for 24 h. After incubation, plates were observed for different isolates based on morphological

features. Morphologically variable colonies picked up and purified on respective media plates. The pure cultures of isolates were made and preserved on the respective media slants. The total fifty eight rhizobacterial isolates were isolated (Ahmad et al., 2008). The rhizobacterial isolates were characterized on the basis of cultural, morphological and biochemical characteristics (Cappuccino and Sherman, 1992).

Screening of soil bacterial strains for plant growth promoting activities

Indole acetic acid production

The IAA production was assayed by the modified method as described by Loper and Scroth (1986). Bacterial cultures were grown for 48 h (*Pseudomonas, Bacillus*), and 72 h (*Azotobacter)* on their respective media at 28°C on rotary shaker. Fully grown cultures were centrifuged at 10000 rpm for 15 min. The 2 ml of supernatant was mixed with 2 to 3 drops of O-phosphoric acid and 4 ml of salkouski reagent solution (1 ml of $FeCl_3$ 0.5 M mixed in 50 ml of 35% $HClO_4$). The samples were incubated for 25 min at room temperature. The development of pink color was observed and optical density was taken at 530 nm with help of spectrophotometer. The concentration of IAA produced by cultures was measured with the help of standard graph of IAA obtained in the range of 20 to 200 µg ml^{-1}.

Ammonia production

The isolates were grown in peptone water in tubes incubated at 30°C for four days. One ml of Nessler's reagent (0.09 mol/L solution of potassium tetra-iodo-mercurate (II) in 2.5 mol potassium hydroxide was added in each test tube. The observed presence of faint yellow color indicates small amount of ammonia and deep yellow to brownish color indicates maximum production of ammonia (Cappuccino and Sherman, 1992).

Phosphorus solubilization

All the isolates were spot inoculated on Pikovskaya agar medium (Yeast extract 0.5 g Dextrose 10 g, Calcium phosphate 5 g , Ammonium sulphate 0.5 g KCl 0.2 g Mg SO4 0.1 g $MnSO_4$0.0001 g, FeSO4 0.001 g , Agar 15 g and 1 L distilled water) with phosphorus source and incubated at 28°C for 4 days. Phosphorus activity was determined by development of clearing zone around the culture spot (Agrawal et al., 2011; Wahyudi et al., 2011).

Siderophore production

Bacterial isolates were tested for siderophore production on chome azurol (60.5 mg CAS,1 Mm FeCl3.6H2O, 10 M HCl, 72.9 mg HDTMA, 1 M Sucrose (3 ml), 1 M $CaCl_2$ (0.4 ml), 1 M $MgSO_4.7H_2O$ (0.8 ml), 2% K_2HPO_4 (10 ml, NaCl (0.2 g), $NaMoO_4$ (0.005 g), PIPES (30.24 g), Difco agar (15 g), 10% Casamino acid (30 ml))) s agar (CAS) medium described by Schwyn and Neilands (1987). Each isolate was streaked on surface of CAS agar medium and incubated at room temperature for 1 to 2 days. The development of orange halo around the growth has been considered as positive for siderophore production.

Antibiotic sensitivity

The isolates were tested against the tetracycline by agar dilution

method as described by Ahmad et al. (2004). The stock solution (5 mg/ml) of antibiotic, that is, tetracycline has prepared and used four different concentrations 1, 5, 10 and 20 µg/ml for antibiotic sensitivity test. The tetracycline was dissolved in 70% ethanol and sterilized with membrane filler (Axiva Scihem biotech). The nutrient agar medium was prepared in 4 L flasks of 500 ml and allowed to cool to 50°C. The diluted tetracycline of different concentrations were mixed in cool molten agar medium and poured in petri plates. The isolates were spot inoculated on solidified agar plate and incubated at 30°C for 48 h. After incubation, the plates were examined for the presence or absence of growth on the spotted area. The strains which were sensitive against tetracycline did not grow on the plate and resistant strains shows the growth on the plates against tetracycline.

