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Full Length Research Paper

Description of one new species of *Tetrastichus* Haliday (Hymenoptera: Eulophidae) reared from the eggs and larvae of tortoise beetle from India

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One new species of *Tetrastichus Haliday* (*Tetrastichus burki* sp. nov.) is illustrated and described from the eggs and larvae of tortoise beetle collected from *Ipomoea* sp. from Pantnagar Uttarakhand, India.

Key words: *Tetrastichus,* tortoise beetle, *Ipomoea*, new species.

INTRODUCTION

The genus *Tetrastichus* Haliday (Hymenoptera: Eulophidae) includes a large number of species of minute chalcid-flies. These may be either primary parasites or hyperparasites, and they attack a wide variety of hosts, including such destructive pests as the Hessian fly and the cotton boll weevil and many kinds of thrips, aphids, midges, leaf miners, scales, tent caterpillars, borers, roaches, beetles, and gall-makers injurious to agriculture, horticulture, and forestry. They have been reared from the eggs, larvae, and pupae of other insects, as well as from many plant galls (Burks, 1943).

Tortoise beetles form a morphologically distinctive sub-family (Cassidinae) of the leaf eating beetles (Chrysomelidae) (Heron, 1992). The eggs are attached singly to the underside of the leaves. Larvae are broad and flattened and adorned with branched spines. Both larvae and adults feed on foliage. The typical form of injury is the creation of numerous small to medium sized. The wasp parasitoid *Tetrastichus cassidus* (Hymenoptera: Eulophidae) and the fly parasitoid *Eucelatoriopsis dimmocki* (Diptera: Tachinidae) are known to attack golden tortoise beetle (Capinera, 2001).

In the present paper, the new species of *Tetrastichus* Haliday attacking the eggs and larvae of golden tortoise beetle is described as *Tetrastichus burki* sp. nov.

MATERIALS AND METHODS

The eggs and larvae of tortoise beetle were collected from the *Ipomoea* sp. from Pantnagar, district Udham singh nagar of Uttarakhand, India. The length of whole specimen is given in millimeters: all other measurements are relative and were taken directly from the divisions of a linear scale of a micrometer placed in the eye piece of a compound microscope for slide mounted parts. Body colour of insect was noted before clearing and mounting the specimen on slide in balsam. The permanent slides were examined under Trinocular microscope in order to make drawings and detailed study of each structure with the help of Camera Lucida.

The terminology given by Gibson (1997) and Graham (1987)

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is followed in this paper. ocellar-ocular distance; POL, post-ocellar distance; SMV, submarginal vein; MV, marginal vein; PMV, Post marginal vein; F1, F2,..., funicle segments 1, 2,...; C1-3, claval segments.

DIAGNOSIS

Description

Female

Body length about 1.71 mm; body colour predominantly dark brown; head black with greenish metallic tinge and eyes brown; antennae brown except scape yellow; thorax brown, reticulated with bluish green metallic tinge; wings hyaline with brown venation, basal cell of forewings broad; legs yellow: except; gaster petiolated, dark brown (Figure 1a to n).

Head (Figure 1c to d): More than 1.3x broader than long in frontal aspect(0.46:0.34), hairy; ocelli arranged in acute angled triangle; POL slightly more than 1.8x times as long as OOL (0.11:0.06); antennal toruli inserted just above the lower level of eye margin; compound eyes large and smooth; malar sulcus prominent, straight; malar space slightly longer than eye width (0.10:0.12), malar space is 1.66 length of eye; mandibles tridentate (Figure 5) with characteristic falcate tooth acute teeth with outermost one being sickle shaped and inner two teeth; maxillary palp and labial palp each single segmented; lower margin of clypeus distinctly bilobed.

Antennae (Figure 1a): 8 segmented excluding 1 anellus; apical tip of antenna with spicule; antennal formula 11133; scape cylindrical about 5.2x as long as wide (0.04:0.21); pedicel as long as first funicle segment (0.07: 0.07); funicle three segmented, FSI 1.4x as long as wide (0.05:0.07), FS2 and FS3 are equal in length and wide about 1.6x as long as wide (0.06:0.07); club 3 segmented, more than 3.1 times as long as wide (0.06:0.19), larger than preceding two funicle segments combined.

Thorax (Figure 1g): Pronotum with distinctly raised spiracles, anterior margin concave in the middle; mesoscutum more than 1.6x as wide as long (0.16:0.27); mesoscutum bearing 3 pairs of setae, a single row of setae at each lateral margin, median longitudinal groove present, notauli complete; axilla advanced; scutellum a little longer than mesoscutum, more than 1.4x times as wide as long (0.17:0.24) with submedian, sublateral longitudinal grooves and 2 pairs of setae; metanotum broad with fine rugose carinae and about 2.4x of the length of propodium; propodeum with median carina and with inverted Y shaped paraspiracular carinae, Propodeum coarsely reticulated, propodeal spiracles large and well separated from the anterior margin of Propodeum by a space less than of its own diameter, spiracle rim not fully exposed.

Fore wings (Figure 1h): More than 2.2x times as long as wide (0.52:1.17), more than 1.2x longer than hind wing length; costal cell long and broad; SMV with one long seta directed upwards and 5 small setae directed downwards, longer than MV (0.34:0.29); MV bearing 10 long strong setae on front edge; PMV rudimentary; MV(0.28) longer than SV (0.07); marginal fringe short; basal vein shortly present with 3 setae, basal cell bare; speculum narrow, closed; cubital vein present, subcubital line of hairs starting from the base of cubital vein.

Hind wings (Figure 1i): More than 5.6x times as long as wide (0.16:0.91) with acute apex; vein length more than 1.7x of the length of wing; marginal fringe long.

Forelegs (Figure 1l): Hairy, tibial spur short.

Mid legs (Figure 1m): Tibial spur small, shorter than basitarsi.

Hind legs (Figure 1n): Tibial spur small, spur shorter than basitarsi.

Gaster (Figure 1j): Elongate, petiolate, petiole broader than long, metasoma longer than mesosoma (0.97:0.57), ovipositor sheath exserted, first valvifers semicircular; anterior margin of basal part of second valvifers much curved; third valvulae distinctly present, outer plates of ovipositor slightly longer than second valvifers; cercal setae with one distinctly long.

Male

Length 1.72 mm. Similar to female except as follows: Malar space 1.53 length of eye; Antenna hairy with long setae on funicle segments and club segments; scape cylindrical about 4x as long as wide (0.06:0.24), funicle 4 segmented, pedicel slightly longer than first funicle segment (0.06:0.05), club more than 5.75 times as long as wide (0.04:0.23). Genitalia with digitus having hardly curved spine (Figure 2).

Type material

Holotype 1 female and 1 male: Dissected and mounted on a slide, India, Uttarakhand., Pantnagar, 16-9-2013. Hym. Eulo. Nr. SR11 (Sweta Rawat).

40 females and 10 males Paratypes: India, Uttarakhand, Pantnagar, 16-9-2013, reared from eggs and larvae of the golden tortoise beetle. Hym. Eulo. Nr. SR11 (Sweta Rawat) (Figure 3 to 8).

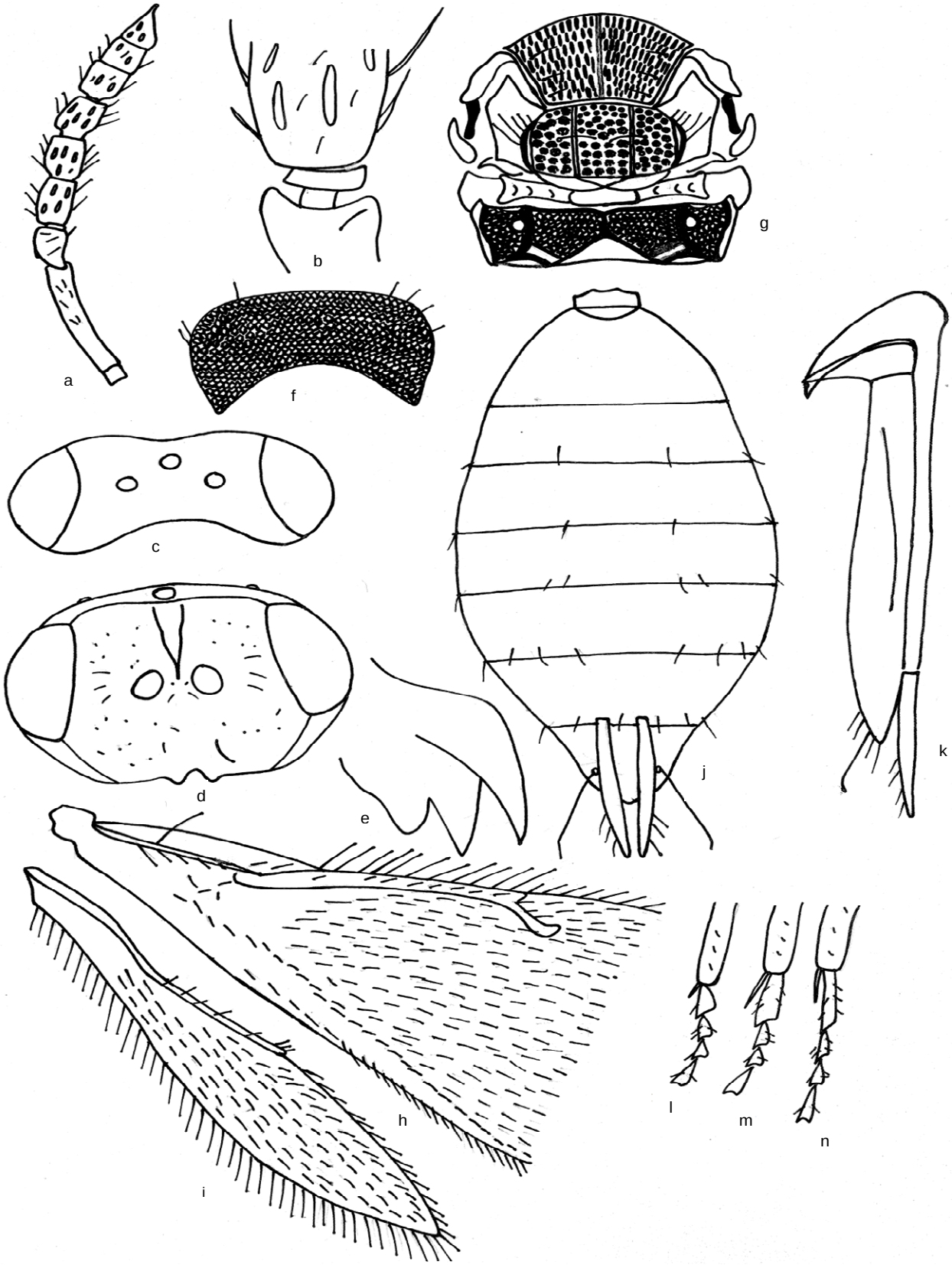

Figure 1. *Tetrastichus burki* sp.nov. female. **a,** Antenna; **b,** Antennal part showing one anellus; **c,** head in dorsal view; **d,** head in frontal view; **e,** mandible; **f,** pronotum; **g,** mesosoma; **h,** fore wing; **I,** hind wing; j, metasoma; **k,** female genitalia; **l,** fore leg; **m,** mid leg; **n,** hind leg.

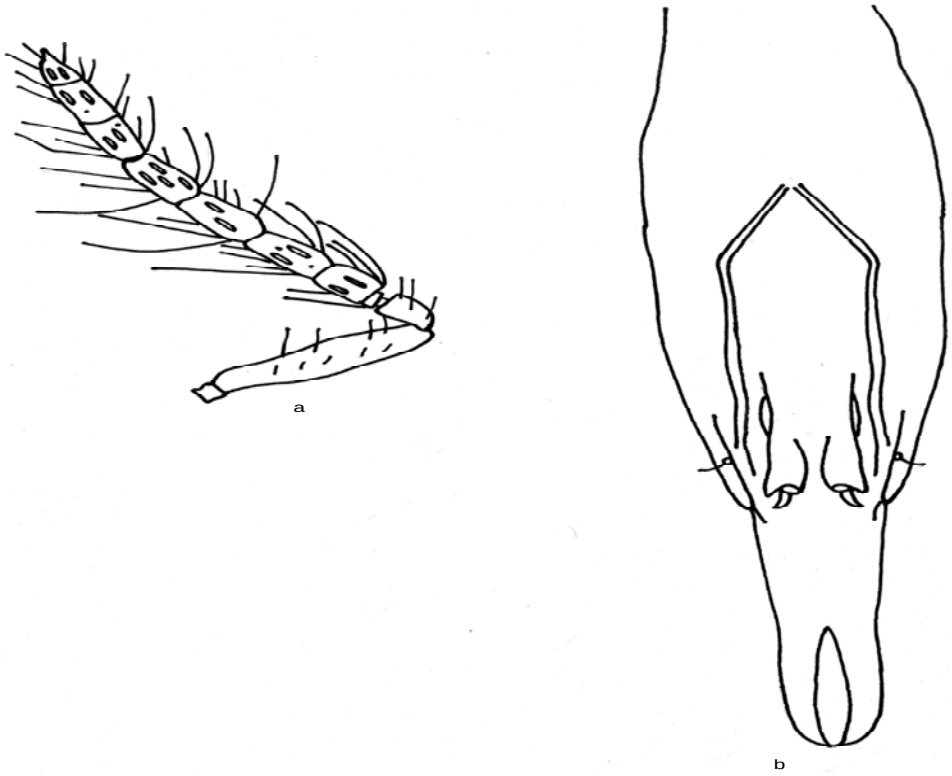

Figure 2. *Tetrastichus burki* sp.nov. male. **a,** Antenna; **b,** Male genitalia.

Figure 3. Parasitized egg case of tortoise beetle. Source: Photographs by Sweta Rawat G.B. Pant University of Agriculture and Technology, Pantnagar

Figure 4. Adult of tortoise beetle. Source: Photographs by Sweta Rawat G.B. Pant University of Agriculture and Technology, Pantnagar

Etymology

The species is named after Dr. B. D. Burks for his outstanding contribution on taxonomy of Tetrastichinae.

Conclusions

T. burki sp. nov. comes close to *T. cassidus* on the following shared characters: Mesoscutum bearing a

Figure 5. Larvae of tortoise beetle. Source: Photographs by Sweta Rawat G.B. Pant University of Agriculture and Technology, Pantnagar

Figure 6. Pupa of tortoise beetle. Source: Photographs by Sweta Rawat G.B. Pant University of Agriculture and Technology, Pantnagar

Figure 7. Damage symptoms of tortoise beetle adult. Source: Photographs by Sweta Rawat G.B. Pant University of Agriculture and Technology, Pantnagar

single row of setae at each lateral margin; surface of propodium is reticulated, legs yellow and all funicle segments equal in length, larval parasitoids of golden tortoise beetle.

T. burki sp. nov. differs from *T. cassidus* as follows: POL slightly more than 1.8x as long as OOL (*T. cassidus* with POL as long as OOL), antennal toruli situated just above the lower level of eye margin (*T. cassidus* with antennal

Figure 8. Larvae of tortoise beetle and damage symptoms of larvae of tortoise beetle. Source: Photographs by Sweta Rawat G.B. Pant University of Agriculture and Technology, Pantnagar

toruli situated at level of ventral margin of compound eyes), pedicl as long as first funicle segment (*T. cassidus* with pedicel slightly shorter than the first funicle segment), paraspicular carinae distinctly present (*T. cassidus* with paraspicular carinae vague or absent), propodeal spiracles are non-contigous with the anterior margin (*T. cassidus* with propodeal spiracles contiguous with anterior margin) and gaster elongate and longer than the thorax (*T. cassidus* with elongate gaster and slightly shorter than the thorax).

Conflict of Interests

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

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Full Length Research Paper

Technical Efficiency of Fadama II Grain Farmers in Taraba State, Nigeria

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The study focused on the productive capacity, technical efficiency of Fadama II grain farmers in Taraba State, Nigeria for the 2008/2009 farming season. Data for the study were obtained from primary source with the aid of interview schedule and analysed using descriptive statistics and stochastic frontier model. Findings revealed that the farmers are within the active farming age (37 years), had average farm size of 5.21 ha, annual income of ₦242,000.00, and 11 years of formal education. Farm size (0.01) and fertilizer (0.05) increased grain output by 44.75 and 17.45% respectively. On the other hand, herbicide (0.05) and labour (0.05) significantly decreased grain output by 67.17 and 98.90% respectively. The inefficiency model showed that while age (-0.05) and sex (-0.01) significantly decreased technical inefficiency by 22.17 and 31.57% respectively, education (0.01) and local crop variety (0.01) increased technical inefficiency by 14.05 and 41.85% respectively. Although, the sigma squared (0.73) indicated the correctness of the specified assumptions of the distributions of the composite error term and gamma was high (0.99) and significant, the mean technical efficiency (0.34) was low. Fadama II achieved the goals of input accessibility and increase in income among farmers. It was recommended that farm size and fertiliser should be increased for farmers; extension should focus attention on herbicide and labour efficient utilisation; and that Fadama II should involve farmers within the age bracket of 37 years in grain production.

Key words: Fadama II, small-scale, grain farmers, stochastic frontier, technical efficiency.

INTRODUCTION

Nigeria is endowed with abundant natural resources. According to Ajakaiye (1993), arable land constitutes about 75% of her total land resources. Matthew (2008) reported that the country is endowed with fresh water source covering 68 million hectares, 960 km of coastline, and an ecological diversity of crop and livestock, forestry and fishery products. Lawanson (2005) observed that Nigeria's agricultural sector contributed about 97.30% to her GDP in the 1960s. Also, the sector employed about 60% of its total population within the same period (Diaz-Bonilla and Gulati, 2003).

Matthew (2008) indicated that, in spite of Nigeria's impressive magnitude of the deposit of primary resources for effective agricultural activities, the sector has continuously stagnated in terms of diminishing productivity. Central Bank of Nigeria (2006) revealed that,

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as from the early 1970s, the contribution of agriculture to GDP began to decline from over 60% to less than 26% by 2003. The utter neglect of the agricultural sector is best captured by Sanni (2006) that its contribution to total export trade remained as low as 4.0% from 1998 to 2004. The implication of this declining productivity is that, agricultural sector could no longer provide decent employment, food and income for those engaged in agricultural production.

It was the realization of the great potential of agriculture that several programmes were launched in the past to reverse the poor trend of productivity and, hence, raise the level of income, productivity, and living standard of rural farmers. Some of the programmes have terminated while others are on-going. An example of the programmes is the Agricultural Development Programme (ADP). The ADP started from 1975 to 1986 and gulped about N1.35 billion. Not less than 6.028 million farm families across the country benefited from the programme (Ayichi, 1995; Ayoola, 2001).

Another programme was the Directorate of Food, Rural Road and Infrastructure (DFRRI) of 1986 to 1992. About ₦2.402 billion was allocated to DFRRI. Under this programme, 289,897.46 km of feeder roads were constructed and rehabilitated; 1,087 rural electricity projects were executed; and 35,281 boreholes were built (Ekpo and Olaniyi, 1995). There was also the Agricultural Credit Guarantee Scheme Fund (ACGSF) which started in 1978. Until 2006, ACGSF had, to its credit, about 497,692 volumes of loan valued at ₦14.9 billion (Central Bank of Nigeria, 2007). The National Special Programme for Food Security, which started in 2001 and National Fadama Development Project have been packaged to tackle poverty and food insecurity problems. By and large, all efforts aimed at enhancing agricultural productivity and farmers' income have not recorded much success. This is depicted in the declining trend in both

national and sectoral productivity measures as reported by Matthew (2008). Fans et al. (2008) also reported that from 1970 to 2000, Nigeria's agricultural sector grew at 1.7% per annum, which is very low in relation to its population growth rate of 2.7%. According to them, this is the principal reason why the country still has one of the highest poverty rates in the world.

Fadama II targeted small-scale farmers as the economic entity that has the best potential to implement agricultural technologies in Nigeria. International Food Policy Research Institute (IFPRI) (2007) indicated that smallholding agriculture is the dominant occupation of rural Nigerians. In spite of their neglect, small scale farmers account for most of the food needs of the entire Nigerian populace. Ayichi (1995) and Ekpo and Olaniyi (1995) showed that rural inhabitants produced 90% of the food marketed and consumed in Nigeria and 2.4% of official export.

The National Fadama Development Project is a major instrument for achieving the Federal Government of Nigeria's poverty reduction objective in the rural areas of Nigeria. The beneficiaries comprise private economic agents who earn their living directly or indirectly by exploiting natural resources in a given fadama area. The project empowers Fadama Community Association (FCAs) and Fadama User Groups (FUGs) with resources, training, and technical assistance or support to properly manage and control these resources for their own development (Abdullahi et al., 2006). Fadama II included capacity building as a project component to reduce poverty through increased productivity and income generation. In its broadest interpretation, capacity building encompasses human resource development as an essential part of overall development. It focuses on a series of actions aimed at assisting participants in the development process to increase knowledge, skills and understanding and to develop the attitudes needed to engender the desired developmental change (Abdullahi et al., 2006).

To an economist, efficiency is a relationship between ends and means (Olaide and Heady, 2006). It also refers to the attainment of a production goal with minimal waste (Arene and Okpukpara, 2006). Theory of production provides the analytical framework for most empirical research on productivity and efficiency (Ajibefun and Daramola, 2003). Technical efficiency measures the relationship between the physical quantities of inputs and outputs. The output-oriented technical efficiency is the ratio between the observed output of the farm firm to the frontier (Battese and Coelli, 1995). In other words, technical efficiency determines the maximum possible output using the same input mix or different combinations of resources.

According to Ekwurke (2005), sorghum, millet, maize and rice are the most important cereals in Nigeria. This is because they are associated with food and drinks throughout the history of humanity as well as animal feed and fodder. In industrialized countries, maize is largely used as livestock feed and as a raw material for industrial products, while in low-income countries, it is mainly used for human consumption (IITA, 2001). Maize is increasingly being utilised for livestock feed, while it remains very important staple food for millions of Nigerians (Oladejo and Adetunji, 2012). Its various uses cut across several ethnic groups in Nigeria (Abdulrahaman and Kolawole, 2006).

The success of a programme depends on the personal characteristics of the key participants. It also depends on the efficiency with which farmers apply available resources to their enterprises. This study was undertaken to determine the technical efficiency of the farmers who benefited from the programme. The outcome of this study will, therefore, serve as a measure of success of Fadama II. Thus, the objectives of this study are to: examine the socio-economic characteristics of grain farmers under Fadama II in Taraba State; evaluate the productivity of the respondents; estimate the level of technical efficiency

of the respondents; and identify the determinants of technical efficiency of the farmers.

METHODOLOGY

Study area

The study area for this research was Taraba State, located within the North-East region of Nigeria. The State is one of the participating States in Fadama II Project. The capital is Jalingo. Taraba State lies between longitudes 9°E and 12°E and within latitudes 6°N and 10°N. The major occupation of the people of Taraba State is agriculture. Cash crops produced in the State include coffee, tea, groundnuts and cotton. Crops such as maize, rice, sorghum, millet, cassava and yam are also produced in commercial quantities (Taraba State Government, 2008; National Population Census, 2009).

Data collection

The population for the study comprised the 16,796 small-scale grain (maize, rice, sorghum and millet) farmers in Taraba State who benefitted from Fadama II. These farmers were located in 10 participating Local Government Areas (LGAs). Simple random sampling technique was used to select respondents from six LGAs, namely Jalingo, Karin-Lamido, Bali, Sardauna, Donga and Wukari. The sample size for the study was determined by applying a fixed sampling proportion of 0.015 to the population of participants to arrive at the total sample size of 252. Up to 235 copies of questionnaire were completed and used for the analysis. Data for the study were obtained from primary source using standard questionnaire. The data were analysed using both descriptive and inferential statistics. Socio-economic characteristics and productivity of the farmers were analysed with descriptive statistics. Stochastic frontier model was used to estimate farmers' level of technical efficiency and identify the determinants of their technical efficiency.

Stochastic frontier analysis

Battese and Coelli (1995) presented the stochastic production frontier as:

$$Y_i = f(x_i, \beta) \quad (1)$$

Where, i = 1,2,3,…,n, Y_i = output of the ith firm, X_i = vector of input, β = vector of parameter to be estimated.
But the stochastic frontier function has two error terms, Vi and Ui, unlike the traditional production function (Amaza and Olayemi, 2002). Thus, the explicit form of the stochastic frontier function is:

$$Y_i = \beta x_i + (V_i - U_i) \quad (2)$$

Where, V_i = random errors assumed to account for measurement error in the output of the firm. The errors are assumed to be normally distributed with zero mean and constant variance $(0, \delta^2)$ which are independent of Ui, and are obtained by the truncation, at zero, of the normal distribution.

U_i = non-negative random errors assumed to account for technical inefficiency in production with zero mean and variances (Coelli and Battese, 1996).