Salt tolerance

The pure cultures of all isolates were streaked on nutrient agar medium, containing 3 to 7% NaCl concentration. Control plates with NaCl amendment were also kept for observation for all strains. All plates were incubated at 30°C for 48 h and observed for the presence or absence of the growth.

RESULTS AND DISCUSSION

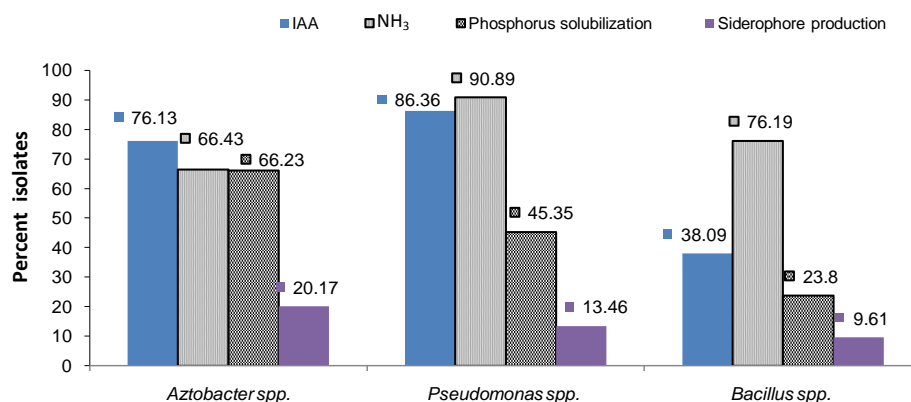### Isolation and biochemical characterization

On the basis of cultural, morphological and biochemical characteristics a total of 58 bacterial isolates were identified as *Azotobacter, Pseudomonas* and *Bacillus* as described in Bergeys manual of determinative bacteriology (Holt et al., 1994). The *Azotobacter, Pseudomonas* and *Bacillus* strains from rhizosphere of different crops were isolated and extensively studied by Kole and Hajra (1997), Gaind and Gaur (1999), Ahmad et al. (2005), Joseph et al. (2007), Fischer et al. (2007), Ahmad et al. (2008), and Wahyudi et al. (2011). The general characteristics of the isolates were illustrated (Table 1).

Screening of rhizobacteria for plant growth promoting traits

In the present study a total of 58 bacterial strains (22 isolates of *Pseudomonas* and 18 of each *Azotobacter* and *Bacillus*) were tested for IAA, ammonia production, phosphorus solubilization and siderophore production (Figure 1). IAA production was shown in most of the *Pseudomonas* isolates (86.36%) followed by *Azotobacter* (76.19%) and *Bacillus* (38.09%). Ammonia production was detected in 90.89% of *Pseudomonas* followed by *Bacillus* (76.19%) *and Azotobacter* (66.43). The *Azotobacter* (66.23%), *Pseudomonas* (45.35%) and *Bacillus* (23.80%) strains were found able to solubilize phosphate. Very few strains of *Azotobacter* (20.17%), *Pseudomonas* (13.46%) and *Bacillus* (9.61%) exhibited siderophore production. Similar to our findings of plant growth promoting activities among Rhizobacteria strains have also been reported by some other workers

Table 1. Morphological and cultural characteristic of rhizobacterial test isolates.

Biochemical characters	*Azotobacter* spp.	*Pseudomonas* spp	*Bacillus* spp
Number of isolates	18	22	18
Grams reaction	-ve	-ve	+ve
Shape	rods	rods	rod
Pigment	Transparent to light milky most isolates become light brown to black after 10 days of incubation	Cream , light to green	Cream
Colony morphology	Watery mucilaginous with smooth margins	Smooth margin, flat to raised	Circular, lobate to serrated margin
Sucrose	+	+	+
Dextrose	+	+	+
Mannitol	+	-	+
H_2S production	-	-	-
Indole	-	-	-
Methyl red	-	-	-
Vogues Prokauer	-	-	-
Citrate Utilization	+	+	+
Starch	+	+	+
Gelatin hydrolysis	-	+	-
Catalase test	+	+	+
Nitrate reduction	-	+	-
Lipid hydrolysis	+	+	+
Casein hydrolysis	+	+	+

Figure 1. Plant growth promoting activities of rhizobacterial test isolate.

(Corsa and Walsh, 2002; Huddedaret al., 2002; Ahmad et al., 2004; Pedraza et al., 2004; Ahmad et al., 2008; Sachdev et al., 2009; Joshi and Bhatt, 2011; Rawat and Asrar, 2011).

Quantitative screening for IAA production by selected rhizobacterial isolates

Total of 18 selected rhizobacterial isolates of

Pseudomonas (nine), *Azotobacter* (six) and *Bacillus* (three) were tested for quantitative IAA production. The production of IAA was recorded highest in isolates of *Azotobacter,* followed by *Pseudomonas* and *Bacillus* respectively. Among *Azotobacter* isolates, Azt-4 and Azt-7 produced highest amount (175.20 µg/ml) of IAA followed by Azt-1>Azt3 >Azt-6. However, *Pseudomonas* rhizobacteria isolates produced IAA in the range of 44.40 to 95.60 µg/ml in the broth culture medium (Tables 2 and 3). Wahyudi et al. (2011) reported that *Bacillus* spp. Cr4

Table 2. Production of Indole Acetic acid (IAA) by selected rhizobacterial isolates grown in respective medium.

S/N	Isolate	IAA Production µg/ml
1	Azt1	130.15
2	Azt2	114.66
3	Azt3	114.66
4	Azt4	175.20
5	Azt5	95.60
6	Azt6	114.60
7	Azt7	175.20
8	Azt8	114.60
9	Azt9	114.60
10	Ps1	95.60
11	Ps2	79.60
12	Ps3	44.40
13	Ps4	66.20
14	Ps5	79.60
15	Ps6	79.60
16	Bc1	70.00
17	Bc2	72.40
18	Bc3	64.00
	CD	22.08
	SEm	7.68

Table 3. Antibiotic sensitivity and salt tolerance of selected rhizobacterial test isolates.

S/N	Isolate	Antibiotic concentration (µg/ml)				NaCl concentration (%)				
		1	5	10	20	3	4	5	6	7
1	Azt1	++	++	-	-	++	-	-	-	-
2	Azt2	+++	-	-	-	++	-	-	-	-
3	Azt3	++	+	-	-	+	-	-	-	-
4	Azt4	+++	-	-	-	-	-	-	-	-
5	Azt5	+++	+++	++	-	+++	++	+	+	-
6	Azt6	-	-	-	-	++	-	-	-	-
7	Azt7	++	++	-	-	++	+	-	-	-
8	Azt8	+++	-	-	-	++	+	-	-	-
9	Azt9	+++	+++	++	++	+	+	+	-	-
10	Ps1	+++	-	-	-	-	-	-	-	-
11	Ps2	+++	+++	+++	++	+	+	-	-	-
12	Ps3	++	++	-	-	+	+	-	-	-
13	Ps4	+++	-	-	-	+	+	-	-	-
14	Ps5	+++	+++	-	-	+	-	-	-	-
15	Ps6	++	++	++	-	+	-	-	-	-
16	Bc1	+++	++	-	-	+++	+++	+++	+++	+
17	Bc2	+++	+++	+++	+++	+++	+	-	-	-
18	Bc3	+++	+++	++	-	+++	+++	++	++	+

Azt = *Azotobacter*, Ps = *Pseudomonas*, Bc = *Bacillus*, Incubation period 36 h; +++ = maximum growth, ++ = medium growth, + = poor growth, - = no growth.

produced 86.82 mg/L IAA in culture medium supplemented with L Tryptophan while 32.80 µg/ml IAA production was reported by Ahmad et al. (2004). The findings of present investigation are outstanding in

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Full Length Research Paper

Effect of salicylic acid on productivity and nutrient uptake of *Brassica species* under different planting durations

Sarita Muhal, N. S. Solanki*, P. Singh and K. B. Shukla

Department of Agronomy, Maharana Pratap University of Agriculture and Technology, Udaipur- 313001, India.