The technical efficiency (TE) of production of the firm is defined as the ratio of the observed output (Yi) to the corresponding stochastic frontier output Y*. Mathematically,

$$TE = \frac{Yi}{Y^*} \quad (3)$$

$$Y^* = \exp(x, B, v_i) \quad (4)$$

Equation (4) described the frontier function. Technical efficiency (TE) is further defined as:

$$TE = \frac{f(x_i,B)\exp(v_i - u_i)}{f(x_i,B)\exp(v_i)} = \exp(-u_i) \quad (5)$$

Empirical specification

The stochastic frontier production function model used in this study is stated explicitly as follows:

$$logy_i = b_o + b_1 logx_1 + b_2 logx_2 + b_3 logx_3 + b_4 logx_4 + b_5 logx_5 + v_i - u_i$$

Where, Yi = output of the ith farmer (tons),
$x_1 = farm\ size\ (ha)$,
$x_2 = seed\ (kg)$,
$x_3 = fertilizer\ (kg)$,
$x_4 = herbicide\ (l)$,
$x_5 = labour\ (man - days)$, $b_s = parameters\ (coefficients) of\ x_1 - x_5\ to\ be\ estimated, v_i =$
$random\ error\ assumed\ to\ be\ normally\ distributed\ with\ (0, \delta^2)$,
u_i
$= technical\ inefficiency\ effect\ independent\ of\ v_i, and\ have\ half - normal\ distribution\ with\ (0, \delta^2)$
Based on the socioeconomic characteristics of the respondents in the study area, and in accordance with the specification of Battese and Coelli (1995), the factors responsible for technical inefficiency were presented as follows:

$$U_i = \partial_0 + \partial_1 z_1 + \partial_2 z_2 + \partial_3 z_3 + \partial_4 z_4 + \partial_5 z_5 + \partial_6 z_6$$

$z_1 = farming\ experience\ (years)$
$z_2 = education\ (years)$
$z_3 = age\ of\ the\ farmers\ (years)$
$z_4 = household\ size$
$z_5 = sex\ (1 = male, 0 = female)$
$z_6 = variety\ of\ crops\ (1 = improved, 0 = local)$
A significant and high value of gamma (γ) would show the presence of inefficiency effects in the data. Having received training in their respective technologies, the level of technical efficiency was expected to be, at least, above average.

RESULTS AND DISCUSSION

Socio-economic characteristics of the respondents

In Table 1, the mean age of the respondents was 37 years. This is the active farming age, and is consistent with Obinne et al. (2009). The average household of respondents was 7 with a standard deviation of 2.749. Average farm size was found to be 5.21 ha which differs positively with IFPRI (2007) that most small-scale farmers in Nigeria are small-holders. Average farm size in this work was also higher than the 1.3 ha reported by Bamire et al. (2007) and the 2.8 ha in Oboh et al. (2007) for sole cropping. By targeting poverty reduction through increased agricultural productivity, Fadama II expectedly

Table 1. Summary statistics of socio-economic characteristics

Variables	Minimum	Maximum	Mean	Standard deviation
Age (years)	17	60	37.14	9.7950
Household size	1	20	6.63	2.75
Farm size (ha)	0.31	13	5.21	2.78
Farm income (N'000)	30	800	241.97	168.35
Farming experience (years)	4	39	13.23	8.55
Education (years)	0	21	11.01	5.77
Post harvest loss (%)	4.00	35	10.80	7.92

Source: Field Study, 2009.

Table 2. Production input per hectare.

Statistics	Minimum	Maximum	Mean	Standard deviation
Input quantity				
Farm size (ha)	0.31	5.55	5.21	2.78
Seed (kg/ha)	0.59	238.7	24.79	30.31
Fertilizer (kg/ha)	24.76	800.00	174.11	131.26
Herbicide (l/ha)	0.23	48.39	5.6011	5.87
Labour (man-days/ha)	89.64	4,988.24	897.72	719.65

Source: Field Study, 2009.

enhanced increased farm size of participants.

The mean annual farm income was ₦242000.00. This annual income of Fadama II grain farmers was large relative to Anozie and Okoronkwo (2009) and Jibril et al. (2009) that small-scale farmers in Nigeria earn an average annual farm income of ₦196685.00 and ₦180000.00 respectively. This finding represented an improvement in the income profile of small-scale farmers which could raise their standard of living and sustain agricultural productivity.

Grain farmers that participated in Fadama II in Taraba State had average farming experience of 13 years, with standard deviation of 8.55, which is lower than 16-20 years in Mbah (2009). Nasiru et al. (2006) stressed that farming experience is an important determinant of profitability because it allows farmers to adjust to changing economic conditions and adopt efficient cultural practices. Average number of years of formal education was found to be 11. This implied that most of the respondents had secondary education. This finding is contrary to most previous research results that small scale farmers in Nigeria have low or no formal education (Nwibo et al., 2009), and thus represents a potential improvement for increased agricultural productivity in Nigeria. However, the result was consistent with Balogun et al. (2007) and Mbah (2009) where 50.0 and 46.67% respectively had 12 years of formal education. Idiong et al. (2006) asserted that education enhances the acquisition and utilisation of information on improved technology by farmers. According to James (2008), education is critical to the attainment of development goals.

Production input per hectare (ha)

In Table 2, findings revealed that the mean seed quantity used was 24.7937 kg/ha. Seed is a critical input in agricultural productivity. As such, most small scale farmers in Nigeria store part of their produce (seed) as planting materials for the next cropping season. According to Umeh (1998), about 80% of farmers in Nigeria make use of farmer-saved-seeds. This probably accounted for the large variation in seed quantities obtained.

The average quantity of fertilizer, which the farmers got for the 2008/2009 farming season, was 174.11 Kg/ha. In spite of the fact that inorganic fertilizer improves/restores soil fertility for greater yield, not all small scale farmers have fully adopted the use of fertilizer. Studies have shown that fertilizer scarcity among small scale farmers has been persistent and remains the bane of crop productivity (Igwe et al., 2009). The mean quantity of herbicide was 5.6011 l/ha, with a standard deviation of 5.8654. The use of herbicide largely reduces the arduous nature of weeding.

In addition, total labour use was 897.72 man-days/ha, with a standard deviation of 5.378, showing large

Table 3. Maximum likelihood estimate for technical efficiency model for Fadama ii farmers in Taraba state for 2008/2009.

Model	Parameter	Coefficient	t-ratio
Production function			
Constant	β_0	2.83	12.55*
Farm size (x_1)	β_1	0.45	3.11*****
Seed (x_2)	β_2	-0.02	-0.43
Fertilizer (x_3)	β_3	0.17	2.67*****
Herbicide (x_4)	β_4	-0.07	-2.15*****
Labour (x_5)	β_5	-0.09	-2.37*****
Technical Inefficiency function			
Constant	δ_0	1.96	0.67
Farming experience (z_1)	δ_1	-0.13	-0.78
Education (z_2)	δ_2	1.41	5.51*****
Age (z_3)	δ_3	-0.22	-2.29*****
Household size (z_4)	δ_4	0.04	0.09
Sex (z_5)	δ_5	-31.57	-4.99*****
Crop variety (z_6)	δ_6	4.19	3.71*****
Diagnostic statistics			
Sigma squared	δ^2	73.17	8.60*****
Gamma	(γ)	0.99	606.66*****
Ln likelihood function		-550.57	-550.56*
LR test		290.47	290.47*

variations. Labour use cuts across all stages of production. Mean labour for this study was large relative to 37 man-days/ha for cassava production in Edeh and Ojemade (2009). This is because in an enterprise combination, labour use would definitely be large. According to Akinpelu and Ogbonna (2005) and Umebali (2007), labour, whose utilisation in man-days varies among men, women and children, is a significant factor of production among small scale farmers.

Maximum likelihood estimates (MLE) for the technical efficiency of Fadama II grain farmers in Taraba State

The stochastic frontier model presented in Table 3 showed that, the sigma squared (δ^2), which indicates the correctness of the specified assumptions of the distributions of the composite error term was high (0.73) and statistically significant at 0.01 level. Furthermore, the variance ratio (γ), which indicates the proportion of total variance attributable to the inefficiency term (Ui), was high (0.99) and statistically significant at 0.01 level. The implication is that one percent of grain output was lost to technical inefficiency. There was the presence of one-sided error (LR) (290) component, thus rendering the use of ordinary least square estimating technique inadequate, and lending credence to the appropriateness of the MLE techniques in representing the data.

The elasticities of farm size and fertilizer were of increasing function. The two variables had positive coefficients and were statistically significant at 1% probability level. Hence, increases in farm size and fertilizer by 1% would lead to increase in grain output by 44.75 and 17.74% respectively. These findings were consistent with Idiong et al. (2006) that farm size and fertilizer are significant resources in production. Herbicide and labour had negative coefficients and were statistically significant at 5% probability level. A one percent increase in these inputs would lead to decrease in grain output by 67.17 and 98.90% respectively. This finding is contrary to Lawal et al. (2008) that labour is positively significant in small-holder agricultural productivity. Akinpelu and Ogbonna (2005) found that labour accounted for high proportion of the variable cost of production. According to Audu et al. (2009), given the ageing trend of our farmers and high rate of rural-urban migration, the high cost of labour is undesirable.

Determinants of technical efficiency among grain farmers

In the technical inefficiency function in Table 4, education (0.01) significantly increased technical inefficiency at 1% probability level. The result implied that 1% increase in the number of years of formal education would reduce technical efficiency by 14.05%. This meant that more educated farmers in the study area were technically

Table 4. Distribution of technical efficiency among Fadama ii farmers in Taraba state, 2008/2009 farming season.

Technical Efficiency class	Frequency	Percentage
< 1.32E-04	34	14.47
0.108 – 0.09	4	1.28
0.101 – 0.020	22	9.36
0.202 – 0.30	31	13.19
0.301 – 0.393	44	18.72
0.405 – 0.497	40	17.02
0.501 – 0.595	33	14.47
≥ 0.607	27	11.49
Total	235	100.00

Mean technical efficiency = 0.342; Minimum technical efficiency = 2.59E-01; Maximum technical efficiency = 0.855.

inefficient. The result is consistent with Ajaero et al. (2008) that education had inverse relationship with technical education. More educated farmers tend to drift away from core farm production activities. Idiong et al. (2006), however, asserted that education enhances acquisition and utilisation of information on improved technology by farmers. Age and sex significantly reduced technical inefficiency at 5 and 1% probability levels respectively.

By implication, increase in the age of farmers by 1% would reduce technical inefficiency by 22.19%. Given the mean age (37 years) in this study, which fell within the active farming age bracket in Nigeria, additional years could still yield more efficient results. This result validates Panwal et al. (2006). Based on indexing for sex, increase in male dominance in the population of farmers would reduce technical inefficiency in grain production by 31.57%. Farming, generally, requires the utilisation of a lot of physical strength. This is the natural endowment of males.

Crop variety (3.71) significantly increased technical inefficiency in grain production at 1% probability level. The positive sign of this variable corresponds with local variety index. Conversely, improved variety reduces technical inefficiency among the farmers. Improved variety is axiomatically associated with high yield. Umeh (1998) had reported that 80% of Nigerian farmers use farmer-saved seeds, invariably local variety, leading to low yield.

Distribution of technical efficiency among respondents

In Table 4, majority of the respondents (18.72%) had technical efficiency ranging from 0.30 to 0.39. Only 11.49% had the highest technical efficiency of 0.61 and above. Mean technical efficiency was 0.34, which was very low. Minimum and maximum technical efficiencies were 2.59 and 0.86 respectively. Following Kebede (2001), the low technical efficiency was an indication that a large proportion of productive inputs could be wasted or misapplied. The low mean technical efficiency is worrisome, given high availability of inputs to farmers. This could only suggest diversion of fund and poor utilisation.

CONCLUSION AND RECOMMENDATIONS

The respondents possessed appropriate socio-economic characteristics to translate their training to useful ends. Prominent among these characteristics are age and high level of education. The latter indicated a departure from the age-long non-formal and illiteracy status of Nigerian farmers. It is important to note that average input utilisation was high among the farmers, which is one of the aims of the capacity building component of Fadama II. Farmers' income level was relatively higher than those of average small-scale farmers in Nigeria, again pointing to the attainment of another aim of Fadama II. While farm size and the quantity of fertiliser used increased grain outputs, herbicide and labour exerted downward pressure on output. Their mean technical efficiency was, however, below average, indicating poor resource combination technique. Education and use of local varieties of grain seeds accounted for the farmers' low technical efficiency level. Based on the findings of this study, the following recommendations have been put forward:

(i) Fadama II grain farmers should be assisted by the programme to acquire more farmland so as to produce more grains for the populace;
(ii) The programme should make more fertiliser available to further assist in the realisation of more output;
(iii) Extension work should focus on the optimum utilisation of herbicide and labour to minimise and eventually eradicate the negative results of these inputs;

(iv) Fadama II should include farmers within the age bracket of 37 years in its grain production programme; and
(v) Similarly, the use of local varieties of grain should be completely replaced by improved varieties.

Conflict of Interest

The authors have not declared any conflict of interest.

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Review

Information sources of knowledge based economic development for fisheries in Turkey

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Fishery is a very important sector in Turkey due to economic, geographic, traditional, and cultural conditions. The sector has being one of the four sub-sectors of the agriculture till recent year when the Ministry of Food, Agriculture, and Livestock separate Fishery as an independent sector. The coastal areas, the amounts of lakes and rivers have been very important for fishery supplies in Turkey. Although Turkey has big potential fishery sector, the production commonly survive as traditional practices. Information sources of fishery sector, water resources, and ecological issues come from master-apprentice connections. The objective of this study is to share information sources of knowledge, science, and technology where stakeholders can reach or create Knowledge Based Economic Development (KBED) in the sector. As a result of the study, the stakeholders of fishery in Turkey as well as in the other countries obtain information sources of fishery from the study where knowledge is shared.

Key words: Fishery, knowledge sources, development, Turkey.

INTRODUCTION

Fishery sector has always been very important activity in coastal areas due to economic, geographic, traditional, and cultural factors. Therefore, in Turkey, as in many other countries, the most intensive users of coastal zone have been fishermen (Unal, 2006). Fishery being one of the four sub-sectors of the agriculture in Turkey. It has been vital importance in contributing beneficial nutrition for human beings, providing raw material for the industrial sector, creating the employment possibilities and high potential for export. Turkey, with its favorable geographic position between the Black Sea and Mediterranean Sea, has access to the fish resources of both water bodies. The country is also endowed with rich inland waters and rivers with significant capture fishery and aquaculture potential (Anonymous, 2008a). Turkey has high potential about catching and growing aquaculture products. But it has not been successful to raise the value of the potential.

Turkey is a country that's surrounded with seas three size of it such as Black Sea, Mediterranean Sea, Aegean

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Sea and Marmara Sea. In addition that Marmara Sea has an inside sea property. Also the amounts of lakes and rivers have been very important for fishery supplies (Tasdan et al., 2010). Total fish production of Turkey is 653,080 tons according to the 2012 fishery statistics. Of the total, 68.2 % is obtained from the marine fisheries, 6.2% from island and 25.6% comes from aquaculture (Anonymous, 2011). Although Turkey has big potential fishery sector, the production commonly survive as traditional practices. Knowledge Based Economic Development (KBED) of the sector has not been created yet. Information sources of fishery sector, water resources, and ecological issues come from master-apprentice connections. The information sources and connection within them are weak to trust in. The objective of this study is to share information sources of knowledge, science, and technology where stakeholders can reach or create KBED in the sector. As result of study, the stakeholders of fishery in Turkey as well as in the other countries obtain information sources of fishery from the study where knowledge is shared.

STAKEHOLDERS OF FISHERIES IN TURKEY

Public

Ministry of Food, Agriculture and Livestock

The Ministry of Food, Agriculture and Livestock (MFAL) is the main state organization responsible for fisheries (including aquaculture) administration, regulation, protection, and promotion and technical assistance through Directorate General of Fisheries and Aquaculture (DFA). “According to the issue No. 27958 of the Official Gazette published on 8 of June in 2011, the Ministry of Agriculture and Rural Affairs (MARA) has been reconstituted as the MFAL while the department of fisheries, previously named for directorate-general, has been reconstituted as the DFA” (Can and Demirci, 2012). All activities in fisheries and aquaculture are based on the Fisheries Law No. 1380, enacted in 1971. With this law, and its related bureaucracy, definitions were codified. Based on this law, regulations and circulars are prepared to regulate fisheries. The Fisheries Law No. 1380 of 1971 is amended by law 3288 of 1986. According to Laws 1380 and 3288 and Continental Waters Law No. 2674 of 1982, foreigners are not allowed to take part in commercial fishing activities (Anonymous, 2008b). The main duties of DFA on fisheries are to:

(1) Determine and to promote the main issues of fishing and aquaculture both in marine and inland water systems for sustainable fishing and aquaculture,
(2) Make up issues to establish, to operate and to control fishing ports and fishing infrastructures,
(3) Protect fisheries resource, to determine marine protected, production and aquaculture areas, and making provisions to protect those areas from harmful activities,
(4) Set up legislative issues for import and export fisheries product,
(5) Conduct facilities that are aimed to improve and to enhance of fisheries and aquatic resources.
(6) Make provisions for inputs that are needed to improve the production of fishing and aquaculture resources.
(7) Set up the legislative issues that are connected to areas where fishing and aquaculture activities are held. To determined the characteristics and conditions of the limits of production tools and their usage and renting bases.
(8) Prepare and implement research projects relating to improving of fishing and aquaculture production,
(9) Set up an information network related to fisheries, fishing and aquaculture activities (Can and Demirci, 2012).

Ministry of Development

The objectives of fisheries management policy in Turkey used to be set up by State Planning Organization (DPT) till 2011 when the Ministry of Development established in Turkey. Managing fishery resources in a sustainable way is the main objective of the fisheries policy. Therefore, region-based preliminary fisheries plans have been designed. The objectives set out for these plans include rebuilding of depleted stocks, long-term resource management, introduction of fishing rights and sustainability of fishing opportunities for fishermen. The Ministry of Development prepares long-term development plans and annual programs conforming to the targets determined by the government. To this end, it coordinates the activities of ministries and public institutions concerning economic, social and cultural policies in order to ensure efficient implementation and advices the government on fishery policy issues. A special Committee for each sector including the stakeholders from fishery and aquaculture gathers every year during the preparation of the Development Plans, and serves as a platform for The Ministry of Development to consult. During the development of the latest development plan, the 9th five year development plan covering the period between 2007 and 2013, the Committee for Fisheries mainly focused on sustainable exploitation of resources, integration of environmental considerations to fishery and institutional restructure for the adoption of the common fishery policy (Can and Demirci, 2012).

Fishery port offices

Turkey has a large number of fishing ports and the MFAL has traditionally not had a presence (office) in any of

these ports. The need to strengthen fisheries protection and control was identified as an important priority. For this purpose, Fishery Port Offices were established in 2006 with 30 ports. All of them will also eventually be linked through the computer-based FIS to Provincial Offices and Headquarters of the Ministry of Food, Agriculture and Livestock in Ankara. The port offices have been operating since the beginning of 2007. These offices are an important aspect of improved fisheries management in Turkey. They will serve to improve the collection, checking and the use of information on the quantities and species of fish landed, and helping to improve compliance with national regulations related to grading and marketing of fish and fishing vessel licensing. A permanent presence in these ports will also help the Ministry of Food, Agriculture and Livestock to investigate fishing offences and take appropriate enforcement action. There will also be an additional benefit for the industry in facilitating easier communication with government officials, enabling more effective communication of industry concerns and needs (Can and Demirci, 2012).

Other organizations

Fisheries production data are gathered and evaluated by the State Statistics Institute in collaboration with the MFAL. The institute uses a complete questionnaire method for large scale fishermen, and sub-sampling for small scale fishermen. The Under Secretariat of Foreign Trade of the Prime Ministry is the other public organization which regulates fish exports and imports regime. The Agricultural Bank of Republic of Turkey and Under Secretariat of the Treasury operate credit and incentive schemes to support the fisheries and aquaculture sectors. The Scientific and Technical Research Council also plays an important role organizing and subsidizing research activities. The Export Promotion Centre of Turkey, which is the only public organization in this field, acts as an intermediary in establishing business contacts between foreign importers and Turkish exporters to develop and to promote Turkish fisheries exports (Duzgunes and Erdogan, 2008).

Universities

Nationally, there are many universities with a fisheries faculty or vocational school, or a fisheries department within an agriculture faculty (Table 1). All fisheries faculties and schools consist of three main departments: aquaculture, capture fisheries and processing, and science.

Cooperatives

Cooperatives have great importance in countries, where small holdings prevail. Since small holdings prevail in Turkish agriculture and fisheries sectors, cooperatives are of vital importance. Most of the cooperatives in Turkey are operating in their small and local markets (Unal et al., 2009). Cooperatives in the small-scale fisheries sector are a way of maximizing long-term community benefits to deal with the threats of fisheries mismanagement, livelihood insecurity and poverty-harsh realities for many of the world's small-scale fishers. Communities with successful community-based organizations are better off than those without (Ostrom, 1990). Cooperatives can: (i) increase fishers' price-negotiating power with market intermediaries, help stabilize markets, improve post-harvest practices and facilities, provide marketing logistics and information, and facilitate investment in shared structures such as ice plants and fish processing facilities; (ii) increase market competition by setting up auctioning systems; (iii) use their greater negotiating power to make cost-saving bulk purchases of fishing gear, engines, equipment and fuel and to advocate with government; (iv) facilitate microcredit schemes for fishers, to reduce their dependency on intermediaries and give them greater freedom in selecting buyers (Anonymous, 2009a). In 1980, there were 229 fishery cooperatives with 14,750 members in Turkey, and 96 of these cooperatives were on the sea coast (Hazar, 1990). In 1992, while there were 8,020 agricultural cooperatives, only 262 fishery cooperatives existed in Turkey (Cikin and Kizildag, 1997). Both number of cooperatives and members have increased in 2009. According to MFAL, there are 528 fishery cooperatives with a total of 28,455 members and 12 fishery cooperatives associations and 1 central union of fishery cooperatives associations (Anonymous, 2012) (Table 2).

Information sources for the development of fisheries sector in Turkey

Information is a basic and fundamentally important element in any development activity. The value of information lies in its ability to affect a behavior, decision, or outcome. Information is an essential ingredient in agricultural development programs (Ozowa, 1995). Fisheries and aquaculture information is produced in different institutions. Much of information is published by commercial publishers and the cost is high and increasing. Efforts such as Access to Global Online Research in Agriculture (AGORA), Health Inter Network Access to Research (HINARI) and Online Access to Research in the Environment (OARE) alleviate the prohibitive costs of access for developing countries (Anonymous, 2009b). Much regional fisheries information is also published as grey literature by intergovernmental organizations such as the Caribbean Regional Fisheries Mechanism, Pacific Islands Forum Fisheries Agency, Secretariat of the Pacific Community, Network of

Table 1. Academic institutions of fisheries or marine science faculties.

Name of University	Name of Faculty	Web Address
Adıyaman University	Kahta Vocational School Department of Fisheries	www.adiyaman.edu.tr
Adnan Menderes University	Faculty of Agriculture Department of Fisheries Engineering	www.adu.edu.tr
Adnan Menderes University	Bozdogan Vocational School Department of Fisheries	www.adu.edu.tr
Akdeniz University	Faculty of Fisheries	www.sufak.akdeniz.edu.tr
Akdeniz University	Finike Vocational School Department of Fisheries	www.finike.akdeniz.edu.tr
Ankara University	Faculty of Agriculture Department of Fisheries Engineering	www.agri.ankara.edu.tr
Atatürk University	Faculty of Fisheries	www.atauni.edu.tr
Atatürk University	Hınıs Vocational School Department of Fisheries	www.atauni.edu.tr
Atatürk University	İspir Hamza Polat Vocational School Department of Fisheries	www.atauni.edu.tr
Bingöl University	Faculty of Agriculture Department of Fisheries	www.bingol.edu.tr
Bingöl University	Genc Vocational School Department of Fisheries	www.bingol.edu.tr
Cumhuriyet University	Gurun Vocational School Department of Fisheries	www.cumhuriyet.edu.tr
Cumhuriyet University	Suşehri Timur Karabal Vocational School Department of Fisheries	www.cumhuriyet.edu.tr
Çanakkale Onsekiz Mart University	Faculty of Marine Sciences and Technology	www.denbiltek.comu.edu.tr
Çanakkale Onsekiz Mart University	Bayramic Vocational School Department of Fisheries	www.bmyo.comu.edu.tr
Çukurova University	Faculty of Fisheries	www.suurunleri.cu.edu.tr
Çukurova University	Feke Vocational School Department of Fisheries	www.fekemyo.cu.edu.tr
Çukurova University	İmamoglu Vocational School, Department of Fisheries	www.imamoglumyo.cu.edu.tr
Dokuz Eylül University	Institute of Marine Sciences and Technology	www.imst.deu.edu.tr
Ege University	Faculty of Fisheries	www.egefish.ege.edu.tr
Ege University	Research and Application Center of Underwater	www.saum.ege.edu.tr
Ege University	Ege Vocational School Department of Fisheries	www.egemyo.ege.edu.tr
Erzincan University	Kemaliye Hacı Ali Akın Vocational School Department of Fisheries	www.erzincan.edu.tr
Erzincan University	Tercan Vocational School Department of Fisheries	www.erzincan.edu.tr
Fırat University	Faculty of Fisheries	www.firat.edu.tr
Fırat University	Keban Vocational School Department of Fisheries	www.firat.edu.tr
Gaziosmanpaşa University	Faculty of Agriculture Department of Fisheries Engineering	www.ziraat.gop.edu.tr
Gaziosmanpaşa University	Almus Vocational School Department of Fisheries	www.almusmyo.gop.edu.tr
Giresun University	Tirebolu Mehmet Bayrak Vocational School Department of Fisheries	www.tmyo.giresun.edu.tr
İstanbul University	Institute of Marine Sciences and Management	www.istanbul.edu.tr
İstanbul University	Faculty of Fisheries	www.suurunleri.istanbul.edu.tr
Kahramanmaraş Sutcu Imam University	Faculty of Agriculture Department of Fisheries	www.su.ksu.edu.tr
Karadeniz Teknik University	Surmene Faculty of Marine Science	www.deniz.ktu.edu.tr
Karadeniz Teknik University	Macka Vocational School Department of Fisheries	www.ktu.edu.tr
Kastamonu University	Faculty of Fisheries	www.su.kastamonu.edu.tr
Kocaeli University	Gazanfer Bilge Vocational School Department of Fisheries	www.gazanferbilge.kocaeli.edu.tr

Table 1. Contd.