A field experiment was conducted at Udaipur during the *Rabi* season of 2011 to 2012 to evaluate the effect of planting duration and salicylic acid (SA) application on yield, quality and nutrient uptake of *Brassica* species. The results revealed that crops sown during SMW (Standard Meteorological Week) 41 recorded significantly ($p < 0.05$) higher seed, stover and oil yields, N and P uptake in seed and stover than SMW 43 and SMW 45 sown crops. Amongst *Brassica* species, *B. juncea* var. Bio-902 proved superiority over *Brassica juncea* var. RGN-73 and *Brassica campestris* var. BSH-1 in terms of seed, stover and biological yield, and N and P uptake in seed and stover. The foliar spray of salicylic acid produced significantly ($p < 0.05$) higher seed yield and oil content compared to water spray.

Key words: Planting duration, *Brassica species*, salicylic acid (SA), nutrient uptake.

INTRODUCTION

Production potentiality of mustard can be fully exploited with suitable agronomic practices and the selection of genotypes. Among the different agronomic practices, optimum sowing time plays an important role to fully exploit the genetic potentiality of a variety as it provides optimum growth conditions such as temperature, light and humidity. The growth phase of the crop should synchronize with optimum environmental conditions for better expression of growth and yield. It is a fact that specified genotypes does not exhibit the same phenotypic characteristics in all environmental conditions. The different genotypes, growth response vary in different environments and their relative ranking usually

differ and this ultimately decides the selection of genotypes for a particular or different sowing dates in other to stabilize higher yields. Out of the various abiotic stresses, high temperature is the most important stress, which can strike a crop at any time and imposed many limitations on growth and development. Developing crop plants with improved thermo tolerance can mitigate the adverse effect of heat stress.

  Brassica is an important oil seed crop and its early sowing implies many important advantages such as escape from aphids infestation, while late sown crop encounters high temperature stress at seed development stage, which causes a great yield reduction (Abolfazl et

al., 2009). The application of plant growth regulators is known to play an important role in plant response to stress (Chakrabarti and Mukherjee, 2003). Salicylic acid (SA) has recently been recognized as a plant growth hormone and plays diverse physiological roles in plants including thermogenesis generate a wide range of metabolic and physiological responses thereby affecting their growth and development (Hayat et al., 2010). SA has been found to be involved in both basal and acquired thermo-tolerance in plants (Dat et al., 1998a, b, 2000; Lopez-Delgado et al., 1998). In the context of the above views, the present study was undertaken to test the hypothesis that SA will mitigate the adverse effects of temperature stress in *Brassica*.

MATERIALS AND METHODS

Plant materials and growth conditions

The field experiment was conducted during Rabi season of 2011 to 2012 at Instructional Farm, Rajasthan College of Agriculture, Udaipur situated at Southern part of Rajasthan at an altitude of 582.0 m above mean sea level, at 24°34' N latitude and 73°42' E longitude. The region falls under agro-climatic zone IV A "Sub-humid Southern Plain and Aravali Hills" of Rajasthan and agro climatic zone VIII (Central plateau and hills) of India. The soils of experimental field was clay loam in texture and slightly alkaline in reaction (pH 7.9) and calcareous in nature. The experiment consisted of 18 treatment combinations under three planting duration (Standard Meteorological Week (SMW) 41, SMW 43 and SMW 45) and three *Brassica* species (*B. juncea* var. Bio-902, *B. juncea* var. RGN-73 and *B. campestris* BSH-1). The experiment was laid out in split plot design with three replications. The crop was sown during SMW 41(12th October), SMW 43 (27th October) and SMW 45 (11th November) with a seed rate of 5 kg/ ha and with 30 cm row to row and 10 cm plant to plant spacing. The crop was fertilized with 60 kg N and 40 kg P_2O_5 ha^{-1}. One half of nitrogen and full dose of phosphorus were given as basal application. The remaining dose of nitrogen was top dressed at first irrigation 30 days after sowing (DAS). The foliar spray of SA 100 ppm was applied at 60 DAS and 75 DAS. The spray solution of 100 ppm SA was prepared by dissolving 100 mg of SA in one litre of water.