Mersin University	Faculty of Fisheries	www.mersin.edu.tr
Mugla Sitkı Kocman University	Faculty of Fisheries	www.mu.edu.tr
Mugla Sitkı Kocman University	Underwater Practice and Research Center	www.mu.edu.tr
Mugğla Sitkı Kocman University	Ortaca Vocational School Department of Fisheries	www.mu.edu.tr
Mustafa Kemal University	Faculty of Marine Sciences and Technology	www.mku.edu.tr
Mustafa Kemal University	Dörtyol Vocational School Department of Fisheries	www.mku.edu.tr
Mustafa Kemal University	Samandağ Vocational School Department of Fisheries	www.mku.edu.tr
Middle East Technical University	Institute of Marine Sciences	www.ims.metu.edu.tr
Recep Tayyip Erdogan University	Faculty of Fisheries	www.suf.rize.edu.tr/tr
Suleyman Demirel University	Egirdir Fisheries Faculty	www.esuf.sdu.edu.tr
Süleyman Demirel University	Water Institute	www.sue.sdu.edu.tr
Sinop University	Faculty of Fisheries	www.sinop.edu.tr
Sinop University	Vocational School Department of Fisheries	www.sinop.edu.tr
Tunceli University	Faculty of Fisheries	www.tunceli.edu.tr
Yalova University	Armutlu Vocational School Department of Fisheries	www.yalova.edu.tr
Yuzuncu Yıl University	Faculty of Fisheries	www.yyu.edu.tr

Table 2. Regional unions of fisheries cooperatives in Turkey.

Regions	Number of cooperatives	Total numbers of partners
Adana Regional Association	9	590
Balıkesir Regional Association	12	674
Çanakkale Regional Association	22	567
Hatay Regional Association	11	xx
İstanbul Regional Association	32	2240
İzmir Regional Association	22	1227
Kocaeli Regional Association	10	607
Marmara Regional Association	14	1529
Mersin Regional Association	9	389
Muğla Regional Association	14	567
Sinop Regional Association	9	394
Tekirdağ Regional Association	8	317

Source: MFAL, 2012.

Aquaculture Centres in Asia-Pacific, Southeast Asian Fisheries Development Center and by regional fishery science bodies such as the North Pacific Marine Science Organization and the International Council for the Exploration of the Sea (Anonymous, 2009b), Tables 3

Table 3. International available information sources about fisheries.

Information sources	Web address	Published by authority
Publications of Fisheries and Aquaculture	http://www.fao.org/fishery/en	Food and Agriculture Organization of the United States
Publications of Fisheries	http://www.eurofish.dk/	U.N. FAO Globefish
Publications of Fisheries	http://www.eurofish.dk/	Eurofish International Organization
Publications of Fisheries Unit	http://ec.europa.eu/fisheries/index_en.htm	European Commission
Publications of Fishery	http://www.apfic.org/modules/wfdownloads	Asia Pacific Fishery Commission
National Agricultural Statistics Service	http://www.nass.usda.gov/	United States Department of Agriculture
Publications of Fisheries Management	http://www.afma.gov.au/	Australian Government Australian Fisheries Management Authority
Publications of FisheriesandOceanography	http://www.vniro.ru/en/	Russian Federal Research Institute of Fisheries and Oceanography
Journal of Northwest Atlantic Fishery Science	http://journal.nafo.int/index.html	Northwest Atlantic Fisheries Organization
American Fisheries Society Journals	http://afsjournals.org/	American Fisheries Society
Scientia Marina	http://www.icm.csic.es/scimar/index.php	Spanish National Research Council
Pan-American Journal of Aquatic Sciences	http://www.panamjas.org/index.htm	Panamjas
Fisheries Journal	http://www.fisheriessciences.com/	www.fisheriessciences.com
Fishery Technology	http://epubs.icar.org.in/ejournal/index.php/FT	Society of Fisheries Technologists
Elsevier Science Fisheris Journals	http://www.elsevier.com/journals/title/a	Elsevier Publishing
The Asian Fisheries Science Journal	http://www.asianfisheriessociety.org/publication/index.php	Asian Fisheries Society
Journal of Taiwan Fisheries Research	http://www.tfrin.gov.tw/	Fisheries Research Institute, Taiwan
African Journals	http://www.ajol.info/index.php	AJOL
Journal of Natural Resources and Life Sciences Education	http://www.jnrlse.org/	American Society of Agronomy
Egyptian Journal of Aquatic Biology and Fisheries	http://www.ejabf.eg.net/index.html	Ain Shams University Faculty of Science Department of Zoology
International Aquatic Research	http://www.intelaquares.com/	Islamic Azad University
Fish farming and fisheries	http://panor.ru/journals/fish	Panorama Publisher
The Journal of Shellfish Research	http://www.shellfish.org/jsr-public	National Shellfisheries Association

and 4 show lists of some international and national available information sources on fisheries, aquaculture and aquatic sciences.

CONCLUSIONS

The Fishery sector has always had a very important activity in coastal areas of Turkey. Therefore, in Turkey, as in many other countries, the most intensive users of coastal zone have been fishery industry sources. The country is also endowed with rich inland waters and rivers with significant capture fishery and aquaculture potential. Although Turkey has big potential fishery sector, the production commonly survive as traditional practices. Knowledge Based Economic Development (KBED) of the sector has not been created yet. The stakeholders of fishery have to reach the source of development for fishery where KBED may create. MFAL, Ministry of Development, Fishery Port Office, Cooperatives, Universities, and Companies are main stakeholders of the sector. Also, national and international available information sources about fisheries as web site are very good source of information. It is necessary to convert information data to knowledge where KBED created in the sector.

Table 4. National available information sources about fisheries.

Information source	Web address	Published by authority
Fisheries Statistics Data General Directorate of Fisheries and Seafood Products	http://www.bsgm.gov.tr/	General Directorate of Fisheries and Seafood Products
Fisheries Statistics Service	http://www.turkstat.gov.tr/	Turkish Statistical Institute
Publications of Fisheries	http://www.sumae.gov.tr/	General Directorate of Agricultural, Researchand Policy
Publications of Fisheries Research	http://www.akdenizsuurunleri.gov.tr/index_en.asp	Mediterranean Fisheries Research Productionand Training Institute
Publications of Fisheries Research	http://www.elazigsuurunleri.gov.tr/default.asp?content=main&lang=en	Elazığ Fisheries Research Station
Publications of Marine Research	http://www.tudav.org/index.php?lang=en	Turkish Marine Research Foundation
Journal of Fisheries	www.egejfas.org	E.U. Fisheries Faculty
Water World Magazine	www.sudunyasidergisi.com	Monthly Journal of Fisheries
Egirdir Journal of Fisheries Faculty	edergi.sdu.edu.tr	S.D.U. Fisheries Faculty
K.U.Journal of Fisheries	su.kastamonu.edu.tr	K.U. Fisheries Faculty
Journal of the Association of Fisheries Engineering	www.suurunleri.org.tr	Association of the Fisheries Engineering
Turkish Journal of Fisheries and Aquatic Sciences	www.trjfas.org	Central Fisheries Research Institute
Dolphin Research Bulletin	www.sumae.gov.tr/yunus	Central Research Institute for Fisheries
Academic Journals of The Scientific and Technological Research Council of Turkey	http://journals.tubitak.gov.tr/	The Scientific and Technological Research Council of Turkey

Conflict of Interest

The authors have not declared any conflict of interest.

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Assessing incidence, development and distribution of banana bunchy top disease across the main plantain and banana growing regions of the Democratic Republic of Congo

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Banana bunchy top disease (BBTD) was first identified in DR Congo in 1958. Previously, the disease's distribution throughout the Congo basin had not been studied, so an initial study, to determine the incidence and severity of BBTD in banana and plantain producing regions of Oriental province, was carried out during 2009 to 2010. Three hundred (300) farms were surveyed across 4 districts and 19 territories, with 30 mats assessed per farm. Visible disease symptoms were recorded and serological tests using triple antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA) were carried out on collected samples. Additional surveys were conducted during 2010 to 2012 in Maniema, Northern Katanga, Eastern and Western Kasai, Bandundu and Equateur provinces to assess the distribution of the aphid vector (*Pentalonia nigronervosa*), BBTD incidence and severity. 92% of mats observed across Oriental province manifested BBTD symptoms but severity levels were low. All plantain and banana cultivars grown in farmers' fields were susceptible to the disease. The vector, *P. nigronervosa*, was found on 89% of all assessed mats. In Tshopo district, all samples collected on plants showing disease severity scores 2, 4 and 5 tested positive for the presence of the virus. However, only 48% of plants with severity score 1 and 33% of plants with score 3 gave positive TAS-ELISA results. More importantly, 40% of symptomless plants (score 0) tested positive. The average BBTD incidences in Bandundu, Equateur, Eastern and Western Kasai, Katanga and Maniema were lower than levels observed in Oriental province. The lowest incidence levels were observed in Equateur (43%) and Katanga (35%). Although, BBTD is widespread in the surveyed provinces, the generally observed low severity levels result in limited impact on production. The generalized spread of BBTD in surveyed areas nevertheless underlines an urgent need to identify virus-free plants for multiplication and distribution of disease-free materials to small-scale farmers.

Key words: Aphid, Bandundu, Equateur, Maniema, oriental province, triple antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA).

INTRODUCTION

Banana (*Musa* spp., including plantain) is an important food crop in the Democratic Republic of Congo (DR Congo). Plantain (*Musa* ABB group) is widely cultivated across the Congo basin, while East African highland banana (*Musa* AAA-EA group) dominates the production landscape in the Eastern Kivu region (De Langhe et al., 1994). *Musa* cultivation in DR Congo is hampered by several pests, including nematodes and weevil (*Cosmopolites sordidus*) (Mobambo and Naku, 1993), and diseases such as banana bunchy top disease (BBTD) (Mobambo, 2010; Kumar et al., 2011), *Fusarium* wilt and *Xanthomonas* wilt (Ndungo et al., 2006).

BBTD is the most severe viral disease affecting banana production in at least 14 African countries (Kumar et al., 2011). No bunches are produced on plants which get infected in their early development stage. In Africa, the disease was first reported in Egypt in 1901 (Fahmy, 1924; Magee, 1927, 1953), in Eritrea in 1964 (Saverio, 1964) and was first identified elsewhere in sub-Saharan Africa, in DR Congo, in 1958 at the "Institut National pour l'Etude Agronomique du Congo" (INEAC) Yangambi research station (Wardlaw, 1961; Fouré and Manser, 1982). Yangambi is centrally located in the Congo basin on the banks of the mighty Congo River. Since 1958, it has been reported in Bas Congo (Mobambo, 2010) and the Kivus (Sebasigari and Stover, 1988) of the DR Congo. The geographical distribution of the disease throughout the vast Congo basin, has, however, not been studied. Banana bunchy top virus (BBTV), a luteovirus, multiplies in the phloem and is transmitted by the aphid vector *Pentalonia nigronervosa* (Magee, 1927; Burns et al., 1995). Once established, it is extremely difficult to eliminate or manage, even in large-scale plantations (Dale, 1987). *P. nigronervosa* is the only known vector able to transmit BBTV (Yasmin et al., 2001). Winged aphids are mainly responsible for short distance spread, while the movement of infected planting materials from farm to farm or village to village also significantly contributes to disease spread (Dale, 1987; CTAHR, 1997).

This study was undertaken to determine incidence and severity of BBTD and aphid colony presence in infected villages across Oriental, Maniema, Katanga, Eastern and Western Kasai, Equateur and Bandundu provinces in DR Congo. In addition, surveys in the districts of Ituri, Haut Uélé and Bas Uélé of Oriental province assessed disease incidence and severity levels and aphid numbers in both older perennial backyard plots and newly-established distant fields, in order to assess a possible gradient in disease parameters across field types. Triple antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA) tests were carried out to confirm BBTV presence in sampled mats across all surveyed provinces.

MATERIALS AND METHODS

In-depth BBTD surveys were carried out during 2009 to 2010 in 4 districts (Tshopo, Ituri, Haut-Uélé and Bas-Uélé) of Oriental province located in north-eastern DR Congo (Figure 1, Table 1). Additional surveys were carried out during 2010 to 2012 in Maniema, Northern Katanga, Eastern and Western Kasai, Bandundu and Equateur provinces (Table 1). In Tshopo district, surveys were carried out in 7 territories in addition to Kisangani town and LubuyaBera (Kisangani outskirts), while in Ituri, Haut Uélé and Bas Uélé surveys were carried out in 3 territories. All visited territories had BBTD. Three villages, with a clear presence of BBTD, were selected per territory in Tshopo district, while 1 village was selected per territory in Ituri, Haut Uélé and Bas Uélé. Ten (10) farms were randomly selected per village giving a total of 390 farms in Oriental province (Table 1). The surveyed villages represented locations with the highest observed incidence and severity levels within a territory. The collected data thus reflects the worst case scenario within a given location. The predominant *Musa* cultivar group and source(s) of planting material were assessed at each surveyed farm in Oriental province. *Musa* plots were mainly located next to the house (that is, backyards) but some were at a distance from the house on cleared primary or secondary forest land. BBTD incidence and severity and aphid colony presence and type were assessed on 30 *Musa* mats per selected farm. Diagonal lines were drawn in each field and 15 mats were selected on each line.

In the districts of Ituri, Haut Uélé and Bas Uélé, half of the 30 mats were selected in perennial plantain fields next to the house, while the other half were selected in plantain fields on cleared forest land. It was postulated that the older perennial backyard plots would have a higher disease incidence and aphid colony presence compared to younger and often newly-established distant fields.

A mat that contained at least one plant with visible BBTD symptoms was regarded as infected. Disease incidence was calculated as the number of infected mats divided by the total number of assessed mats. Disease severity was assessed using a scale from 0 to 5 (0: no symptoms, 1: dark green streaks on the leaf lamina, 2: dark green streaks on the leaf petiole, 3: chlorosis of the leaf margin, 4: reduction in leaf size and 5: bunchy top appearance). Aphid colony type was assessed using a scale from 0 to5 (0: no aphids; 1: a single simple colony; 2: several simple colonies; 3: a large colony with one or more winged individuals; 4: several colonies with one or more winged individuals and 5: generalized colonies at the level of the leaves and the pseudostem) (Niyongere et al., 2011).

The surveys carried out in Maniema, Northern Katanga, Eastern and Western Kasai, Bandundu and Equateur provinces during the period 2010 to 2012 focused on disease presence and current geographical distribution, disease incidence and severity, and aphid presence and colony type. Aphid colony type was, however, not assessed in Bandundu province. A total of 25 territories were surveyed across these 6 provinces in 2010 to 2012 (Table 1). As in Oriental province, the surveyed farms and villages represent locations with highest observed incidence and severity levels within a territory. Disease presence was recorded in three villages per territory. In addition, a further in-depth survey was carried out in

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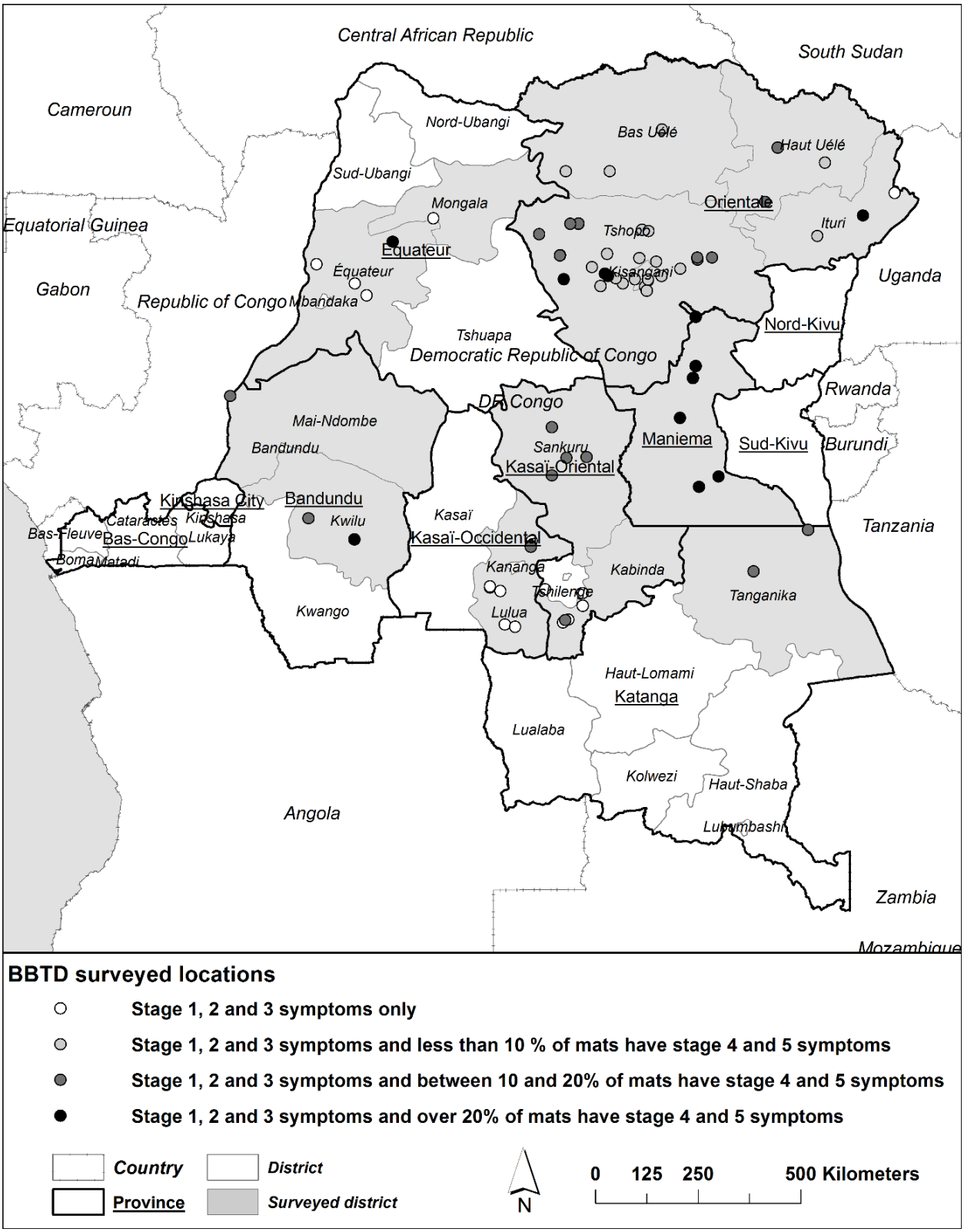

Figure 1. Map indicating the surveyed districts and study provinces (Oriental, Maniema, Katanga, Eastern and Western Kasai, Bandundu and Equateur) in DR Congo. The predominant mean BBTD severity levels for the surveyed villages are indicated with symbols (see map legend). The surveyed villages represent locations with highest observed incidence and severity levels within a territory or district. Disease severity was assessed using a scale from 1 to 5 (1: dark green streaks on the leaf lamina, 2: dark green streaks on the leaf petiole, 3: chlorosis of the leaf margin, 4: reduction in leaf size and 5: bunchy top appearance).

Table 1. Overview of number of surveyed farms, villages, territories, districts and provinces across the Congo basin of the Democratic Republic of Congo.

Period of survey	Province	Districts	Territories	No of villages with a clear presence of BBTD which were selected per territory	Total number of surveyed villages per district	No of farms randomly selected per village	Total number of farms per district
2009 - 2010	Oriental province	Tshopo	Isangi, Basoko, Yahuma, Opala, Ubundu I and II, Bafwasende, Banalia, Kisangani town and Lubuyabera (Kisangani outskirts)	3	30	10	300
		Ituri	Mambasa, Djugu and Mahagi	1	3	10	30
		Haut-Uélé	Wamba, Watsa and Niangara	1	3	10	30
		Bas-Uélé	Buta, Aketi and Ango	1	3	10	30
				No of villages where disease presence was recorded (3 villages per territory)	Further in-depth surveys (1 village per per territory)	One farm was selected per village representing the highest observed incidence and severity levels	Total number of farms per province
2010 - 2012	Maniema	-	Kailo, Kasongo, Kibombo, Lubutu, Pangi and Punia	18	6	1	6
	Northern Katanga	Tanganyika	Kalemie and Nyunzu	6	2	1	2
	Eastern Kasai	Kabinda	Katako-kombe, Kole, Lodja, Lomela, Luilu and Ngandajika	18	10	1	10
	Western Kasai	Lulua	Dimbelenge, Kazumba and Luiza	9	9	1	9
	Bandundu	Plateau	Bolobo	3	1	1	1
		Kwilu	Bulungu and Masi-manimba	6	2	1	2
	Equateur	Equateur	Basankusu, Bikoro, Bolomba, and Ingende	12	4	1	4
		Tshuapa	Monkoto	3	1	1	1

one village per territory - in Bandundu, Maniema, Equateur, North Katanga and the Katako-kombe, Kole, Lodja and Lomela territories in Eastern Kasai, while 3 villages were selected in Western Kasai and in the Luilu and Ngandajika territories in Eastern Kasai, thus giving a total of 35 surveyed villages (Table 1). No disease incidence and severity assessments were carried out in Sankura district of Eastern Kasai. Surveys and field assessments were carried out on one farm per village representing the highest observed incidence and severity levels. A total of 30 mats were assessed on each farm in all villages, except in Western Kasai and in the Luilu and Ngandajika territories in Eastern Kasai, where 10 mats were assessed per farm in 3 different villages to achieve a total of 30 mats.

Niyongere et al. (2013) reported that an increase in altitude and the associated lower temperatures negatively influence the virus transmission rate and lengthen the disease incubation period. In order to assess a possible relationship between altitude and BBTD incidence and aphid presence, the altitude of each surveyed farm across all the surveyed provinces was measured using a global positioning system (GPS) receiver.

BBTV presence was confirmed using the commercial AGDIA (Agdia-Biofords, Evry, France) kit for TAS-ELISA. TAS-ELISA tests were carried out at the laboratory of the University of Kisangani (UNIKIS). Four plantain maiden sucker samples were collected in the 7 territories of Tshopo district, Oriental province for each of the five disease severity levels in each territory to confirm field observations of visible symptoms, and especially for the minor streak symptoms of severity levels 1 and 2, and for leaf lamina chlorosis symptoms of severity level 3. In addition, four symptomless suckers were collected in all the 7 territories of Tshopo district from symptomless mats in infected fields. In addition, four stage 5 sword suckers were collected in the other 3 districts (Ituri, Haut Uélé and Bas Uélé) of Oriental province, while three stage 4 or 5 suckers were collected in all the other surveyed provinces in order to confirm disease presence. Finally, eight visibly diseased suckers (severity level 5) were collected in Butembo town, North Kivu in order to confirm the presence of the disease in this province.

All the collected suckers were first treated with a systemic insecticide (AMBUSH 500EC containing synthetic pyrethroid), to kill putative viruliferous aphids that could transmit the disease, before being planted out in pots at the UNIKIS screenhouse, which is free of aphids. Samples for TAS-ELISA testing were taken from the midrib of the youngest expanded leaf at 2 to 3 weeks after sucker establishment in the screen house.

Statistical analysis was carried out using the R language

Table 2. BBTD incidence and severity across territories and districts of Oriental province, north-eastern DR Congo. TAS-ELISA results are presented for samples collected from plants showing the full range of symptoms in Tshopo district and stage five symptoms in the other districts.

District	Territory	Altitude (masl)	Predominant genome group	Average BBTD incidence (% of assessed mats)	Average BBTD severity score (0 - 5)# (%)						TAS-ELISA (severity level) (%)					
					0	1	2	3	4	5	0 (n = 4)	1 (n = 4)	2 (n = 4)	3 (n = 4)	4 (n = 4)	5 (n = 4)
Tshopo	Basoko	390	AAB plantain	96.6	3.4	33.4	25.4	22.2	14.2	1.1	50	50	100	50	100	100
	Kisangani	416	AAB plantain	98.7	1.3	39.4	2.3	29.7	19.2	7.9	25	25	100	25	100	100
	Isangi	429	AAB plantain	92.1	7.8	7.7	72.6	6.2	4.3	1.2	50	75	100	25	100	100
	Banalia	433	AAB plantain	97.6	2.3	5.1	85.7	4.1	2.2	0.4	50	50	100	25	100	100
	Lubuyabera	435	AAB plantain	97.0	3.0	41.2	5.3	41.6	6.5	2.3	25	25	100	50	100	100
	Ubundu II	436	AAB plantain	94.0	6.0	35.8	16.0	37.8	4.0	0.3	50	25	100	25	100	100
	Yahuma	445	AAB plantain	97.9	2.2	25.0	19.7	32.9	19.0	0.9	50	25	100	25	100	100
	Opala	453	AAB plantain	97.6	2.5	1.3	88.3	4.1	3.7	0.0	25	50	100	50	100	100
	Ubundu I	457	AAB plantain	94.2	6.0	30.6	8.2	47.0	8.0	0.1	25	75	100	25	100	100
	Bafwasende	562	AAB plantain	99.1	1.1	58.6	8.6	20.4	9.4	1.9	50	75	100	25	100	100
	Mean			96.5	3.6	27.8	33.2	24.6	9.1	1.6	40.0	47.5	100.0	32.5	100	100
	SE			6.4	6.4	51.9	98.9	45.1	18.0	6.6						
																(n = 3*)
Ituri	Mambasa	901	AAB plantain	67.7	32.3	54.7	5.0	6.7	0.7	0.7	-	-	-	-	-	100
	Djugu	1117	AAA-EA	99.7	0.3	28.7	4.3	31.7	19.0	16.0	-	-	-	-	-	100
	Mahagi	1703	AAA-EA	43.3	56.7	40.7	0.7	2.0	0.0	0.0	-	-	-	-	-	100
	Mean			70.2	29.8	41.3	3.3	13.4	6.6	5.6	-	-	-	-	-	100
	SE			48.9	48.9	22.5	4.0	27.6	18.7	15.7						
																(n = 3*)
Haut Uélé	Niangara	724	AAB plantain	85.3	14.7	46.7	3.0	24.0	7.7	4.0	-	-	-	-	-	100
	Wamba	779	AAB plantain	96.0	4.0	59.0	2.7	23.3	8.7	2.3	-	-	-	-	-	100
	Watsa	988	AAB plantain	74.7	25.3	43.3	6.7	15.3	6.0	3.3	-	-	-	-	-	100
	Mean			85.3	14.7	49.7	4.1	20.9	7.4	3.2	-	-	-	-	-	100
	SE			18.5	18.5	14.3	3.8	8.4	2.3	1.5						
																(n = 4)
Bas Uélé	Buta	413	AAB plantain	83.7	16.3	55.7	5.0	19.3	2.0	1.7	-	-	-	-	-	50
	Aketi	414	AAB plantain	69.7	30.3	46.7	8.7	13.7	0.7	0.0	-	-	-	-	-	50
	Ango	611	AAB plantain	68.3	31.7	50.0	6.7	8.3	1.7	1.7	-	-	-	-	-	50
	Mean			73.9	26.1	50.8	6.8	13.8	1.4	1.1	-	-	-	-	-	50
	SE			14.7	14.7	7.9	3.2	9.5	1.2	1.7						
	Overall mean			91.9	8.2	32.3	26.6	22.6	8.2	2.0	-	-	-	-	-	91.4
	SE			75.6	9.6	29.8	60.8	31.2	12.4	4.1						

#: 0: no symptoms, 1: dark green streaks on the leaf lamina, 2: dark green streaks on the leaf petiole, 3: chlorosis of the leaf margins, 4: reduction in leaf size and 5: bunchy top appearance.
*: although four suckers were collected, some suckers did not survive the long journey back to Kisangani.

and environment (R Development Core Team, 2010). The relationship between altitude and BBTD incidence and aphid presence was assessed through correlation analysis. The Student test ($P \leq 0.05$) was used to compare BBTD incidence and severity, aphid presence and type of aphid colony between districts or between provinces. In addition, comparisons were also made between backyard plots adjacent to the house and distant plots on cleared forest land for the Ituri, Haut Uélé and Bas Uélé districts of Oriental province.

RESULTS

Surveys in Oriental province

Plantains are predominantly cultivated across the Tshopo, Haut Uélé and Bas Uélé districts of Oriental province (Table 2). These districts are also characterized by lower elevations, ranging from 390 to 988 m above sea level (masl). In contrast, East African highland cultivars dominate the production landscape in the Djugu and Mahagi territories of Ituri district (Table 2). These

Table 3. Source of planting material in surveyed districts and territories of Oriental province, north-eastern DR Congo.

District	Territory	Altitude (masl)	Predominant genome group	Source of planting material (% of farmers)[#]				
				1	2	3	4	5
Tshopo	Basoko	390	AAB plantain	82.7	10.3	7.0	0.0	0.0
	Kisangani	416	AAB plantain	56.2	43.2	0.6	0.0	0.0
	Isangi	429	AAB plantain	89.3	10.4	0.0	0.0	0.0
	Banalia	433	AAB plantain	90.3	9.7	0.0	0.0	0.0
	Lubuyabera	435	AAB plantain	82.7	17.3	0.0	0.0	0.0
	Ubundu II	436	AAB plantain	88.7	10.3	1.0	0.0	0.0
	Yahuma	445	AAB plantain	94.2	4.2	1.6	0.0	0.0
	Opala	453	AAB plantain	96.1	3.9	0.0	0.0	0.0
	Ubundu I	457	AAB plantain	93.4	6.4	0.0	0.0	0.0
	Bafwasende	562	AAB plantain	96.8	3.4	0.0	0.0	0.0
Ituri	Mambasa	901	AAB plantain	62.9	25.9	11.1	0.0	0.0
	Djugu	1117	AAA-EA	79.2	12.5	8.3	0.0	0.0
	Mahagi	1703	AAA-EA	56.5	34.8	8.7	0.0	0.0
Haut Uélé	Niangara	724	AAB plantain	61.9	28.6	9.5	0.0	0.0
	Wamba	779	AAB plantain	68.2	18.2	13.6	0.0	0.0
	Watsa	988	AAB plantain	63.7	18.2	18.2	0.0	0.0
Bas Uélé	Buta	413	AAB plantain	52.4	23.8	23.8	0.0	0.0
	Aketi	414	AAB plantain	55.0	25.0	20.0	0.0	0.0
	Ango	611	AAB plantain	68.0	25.0	7.0	0.0	0.0
Mean				81.5	14.6	3.9	0.0	0.0

#: 1: From own farm, 2: from neighbouring farm (<1 km), 3: from a friend's farm (>1 km), 4: tissue culture plantlets distributed by a government agency and 5: tissue culture plantlets bought from a private laboratory.

territories are located at higher elevations, which range from 1,117 to 1,703 masl. All the *Musa* cultivars grown in farmers' fields across Oriental province were susceptible to BBTD.

Farmers mainly obtain planting material from their own fields (81% of respondents), or from neighboring farms located less than 1 km away (15%) (Table 3). A few farmers (4%) obtained suckers from distant farms, while no tissue culture plantlets were used. The movement of planting materials is not regulated though quarantine agencies in DR Congo and diseased suckers are often selected as planting material.

An overall average disease incidence of 92% was observed across Oriental province (Table 2). Disease severity levels were, however, low and 81.5% of all assessed mats had a disease severity score ranging from 1 to 3 (Table 2). Only 10% of mats had advanced disease symptoms (that is, dwarfing of leaves, bunched leaves which stand upright and are brittle) corresponding to disease severity scores 4 and 5. Disease incidence was above 90% in all surveyed territories of Tshopo district (Table 2) and reached almost 100% in Djugu (Ituri district) and Bafwasende (Tshopo district).

The territories of Djugu (Ituri), Kisangani (Tshopo), Yahuma (Tshopo) and Basoko (Tshopo) had the highest proportion of mats (respectively, 35, 27, 20 and 15%) with advanced disease symptoms (scores 4 plus 5). In contrast, very few mats with advanced disease symptoms were observed especially in Bas Uélé (Buta, Aketi and Ango), but also in Mahagi and Mambasa (Ituri district) and in the Banalia and Opala territories of Tshopo district (Table 2).

The aphid vector was present in all districts and surveyed territories and was found on 89% of all assessed mats across the province. Aphid presence was at least 92% across the territories of Tshopo district, while a lower aphid presence was observed across the three other districts (Table 4). A single simple aphid colony without winged insects was most frequently observed in Ituri (37% of mats), Haut Uélé (49%) and Bas Uélé (43%), while Tshopo district had a considerable presence (43%) of several simple colonies without winged aphids.

A slightly higher BBTD incidence was observed in home gardens (79%) compared to the distant plots on cleared forest land (74%) across Ituri, Haut and Bas Uélé

Table 4. Aphid vector presence and colony typology across districts and territories of Oriental province, north-eastern DR Congo.

District	Territory	Altitude (masl)	Predominant genome group	Aphid presence (% of assessed mats)	Aphid colony typology[#] (%)					
					0	1	2	3	4	5
Tshopo	Basoko	390	AAB plantain	93.3	3.5	25.1	56.2	9.8	3.7	1.6
	Kisangani	416	AAB plantain	98.7	1.3	31.2	42.9	20.3	4.1	0.0
	Isangi	429	AAB plantain	92.2	7.8	29.0	32.6	26.9	3.7	0.0
	Banalia	433	AAB plantain	97.6	2.4	32.7	43.9	13.2	7.4	0.2
	Lubuyabera	435	AAB plantain	97.0	3.0	31.7	35.2	20.8	8.8	0.7
	Ubundu II	436	AAB plantain	94.0	5.9	37.9	40.6	10.9	4.4	0.0
	Yahuma	445	AAB plantain	97.8	2.2	38.8	52.3	4.8	1.7	0.1
	Opala	453	AAB plantain	97.4	2.6	42.7	31.2	21.1	2.3	0.0
	Ubundu I	457	AAB plantain	94.2	5.8	32.1	41.9	14.1	3.5	2.4
	Bafwasende	562	AAB plantain	96.0	0.9	29.3	54.5	12.1	2.9	0.2
	Mean			95.8	3.5	33.1	43.1	15.4	4.3	0.5
	SE			6.3	6.3	15.0	25.3	19.0	6.3	2.4
Ituri	Mambasa	901	AAB plantain	60.3	39.7	39.7	13.7	6.3	0.7	0.0
	Djugu	1117	AAA-EA	78.3	21.7	35.0	21.7	16.0	5.7	0.0
	Mahagi	1703	AAA-EA	36.3	63.7	35.3	1.0	0.0	0.0	0.0
	Mean			58.3	41.7	36.7	12.1	7.4	2.1	0.0
	SE			36.5	36.5	4.5	18.0	14.0	5.4	0.0
Haut Uélé	Niangara	724	AAB plantain	70.7	29.3	46.3	11.3	11.0	2.0	0.0
	Wamba	779	AAB plantain	87.7	12.3	54.7	12.0	19.3	1.7	0.0
	Watsa	988	AAB plantain	60.0	40.0	46.7	5.3	6.0	2.0	0.0
	Mean			72.8	27.2	49.2	9.6	12.1	1.9	0.0
	SE			24.2	24.2	8.2	6.4	11.7	0.3	0.0
Bas Uélé	Buta	413	AAB plantain	74.3	25.7	43.3	18.3	9.7	3.0	0.0
	Aketi	414	AAB plantain	67.7	32.3	41.7	17.0	8.7	0.3	0.0
	Ango	611	AAB plantain	70.0	30.0	45.3	14.7	10.0	0.0	0.0
	Mean			70.7	29.3	43.4	16.7	9.4	1.1	0.0
	SE			5.9	5.9	3.2	3.2	1.2	2.8	0.0
	Overall mean			89.2	10.3	35.4	36.1	14.1	3.7	0.4
	SE			78.8	10.7	21.4	43.3	16.4	5.2	1.4

[#]: 0: no aphids; 1: a single simple colony; 2: several simple colonies; 3: a large colony with one or more winged individuals; 4: several colonies with one or more winged individuals and 5: generalized colonies at the level of the leaves and the pseudostem.

(Table 5). Disease severity was similar for both plot types, with about half of the symptomatic plants showing dark green streaks on the leaf lamina (severity score 1) (Table 5). Leaf margin chlorosis was the second most observed symptom and was recorded on 17% of symptomatic plants in the home gardens and on 15% of plants in the distant plots.

Aphid colonies were observed on 71% of mats in the home gardens compared with 65% of mats in the distant plots (Table 5). Single simple aphid colonies (score 1) clearly dominated in both plot types (46 and 41% for, respectively, home and distant plots), while multiple aphid colonies with one or more winged individuals (score 4) were rare and generalized aphid colonies at the level of the leaves and pseudostem (score 5) were totally absent (Table 5). A total of 12 and 11% of mats in, respectively, the home gardens and distant plots harbored winged aphids.

A very strong relationship was observed between BBTD incidence and aphid presence in Oriental province ($R = 0.93$, $p < 0.01$). The correlation coefficient between site altitude and percentage BBTD incidence was -0.65 ($p < 0.01$) when taking into account all the surveyed territories in Oriental province, while the correlation coefficient between altitude and aphid vector presence (%) was $R=-0.79$ ($p < 0.01$). The altitude across all the

Table 5. BBTD incidence and severity, and aphid presence according to the location of a plantain/banana plot in the districts of Ituri, Haut Uélé and Bas Uélé, Oriental province, north-eastern DR Congo.

Location	District	Territory	Altitude (masl)	Predominant genome group	Average BBTD incidence (% of assessed mats)	Average BBTD severity score (1-5)[#] (%)					Aphid presence (% of assessed mats)	Aphid colony typology* (%)				
						1	2	3	4	5		1	2	3	4	5
Plots adjacent to the house	Ituri	Mambasa	901	AAB plantain	75.3	64.0	6.0	4.7	0.7	0.0	62.0	37.3	15.3	8.7	0.7	0.0
		Djugu	1117	AAA-EA	100.0	39.3	5.3	32.0	14.7	8.7	77.3	34.7	23.3	14.7	4.7	0.0
		Mahagi	1703	AAA-EA	40.0	36.7	0.7	2.7	0.0	0.0	33.3	32.0	1.3	0.0	0.0	0.0
	Haut Uélé	Niangara	724	AAB plantain	88.0	45.3	2.7	25.3	10.7	4.0	78.0	53.3	13.3	8.7	2.7	0.0
		Wamba	779	AAB plantain	98.7	66.0	1.3	21.3	8.7	1.3	90.0	60.0	10.7	17.3	2.0	0.0
		Watsa	988	AAB plantain	-	-	-	-	-	-	-	-	-	-	-	-
	Bas Uélé	Buta	413	AAB plantain	88.7	60.0	3.3	19.3	2.7	3.3	78.7	46.0	14.0	12.7	6.0	0.0
		Aketi	414	AAB plantain	73.3	36.7	14.0	22.0	0.7	0.0	78.0	54.0	14.0	9.3	0.7	0.0
		Ango	611	AAB plantain	70.0	53.3	3.3	8.7	2.0	2.7	72.7	47.3	14.7	10.7	0.0	0.0
Mean					79.2	50.2	4.6	17.0	5.0	2.5	71.2	45.6	13.3	10.2	2.1	0.0
SE					10.3	6.5	2.2	5.6	2.9	1.6	9.1	5.4	3.2	2.7	1.2	0.0
Distant plots on cleared forest land	Ituri	Mambasa	901	AAB plantain	60.0	45.3	4.0	8.7	0.7	1.3	58.7	42.0	12.0	4.0	0.7	0.0
		Djugu	1117	AAA-EA	99.3	18.7	3.3	31.3	23.3	23.3	79.3	35.3	20.0	17.3	6.7	0.0
		Mahagi	1703	AAA-EA	46.7	44.7	0.7	1.3	0.0	0.0	39.3	38.7	0.7	0.0	0.0	0.0
	Haut Uélé	Niangara	724	AAB plantain	82.7	48.0	3.3	22.7	4.7	4.0	63.3	39.3	9.3	13.3	1.3	0.0
		Wamba	779	AAB plantain	93.3	52.0	4.0	25.3	8.7	3.3	85.3	49.3	13.3	21.3	1.3	0.0
		Watsa	988	AAB plantain	74.7	43.3	6.7	15.3	6.0	3.3	60.0	46.7	5.3	6.0	2.0	0.0
	Bas Uélé	Buta	413	AAB plantain	78.7	51.3	6.7	19.3	1.3	0.0	70.0	40.7	22.7	6.7	0.0	0.0
		Aketi	414	AAB plantain	66.0	56.7	3.3	5.3	0.7	0.0	57.3	29.3	20.0	8.0	0.0	0.0
		Ango	611	AAB plantain	66.7	46.7	10.0	8.0	1.3	0.7	67.3	43.3	14.7	9.3	0.0	0.0
Mean					74.2	45.2	4.7	15.3	5.2	4.0	64.5	40.5	13.1	9.5	1.3	0.0
SE					12.6	9.8	2.8	4.9	3.7	3.7	13.5	8.3	4.5	3.7	1.1	0.0

[#]: 0: no symptoms, 1: dark green streaks on the leaf lamina, 2: dark green streaks on the leaf petiole, 3: chlorosis of the leaf margins, 4: reduction in leaf size and 5: bunchy top appearance. *: 0: no aphids; 1: a single simple colony; 2: several simple colonies; 3: a large colony with one or more winged individuals; 4: several colonies with one or more winged individuals and 5: generalized colonies at the level of the leaves and the pseudostem.

territories in the province ranges from 390 to 1,703 masl. The lowest average disease incidence and severity level was observed at Mahagi, Ituri district (1,703 masl) which is by far the highest altitude location (Table 2).

All samples collected in Tshopo district on plants showing dark green streaks on the leaf midribs and leaf petioles (that is, severity score 2), and leaf dwarfing and a typical bunchy top appearance (scores 4 and 5) had positive TAS-ELISA results (Table 2). However, only 48% of plants with dark green streaks on the leaf lamina veins (severity score 1) and 33% of plants with chlorosis of the leaf margins (score 3) gave positive TAS-ELISA results. In addition, 40% of symptomless plants tested positive. The samples collected in Ituri and Haut-Uélé, from plants having a typical bunchy top appearance (severity score 5) all gave positive

TAS-ELISA results, while only 50% of samples collected from stage 5 plants in Bas Uélé tested positive (Table 2).

Surveys in Bandundu, Equateur, Eastern and Western Kasai, Katanga and Maniema provinces

Plantains are predominantly cultivated in Bandundu, Eastern Kasai, Equateur and Maniema, while east African highland cultivars dominate the production landscape in Katanga (Table 6). The cultivation of AAA dessert bananas is widespread in Kwilu district, Bandundu and in Lulua district in Western Kasai. All the *Musa* cultivars grown in farmers' fields across these six provinces are susceptible to BBTD. The average BBTD incidences in Bandundu, Equateur, Kasai, Katanga and Maniema were lower than the incidence levels observed in Oriental province (Tables 2 and 6). The lowest incidence levels were observed in Equateur (43%) and Katanga (35%). Disease severity level 1 was most commonly observed across these provinces (Table 6). However, just as in Djugu territory (35%) in Oriental province, relatively high disease severity levels (scores 4 plus 5) were also recorded in most territories of Maniema (ranging from 20 to 53%), Bandundu (ranging from 10 to 20%) and Katanga (13%). All suckers/lateral shoots with stage 4 or 5 symptoms collected across Bandundu, Equateur, Katanga, Maniema and in Butembo, North Kivu gave positive TAS-ELISA results, while only 50% of samples from suckers collected in Eastern and Western Kasai tested positive (Table 6). The aphid vector was present in all districts and surveyed territories of Bandundu, Equateur, Kasai, Katanga and Maniema (Table 7). Single simple aphid colonies without winged insects and multiple simple colonies without winged insects were most frequently observed across these provinces. However, 20% of mats in Maniema contained winged aphids (colony types 4 and 5) (Table 7). This corresponds with the high disease incidence and especially severity levels which were observed in this same province (Table 6).

A high correlation was observed between disease incidence and aphid occurrence when analyzing data from all the 7 surveyed provinces ($R = 0.63$, $p < 0.001$). A significant negative correlation was observed between altitude and aphid presence ($R = -0.44$, $p = 0.002$), while no significant correlation was observed between BBTD incidence and altitude ($R = -0.08$, $p = 0.57$).

TAS-ELISA results confirmed the presence of BBTV in all stage 5 suckers collected in Butembo town. In contrast, no BBTV infected plants were observed in the countryside around Butembo (Charles Sivirihauma, personal communication, 2013).

DISCUSSION

BBTD and the aphid vector were observed in all surveyed provinces, districts and territories. It is no surprise to have observed the BBTV aphid vector across the whole of north-eastern DR Congo as this aphid has been reported to be present in all banana-growing regions of the tropical world (Hill, 1983; Robson et al., 2007). Highest disease incidence levels were observed in Oriental, Eastern and Western Kasai and Maniema provinces. The lowest average disease incidence and severity level, in Oriental province, was observed at Mahagi, Ituri district (1,703 masl) which is the highest altitude location that was surveyed. Very low disease incidence levels are currently also observed in the highland regions of North and South Kivu provinces (FAO, 2010; Charles Sivirihauma and Célestin Niyongere, personal communication, 2013). An increase in altitude and corresponding lower temperatures negatively influences vector presence and disease incidence; *P. nigronervosa* is known to have a preference for warmer climates. A negative correlation has, for instance, been found between aphid presence and altitude (cooler temperatures) in the Great Lakes region of Africa (Niyongere et al., 2012). Moreover, a high temperature is more favorable for aphid transmission of the BBTV than a low temperature (Wu and Su, 1990).

The altitude across all the territories of the 7 provinces ranges from 320 to 1,117 masl, with one outlier of 1,703 masl for Mahagi territory in Ituri district, Oriental province. The weak altitude effect, when analyzing the data from all 7 provinces together, may have arisen from the fact that most territories are located at altitudes below 1,000 masl.

Highest disease severity levels were observed in Djugu, Kisangani and Yahuma territories in Oriental province, and in the majority of territories in Maniema province. However, disease severity in the majority of surveyed districts is predominantly limited to streaks on the leaf veins and petioles, which has not been reported as having an influence on bunch weight or yield. In addition, infected mats can produce numerous healthy looking and productive plants over prolonged periods of time (Benoit Dhed'a and Bonaventure Ibanda, personal communication, 2013).

Home garden plantations or backyards are generally older than those found on cleared forest land. However, high values of disease incidence and aphid presence were observed in most plots on cleared land, which most likely resulted from the use of infected planting material when establishing a new distant plot. In addition, the surveys revealed that famers do not remove any aphids when preparing planting material, as they are simply unaware of the presence and role of these aphids.

The positive results from the TAS-ELISA analysis of samples from Butembo, North Kivu confirm the presence of BBTD in this Eastern province. A *Musa* diseases survey that was carried out by the International Institute of Tropical Agriculture (IITA) in 2009 in North Kivu reported a BBTD incidence of 29% across backyards of Butembo town (1,600 to 1,800 masl), while surveys carried out by FAO in 2010 (FAO, 2010) in the Beni and

Table 6. BBTD incidence and severity across Maniema, northern Katanga, Eastern and Western Kasai, Bandundu and Equateur provinces. TAS-ELISA results are presented for samples collected from plants exhibiting severity levels 4 or 5.

Province	District	Territory	Altitude (masl)	Predominant genome group	Average BBTD incidence (% of assessed mats)	Average BBTD severity score (0-5)# (%)						TAS-ELISA (severity level 4 and 5) (%)
						0	1	2	3	4	5	
												(n = 3)
Bandundu	Kwilu	Masi-manimba	413	AAB plantain	60.0	40.0	20.0	3.3	20.0	3.3	13.3	100
		Bulungu	445	AAA dessert	70.0	30.0	26.7	3.3	20.0	16.7	3.3	100
	Plateau	Bolobo	336	AAB plantain	50.0	50.0	26.7	0.0	13.3	3.3	6.7	100
	Mean				60.0	40.0	24.4	2.2	17.8	7.8	7.8	100
	SE				1.7	1.7	0.7	0.3	0.7	1.3	0.9	
												(n = 2*)
Eastern Kasai	Kabinda	Ngandajika	766	AAB plantain	66.7	33.3	33.3	26.7	6.7	0.0	0.0	50
		Luilu	832	AAB plantain	73.3	26.7	30.0	30.0	10.0	3.3	0.0	50
	Mean				70.0	30.0	31.7	28.3	8.3	1.7	0.0	50
	SE				1.0	1.0	0.5	0.5	0.5	0.5	0.0	
												(n = 3)
Equateur		Bikoro	320	AAB plantain	46.7	53.3	23.3	0.0	23.3	0.0	0.0	100
	Equateur	Ingende	332	AAB plantain	36.7	63.3	20.0	0.0	16.7	0.0	0.0	100
		Bolomba	343	AAB plantain	33.3	66.7	26.7	0.0	6.7	0.0	0.0	100
		Basankusu	366	AAB plantain	73.3	26.7	23.3	0.0	16.7	16.7	16.7	100
	Tshuapa	Monkoto	375	AAB plantain	26.7	73.3	10.0	0.0	16.7	0.0	0.0	100
	Mean				43.3	56.7	20.7	0.0	16.0	3.3	3.3	100
	SE				2.4	2.4	0.9	0.0	0.8	1.0	1.0	
												(n = 3)
Katanga	Tanganyika	Nyunzu	641	AAA-EA	40.0	60.0	20.0	6.7	0.0	6.7	6.7	100
		Kalemie	1011	AAA-EA	30.0	70.0	3.3	10.0	3.3	10.0	3.3	100
	Mean				35.0	65.0	11.7	8.3	1.7	8.3	5.0	100
	SE				1.5	1.5	2.5	0.5	0.5	0.5	0.5	
												(n = 3)
Maniema		Lubutu	512	AAB plantain	76.7	23.3	13.3	0.0	40.0	10.0	13.3	100
		Kailo	517	AAB plantain	60.0	40.0	10.0	3.3	13.3	26.7	6.7	100
		Kibombo	534	AAB plantain	66.7	33.3	0.0	0.0	26.7	30.0	10.0	100
		Punia	547	AAB plantain	50.0	50.0	13.3	13.3	3.3	16.7	3.3	100
		Pangi	548	AAB plantain	76.7	23.3	13.3	0.0	40.0	10.0	13.3	100
		Kasongo	554	AAB plantain	86.7	13.3	6.7	0.0	26.7	26.7	26.7	100
	Mean				69.4	30.6	9.4	2.8	25.0	20.0	12.2	100
	SE				1.6	1.6	0.7	0.7	1.8	1.1	1.0	

Table 6. Contd.

													(n = 2*)
Western Kasai	Lulua	Dimbelenge	624	AAB plantain	80.0	20.0	36.7	26.7	10.0	6.7	0.0		50
		Kazumba	705	AAA dessert	73.3	26.7	40.0	23.3	10.0	0.0	0.0		50
		Luiza	831	AAA dessert	76.7	23.3	26.7	36.7	13.3	0.0	0.0		50
	Mean				76.7	23.3	34.4	28.9	11.1	2.2	0.0		50
	SE				0.6	0.6	1.2	1.2	0.3	0.7	0.0		
	Overall mean				59.7	40.3	20.2	8.7	16.0	8.9	5.9		91.4
	SE				1.2	1.2	0.7	0.8	0.7	0.6	0.5		

#: 0: no symptoms, 1: dark green streaks on the leaf lamina, 2: dark green streaks on the leaf petiole, 3: chlorosis of the leaf margins, 4: reduction in leaf size and 5: bunchy top appearance. *: although three suckers were collected, some suckers did not survive the long journey back to Kisangani.

Table 7. Aphid presence and aphid colony type across Maniema, northern Katanga, Eastern and Western Kasai, Bandundu and Equateur provinces.