Nutrient estimation

Nitrogen content in stover and seed was determined by using Nesseler's reagent colorimetric method (Linder, 1944) and phosphorus by Vandomolybdo phosphoric acid yellow colour method (Jackson, 1967). Nitrogen and phosphorus uptake by plant at harvest was calculated by using following formula:

$$\text{Nutrient uptake by seed/stover (kg ha}^{-1}) = \frac{\text{Nutrient content in seed/stover (\%)} \times \text{Seed/stover yield (kg ha}^{-1})}{100}$$

The total nutrient uptake by the crop was estimated through summing up nitrogen and phosphorus uptake by seed and stover.

Yield components

Biological yield was recorded by weighing sun-dried plants of net plot along with siliquae and expressed in kg ha^{-1} for biological yield. The stover yield was obtained by subtracting seed yield from biological yield. Harvest index was worked out by the formula of Singh and Stoskopf (1971).

Harvest index (%) = (Economic yield/ Biological yield) × 100

Statistical analysis of data

In order to test the significance of variation in experimental data obtained for various treatment effects, data were statistically analyzed as described by Panse and Sukhatme (1989). The critical difference was calculated to assess the significance of treatment mean wherever the 'F' test was found significant at 5% level. To estimate inter-relationship between various characters, correlation coefficients were computed.

RESULTS AND DISCUSSION

Planting duration

Data on seed, stover and biological yields and harvest index presented in Table 1 shows that the highest seed yield of 1971.11 kg ha^{-1} was obtained under SMW 41 sown crop which was significantly ($p < 0.05$) superior over SMW 43 and SMW 45 sown crop by 7.8 and 34.1%, respectively. Further, it was noted that the crop sown on SMW 43 gave significantly ($p < 0.05$) higher seed yield (1827.83 kg ha^{-1}) by 24.3% over SMW 45 sown crop (1470.26 kg ha^{-1}). Similarly, SMW 41 sown crop recorded the highest stover (4877.99 kg ha^{-1}) and biological yields (6849.10 kgha[-1]), which was significantly ($p < 0.05$) higher over SMW 43 by 10.3 and 9.6% and SMW 45 sown crop by 14.8 and 19.7%, respectively. Furthermore, the crop during SMW 43 recorded the maximum harvest index (29.4%) which was significantly ($p < 0.05$) superior over SMW 41 and SMW 45 sown crops by 2.4 and 13.1%, respectively. Similarly, SMW 41 sown crop recorded significantly ($p < 0.05$) higher harvest index over SMW 45 sown crop by 10.4%. Higher temperature prevailed during later phase of the crop growth which caused shortening of crop period and forced maturity resulting into reduced unit weight of seed and ultimately low seed yield under delayed sowings. The seed yield was negatively correlated with maximum, minimum and mean temperatures during 90 to 105 DAS (r = -0.654, -0.612, -0.639). In the present investigation, it was observed that an increase in mean temperature by 1°C during 90 to 105 DAS caused reduction in seed yield of about 250 kg ha^{-1} (Figure 1).

Interactive effects between planting duration and foliar spray on seed yield (Table 2) shows that the maximum seed yield of 1974.15 kg ha^{-1} was obtained under SMW 41 sown crop with 100 ppm SA which was at par with treatment combinations viz. SMW 43+100 ppm SA, SMW 41+water spray and SMW43+water spray. Under SMW 41 and SMW 43, foliar spray of 100 ppm SA was at par with water spray. However, under SMW 45, foliar spray of

Table 1. Effect of planting duration and salicylic acid (SA) on yields and harvest index of *Brassica* species.