Province	District	Territory	Altitude (masl)	Predominant genome group	Aphid presence (% of assessed mats)	Aphid colony typology# (%)					
						0	1	2	3	4	5
Bandundu	Kwilu	Masi-manimba	413	AAB plantain	76.7	23.3	-	-	-	-	-
		Bulungu	445	AAA dessert	60.0	40.0	-	-	-	-	-
	Plateau	Bolobo	336	AAB plantain	73.3	26.7	-	-	-	-	-
	Mean				70.0	30.0	-	-	-	-	-
	SE				1.5	1.5					
Eastern Kasai	Kabinda	Ngandajika	766	AAB plantain	66.7	33.3	40.0	26.7	0.0	0.0	0.0
		Luilu	832	AAB plantain	66.7	33.3	50.0	16.7	0.0	0.0	0.0
	Sankuru	Lodja	374	AAB plantain	63.3	36.7	43.3	10.0	3.3	3.3	3.3
		Katako-kombe	475	AAB plantain	73.3	26.7	70.0	0.0	3.3	0.0	0.0
		Lomela	571	AAB plantain	73.3	26.7	56.7	3.3	3.3	6.7	3.3
		Kole	575	AAB plantain	80.0	20.0	56.7	0.0	3.3	10.0	10.0
	Mean				70.6	29.4	52.8	9.4	2.2	3.3	2.8
	SE				0.7	0.7	1.3	1.3	0.2	0.5	0.5
Equateur	Equateur	Bikoro	320	AAB plantain	80.0	20.0	63.3	16.7	0.0	0.0	0.0
		Ingende	332	AAB plantain	80.0	20.0	33.3	43.3	3.3	0.0	0.0
		Bolomba	343	AAB plantain	76.7	23.3	56.7	20.0	0.0	0.0	0.0
		Basankusu	366	AAB plantain	56.7	43.3	36.7	16.7	3.3	0.0	0.0
	Tshuapa	Monkoto	375	AAB plantain	73.3	26.7	53.3	20.0	0.0	0.0	0.0
	Mean				73.3	26.7	48.7	23.3	1.3	0.0	0.0
	SE				1.3	1.3	1.7	1.5	0.2	0.0	0.0

Table 6. Contd.

Katanga	Tanganyika	Nyunzu	641	AAA-EA	50.0	50.0	30.0	10.0	10.0	0.0	0.0
		Kalemie	1011	AAA-EA	16.7	83.3	10.0	6.7	0.0	0.0	0.0
	Mean				33.3	66.7	20.0	8.3	5.0	0.0	0.0
	SE				5.0	5.0	3.0	0.5	1.5	0.0	0.0
Maniema	-	Lubutu	512	AAB plantain	20.0	80.0	3.3	13.3	0.0	0.0	3.3
		Kailo	517	AAB plantain	53.3	46.7	10.0	6.7	13.3	16.7	6.7
		Kibombo	534	AAB plantain	63.3	36.7	6.7	6.7	13.3	26.7	10.0
		Punia	547	AAB plantain	46.7	53.3	26.7	16.7	3.3	0.0	0.0
		Pangi	548	AAB plantain	66.7	33.3	26.7	13.3	16.7	10.0	0.0
		Kasongo	554	AAB plantain	86.7	13.3	10.0	20.0	10.0	13.3	33.3
	Mean				56.1	43.9	13.9	12.8	9.4	11.1	8.9
	SE				2.7	2.7	1.2	0.7	0.8	1.3	1.5
Western Kasai	Lulua	Dimbelenge	624	AAB plantain	73.3	26.7	56.7	13.3	3.3	0.0	0.0
		Kazumba	705	AAA dessert	66.7	33.3	43.3	20.0	3.3	0.0	0.0
		Luiza	831	AAA dessert	70.0	30.0	46.7	23.3	0.0	0.0	0.0
	Mean				70.0	30.0	48.9	18.9	2.2	0.0	0.0
	SE				0.6	0.6	1.2	0.9	0.3	0.0	0.0
	Overall mean				64.5	35.5	37.7	14.7	4.2	3.9	3.2
	SE				1.0	1.0	1.3	0.6	0.3	0.5	0.5

[#]: 0: no aphids; 1: a single simple colony; 2: several simple colonies; 3: a large colony with one or more winged individuals; 4: several colonies with one or more winged individuals and 5: generalized colonies at the level of the leaves and the pseudostem.

Lubero territories of North Kivu province reported a 19% disease incidence in the town of Butembo. A survey carried out by the Consortium for Improving Agriculture-based Livelihoods in Central Africa (CIALCA) in 2011 recorded the disease on 36% of assessed mats, mainly in the Kitulu neighborhood of Butembo town **(**Charles Sivirihauma, personal communication, 2012). This may indicate that town residents brought diseased planting material into Butembo town upon their return from the Congo basin region where the disease is omnipresent. More and more farmers from Butembo town and surrounding villages have started buying large farms in the Mambasa and Irumu territories of Ituri district (960 masl) for the cultivation of cacao, coffee, banana, palm oil and cassava (Charles Sivirihauma, personal communication, 2013). This trend could indeed increase the likelihood of *Musa* seed movements and could lead to further BBTD spread into the highland regions around Butembo town. Surveys carried out initially by Walangululu et al. (2010) and subsequently by Niyongere et al. (2013) confirmed the presence of BBTD and its aphid vector in predominantly mid-altitude regions of South Kivu province bordering the Rusizi valley. An average disease incidence of 23 and 29% was observed in Kamanyola (895 to 972 masl) and Nyangezi (1,254 to 1,937 masl) districts, respectively (Niyongere et al., 2012). Mats with severe disease symptoms (scores 4 and 5) attained a 15 and 17% frequency in Kamanyola and Nyangezi and aphid vectors were observed on 40 and 41% of mats, respectively (Niyongere et al., 2012). Aphid populations containing winged aphids (colony type 3 to 5) were observed on 15 and 17% of mats in Kamanyola and Nyangezi districts.

An effective quarantine service needs to be established to prevent the movement of planting materials into areas where the disease is currently non-existent or rare (e.g. in the largest parts of

North Kivu and South Kivu, which are predominantly high elevation sites). In addition, there is an urgent need to carry out serological tests (TAS-ELISA) in order to identify BBTD-free plants for multiplication and distribution of disease-free planting materials, while information on disease epidemiology and control needs to be disseminated on a large scale.

Conflict of Interests

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

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Essential oils for the control of bacterial speck in tomato crop

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Bacterial speck (*Pseudomonas syringae* pv. *tomato*) is considered one of the major diseases of tomato crop worldwide and alternative methods to control it are desirable. The objective of this study was to evaluate the effect of essential oils (EOs) on *P. syringae* pv. *tomato* strain ufv-1 growth, in controlling bacterial speck in tomato plants, as well as to find the best application time of the EOs. The EOs used in this study were thyme (TH), eucalyptus (EU), tea tree (TT), clove (CL), cinnamon (CN), citronella (CI), and lemon grass (LE). An *in vitro* test using EOs were conducted to verify the ufv-1 inhibition, and two tests were carried out in a greenhouse to evaluate the effect of EOs before and after inoculation with *Pst*. Inhibition zones were observed for EU, CI, CL, and CN at a concentration of 1%. Plants pre-treated with EOs showed lower disease severity than that in plants post-treated with EOs ($P<0.05$), whereas higher efficacy was observed using acibenzolar-*S*-methyl and CI (91 and 83%, respectively). Regarding post-treatment, TT, TH, CI, EU, LE, and commercial fungicide, resulted in reducing disease severity by 8 to 40% compared to control (water). Results from this study showed the potential use of EOs in controlling bacterial speck in tomato and suggest the induced resistance as the major mode of action.

Key words: *Pseudomonas syringae* pv. *tomato,* tomato diseases, alternative control of plant diseases, *Solanum lycopersicum.*

INTRODUCTION

Bacterial speck (*Pseudomonas syringae* pv. *tomato*, *Pst*) is considered one of the major diseases of tomato crop in many countries around the world (Cai et al., 2011), including some located in North and South America (Junior, 2013), in Europe (Milijašević et al., 2009b; Quattrucci et al., 2013), as well as in Africa (Shenge et al., 2008a, b). Dark lesions may be present in infected plant parts, such as leaves, stems, and fruits resulting in depreciation of fruit quality and consequently a decrease in commercialization (Herman et al., 2008; Pietrarelli et al., 2006).

Although there are some tomato cultivars resistant to bacterial speck there is still the chance of disease overcome by new strains of *P. syringae* pv. *tomato*, which continue evolving (Milijasevic et al., 2009a). The use of antibiotics has also been considered as ineffective against several pathogens and many countries do not permit its use. On the other hand, the use of chemicals such as copper-based products may result in phytotoxicity, as well as require frequent applications, causing accumulation in the soil due to its continuous use (Balestra et al., 2009). The use of chemicals products are

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also a risk to human health because frequently, the post chemical application waiting period is not respected by many growers before harvest, which means that tomato could be carrying high levels of chemicals residues. For instance, high levels of Cu was found in tomato samples after exposing them to different levels of the metal, indicating a food contamination as a result of the metal exposure (Granata et al., 2011).

In this context, the use of essential oils (EOs) for disease control may serve as an alternative method for decreasing or replacing the use of chemical products in agricultural crops. According to Bakkali et al. (2008), EOs are volatile, natural, complex compounds coming from secondary metabolites of aromatic plants and characterized by a strong odor. EOs control bacteria by directly imparting toxic effects which inhibit bacterial growth (Vigo et al., 2009; Lucas et al., 2012), as well as promote plant resistance (Vigo et al., 2009). However, there are only few studies focused on the best time to apply EOs for controlling plant diseases, and some of them show the application of EOs as a preventative method (that is, applied before pathogen inoculation) (Da Silva et al., 2012).

To identify an alternative and environmentally friendly method of managing bacterial speck in tomato plant, we aimed at: (i) evaluating the *in vitro* inhibition of *Pst* when using EOs; (ii) evaluating the effect of EOs in controlling bacterial speck in tomato plants under greenhouse conditions; and (iii) assessing the best time of application of EOs: pre-treatment (before pathogen inoculation) or post-treatment (after pathogen inoculation).

MATERIALS AND METHODS

Three experiments were performed at the Federal University of Lavras (UFLA) in Lavras, Minas Gerais, Brazil. The first experiment comprised an *in vitro* test to evaluate *Pst* growth inhibition and the two other experiments were conducted in a greenhouse to evaluate the effect of EOs in the control of bacterial speck in tomato plants and to determine the best time of application of the natural product.

The pathogen used in this work was the strain ufv-1 of *P. syringae* pv. *tomato,* (hereafter ufv-1), provided by the Laboratory of Plant Pathology of Universidade Federal de Viçosa, Minas Gerais, Brazil. This strain was isolated from tomato leaves and preserved in mineral water, where it was recorded from before the experiments. For the *in vitro* inhibition experiment, the pathogen was cultured on medium 523 (Kado and Heskett, 1970) by using the parallel streak method and incubating at 28°C for 48 h. The inoculum was prepared by suspending the bacterial colonies in autoclaved distilled water, followed by adjustment of the concentration of the bacterial suspension by using a spectrophotometer to A_{540nm} = 0.20, which corresponded approximately to 10^8 CFU mL^{-1} (Silva et al., 2008). Approximately 200 µL of bacterial suspension was inoculated onto 9-cm diameter Petri dishes containing 20 mL of culture medium 523 and spread using a sterilized Drigalski spatula.

The EOs used in this study includes thyme (TH; *Thymus vulgaris* L.), clove (CL; *Syzygium aromaticum* (Linne) Merril), eucalyptus (EU; *Corymbia citriodora* Hill & Johnson), cinnamon (CN; *Cinnamomum zeylanicum* Blume), citronella (CI; *Cymbopogon nardus* (L.) Rendle.), tea tree (TT; *Melaleuca alternifólia* Cheel), and lemon grass (LE; *Cymbopogon citratus* (D.C.) Stapf) at concentrations (v/v) of 0.1, 1 and 10% (essential oil/powdered milk solution at 1%). To prepare the powdered milk emulsifier 1% (w/v), 1 g of powdered milk was added in 99 mL of water. As positive controls, streptomycin sulfate (25 mg mL^{-1}) and Recop® (copper oxychloride; 2.0 mg mL^{-1}), were used. Sterilized water was used as negative control. The EOs obtained from Brasil Portrait (Sorocaba, SP, Brazil) were separated by steam drag distillation from plant leaves.

Autoclaved filter paper discs of 6-mm diameter were soaked in 20 µL of each of the tested oils, dried at room temperature, and placed over ufv-1 suspension. Plates were incubated in BOD at 28°C, and after 24 h of incubation, the presence of inhibition halos were evaluated. The experiment was conducted using a completely randomized design (CRD) with six replicates, with each experimental unit consisting of one Petri dish containing four filter paper discs.

In the second experiment, two tests were prepared under greenhouse conditions to evaluate the plant's defense response to EOs when applied before (pre-treatment) or after (post-treatment) pathogen inoculation. Treatments included in this experiment comprised the EOs previously described at a concentration of 0.1%. Two commercial products were used as standard substances for the induction of plant resistance: Bion® (acibenzolar-S-methyl, ASM; 0.2 mg·mL^{-1}) and Recop® (copper oxychloride; 2.0 mg·mL^{-1}). Powdered milk (PM, 1%) and water were used as inoculated and non-inoculated controls. For all EO-based treatments, 1% PM was used as the emulsifier.

Tomato seeds of cultivar Santa Cruz Kada, which is susceptible to *Pst*, were sown in trays with 128 cells filled with a commercial substrate Plantmax® HT. Approximately 20 days after sowing (DAS), two transplants were placed in 5-kg pots containing the same substrate. The plants were maintained under greenhouse conditions and watered whenever necessary until the end of the trials. After 30 DAS, tomato plants previously placed in a moist chamber for 24 h were spray inoculated with ufv-1 until soaked, and returned to the moist chamber for 24 h to facilitate bacterial infection (Kado and Heskett, 1970).

For the pre-treatment trial, spraying with the previously mentioned 11 products was performed one week before inoculation with *Pst* (23 DAS), whereas for the post-treatment, spraying was performed one week after inoculation (37 DAS), as described by Lucas et al. (2012).

After 37 DAS for the pre-treatment and 44 DAS for the post-treatment, five evaluations of disease severity for both trials were conducted weekly according to an index scale described by Yunis et al. (1980), in which 0 = no symptoms; 1 = 2–5 specks together or spread all over the leaf; 2 = 6–10 specks; and 3 = more than 11 specks per leaf. For the controls, disease severity data were transformed using MCKINNEY's index (Mckinney, 1923) to calculate the area under the disease progress curve (AUDPC) (Shaner and Finney, 1977).

The experiment was conducted using a random block design with five replicates, and three plants per pot, being the mean of these three plants the experimental unit. Data of AUDPC was subjected to one-way ANOVA analysis and the means were compared using Tukey's test, with 5% probability. The Shapiro–Wilk test was used to verify normality of the data; the transformation of data was deemed not necessary. For statistical analysis, software Sisvar was used (Build 72) Copyright Daniel Furtado Ferreira 1999–2007 version 5.1 (Ferreira, 2011).

RESULTS AND DISCUSSION

No inhibition halos were observed for any of the products under study at the 0.1% concentration. At a concentration of 1%, EOs from EU, CN, CI, and LE were observed to be toxic to ufv-1. At a concentration of 10% or higher, all

Table 1. *In vitro* inhibition of *Pseudomonas syringae* pv. *tomato* growth by using various concentrations of essential oils, powdered milk, copper oxychloride, or streptomycin sulfate.

Treatments	Concentration of essential oils (%)			
	0.1	1.0	10	100
Citronella	-[1]	+	+[2]	+
Lemon grass	-	+	+	+
Eucalyptus	-	+	+	+
Cinnamon	-	+	+	+
Thyme	-	-	+	+
Tea tree	-	-	+	+
Clove	-	-	+	+
Powdered milk (1 mg·mL^{-1})[3]	-	-	-	-
Copper oxychloride (2 mg·mL^{-1})[3]	-	-	-	-
Streptomycin sulfate (25 mg·mL^{-1})[3]	-	-	-	+
Sterile water (control)	-	-	-	-

[1] Absence of inhibition halo. [2] Presence of inhibition halo. [3] The same concentration was used in all treatments in which essential oils were not added.

EOs inhibited the pathogen's growth, whereas bacterial proliferation was observed in treatments using sterile water, PM or copper oxychloride, a contact fungicide which are known to show toxic effect against phytobacteria in general. On the other hand, streptomycin sulfate also inhibited bacterial growth at 100% concentration (Table 1).

Previous studies have also shown *in vitro* antimicrobial activity of substances obtained from medicinal plants. Zabka et al. (2009) found an *in vitro* activity of some EOs, being *Carum carvi*, *Cymbopogonnardus*, *Pelargonium roseum*, *Pimenta dioica*, and *Thymus vulgaris* the most effective ones against six important pathogenic and toxinogenic fungal species: *Fusarium oxysporum*, *Fusarium verticillioides*, *Penicillium expansum*, *Penicillium brevicompactum*, *Aspergillus flavus,* and *Aspergillus fumigatus*. In addition, Lucas et al. (2012) reported *in vitro* inhibition of *Xanthomonas vesicatoria* by using EOs of CI, CL, CN, LE, EU, TH, and TT at a concentration of 10%.

When the two time points of EOs application against bacterial speck in tomato were analyzed under greenhouse conditions we found a significant statistic effect for pre-treatment trial (a week before pathogen inoculation) compared to post-treatment (P<0.05) (a week after pathogen inoculation), with the use of EOs showing high disease control (low AUDPC value).

When treatments means of pre-treatment trial were compared using Tukey's test, ASM and CI were found to be the most effective ones in controlling bacterial speck in the tomato plant, with disease controls of 91% and 83%, respectively (Figure 1).

In addition, EOs of LE, TH, TT, EU, and CN were also statistically different from the inoculated controls treated with water and PM, resulting in disease controls of 63, 56, 55, 32 and 30%, respectively.

In regard to post-treatment trial, fungicide treatment was most effective in the disease control, with a control of 40%, followed by the EOs of LE, EU, CI, TH, and TT, with disease controls of 25, 19, 13, 9 and 8%, respectively (Figure 2).

No plants showed any signs of phytotoxicity that may be attributable to the use of the products in the greenhouse test, including copper oxychloride or ASM, although such effects have been reported in previous studies that used the same products on tomato plants (Gilardi et al., 2010).

Not all previous studies have shown an association between *in vitro* inhibition tests and *in vivo* treatment tests (Fravel, 2005; Medeiros et al., 2012). However, in the current study, the EOs of LE and CI showed *in vitro* inhibition of ufv-1 at the second lowest concentration tested (1%) and were also efficient in the *in vivo* control of the disease, in both preventive and curative forms. On the other hand, the treatments using ASM and CN showed control of the disease when applied before the inoculation of ufv-1 but were not statistically different from the control when applied after pathogen inoculation.

Results from this study showed that EOs of these specific medicinal plants have characteristics that resemble products with plant resistance induction activity, because best results were obtained when treatments were applied before inoculation of pathogen. For instance, the EO of CI decreased bacterial speck severity in tomato plants when applied before pathogen inoculation (Figure 1), suggesting that induction of resistance is the main mechanism of action in the study pathosystem once these two EOs showed characteristics similar to the commercial standard product ASM, a benzothiadiazole (BTH) known to mimic the pathogen–host interaction and then resulting in systemic acquired resistance in plants. Also, Veloso et al. (2012) identified 24 chemical compounds

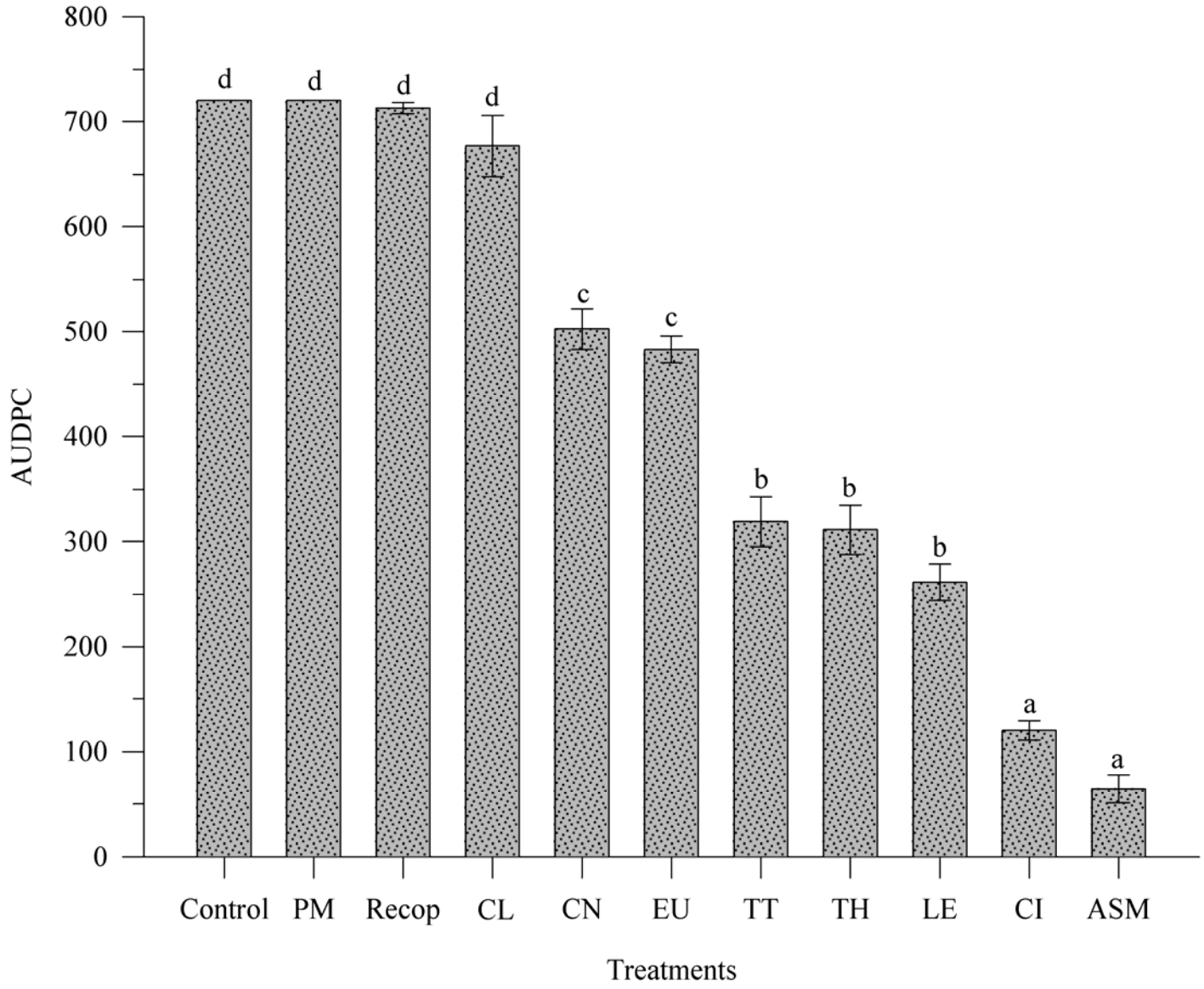

Figure 1. Area under the disease progress curve (AUDPC) in tomato plants of the cultivar Santa Cruz Kada with pre-treatment (7 days before pathogen inoculation) by using essential oils of citronella (CI); lemongrass (LE), thyme (TH), tea tree (TT), eucalyptus (EU), cinnamon (CN), clove (CL), and the products: copper oxychloride (Recop®), acibenzolar-*S*-methyl (ASM), powdered milk (PM), and water (control). Coefficient of variation (CV) = 9.31. Treatments followed by the same letter were not significantly different at 5% according to Tukey's test. The vertical bars represent the standard error of the mean.

present in the citronella EO that belonged to the terpene and terpenoid compound groups. Some studies have shown evidence that these compounds, which are produced as secondary metabolites in plants, have an important role in the plant's defense against various pathogens (Attaran et al., 2008; Henriquez et al., 2012).

The curative control of bacterial speck disease was observed by applying EOs, suggesting that direct toxicity mechanism may also be involved in the plant's defense system, thereby optimizing the efficiency of EOs, once one of the possibilities of increasing disease control in plants is using products that have more than one action mechanism (Fravel, 2005).