Treatments	Seed yield (kg ha^{-1})	Stover yield (kg ha^{-1})	Biological yield (kg ha^{-1})	Harvest index (%)
Planting duration				
SMW 41	1971.11	4877.99	6849.10	28.7
SMW 43	1827.83	4420.93	6248.76	29.4
SMW 45	1470.26	4249.37	5719.63	26.0
SEm±	46.15	127.47	172.09	0.16
CD (P=0.05)	138.36	382.16	515.95	0.50
Brassica spp.				
Brassica juncea var. Bio-902	1945.59	4955.52	6901.11	28.1
Brassica juncea var. RGN-73	1875.40	4881.37	6756.77	27.7
Brassica campestris var. BSH-1	1448.21	3711.39	5159.60	28.2
SEm±	46.15	127.47	172.10	0.17
CD (P=0.05)	138.36	382.16	515.95	NS
Foliar spray				
Water spray	1713.68	4426.22	6139.91	27.8
100 ppm SA	1799.12	4605.97	6405.09	28.2
SEm±	20.97	60.24	78.68	0.13
CD (P=0.05)	62.29	178.98	233.76	NS
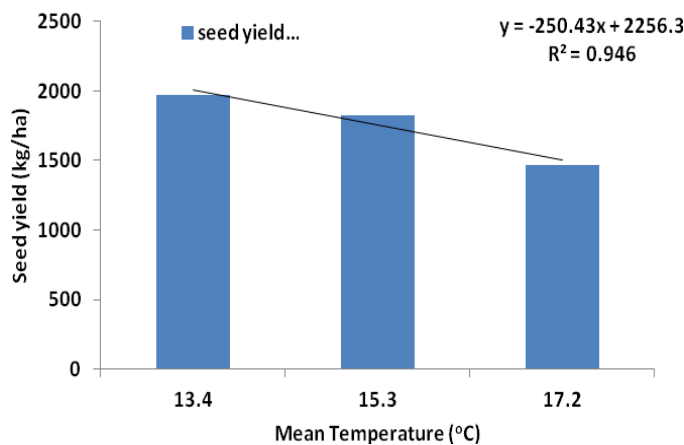
Figure 1. Effect of mean temperature during 90 – 105 DAS on seed yield of *Brassica*.

SA produced significantly higher seed yield over water spray by 14.7%. Foliar spray of SA might reverse the effect of heat stress under SMW 45 sown crop resulting in significantly ($p < 0.05$) higher seed yield. It has been reported that exogenous application of SA enhanced photosynthesis efficiency, metabolism and growth of mustard plant under elevated temperatures (He et al., 2005; Kaur et al., 2009; Chhabra et al., 2013).

Data pertaining to oil yield (Table 3) revealed that the crop sown on SMW 41 kept maximum oil content (38.10%) followed by SMW 43 (37.60%) and SMW 45 (36.48%). The crop sown on SMW 41 produced maximum oil yield (753.21 kg ha^{-1}) followed by SMW 43 (689.74 kg ha^{-1}) and SMW 45 (538.25 kg ha^{-1}).

Planting duration did not influence N and P content in seed and stover (Table 3). However, the crop sown on SMW 41 recorded maximum N uptake (63.02 kg ha^{-1}) which was significantly ($p < 0.05$) superior over SMW 43 (57.63 kg ha^{-1}) and SMW 45 (46.00 kg ha^{-1}). Similar trends were noted with respect to P uptake (Table 4). The crop sown on SMW 41 recorded maximum total N and P uptake (105.26 and 19.69 kg ha^{-1}) which was significantly

Table 2. Interaction effect of planting duration and foliar spray on seed yield (kg ha^{-1}).

Treatment	Foliar spray	
	Water spray	100 ppm SA
Planting duration		
SMW 41	1968.07	1974.15
SMW 43	1803.33	1852.33
S SMW 45	1369.64	1570.89
	SEm±	CD (P = 0.05)
Same planting duration for different foliar spray	36.31	107.89
Same foliar spray and different planting duration	74.68	221.91

Table 3. Effect of planting duration and *Brassica* species on quality and nutrient content.

Treatments	Oil content (%)	Oil yield (kg ha^{-1})	N content (%)		P content (%)	
			Seeds	Stover	Seeds	Stover
Planting duration						
SMW 41	38.10	753.21	3.194	0.861	0.356	0.258
SMW 43	37.60	689.74	3.152	0.850	0.355	0.254
SMW 45	36.48	538.25	3.126	0.840	0.346	0.251
SEm±	0.34	21.32	0.027	0.008	0.004	0.004
CD (P=0.05)	1.03	63.92	NS	NS	NS	NS
***Brassica* spp.**						
Brassica juncea var. Bio-902	38.11	745.51	3.212	0.874	0.352	0.264
Brassica juncea var. RGN-73	37.53	705.21	3.167	0.838	0.359	0.252
Brassica campestris var. BSH-1	36.54	530.49	3.093	0.839	0.345	0.247
SEm±	0.34	21.32	0.027	0.008	0.004	0.004
CD (P=0.05)	1.03	63.92	0.082	0.025	NS	0.011
Foliar spray						
Water spray	36.82	635.03	3.127	0.846	0.352	0.255
100 ppm SA	37.96	685.78	3.188	0.855	0.352	0.254
SEm±	0.14	8.92	0.021	0.006	0.002	0.003
CD (P=0.05)	0.42	26.49	NS	NS	NS	NS

($p < 0.05$) superior over SMW 43 (95.17 and 17.48 kg ha^{-1}) and SMW 45 (82.05 and 15.95 kg ha^{-1}).

The maximum net returns of ₹ 54469 ha^{-1} were obtained under SMW 41 sown crop which was significantly higher over SMW 43 and SMW 45 sown crops by ₹ 4943 and ₹ 17279 ha^{-1}, respectively. Further, SMW 43 planting duration gave significantly ($p < 0.05$) higher net return over SMW 45 by ₹ 12336 ha^{-1} (Table 4).

Brassica species

The highest seed yield of 1945.59 kg ha^{-1} was recorded in *B. juncea* var. Bio-902 which was significantly ($p <$ 0.05) superior over *B. campestris* var. BSH-1 by 34.3% (Table 1). Further, it was noted that *B. juncea* var. RGN-73 gave significantly ($p < 0.05$) higher seed yield (1875.40 kg ha^{-1}) over *B. campestris* var. BSH-1 by 29.5%. However, *B. juncea* var. Bio-902 was at par with *B. juncea* var. RGN-73 in respect to seed yield. Similar trends were recorded in stover and biological yields. However, *Brassica species* did not influence harvest index. The superiority of *B. juncea* var. Bio-902 seems to be on account of efficient translocation of metabolites towards sink. The *B. juncea* var. Bio-902 recorded significantly higher biological yield of 6901.11 kg ha^{-1} and was 33.7% higher compared to varieties *B. campestris* var. BSH-1. This was attributed to significantly ($p < 0.05$)

Table 4. Effect of planting duration, *Brassica* species and salicylic acid on nutrient uptake and economics.

Treatments	N uptake (kg ha⁻¹)			P uptake (kg ha⁻¹)			Net returns (₹ ha⁻¹)
	Seeds	Stover	Total	Seeds	Stover	Total	
Planting duration							
SMW 41	63.02	42.00	105.26	7.04	12.65	19.69	54469
SMW 43	57.63	37.54	95.17	6.41	11.07	17.48	49526
SMW 45	46.00	36.05	82.05	5.12	10.83	15.95	37190
SEm±	1.44	1.02	2.38	0.17	0.36	0.51	1592
CD (P=0.05)	4.32	3.07	7.14	0.52	1.07	1.54	4773
***Brassica* spp.**							
Brassica juncea var. Bio-902	62.25	43.26	105.51	6.86	13.10	19.96	53588
Brassica juncea var. RGN-73	59.56	40.93	100.49	6.72	12.29	19.01	51167
Brassica campestris var. BSH-1	44.83	31.41	76.24	5.00	9.15	14.15	36429
SEm±	1.44	1.02	2.38	0.17	0.36	0.51	1592
CD (P=0.05)	4.32	3.07	7.14	0.52	1.07	1.54	4773
Foliar spray							
Water spray	53.72	37.56	91.28	6.05	11.29	17.34	45670
100 ppm SA	57.38	39.51	96.88	6.33	11.74	18.07	48453
SEm±	0.76	0.63	1.28	0.08	0.18	0.23	723
CD (P=0.05)	2.26	1.86	3.81	0.23	NS	0.69	2149