EOs have been used in the control of not only phytopathogens of the aerial plant parts, as shown in this and other studies (Balestra et al., 2009), but also in the control of pathogens from other pathosystems, such as post-harvested fruits and vegetables, as well as in seed and grain production. Santos et al. (2012) observed that the application of the EO of *Origanum vulgare* L. together with chitosan-inhibited spore germination and growth of *Rhizopus stolonifer* URM 3728 and *Aspergillus niger* URM 5842 fungi in artificially inoculated grapes. The use of *C. citriodora* oil at a concentration of 0.5% resulted in

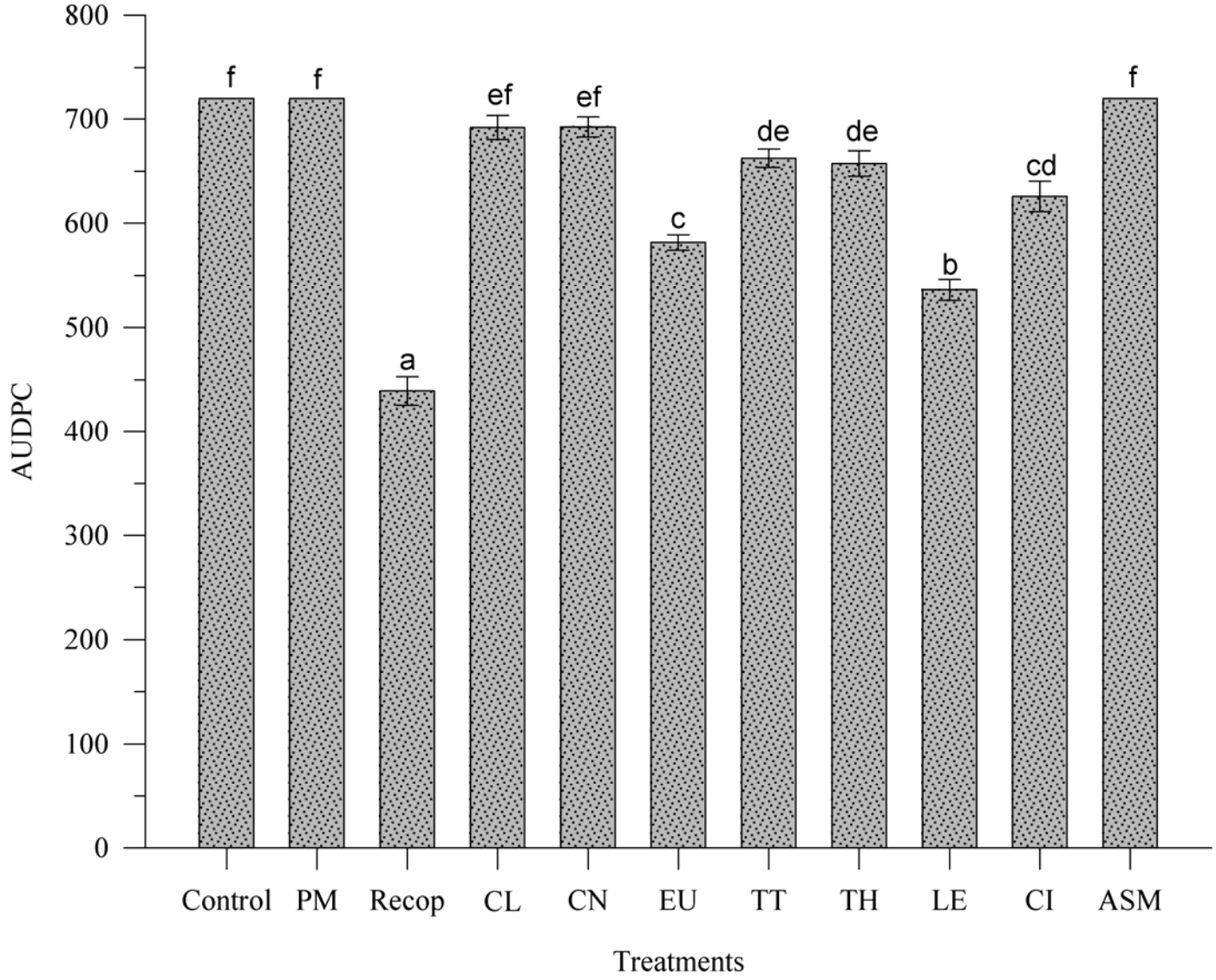

Figure 2. Area under the disease progress curve (AUDPC) in tomato plants of the cultivar Santa Cruz Kada with post-treatment (7 days after pathogen inoculation) by using the essential oils of citronella (CI), lemon grass (LE), thyme (TH), tea tree (TT), eucalyptus (EU), cinnamon (CN), clove (CL), and the products: copper oxychloride (Recop®), acibenzolar-*S*-methyl (ASM), powdered milk (PM), and water (control). Coefficient of variation (CV)= 3.53. Treatments followed by the same letter are not significantly different at 5% according to Tukey's test. The vertical bars represent the standard error of the mean.

an 83% decrease in the occurrence of soft-rot fungus (*Pectobacterium carotovorum* subsp. *carotovorum*) in lettuce (Silva et al., 2012). Amaral and Bara (2005) studied the effect of the EO of clove (*S. aromaticum*) on fungal growth in seeds of rice, soybean, corn, and beans and observed that clove oil imparted a fungicidal effect at concentrations of 0.5 to 0.1%.

Our findings suggest the potential role of EOs of medicinal plants in the alternative management of plant diseases and the importance of conducting further research in this area.

The EOs utilized in this study inhibited bacterial growth and the best time point of application identified was before pathogen inoculation being the citronella oil the most effective one. Also, a better disease control was obtained by pre-treatment than post-treatment. The study results showed that the induction of plant resistance may be the main mode of action of EOs in the tomato plant.

Conflict of Interest

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

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Full Length Research Paper

Optimal conditions for germination of seeds of *Epiphyllum oxypetalum*

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Queen of the night (*Epiphyllum oxypetalum*), an ornamental cactus, is widespread in many countries. The demand for this species is increasing because esthetic quality of its flowers is appreciated. This study aimed to analyze the influence of temperature, substrate and luminosity on the germination of *E. oxypetalum* seeds. A completely randomized design with four replications was used in a 3 x 2 x 2 factorial scheme, corresponding to three temperatures (20, 25 and 30°C), two substrates (blotting paper and sand of average particle size) and two luminosity conditions (light and dark). The percentage germination, germination speed index (GSI) and mean germination time (MGT) were evaluated. The 20°C treatment combined with the sand substrate and the presence of light provided favorable conditions for the germination of the seeds of *E. oxypetalum*, yielding a higher GSI and a shorter MGT; however, the species may be considered as preferentially photoblastic because of its ability to germinate in darkness.

Key words: Cactaceae, luminosity, ornamental plant, substrate, temperature, vigor.

INTRODUCTION

The family Cactaceae (eudicotyledon) comprises between 120 and 200 genera and between 1,500 and 2,000 species, of which approximately 40 genera and 200 species occur in Brazil; many of these species are used as ornamental plants (Souza and Lorenzi, 2012). Queen of the night [*Epiphyllum oxypetalum* (A.P. de Candolle) Haworth] is an ornamental plant that is native to Mexico, widely cultivated in the tropics and distributed in Mexico, Guatemala, Honduras, Nicaragua, El Salvador, Costa Rica, Venezuela and Brazil (Anderson, 2001).

The genus *Epiphyllum* comprises epiphytic cacti that thrive in shaded environments and acid substrates (Mace and Mace, 2009). The species *E. oxypetalum* is a robust and branched plant with a cylindrical primary branch (2 to 3 m long). The secondary branches, which are approximately 30 cm in length and between 10 and 12 cm in width, are flattened, elliptical, thin, marginally cut, wavy and leafy in appearance. The flowers are aromatic with nocturnal anthesis, 25 to 30 cm long and 12 to 17 cm in diameter, and have a perianth that is externally reddish and white on the inside. The buds begin to form

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in early spring and bloom in the fall (Anderson, 2001).

Because of its ease of cultivation and attractiveness stemming from the abundance and size of its flowers, the demand for this species is increasing. Research indicates that the stem of *E*. *oxypetalum* has a broad spectrum of activity against pathogenic bacteria and a high nutritional value and may thus be employed for medical and nutritional purposes (Upendra and Khandelwal, 2012).

The temperature, substrate and lighting conditions are of significant importance for germination and can be manipulated to optimize the percentage, speed and uniformity of germination, resulting in vigorous seedlings and reduced production costs (Pacheco et al. 2006).

Temperature acts as an inducer of germination and plant development and may change the speed of water absorption and chemical reactions that trigger the unfolding, reserve transportation and synthesis of substances for the seedling (Coelho et al., 2008). The optimum temperature interval for most tropical species is between 15 and 30°C, wherein the seed expresses its maximum potential for germination in the shortest time, whereas higher or lower temperatures tend to inhibit this process. The best germination temperatures are not sharply defined, but critical points can be identified, such as the maximum and minimum values that hinder the process (Stefanello et al., 2006; Renner et al., 2007; Carvalho and Nakagawa, 2012).

The cardinal temperatures for seed germination help explain the biogeographic peculiarities of Neotropical species (Borghetti, 2005). The study of these temperatures is important to provide useful information on seed technology and for understanding the physiological ecology of plant species (Ferreira and Borghetti, 2004).

The substrate also affects germination because the structure, aeration, water retention capacity, degree of infestation by pathogens and other factors may vary according to the type of material used (Popinigis, 1985). According to Abreu et al. (2005), the substrate is a factor that affects both the speed and percentage of germination; thus, in choosing the substrate, the size of the seed, the moisture and light requirements and the effort required to install and evaluate the seedlings must be considered.

Species have different luminosity demands because light is required for the germination of seeds (positively photoblastic seeds) in some species, inhibits the germination process in other (negatively photoblastic seeds) and, in some species, germination occurs either with or without light because the seeds are insensitive to this factor (neutrally photoblastic) (Ferreira and Borghetti, 2004; Yamashita, 2008).

Temperature and substrate are basic components for conducting germination tests (Stockman et al., 2007). As the seeds present variable physiological responses upon exposure to different temperatures, substrates and luminosities, it is recommended that the influence of these components on the germination of each species of interest be studied, thus providing support for the analysis of these influences (Guedes et al., 2009). Therefore, this study aimed to analyze the influence of temperature, substrate and luminosity on the germination of *E. oxypetalum* seeds.

MATERIALS AND METHODS

The experiment was conducted at the Seed Laboratory of the Londrina State University (Universidade Estadual de Londrina - UEL), which is located in the northern part of the State of Paraná, Brazil (23°23'S and 51°11'W). Queen of night (*E. oxypetalum*) seeds from mature fruits produced in the experimental area of the Department of Agronomy of UEL were used. The fruits were manually pulped with a spoon, and the pulp with the seeds was transferred to a 2 L beaker containing an aqueous sugar (25 g L^{-1}) solution. This mixture was left for 48 h at room temperature to encourage fermentation. After 2 days of fermentation, the pulp-seed mixture was placed in a fine-mesh sieve and washed in running water to remove the mucilage from the seeds, which were placed on filter paper to dry for 48 h in the shade at room temperature.

For the germination study, three temperatures (20, 25 and 30°C), two substrates (blotting paper and sand of average grain size) and two luminosity conditions (light and dark) were used in a completely randomized 3 x 2 x 2 factorial design with four replications. The substrates were placed in covered crystal polystyrene boxes with lids (L11 x l11 x h3 cm) that each contained 50 seeds and constituted one replicate. For the treatment with the paper substrate, a sheet of white blotting paper (10.5 x 10.5 cm, 250 mg) moistened with distilled water (2.5 times the dry weight of the paper) was used in each box (Brazil, 2009); for the treatment with the sand substrate, each box contained 200 g of sand (particles size of 0.25 to 0.50 mm) and 50 ml of distilled water. The chemical properties of the sand are $pH(CaCl_2)$ = 6.7; Al^{3+} = 0.0 $cmol_c$ dm^{-3}; (H+Al) = 1.51 $cmol_c$ dm^{-3}; Ca^{2+} = 0.34 $cmol_c$ dm^{-3}; Mg^{2+} = 0.16 $cmol_c$ dm^{-3}; K^+ = 0.005 $cmol_c$ dm^{-3}; $CTC_{pH=7.0}$ = 2.015 $cmol_c$ dm^{-3}.

The treatments subject to darkness were packed in plastic boxes, which were wrapped in foil and black plastic; whereas the other were maintained in constant light, using a fluorescent lamps of the photon flux density of 9.84 µmol m^{-2} s^{-1} (±0.69 of standard deviation), according to the average of 10 measurements. The treatments subject to darkness were evaluated in a dark room with the aid of green lighting, using a lamps of the intensity of quantum irradiation of 1.07 µmol m^{-2} s^{-1} (±0.13 of standard deviation), according to the average of 10 measurements, at a distance of 47 cm from the sensor and light source. Each replicate was always maintained in germinators at the 3 chosen temperatures.

The treatments were evaluated daily up to stabilization of germination, 26 days in this experiment. The seeds with a root length equal to or greater than 2 mm were considered to have germinated. The evaluated variables were the percentage of the seeds that germinated (G), the germination speed index (GSI), calculated according to Maguire's formula (1962), and the mean germination time (MGT) in days, calculated according to Lima et al. (2006). The data were subjected to analysis of variance, and the means were compared by Tukey's test.

RESULTS AND DISCUSSION

The three-way interaction among the temperature, substrate and luminosity factors was statistically significant for each of the analyzed variables (Table 1). In

Table 1. Germination (G), germination speed index (GSI) and mean germination time (MGT) of *E. oxypetalum* seeds subjected to different temperature, substrate and luminosity conditions.

Source of variation	Variable		
	G (%)	GSI	MGT (d)
Temperature (T)			
20°C	99.25[a]	016.65[a]	007.01[c]
25°C	91.37[b]	013.72[b]	008.52[b]
30°C	79.75[c]	011.87[c]	012.29[a]
Substrate (S)			
Sand	92.83[a]	015.47[a]	008.68[b]
Paper	87.42[b]	012.69[b]	009.87[a]
Luminosity conditions (L)			
Light	98.50[a]	020.72[a]	005.05[b]
Dark	81.75[b]	007.44[b]	013.50[a]
F Value			
Temperature (T)	35.37[*]a	0073.06[*]ai	0522.31[*]ai
Substrate (S)	18.09[*]a	0073.35[*]ai	0075.79[*]ai
Luminosity conditions (L)	77.35[*]a	1668.27[*]ai	3788.15[*]ai
T*S	012.44[ns]a	0000.70[nsii]	0003.47[*]ai
T*L	28.30[*]a	0031.21[*]ai	0420.23[*]ai
S*L	15.16[*]a	0003.23[nsii]	0001.90[ns]a
T*S*L	012.74[**]ai	0007.12[*]ai	021.60[*]i
CV (%)	07.32ai	008.00ai	005.13ii

[ns] Not significant, * Significant at $p < 0.05$, ** Significant at $p < 0.07$.

Tables 2, 3 and 4, the interaction of the various factors (temperature, substrate and luminosity) is shown for G. The effect of the temperature factor on G is shown in Table 2 at each level of the substrate and luminosity factors. For the sand substrate, the 20 and 25°C treatments produced better G than the 30°C treatment in the dark, but no significant difference occurred among the temperature treatments in the light. For the paper substrate, the best and worst G occurred in darkness at 20 and 30°C, respectively, but no differences occurred in the light. For Rojas-Aréchiga and Vázquez-Yanes (2000), the optimum temperature interval to get over 75% germination for some cactus species is between 15 and 35°C, but 20°C provide appropriate conditions for germination rate in a wide range of genera. However, the optimum temperature for most cactus seed germination is 25°C (Rojas-Aréchiga and Vázquez-Yanes, 2000; Nobel, 2003). Other studies show that both 20 and 25°C are favorable for certain species, since the better performance was observed at 25°C in two cactaceous species, *Melocactus bahiensis* (Britton and Rose) and *Schlumbergera truncate* (Haw.) Moran (Lone et al., 2007, 2010). By contrast, Almeida (2008) evaluated the germination of *Cereus fernambucensis* Lem., other cactaceous species, and found that 20°C provided the best germination, result that agrees with those obtained in the present study (Table 2).

The substrate factor effect at each level of the luminosity and temperature factors is shown in Table 3. The paper substrate showed higher germination in the light at 30°C, with no significant difference between substrates at the other temperatures. In the dark, the sand substrate showed higher germination when subjected to 25 and 30°C, but there was no difference at 20°C. Lone et al. (2007), who examined the seeds of *M. bahiensis*, observed greater germination using sand as the substrate. According to Cavalcanti and Resende (2007), sand is the standard substrate for the germination of several species of cacti. However, Andrade et al. (2008), who worked with the seed germination of the cactus *Hylocereus undatus* (Haworth) Britton and Rose on different substrates, obtained better results with paper than with sand.

Varela et al. (2005), who studied the influence of temperature (20, 25, 30 and 35°C) and substrate (sand, paper and vermiculite) on the seeds of *Acosmium nitens* (Vog) Yakovlev, found greater germination for the sand substrate at temperatures of 20, 25 and 30°C.

In Table 4, the luminosity factor effects on G are shown at each level of the substrate and temperature factors. In

Table 2. Temperature factor effect on the percentage of *E. oxypetalum* seeds germinating (G) at each level of the substrate and luminosity factors.

Temperature	Sand		Paper	
	Light	Dark	Light	Dark
20°C	98.5[a]	100.0[a]	100.0[a]	98.5[a]
25°C	98.0[a]	195.0[a]	100.0[a]	72.5[b]
30°C	96.0[a]	169.5[b]	198.5[a]	55.0[c]

Means within columns followed by the same letter do not differ by Tukey's test at $p < 0.07$.

Table 3. Substrate factor effect on the percentage of *E. oxypetalum* seeds germinating (G) at each level of the luminosity and temperature factors.

Substrate	Light			Dark		
	20°C	25°C	30°C	20°C	25°C	30°C
Sand	198.5[a]	198.0[a]	96.0[b]	100.0[a]	95.0[a]	69.5[a]
Paper	100.0[a]	100.0[a]	98.5[a]	198.5[a]	72.5[b]	55.0[b]

Means within columns followed by the same letter do not differ by Tukey's test at $p < 0.07$.

Table 4. Luminosity factor effects on the percentage of *E. oxypetalum* seeds germinating (G) at each level of the substrate and temperature factors.

Luminosity	Sand			Paper		
	20°C	25°C	30°C	20°C	25°C	30°C
Light	198.5[a]	98.0[a]	96.0[a]	100.0[a]	100.0[a]	98.5[a]
Dark	100.0[a]	95.0[a]	69.5[b]	198.5[a]	172.5[b]	55.0[b]

Means within columns followed by the same letter do not differ by Tukey's test at $p < 0.07$.

the sand, G was greater in the light than in the dark for seeds held at 30°C, but there were no luminosity effects at the lower temperatures. However, light improved G on the paper at both 25 and 30°C compared with darkness, with no significant luminosity effect on the paper at 20°C. Previous studies with *E. phyllanthus* (L.) Haworth seeds also demonstrated their sensitivity to light, which promotes their germination (Simão et al., 2010).

A total germination was observed both in the presence and absence of light. Similar results were obtained by Cotá-Sanchez and Abreu (2007), who obtained values greater than 90% in studies on the germination of *E. phyllanthus*. Thus, it is possible to achieve high germination percentages by adjusting the temperature, substrate and luminosity according to the needs of each species. Light has been recognized as necessary for the germination of many species and is considered by some authors to be a factor overcoming seed dormancy (Bewley and Black, 1994; Baskin and Baskin, 2001). Positive photoblastism is a mechanism for the preservation of species that prevents germination at great depths, where seedling emergence would not succeed because of limited nutritional reserves (Bewley and Black, 1994). The seeds of *E. oxypetalum* were able to reach the peak of germination both in the dark and the light; however, because of its lower percentage of germination in darkness, the species displays a preferential photoblastism (Table 4).

In Tables 5, 6 and 7, the interaction of the various factors (temperature, substrate and luminosity) is shown for the GSI. The analysis of the effect of temperature at each level of the luminosity and substrate factors is presented in Table 5. The highest GSI occurred at 20°C for the seeds germinated on the paper substrate, both in the light and in darkness, but only in darkness in the sand substrate. However, in the sand substrate, both 20 and 30°C in the presence of light provided higher GSIs.

According to Bewley and Black (1994); Carvalho and Nakagawa (2012), the higher the temperature (up to a certain limit), the faster and more efficient the germination process. Lone et al. (2007) obtained a higher GSI at 25°C for *M. bahiensis*, and Almeida (2008), evaluating the influence of temperature and luminosity on the germination of *Himatanthus drasticus* (Mart.) Plumel

Table 5. Temperature factor effect on the germination speed index (GSI) of *E. oxypetalum* seeds at each level of the substrate and luminosity factors.

Temperature	Sand		Paper	
	Light	Dark	Light	Dark
20°C	23.49[a]	12.05[a]	21.16[a]	9.88[a]
25°C	20.69[b]	19.73[b]	18.31[b]	6.14[b]
30°C	23.02[a]	13.84[c]	17.63[b]	3.00[c]

Means within columns followed by the same letter do not differ by Tukey's test at $p < 0.05$.

Table 6. Substrate factor effects on the germination speed index (GSI) of *E. oxypetalum* seeds at each level of the luminosity and temperature factors.

Substrate	Light			Dark		
	20°C	25°C	30°C	20°C	25°C	30°C
Sand	23.49[a]	20.69[a]	23.02[a]	12.05[a]	9.73[a]	3.84[a]
Paper	21.16[b]	18.31[b]	17.63[b]	19.88[b]	6.14[b]	3.00[a]

Means within columns followed by the same letter do not differ by Tukey's test at $p < 0.05$.

Table 7. Luminosity factor effects on the germination speed index (GSI) of *E. oxypetalum* seeds at each level of the substrate and temperature factors.

Luminosity	Sand			Paper		
	20°C	25°C	30°C	20°C	25°C	30°C
Light	23.49[a]	20.69[a]	23.02[a]	21.16[a]	16.31[a]	17.63[a]
Dark	12.05[b]	29.73[b]	23.84[b]	29.88[b]	16.14[b]	13.00[b]

Means followed within columns by the same letter do not differ by Tukey's test at $p < 0.05$.

seeds, observed that 25 and 30°C in darkness promoted a higher GSI, which differed from the results obtained for the species under study here (Table 5). Temperature variations affect the percentage, speed and uniformity of germination. Therefore, the optimum temperature is that which allows the most efficient combination of the percentage and speed of germination (Marcos Filho, 2005), which was 20°C in the present study.

The analysis of the substrate factor at each level of luminosity and temperature is shown in Table 6. The sand substrate showed a higher GSI than the paper at the evaluated temperatures both in the presence and absence of light, but the difference was not statistically significant at 30°C in darkness. Iossi et al (2003), evaluating the effect of substrate and temperatures in the germination of *Phoenix roebelenii* O'Brien seeds, had a higher GSI at 30°C using sphagnum or sand.

In Table 7, the luminosity factor effects on the GSI are shown at each level of the substrate and temperature factors. Light produced a higher GSI than darkness regardless of the evaluated temperature and substrate. For *Pimpinella anisum* L. seeds, both in the light and in darkness, the highest GSIs occurred at temperatures of 20 and 25°C (Stefanello, 2005).

In Tables 8, 9 and 10, the interaction of the various factors (temperature, substrate and luminosity) is shown for the MGT. Table 8 presents the analysis of the temperature factor effects at each level of the substrate and luminosity factors. The 20°C treatment produced a shorter MGT under the evaluated luminosity and substrate conditions, although the difference was not statistically significant in the sand substrate in light.

While evaluating the influence of temperature, luminosity and substrate on the germination of *Caesalpinia echinata* Lam. seeds, Mello and Barbedo (2007) observed that the MGT tended to decrease in both light and darkness from 15 to 35°C; thus, the seeds proved indifferent to luminosity for germination. According to Almeida (2008) and Lone et al. (2007), 25°C for *C. fernambucensis* and 30°C for *M. bahiensis* caused the fastest germination.

The analysis of the substrate factor effects at each level of the luminosity and temperature factors is shown in Table 9. The sand substrate provided the shortest

Table 8. Temperature factor effects on the mean germination time (MGT) of *E. oxypetalum* seeds at each level of the substrate and luminosity factors.

Temperature	Sand		Paper	
	Light	Dark	Light	Dark
20°C	4.33[a]	18.51[c]	4.80[b]	10.40[c]
25°C	4.93[a]	10.46[b]	5.64[a]	13.05[b]
30°C	4.36[a]	19.46[a]	6.20[a]	19.14[a]

Means within columns followed by the same letter do not differ by Tukey's test at $p < 0.05$.

Table 9. Substrate factor effects on the mean germination time (MGT) of *E. oxypetalum* seeds at each level of the luminosity and temperature factors.

Substrate	Light			Dark		
	20°C	25°C	30°C	20°C	25°C	30°C
Sand	4.33[a]	4.93[b]	4.36[b]	18.51[b]	10.46[b]	19.46[a]
Paper	4.80[a]	5.64[a]	6.20[a]	10.40[a]	13.05[a]	19.14[a]

Means within columns followed by the same letter do not differ by Tukey's test at $p < 0.05$.

Table 10. Luminosity factor effects on the mean germination time (MGT) of *E. oxypetalum* seeds at each level of the substrate and temperature factors.

Luminosity	Sand			Paper		
	20°C	25°C	30°C	20°C	25°C	30°C
Light	4.33[b]	14.93[b]	14.36[b]	14.80[b]	15.64[b]	16.20[b]
Dark	8.51[a]	10.46[a]	19.46[a]	10.40[a]	13.05[a]	19.14[a]

Means within columns followed by the same letter do not differ by Tukey's test at $p < 0.05$.

MGT in the presence of light at temperatures of 25 and 30°C, but did not differ from the paper substrate at 20°C. In darkness at 20 and 25°C, the sand substrate provided better performance than the paper, without differing at 30°C.

In Table 10, the luminosity factor effects are shown at each level of the substrate and temperature factors. Germination in light provided the shortest MGT for the evaluated temperatures and substrates. However, Amaro et al. (2006) observed that 25 and 30°C in darkness reduced the MGT in *H. drasticus*.

Studies such as this verify the importance of determine the temperature, substrate and luminosity factors and provide an account of the influence of these factors on the percentage, speed and uniformity of seed germination. So, the results indicated that the germination of *E. oxypetalum* seeds is favored by 20°C, a sand substrate and the presence of light; however, the species may be considered as preferentially photoblastic because of its ability to germinate in darkness.

Conclusion

The temperature of 20°C combined with the sand substrate and the presence of light provided optimal conditions for the germination of *E. oxypetalum* seeds measured by a higher GSI and a shorter MGT.

Conflict of Interest

The authors have not declared any conflict of interest.