higher seed yield (1945.59 kg ha⁻¹) and stover yield (4955.52 kg ha⁻¹). The results are in agreement with that of Chaplot et al. (2012).

The results (Table 3) revealed that *B. juncea* var. Bio-902 recorded maximum oil content (38.11%). This was significantly higher over *B. campestris* var. BSH-1 by 4.3%. However, *B. juncea* var. Bio-902 was at par with *B. juncea* var. RGN-73 (37.53%). Similarly, *B. juncea* var. Bio-902 produced maximum oil yield (745.51 kg ha⁻¹). This was significantly superior over *B. campestris* var. BSH-1 by 40.5%, respectively. However, *B. juncea* var. Bio-902 was at par with *B. juncea* var. RGN-73 (705.21 kg ha⁻¹). Further, *B. juncea* var. RGN-73 recorded significantly higher oil yield over *B. campestris* var. BSH-1 by 32.9%. The results are in close conformity with the findings of Rana and Pachauri (2001).

B. juncea var. Bio-902 recorded maximum N content (3.212%) which was significantly higher over *B. campestris* var. BSH-1 by 3.8% (Table 3). However, *B. juncea* var. Bio-902 was at par with *B. juncea* var. RGN-73 (3.167%). Further, *B. juncea* var. Bio-902 recorded maximum N uptake (43.26 kg ha⁻¹) which was significantly higher over *B. campestris* var. BSH-1 by 37.7 %. However, *B. juncea* var. Bio-902 was at par with *B. juncea* var. RGN-73 (40.93 kg ha⁻¹).

Salicylic acid

Data on yield presented in Table 1 indicate that foliar

spray of 100 ppm SA produced significantly higher seed yield (1799.12 kg ha⁻¹) by 5.0% as compared to water spray (1713.68 kg ha⁻¹). Foliar spray of 100 ppm SA produced significantly higher stover and biological yields by 4.1 and 4.3%, respectively as compared to water spray. Increase in seed yield with foliar spray of SA could be ascribed to the fact that crop yield is not an abstract entity but it is an outcome of positive interaction between vegetative and reproductive growth of the crop. Significant improvement in yield components of wheat crop under the influence of SA was also reported by Hassanein et al. (2012).

Foliar spray of 100 ppm SA registered significantly higher oil content and oil yield by 3.1 and 8.0%, respectively as compared to water spray (Table 3). However, N and P content in seed and stover were not affected due to foliar application of SA. Foliar spray of 100 ppm SA significantly influenced N and P uptake by seed and stover as well as total uptake of N and P. Total N uptake by was higher under 100 ppm SA by 6.1% over water spray (91.28 kg ha⁻¹). While total P uptake was 4.2% higher over water spray (17.34 kg ha⁻¹) under the 100 ppm SA. The positive impact of nutrient uptake in seed and stover seems to be on account of better development of canopy which might have maintained adequate supply of metabolites for better growth. Thus better developed root system might have facilitated in more extraction of nutrients from soil and translocation to plant parts. Higher concentration of nutrients in seed along with higher seed yield under foliar spray of SA

resulted in higher uptake of nutrients (Nafees et al., 2010).

Foliar spray of 100 ppm SA recorded significantly higher net returns of ₹ 48453 ha^{-1} over water spray by ₹ 2783 ha^{-1} (Table 4).

Conclusion

It can be concluded from the present study that *Brassica juncea* var. Bio-902 sown during SMW 41 gave maximum yield and foliar spray of 100 ppm SA enhanced seed yield in delayed sowing of *Brassica*.

Conflict of Interest

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

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