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Effect of organic manure and nitrogen on growth yield and quality of kinnow mandarin in sandy soils of hot arid region

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The findings of present investigation revealed that the application of 80 kg farm yard manure (FYM) per plant significantly increased trunk diameter (9.47%), fruit yield (25.22 kg/tree), number of fruits (212.75 fruits/tree), average fruit weight (118.22 g), fruit diameter (5.96 cm), fruit length (5.58 cm), volume of fruit (129.71 cc), peel weight (32.95 g), weight of sacs (85.27 g), juice percentage (48.30%), TSS (12.11 °B), ascorbic acid (26.37 mg/100 g edible portion), total sugar (6.63%), reducing sugar (2.92%), non-reducing sugar (3.71%), juice acidity (0.79%) (significantly minimum), phosphorus soil at 15 to 30 cm depth (12.50%), soil potassium at 0 to 15, 15 to 30 and 30 to 60 cm depths (0.74, 2.27 and 0.75%, respectively), leaf nitrogen (28.17%), leaf potassium (6.28%), leaf zinc (27.88%), leaf iron (5.47%) and minimum 29.92 days to 75% flowering, 52.58 days to fruit set at initial stage and 6.33% fruit drop at maturity. Whereas, application of FYM 60 kg per plant gave maximum B:C ratio (2.30) and net return (38472.31 Rs/ha). The application of 750 g nitrogen per plant gave significant maximum trunk diameter (8.99%), average weight (118.19 g), diameter (6.06 cm) and length of fruit (5.53 cm), peel weight (33.38 g), weight of sacs (84.80 g), volume of fruit (132.31 cc), titrable acidity (0.83%), leaf nitrogen (25.25%), leaf zinc (24.37%), leaf iron (3.46%) and minimum 55.60 days to fruit set at initial stage and 6.79% fruit drop at maturity. While the application of 500 g nitrogen per plant increased number of fruits (204.20 per plant), yield (23.19 kg per plant, TSS (11.37 °B), ascorbic acid (25.66 mg/100 g edible portion), total sugar (6.30%), reducing sugar (2.83%), non-reducing sugar (3.48%), B:C ratio (2.55), net return (39212.93 Rs./ha) and minimum 31.33 days to 75% flowering. The combined application of 80 kg FYM and 750 g nitrogen per plant led to significant increase in plant height (15.20%), spread (N-S, 18.03%; E-W, 18.99%), canopy volume (81.81%), soil nitrogen at different depths (0 to 15 cm, 41.78%; 15 to 30 cm, 51.36% and 30 to 60 cm, 27.71%) over initial level.

Key words: Farm yard manure (FYM), nitrogen, kinnow mandarin, soil and leaf analysis, hot arid region, economical treatments, sandy soils, fruit yield.

INTRODUCTION

Citrus is the leading fruit crop of the world. It belongs to the family Rutaceae and sub-family Aurantoideace. A large number of citrus species are widely grown in India. Kinnow is an economical important subtropical fruit grown

almost all over the arid and semi-arid regions of India, where irrigation facilities are available. Among fruit crops, Kinnow mandarin is an important crop of hot arid region of Rajasthan. In Rajasthan, total area under fruit crops is 46.5 thousand ha with production of 716.8 thousand MT. For kinnow cultivation, Sriganganagar District is on prime position with 15.2 000 ha area and 27.2 000 MT production followed by Hanumangarh and Bikaner districts of North-west Rajasthan (Anonymous, 2013).

Nowadays, its production as well as quality is deteriorating day by day because farmers do not know the nutritional value of fruit orchards; and also because the soil of hot arid regions is not fertile; it has low carbon and nitrogen contents, which are essential for growth and development of plants. To increase the fruit production in terms of quantity and quality, farmers have to meet the international standards in order to compete in the global market.

Farm yard manure (FYM) provides essential nutrients along with organic matter to the plant rhizosphere. FYM also enhances soil porosity and water holding capacity of the soil. It also limits the losses caused by leaching and maintains balanced nutrient status of the soil. However, nitrogen is the most important essential plant nutrient which plays a great role in increasing vegetative growth and fruit production of the plant. Nitrogen causes early vigorous vegetative growth and green colour, which triggers the physiological activities of the plant. Sharma and Chopra (2000) observed that nitrogen played an important role in increasing the growth and yield of sweet orange.

Thus, there is ample scope for increasing the growth and production parameters by using FYM and nitrogen, especially by standardizing the economic doses. Hence, this work was carried out to study the effect of FYM and nitrogen levels on growth, yield and quality of Kinnow mandarin in sandy soils of hot arid region.

MATERIALS AND METHODS

The experiment was carried out at the Research Farm, Central Institute for Arid Horticulture, Bikaner and Laboratory, Department of Horticulture, College of Agriculture, SKRAU-Bikaner (Rajasthan) from February to December 2008. Seven years old uniform and healthy Kinnow trees were used; they were spaced at 6 m apart. There were 20 treatment combinations consisting of five levels of FYM (0, 20, 40, 60 and 80 kg per plant) and four levels of nitrogen (0, 250, 500 and 750 g per plant). This was done to find out their effect on soil and plant nutrient and to standardize the best economical dose. The experiment was laid out in factorial randomized block design with three replications. Full dose of FYM and half dose of nitrogen were applied in the second week of February through basal dose and the remaining half dose of nitrogen was applied in the second week of July in 2008. The nitrogen was applied though urea. Urea contains 46% nitrogen, whereas FYM contains 0.50% nitrogen, 0.25% phosphorus and 0.50% potassium. The orchard soil contains 86.41, 22.91 and 234.00 kg/ha nitrogen, phosphorus and potassium, respectively. The uniform doses of phosphorus (250 g/plant), potassium (100 g/plant) and zinc (25 g/plant) were applied through basal dose per plant.

Growth parameters in terms of plant height, spread (N-S and E-W), trunk diameter, canopy volume, soil nutrient status related to nitrogen, phosphorus and potassium at three consecutive depths (0 to 15, 15 to 30 and 30 to 60 cm) and analysis of leaves in relation to nitrogen, phosphorus, potassium, zinc and Iron of Kinnow plants were measured two times. That is, before imposing treatment (February 2008) and after completion of experiment (December 2008). The percent increase in last observation was calculated on the basis of initial observation. Finally, results are presented as percent increase over the initial one. Yield parameter in terms of fruit yield was noted at various intervals of harvesting. Number of fruits was counted in November and it was again confirmed at the time of harvesting (end of December). Average weight, diameter, length of fruit, peel weight, weight of sacs, number of seeds, juice percentage and volume of fruit were recorded by taking five representative fruits from each tree and were averaged. Growth and development parameter of fruits like percentage of fruit drop at maturity was counted and divided by total number of fruits and then multiplied by 100. The dates of fruit set at initial stage and flowering were recorded by visual observation. They were expressed as number of days required to attain the particular stage on the day the treatment was applied, that is, 8th February, 2008.

Qualitative attributes like TSS was determined with a hand refractometer. Ascorbic acid was estimated by titration method. Acidity was estimated according to the method suggested by official and tentative methods of analysis. Total sugar was estimated by colorimetric method. Reducing sugar was measured by aresenomolybdate reagent colour development method. Non-reducing sugar was calculated by reducing sugar from total sugar.

The available soil nitrogen was determined by Alkaline Potassium Permanganate Method. Available soil phosphorous was extracted using 0.5M $NaHCO_3$ solution and thereafter determined by Dickmon and Bray method. Available soil and leaves potassium was determined by Flame photometer. Estimation of leaf nitrogen was done with wet colorimetric method. Phosphorus in plant samples was analyzed with wet digestion of triacid mixture using Vanado molybdo-phosphric yellow colour method on spectrophotometer. Estimation of iron and zinc in plant was determined by Atomic Absorption Spectrophotometer.

In order to evaluate the economic feasibility of the treatments, net returns and B:C ratio were worked out on the basis of prevailing market prices so that most remunerative treatment could be recommended.

Climate and weather conditions

Bikaner District extends from 27°15' to 29.5° north latitudes and 71°54' to 74°12' east longitudes. Bikaner has arid climate with an annual average rainfall of about 260 mm. More than 80% rainfall is received during South-west monsoon season. During summer, the maximum temperature is as high as 48°C, while in the winter it falls to 0°C and sometimes sub-zero. This region is prone to high wind velocity and soil erosion. Soil drifting due to high speed winds leads to soil erosion. This is the major problem in summer. The weekly mean weather parameters for the period of the experimentation

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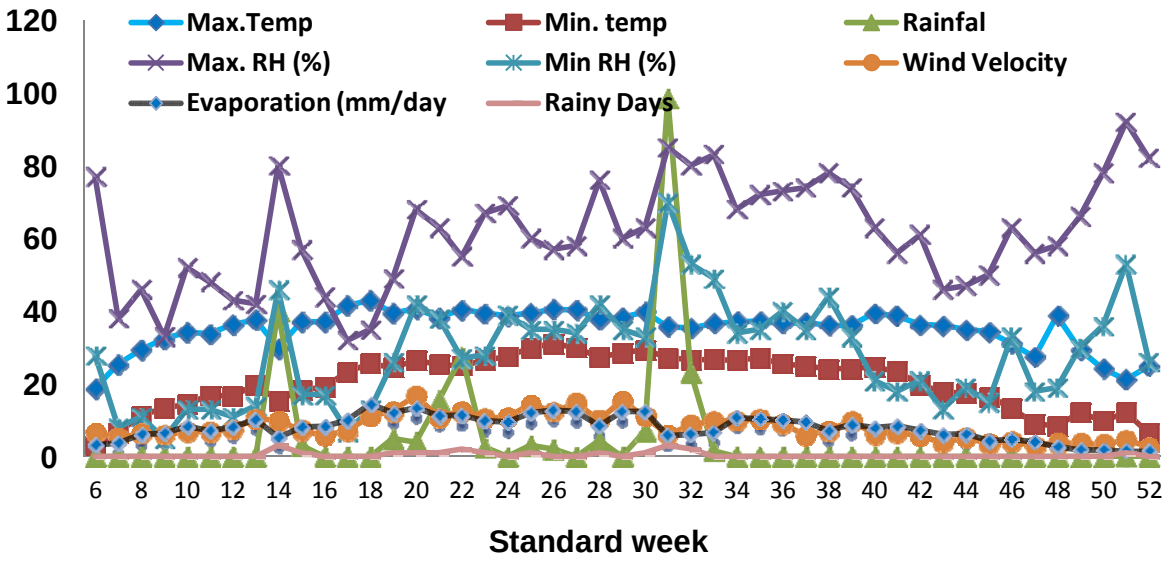

Figure 1. Mean weekly meteorological data recorded during the experiment.

were recorded from the Meteorological Observatory of Agricultural Research Station, Beechwal, Bikaner (Figure 1).

RESULTS AND DISCUSSION

Growth parameters

The data in Tables 1 and 2 show that application of 80 kg FYM per plant led to significant percentage increase in plant height (12.48%), plant spread (North-South, 13.60%; East-west; 14.71%), canopy volume (58.57%) and trunk diameter (9.47%). This might be due to improved nutritional status and physical properties of the soil caused by the addition of FYM. This made the plant to uptake water and mineral nutrients better, resulting in its increased growth rate. Similarly, significant growth of Kinnow mandarin by the application of FYM has been earlier reported by Dudi et al. (2003).

The data on the effect of nitrogen revealed that the application of 750 g nitrogen per plant led to significant percent increase in plant height (13.05%), plant spread (North-South, 13.54%; East-West, 14.67 %), canopy volume (57.16%) and trunk diameter (8.99%). Application of nitrogen resulted in vigorous vegetative growth of the plant and gave the dark green colour of the foliage. This favoured the photosynthetic activity of the plants and greater synthesis of carbohydrate, which led to the formation of amino acids, nucleo-proteins, chlorophyll, alkaloids and amides. These complex compounds are responsible for building up of new tissues and are associated with a number of metabolic processes, which in turn favour better development of plants. The increase in growth as a result of nitrogen application is obvious. Similarly, increase in vegetative growth of fruit plants by the application of nitrogen has also been reported earlier by Kaul and Bhatnagar (2006) in Kinnow mandarin and Dhomane et al. (2011) in guava.

The combined application of 80 kg FYM and 750 g nitrogen per plant significantly led to maximum percentage increase over the initial one. It is seen in plant height (15.20%), plant spread (North-South, 18.03%; East-West, 18.99%) and canopy volume (81.81%). This might be due to the fact that combined application of nitrogen and FYM enhances leaf expansion and its dark green colour, which favours photosynthesis and respiration; hence, growth is enhanced by application of nitrogen and balanced nutrition provided by FYM. FYM improves physico-chemical properties of soil, which provides better conditions for plant growth and development. The findings are in line with the results obtained in Kinnow (Dudi et al., 2003; Kaul and Bhatanagar, 2006). Data also revealed that plant spread was more in E-W direction compared to N-S direction. This might be due to the fact that the foliage receives sunlight directly.

Yield characters

The data presented in Table 2 revealed that fruit yield (25.22 kg/tree), number of fruits (212.75 fruits/tree), average weight (118.22 g), diameter (5.96 cm), length of fruit (5.58 cm), volume of fruit (129.71 cc), peel weight (32.95 g), weight of sacs (85.27 g) and juice percentage (48.83%) significantly increased in plants receiving 80 Kg FYM followed by 60 kg FYM. This may be due to increased vegetative and reproductive growth of plant and better nutrient supply as a result of the application of FYM. It does not only add organic matter and macro and

Table 1. Effect of FYM and nitrogen levels on per cent increase in plant height, plant spread (North-South) and (East- West) and canopy volume of Kinnow mandarin.

Nitrogen (g/plant)	Percent increase in plant height						Percent increase in plant spread (N-S)					
	FYM (kg/plant)						FYM (kg/plant)					
	0	20	40	60	80	Mean	0	20	40	60	80	Mean
0	3.08	3.71	5.33	8.07	9.45	5.93	3.05	4.18	5.06	7.22	7.91	5.49
250	6.04	8.44	8.54	10.35	11.67	9.01	5.6	7.50	9.09	11.24	12.38	9.16
500	8.84	10.07	9.74	11.42	13.61	10.74	8.98	10.68	10.75	12.46	16.08	11.79
750	9.59	11.31	14.02	15.14	15.2	13.05	9.91	11.43	13.26	15.07	18.03	13.54
Mean	6.89	8.38	9.41	11.25	12.48		6.89	8.45	9.54	11.5	13.6	
	Nitrogen		FYM		Nitrogen x FYM		Nitrogen		FYM		Nitrogen x FYM	
S.Em. ±	0.07		0.08		0.16		0.17		0.19		0.39	
CD at 5%	0.21		0.23		0.46		0.50		0.55		1.11	

Nitrogen (g/plant)	Percent increase in Plant spread (E- W)						Percent increase in canopy volume					
	FYM (kg/plant)						FYM (kg/plant)					
	0	20	40	60	80	Mean	0	20	40	60	80	Mean
0	3.38	4.46	5.80	6.67	6.75	5.41	10.25	14.08	18.11	23.82	25.31	18.31
250	6.12	8.18	10.45	15.16	15.16	10.52	19.72	27.49	35.55	45.39	54.84	36.60
500	8.85	11.73	12.78	17.94	17.94	12.98	31.78	41.84	44.79	54.03	72.34	48.96
750	10.88	12.95	14.80	18.9	18.99	14.67	38.20	46.53	55.98	63.29	81.81	57.16
Mean	7.31	9.33	10.96	12.16	14.71		24.99	32.49	38.61	46.63	58.57	
	Nitrogen		FYM		Nitrogen x FYM		Nitrogen		FYM		Nitrogen x FYM	
S.Em. ±	0.11		0.12		0.24		0.56		0.63		1.26	
CD at 5%	0.31		0.35		0.70		1.62		1.81		3.62	

micro nutrients to soil, but also improves the physico-chemical properties of soil; and hence provides better conditions for plants' growth and development (Dudi et al., 2004). Similar results were earlier obtained by Hiwale et al. (2010) in sapota and Kashyap et al. (2012) in pomegranate. Average number of seeds per fruit was found statistically non – significant in various combined treatments of FYM and nitrogen. It is evident from the data in Table 2 that significant maximum fruit yield (23.19 kg/tree), number of fruits (204.20 fruits/tree) and juice percentage (48.83%) were observed in plants receiving 500 g nitrogen. Whereas, other characters such as average weight (118.19 g), diameter (6.06 cm), length (5.53 cm) volume (132.31 cc), peel weight (33.38 g) and weight of sacs (84.80 g) of fruit increased significantly by the application of 750 g nitrogen per plant. It is due to the fact that increases in nitrogen doses at 750 g per plant causes excessive and imbalance vegetative growth. This is caused by the diversion of reserved food material to vegetative growth instead of reproductive growth (Dudi et al., 2004 in kinnow). It is in accordance with the findings of Dhomane et al. (2011) in guava and Kashyap et al. (2012) in pomegranate. Nitrogen leads to increased

Table 2. Effect of FYM and nitrogen levels on trunk diameter, fruit yield, number of fruits, average weight, diameter and length, peel weight, weight of sacs, no. of seeds and volume of Kinnow mandarin fruits.

Treatment symbol	% increase in trunk diameter	Fruit yield kg plant^{-1}	No. of fruits plant^{-1}	Av. weight of fruit (g)	Diameter of fruit (cm)	Length of fruit (cm)	Peel weight of fruit (g)	weight of sacs fruit^{-1} (g)	Av. No. of seeds fruit^{-1} (g)	Juice percent	Volume of fruit (CC)
Nitrogen (g/plant)											
N_0	3.61	17.81	181.40	93.95	5.33	5.00	26.84	67.11	11.05	42.99	104.15
N_{250}	5.43	19.74	192.00	102.13	5.60	5.15	29.25	72.89	11.30	46.03	114.32
N_{500}	7.19	23.19	204.20	112.79	5.95	5.40	31.51	81.28	11.47	48.83	124.95
N_{750}	8.99	23.42	197.00	118.19	6.06	5.53	33.38	84.80	11.81	47.44	132.31
S.Em.±	0.16	0.15	0.98	0.50	0.02	0.02	0.21	0.57	0.35	0.20	0.63
CD 5%	0.47	0.43	2.82	1.43	0.07	0.06	0.61	1.62	NS	0.57	1.79
FYM (kg/plant)											
FYM_0	3.76	15.97	169.75	93.72	5.44	5.00	26.45	67.27	12.16	44.16	105.27
FYM_{20}	4.70	18.46	184.75	99.48	5.66	5.07	29.14	70.35	11.56	45.28	114.54
FYM_{40}	5.92	20.98	193.50	108.05	5.76	5.26	30.89	77.16	11.36	46.34	119.97
FYM_{60}	7.67	23.78	207.50	114.35	5.86	5.46	31.80	82.55	11.22	47.53	125.18
FYM_{80}	9.47	25.22	212.75	118.22	5.96	5.58	32.95	85.27	10.73	48.30	129.71
S.Em. ±	0.18	0.17	1.10	0.56	0.03	0.02	0.24	0.63	0.39	0.22	0.70
CD 5%	0.52	0.48	3.15	1.60	0.07	0.06	0.69	1.81	NS	0.64	2.00

average weight, length, diameter, volume, peel weight and weight of sacs of kinnow fruits. This is due to the fact that nitrogen increases the efficiency of metabolic process of the plants; and thus encourages the growth of the plant and consequently increases the size and weight of the fruit. Another probable cause could be greater mobility of nutrients to the developing fruits which act as strong metabolic sink; examples are pomegranate (Prasad and Mali, 2000), Kinnow (Dudi et al., 2004) and guava (Kashyap et al., 2012). The reduction in juice content caused by high doses of nitrogen might be due to the thickness of the peel; example is sweet orange (Sharma and Chopra, 2000). Carranca et al. (1992) reported that the production of sweet oranges and mandarins flower and fruit were high by the application of the highest level of nitrogen but the total yield was maximum by the application of medium level of nitrogen. These results are in accordance with the findings of Prasad and Mali (2000) in pomegranate, Dudi et al. (2004) in Kinnow mandarin, Kaul and Bhatnagar (2006) in kinnow, Hiwale et al. (2010) in sapota and Kashyap et al. (2012) in pomegranate.

Fruit growth and development

The data in Table 3 show that the application of 80 kg FYM per plant led to significant decrease in 29.92 days to 75% flowering, 55.58 days to fruit set at initial stage and 6.33% fruit drop at maturity, over the control. This is because FYM not only adds organic matter and macro and micro nutrients to soil, but also improves the physico–chemical properties of soil; and hence causes nutritional balance of the soil as well as the plant. Thus, the improved plant growth and development caused by nutritional balance reduces the days taken to have 75% flowering and the days taken to have fruit set at initial stage and fruit drop at maturity stage.

It is also clarified from the data in Table 3 that application of 500 g nitrogen per plant significantly

Table 3. Effect of FYM and nitrogen levels on total soluble solids (TSS), titrable acidity, ascorbic acid, total sugar, reducing sugar, non-reducing sugar, days to 75% flowering, days to fruit set at marble stage, fruit drop at maturity, B:C ratio and net returns of Kinnow mandarin.

Treatment symbol	TSS (°B)	Titrable acidity (%)	Asco. acid (mg/100 g)	Total sugar (%)	Reducing sugar (%)	Non-reducing sugar (%)	Days to 75% flowering	Days to fruit set at marble stage	Fruit drop at maturity (%)	B:C ratio	Net returns (Rs/ha)
Nitrogen (g/plant)											
N_0	9.78	0.78	23.50	5.61	2.51	3.10	33.80	58.93	7.67	2.01	24024.94
N_{250}	10.56	0.80	24.42	5.95	2.65	3.30	33.07	58.00	7.37	2.24	30387.23
N_{500}	11.37	0.81	25.66	6.30	2.83	3.48	31.33	57.07	7.03	2.55	39212.93
N_{750}	11.10	0.83	25.06	6.08	2.74	3.34	32.20	55.60	6.79	2.50	39093.07
S.Em.±	0.08	0.0047	0.17	0.09	0.07	0.07	0.24	0.26	0.14	---	413.92
CD 5%	0.23	0.013	0.49	0.25	0.20	0.21	0.69	0.75	0.41	---	1185.39
FYM (kg/plant)											
FYM_0	9.37	0.82	23.00	5.40	2.39	3.00	35.42	61.42	7.94	2.29	25079.85
FYM_{20}	10.02	0.81	23.81	5.64	2.58	3.05	33.58	59.75	7.66	2.31	29224.29
FYM_{40}	10.62	0.80	24.59	5.95	2.69	3.27	32.42	57.83	7.35	2.34	33450.57
FYM_{60}	11.40	0.80	25.51	6.32	2.83	3.49	31.67	55.42	6.81	2.39	38472.31
FYM_{80}	12.11	0.79	26.37	6.63	2.92	3.71	29.92	52.58	6.33	2.30	39670.69
S.Em. ±	0.09	0.0052	0.19	0.10	0.08	0.08	0.27	0.29	0.16	---	462.78
CD 5 %	0.26	0.015	0.55	0.28	0.23	0.23	0.77	0.84	0.46	---	1325.30

curtailed days taken to have 75% flowering (31.33 days). By increasing nitrogen levels to 750 g/plant further, the days were recorded more (32.30). This might be due to the fact that application of 750 g nitrogen per plant causes the diversion of reserved food material to vegetative growth instead of reproductive growth; it also causes nutritional imbalance for flower production. Thus, adequate dose of nitrogen may be required for flower production. Dayal et al (2006) reported that flower production could be regulated by the nitrogen status in grape. Sharma and Chopra (2000) reported that nitrogen is considered to be of prime importance in the production of leaves, flowers and fruits in Blood Red sweet orange.

However, days taken to have fruit set at initial stage and fruit drop percentage at maturity stage were minimum 55.60 and 6.79 by the application of 750 g nitrogen per plant, over previous levels. The data revealed that days taken to have fruit set at initial stage significantly decreased with increasing levels of nitrogen. This is because nitrogen as an important constituent of nucleo-protiens, amino acids and amino sugars is responsible for cell division and cell elongation. The fruit drop percentage at maturity stage significantly decreased over control by the application of 750 g nitrogen. Nitrogen applications usually enhance micronutrients uptake and utilization (Gupta, 1999). Increased nitrogen rates resulted in more absorption of water and minerals from the soil. This results in the maintenance of nutritional and water requirement of the plant which reduce the fruit drop. Singh et al. (2003) reported the increase in fruit set and decrease in fruit drop as a result of nitrogen application in sapota. This might be due to increase in auxin content. Sharma et al. (2003) reported that the increased doses of nitrogen in phalsa significantly reduced fruit drop.

Quality attributes

The data in Table 3 revealed that the application of 80 kg FYM per plant resulted in significant increase in TSS (12.11 °B), ascorbic acid (26.37 mg/100 g edible portion), total sugar (6.63%), reducing sugar (2.92%), non-reducing sugar (3.71%), but significant decrease in juice acidity (0.79%). This might be due to good nutrient

status, improved plant conditions, efficient functioning of leaf area and increased photosynthetic activity. These results are in conformity with the results obtained by Sharma et al. (2003) in pomegranate and Singh and Banik (2011) in mango.

It was further observed that significant increase in TSS (11.37 °B) was recorded by the application of 500 g nitrogen per plant. This is because adequate dose of nitrogen stimulates the functioning of number of enzymes in the physiological process which may have increased the total soluble solid content of the fruits. The highest ascorbic acid content (25.66 mg/100 g edible portion) was found significant by the application of 500 g nitrogen per plant. This might be due to the catalytic activity of several enzymes which participate in the biosynthesis of ascorbic acid and precursor. The sugars (total, reducing and non-reducing) (6.30, 2.83 and 3.48%) increased significantly by the application of 500 g nitrogen per plant.

The highest mean values for sugars with the application of nitrogen could be attributed to the involvement of nitrogen in various energy sources like amino acids and amino sugars. Application of 750 g nitrogen per plant decreased the TSS, ascorbic acid, sugars (total, reducing and non-reducing). This is because when it reaches the toxicity level, it decreases other enzymes and nutrient molecules which help in the synthesis of these quality attributes. These results are in conformity with the results obtained by Prasad and Mali (2000) in pomegranate, Kaul and Bhatanagar (2006) in Kinnow and Kashyap et al. (2012) in pomegranate.

The acidity of fruit juice (0.83%) significantly increased by the application of the highest dose of nitrogen (750 g per plant). This is due to increased synthesis and translocation of organic acids in the fruits as cited by Prasad and Mali (2000) in pomegranate. Similar findings were earlier reported by Sharma et al. (2013) in guava.

Soil analysis

An analysis was carried out on the soil's three distinct layers of nitrogen content: 0 to 15, 15 to 30 and 30 to 60 cm. Data in Table 4 revealed that nitrogen content increased significantly with increased levels of FYM. The maximum increase in nitrogen content (37.88, 46.23 and 25.44%) was found in 0 to 15, 15 to 30 and 30 to 60 cm soil depths, respectively by the application of 80 kg FYM per plant followed by 60 kg FYM per plant. But, it was minimum in control.

The data on soil nitrogen content at different soil depths showed that soil nitrogen content increased significantly with continuous increase in the levels of nitrogen. The maximum increase in nitrogen content (36.11, 46.03 and 25.73%) was found in 0 to 15, 15 to 30 and 30 to 60 cm soil depths, respectively by the application of 750 g nitrogen per plant followed by 500 g nitrogen.

The interaction between FYM and nitrogen significantly increased soil nitrogen content percentage over initial level. Maximum increase of 41.78, 51.36 and 27.71% was found in 0 to 15, 15 to 30 and 30 to 60 cm soil depths, respectively by the combined application of 750 g nitrogen and 80 kg FYM per plant followed by 750 g nitrogen and 60 kg FYM per plant treatment.

It is evident from Table 5 that percent increase in phosphorus content of soil was not significantly influenced by FYM application at 0 to 15 and 30 to 60 cm soil depths. However, a general trend of increase in phosphorus content was observed with increasing levels of FYM. At 15 to 30 cm soil depth, the phosphorus content (12.50%) was significantly increased by the application of 80 kg FYM per plant.

The data on potassium content in Table 5 showed that maximum increase in potassium (0.74, 2.27 and 0.75%) was observed at 0 to 15, 15 to 30 and 30 to 60 cm soil depths, respectively by the application of 80 kg FYM per plant, followed by 60 kg FYM. However, minimum increase was recorded in control. The data on soil phosphorus and potassium were not significantly influenced by the application of nitrogen at different soil depths (0 to 15, 15 to 30 and 30 to 60 cm).

The nitrogen content in soil at different depths increased with increasing doses of nitrogen and FYM in combination and separately due to increased concentration of supplied sources (FYM and Urea) and mobility character of nitrogen in soil. Therefore, higher percent increase of nitrogen was observed at different depths. The application of 80 kg FYM in soil significantly increased the potassium content of soil at different depths, but phosphorus content increased significantly only at 15 to 30 cm soil depth. This is because this depth has residual effect of FYM, which increases the level of phosphorus. But phosphorus content decreased at 0 to 15 and 30 to 60 cm depth compared to nitrogen and potassium content. This is because FYM has very low quantity of phosphorus (0.25%) compared to nitrogen and potassium (0.50%), respectively. The maximum increase of nitrogen, phosphorus and potassium was observed at 15 to 30 cm soil depth because FYM and urea (only for nitrogen) were applied at this depth. Therefore, residual effects of supply sources could enhance the concentration of these nutrients. Similar results were also found by Sharma et al. (2003) in pomegranate and Sharma et al. (2009) in pomegranate variety Jalore seedless.

Leaf analysis

The data presented in Table 5 showed that leaf nitrogen (28.17%), potassium (6.28%), zinc (27.88%) and iron (5.47%) significantly increased by the application of 80 kg FYM per plant against lower levels of FYM. This might be due to improved soil texture, structure and moisture level which facilitate the absorption of mineral nutrition

Table 4. Effect of FYM and nitrogen levels on percent increase in nitrogen content at different depths of Kinnow orchard soil.

Nitrogen (g/plant)	At 0-15 cm soil depth						At 15-30 cm soil depth					
	FYM (kg/plant)						FYM (kg/plant)					
	0	20	40	60	80	Mean	0	20	40	60	80	Mean
0	-1.51	26.43	28.08	30.83	33.67	23.50	-1.81	34.02	36.34	39.04	41.26	29.77
250	27.62	28.67	31.71	33.80	36.68	31.70	36.49	36.34	39.05	42.77	44.56	39.84
500	28.79	31.16	34.09	36.08	39.41	33.90	38.83	38.75	42.59	46.90	47.73	42.96
750	29.38	33.30	36.79	39.29	41.78	36.11	41.08	41.17	45.96	50.57	51.36	46.03
Mean	21.07	29.89	32.67	35.00	37.88		28.65	37.57	40.99	44.82	46.23	

	Nitrogen	FYM	Nitrogen x FYM	Nitrogen	FYM	Nitrogen x FYM
S.Em. ±	0.39	0.43	0.87	0.35	0.39	0.78
CD at 5%	1.11	1.25	2.49	1.00	1.12	2.24

Nitrogen (g/plant)	At 30-60 cm soil depth					
	FYM (kg/plant)					
	0	20	40	60	80	Mean
0	1.98	20.49	21.36	22.15	23.28	17.85
250	21.49	22.04	22.56	23.63	24.67	22.88
500	22.58	23.60	24.03	25.03	26.08	24.26
750	23.91	25.02	25.43	26.56	27.71	25.73
Mean	17.49	22.78	23.35	24.34	25.44	

	Nitrogen	FYM	Nitrogen x FYM
S.Em. ±	0.25	0.28	0.56
CD at 5%	0.71	0.80	1.60

from the soil. Secondly, farm yard manure being a good source of all nutrients certainly improves nutrient contents.

The phosphorus content of leaves was found non- significant with the application of FYM. This is due to the low content of FYM in the soil and its immobility. Similar results were obtained by Sharma et al. (2003) in pomegranate.

It is also evident from the data that the leaf nitrogen (25.25%), zinc (24.37%) and iron (3.46%) increased significantly by the application of 750 g nitrogen per plant, against that of control. The leaf nitrogen increased with urea application due to nitrogenous fertilizer. Zinc and iron contents increased with nitrogen application. This is because nitrogen application usually enhances micronutrients uptake and utilization (Gupta, 1999); secondly, increased nitrogen rates resulted in more absorption of water and minerals from the soil, which enhanced the zinc and iron contents in leaves.

These results are in accordance with the findings of Singh et al. (2003) in sapota. Phosphorus and potassium content of leaves decreased with increasing levels of nitrogen. Similar results were earlier found by Intrigliolo and Intelisano (1997) in lemon tree.

Table 5. Effect of FYM and nitrogen levels on percent increase in soil phosphorus and potassium content at different depths, leaves nitrogen, phosphorus and potassium contents Kinnow mandarin orchard.

Treatment symbol	Phosphorus at different soil depths (cm)			Potassium at different soil depths (cm)			Percent increase in leaves contents				
	0-15	15-30	30-60	0-15	15-30	30-60	Nitrogen	Phosphorus	Potassium	Zinc	Iron
Nitrogen (g/plant)											
N_0	3.26	10.36	2.18	0.54	1.80	0.55	9.06	20.24	5.14	23.50	2.47
N_{250}	3.29	10.27	2.20	0.53	1.80	0.54	14.34	19.73	4.81	23.79	2.76
N_{500}	3.31	10.25	2.22	0.53	1.79	0.54	19.73	19.19	4.61	24.09	3.11
N_{750}	3.32	10.41	2.20	0.53	1.80	0.55	25.25	18.66	4.30	24.37	3.46
S.Em.±	0.15	0.13	0.07	0.0007	0.0018	0.0010	0.55	0.41	0.21	0.05	0.02
CD 5%	NS	NS	NS	NS	NS	NS	1.58	NS	NS	0.16	0.06
FYM (kg/plant)											
FYM_0	2.93	7.34	2.04	0.28	1.32	0.34	6.27	18.95	2.92	19.67	0.30
FYM_{20}	3.16	9.31	2.12	0.48	1.58	0.47	11.70	19.21	4.12	22.02	1.31
FYM_{40}	3.30	10.61	2.21	0.53	1.78	0.53	16.57	19.50	4.83	24.03	3.01
FYM_{60}	3.53	11.46	2.29	0.63	2.03	0.65	22.77	19.74	5.42	26.08	4.37
FYM_{80}	3.56	12.50	2.34	0.74	2.27	0.75	28.17	19.91	6.28	27.88	5.74
S.Em. ±	0.17	0.15	0.08	0.0008	0.0020	0.0011	0.62	0.46	0.24	0.06	0.02
CD 5 %	NS	0.43	NS	0.0023	0.0058	0.0032	1.76	NS	0.68	0.17	0.06

Economics

The data on benefit- cost ratio (Table 3) revealed that maximum B : C ratio of 2.39 and 2.55 was recorded in the plants receiving 60 kg FYM and 500 g nitrogen per plant followed by lower levels. The data on net returns (Table 3 and Figure 2) show that significant maximum net returns of 39670.69 and 39212.93 Rs./ha were recorded in the plants receiving 80 kg FYM and 500 g nitrogen per plant; and it was also found at par with the application of FYM. That is, 60 Kg per plant (38472.31 Rs./ha) over lower levels of FYM. The minimum net return was recorded in untreated plants.

The highest B : C ratio and net returns were obtained with the application of 500 g nitrogen per plant. Whereas in FYM application, the maximum B : C ratio was recorded in the application of 60 kg FYM; maximum net returns were found in the application of 80 kg FYM per plant. The increase in net returns from 60 to 80 kg FYM was not significant; therefore, application of 60 kg FYM per plant is the best economical dose. Similar economical returns have been reported by Luhach et al. (2007) in mango and Luhach et al. (2007) in guava.

Conclusion

It can be concluded that application of 60 kg FYM and 500 g nitrogen per plant is the best doses among all the treatment combinations for Kinnow mandarin fruit crop. Hence, these doses of FYM and nitrogen are recommended particularly in sandy soils of hot arid region in North – west Rajasthan. However, these results are only indicative and require further experimentation to

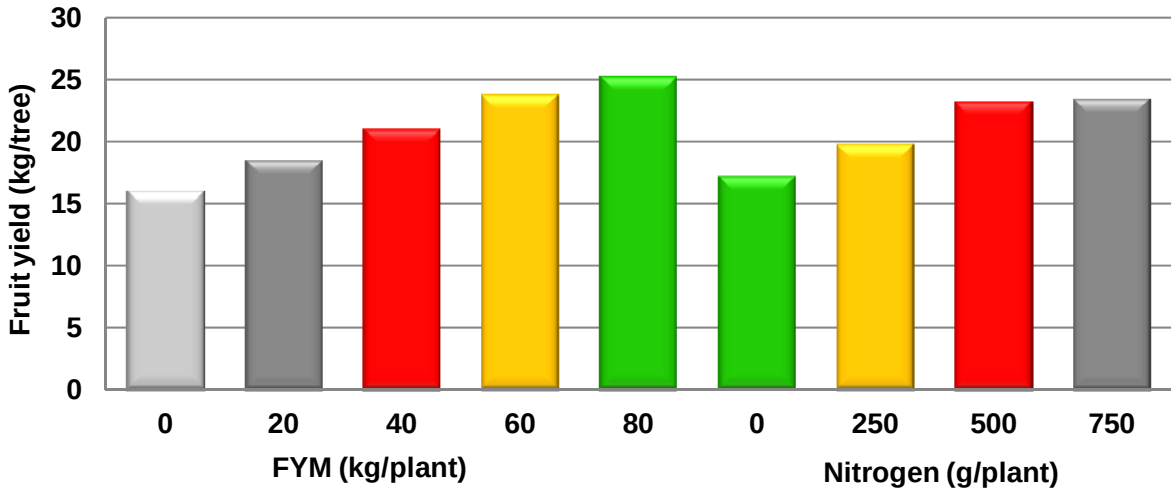

Figure 2. Effect of FYM and Nitrogen levels on fruit yield of Kinnow mandarin.

arrive at a final conclusion.

Conflict of Interest

The authors have not declared any conflict of interest.

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Effect of rates and forms of nitrogen splitting on corn in the Brazilian Cerrado of Piauí State

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Zea mays **crop has been standing out in the Brazilian Cerrado of Piauí State, located in the Northeastern Brazil. However, despite the high technology level utilized, yield levels are still below the expectation, mainly due to the mismanagement of nitrogen (N). This experiment was conducted in field using single-cross hybrid 30F53 from Pioneer®. The experiment was conducted as completely randomized blocks design with nine treatments and four replications. The treatments consisted of five rates of N as urea, equivalent to 0, 60, 120, 180 and 240 kg N ha^{-1}. It was applied twenty kg N ha^{-1} at sowing and the rest as topdressing. The splits comprised the application of 50% at the four expanded leaf stage (V1) + 50% at the eight expanded leaf stage (V2) or total rates at four expanded leaf stage. Leaf N content, leaf relative chlorophyll content, plant height, ear height, stem diameter, yield, grains per ear and rows per ear, grains per row, 1000 grains weight and agronomic efficiency index were measured. Nitrogen significantly increased agronomic characteristics of corn and the highest grain yield was obtained with 190.65 kg ha^{-1} N. Plant height, relative chlorophyll content, leaf N content, plant biomass, stem diameter, thousand seed weight, yield and agronomic efficiency index were positively influenced by the rates of nitrogen. The split of topdressing in twice was not suitable in the dystrophic Yellow Latosol of the Piauí Cerrado. Plant height, relative chlorophyll content, ear insertion height, stem diameter, grains per ear and rows per ear, grains per row and agronomic efficiency index positively correlated with grain yield.**

Key words: Agronomic efficiency index, yield, relative chlorophyll content.

INTRODUCTION

The Brazilian Cerrado of Piauí has been standing out in the national scenario with an extensive acreage favorable to grain Yuction, in particular for the corn and *Glycine max* crops. In Piauí state, the corn crop in 2011/2012 had an area planted of 351.6 thousand ha, with estimated total Yuction of 787.2 M t and an average yield of 2,239 kg ha^{-1} (Conab, 2012).

In this region, most soils present flat relief and excellent physical conditions for agricultural expansion (Pragana et al., 2012). Nevertheless, they present low clay contents and low cation exchange capacity (CEC), mainly owing to low organic matter contents (OM), which for these soils

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may stand for up to 80% of the CEC. Owing to their low OM contents, they also present low N supply capacity for corn.

Corn crop presents varying demands for fertilizers, with an emphasis on N (Silva et al., 2012), which has its dynamics in soils conditioned by the management system, climatic conditions and soil characteristics, among others (Silva et al., 2005b). The importance of N as to its functions in the metabolism of plants, participating as a constituent of molecules of proteins, coenzymes, nucleic acids, cytochromes, chlorophyll etc., in addition to being one of the nutrients most relevant to the increased grain yield (Varvel et al., 1997; Ferreira et al., 2001). In corn, N is a nutrient absorbed in greatest amount, became it one of the greatest influence on yield, due to the several relevant functions in its physiological activities (Silva et al., 2005a, 2005c; Farinelli and Lemos, 2012) and the one which burdens the most on the Yuction cost of the crop.

The nitrogen fertilization management is one of the most complexes due to the nutrient possessing one of the greatest indices of losses, which may occur by leaching, erosion, ammonia volatilization and denitrification (Queiroz et al., 2011). The form of N management exerts a great influence on the use of this nutrient by corn (Silva et al., 2005a). Some studies demonstrate distinct responses as to the rate, the number of splits and its time of application (Silva et al., 2006; Duete et al., 2008), a fact conditioned to the N transformation in soil, which are mediated by microorganisms dependent on the edaphoclimatic conditions (Hurtado et al., 2009).

The nitrogen management should supply the plant demands in critical periods, maximize the percentage of N recovery (R%) and minimize the impact on the environment by the reduction of losses (Fernandes and Libardi, 2007). In this sense, the split and the time of application of the nitrogen fertilizer constitute alternatives to increase the efficiency of nitrogen fertilizers by corn, due to, among other factors, the reduction of the losses by the increased use of N, resulting from the timing between the applications and period of high demand of the nutrient (Silva et al., 2005a; Duete et al., 2008; Hurtado et al., 2009). Bastos et al. (2008) evaluating the effect of both rates and forms of N split for corn Yuction under no-till farming, observed the highest grain yield (7.74 t ha^{-1}) in the treatments in which N fertilizer was split into three times, obtained with the rate of 180 kg of N ha^{-1}.

In State of Piauí, there are shortage studies which aim to identify the effects of the combination between rates and forms of splits of nitrogen fertilization, which many times, limits the development of the plant. Also, is necessary to identify the right moment and at the adequate rate of N, it maximizes the expression potential of the crop. It was intended to verify the effect of the rates and splits of N in relation to the Yuction and yield components of corn in the Brazilian Cerrado of Piauí, northeastern region.

MATERIALS AND METHODS

The experiment was conducted on the Fazenda União, municipality of Currais - PI (09° 37 '27 of "latitude and 44° 40' of 52" longitude and altitude of 541 m) in January-May 2012. The climate of the region is of the Aw type according to Jacomine et al. (1986), with two well distinct seasons, one being dry which goes from May to September and a rainy season which goes from October to April. The average temperature of the region is 26.5°C (Viana et al., 2002). The data of rainfall during the period of the carrying out of the experiment are in Figure 1.

The predominant soil in the region is the Distrophic Red Yellow Latosol (Typic Haplorthox), characterized by presenting high acidity and low fertility, with appropriate physical conditions. It is situated on a flat to gently wavy relief, and it is suitable for agricultural activity (Pragana et al., 2012).

The chemical and textural characterization of the soil prior to the establishment of the experiment, and soil sampling in the second layer of 0-0.20 m (EMBRAPA, 1997) are shown in Table 1. Soil preparation was done by means of the passing of a heavy harrow and two light harrows to level the ground and eliminate weeds. The correction of the soil acidity was performed three months prior to the establishment of the experiment, using dolomitic limestone (RPTN = 90%) at the rate of 1.31 ton ha^{-1}.

Corn (single-cross hybrid 30F53 Pioneer®) was sown manually in January, 2012. This cultivar characterized as early, high yielding and capacity of adaptation to both the low and high lands of Central Brazil. At planting, the seeds were distributed with the help of a ruler, leaving every 0.3 m a seed, for the obtaining of a final population of approximately sixty-six thousand (66,000) plants per hectare.

Fertilizers (70 kg ha^{-1} P_2O_5 and 60 kg ha^{-1} of K_2O) were applied in the furrow at sowing time according to the soil analysis (Table 1), using simple superphosphate and potassium chloride, respectively. In relation to the N fertilizer, urea was utilized, with the amount applied according to the treatments of rates and splitting of N (Table 2). Nitrogen topdressing (Table 2) was distributed manually on a band at 15 cm away from the seeds.

The disease control treatments such as weed and pest insect management were employed using Yucts recommended to the crop. Before sowing the corn, the herbicides glyphosate and 2,4-D were applied at rates of 1,440 and 806 g ha^{-1} ai, respectively. The weed control in post-emergence was conducted with corn in the stadium of five expanded leaves (V5), using the herbicide Atrazine (1,500 g ha^{-1}) ai. In the pre-blossoming period proceeded to application of the fungicides Epoxiconazole + pyraclostrobin (99.7 + 87.5 g ai ha^{-1}) and insecticides Methomyl (12.9 g ai ha^{-1}) and Imidacloprid + thiodicarb (45 + 135 g ai ha^{-1}).

The experiment was conducted as completely randomized blocks design with four replications. The treatments consisted of five rates of N (0, 60, 120, 180 and 240 kg ha^{-1}) as urea. Twenty kg ha^{-1} N were applied at sowing and the rest as split at topdressing. The splits comprised the application of 50% at the four expanded leaf stage (V1) + 50% at the eight expanded leaf stage (V2) or total rates at four expanded leaf stage. The experimental plot consisted of an area of 15 m^2 (3 × 5 m) with six rows of corn spaced 0.5 m and two central rows were considered.

For leaf N content (LNC), measured on the blossoming stage, the central third of 3 leaves of the base of the main ear was collected. Afterwards, the material was washed with tap and distilled water, dried in an oven with air forced circulation and air renewal at 60°C, and then ground into a Welley type mill for determination of N by Kjeldal method, according to the Malavolta et al. (1997). Simultaneously two plants of each plot were also collected for determination of plant biomass, which were dried at 60°C till constant weight. The reading of the relative content of chlorophyll (RCC) (SPAD index) at blossoming stage was obtained using a portable chlorophyll meter, three readings being obtained by plot,

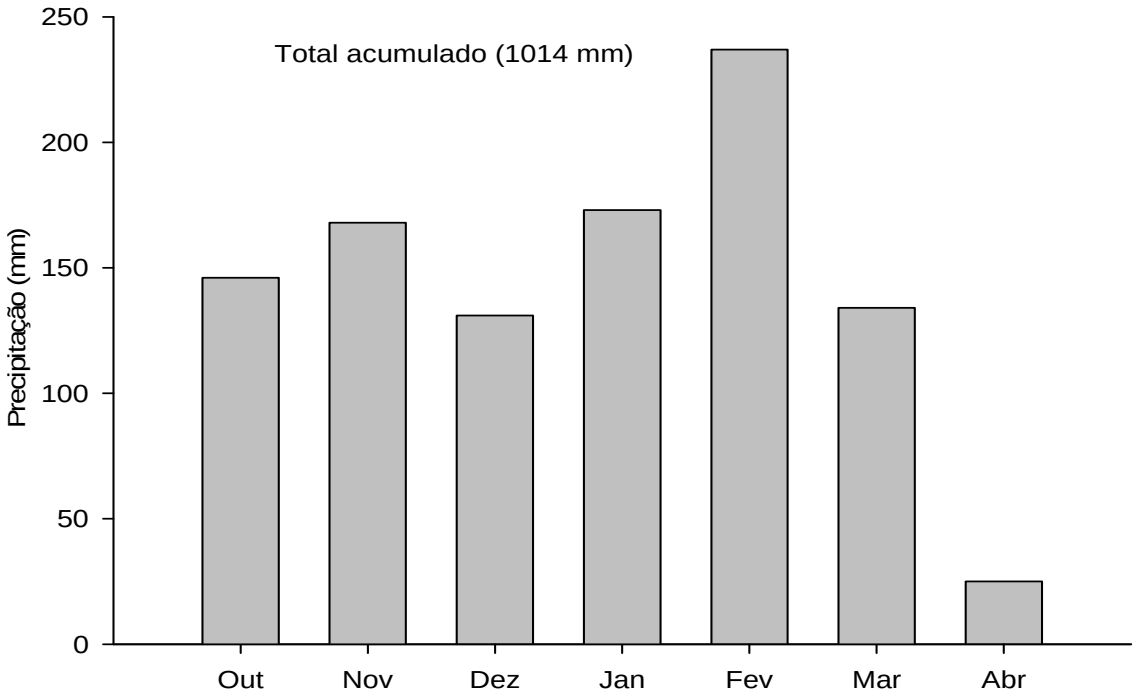

Figure 1. Average rainfall in the Farm União, Serra das Laranjeiras, municipality of Currais – PI, 2012. Accumulated total (1014 mm) precipitação = raiLNCall (mm) Oct, Nov, Dec, Jan, Feb, Mar, Ap.

Table 1. Contents of phosphorus (P), organic matter (OM), clay (Arg), silt (Sil), sand (Are), pH, exchangeable acidity (Al^{3+}), potential acidity (H+Al), calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^{+}), sum of bases (SB), cation exchaGPE capacity (CEC), base saturation (V) and aluminum saturation of Distrophic Red Yellow Latosol (Typic Haplorthox) on the Piauí Cerrado.

pH	P	K^+	Ca^{2+}	Mg^{2+}	Al^{3+}	H+Al	SB	CTC	V	m	MO	Are	Sil	Are
$CaCl_2$	mg kg^{-1}	.....$cmol_c$ dm^{-3}.....							.....%.....		g kg^{-1}	……g kg^{-1}……		
4.6	53	0.19	2.10	1.0	0.2	3.10	3.29	6.39	51.49	5.73	15	790	50	160

pH em $CaCl_2$-Ratio 1: 2.5; P e K^+ – Mehlich Extractor; Ca^{2+}, Mg^{2+} e Al^{3+} – Extractor: KCl 1 mol/L; H + Al – Calcium Acetate Extrator 0.5 mol/L– pH 7.0.

Table 2. Description of the treatments used: splitting rates and strategies of N.

Treatment	Rates of N (kg ha^{-1})			Total applied N (kg ha^{-1})
	Planting	4 leaves	8 leaves	
01	-	-	-	-
02	20	20	20	60
03	20	40	-	60
04	20	50	50	120
05	20	100	-	120
06	20	80	80	180
07	20	160	-	180
08	20	110	110	240
09	20	220	-	240

always performed in the central third of the opposite leaf and below the first ear, as the method proposed by Argenta et al. (2002). The evaluations of measurement of the plant height (H), height of ear insertion (EIH) and stem diameter (D) on three plants per plot were carried out at physiological maturity stage, using a measuring tape. Two central rows were harvested and the ears were threshed

Table 3. Table of contents of the descriptive and variance analysis for agronomic characteristics**,** Yuction and yield components of corn in response to nitrogen rates and splits in Distrophic Red Yellow Latosol (Typic Haplorthox) in the Piaui Cerrado.

Variable	H	RCC	LNC	PB	EIH	D	RPC	GPE	GPR	TSW	Y	AEI
	cm		g kg^{-1}	g	cm	mm				g	kg ha^{-1}	%
Treatment	*	***	***	***	ns	***	ns	ns	ns	*	*	*
CV (%)	5.77	3.98	8.37	11.24	10.22	7.50	4.73	12.01	12.48	4.51	19.19	66.04
Mean	2.16	64.18	24.34	196.44	90.94	20.09	14.89	416.25	27.95	265.10	6210	12.94

Subtitle: H: plant height; RCC: relative chlorophyll content, LNC: leaf N content; PB: plant biomass; EIH: ear insertion height; D: stem diameter; RPC: rows per cob; GPE: grains per ear; GPR: grains per row; TSW: thousand seed weight; Y: yield, AEI: Agronomic efficiency index, CV: coefficient of variation (%), * P < 0.05, ** P < 0.01, *** P < 0.001, and ns: non-significant.

Table 4. Table of contents of the analysis of variance for the agronomic characteristics, yield components and grain yield in response to different rates and splitting strategies of N in Distrophic Red Yellow Latosol (Typic Haplorthox) in the Piaui Cerrado.

Variable	H	RCC	LNC	PB	EIH	D	RPC	GPE	GPR	TSW	Y	AEI
	cm		g kg^{-1}	g	cm	mm				g	kg ha^{-1}	%
(Fc) Splits	2.62^{ns}	1.97^{ns}	1.39^{ns}	0.01^{ns}	2.68^{ns}	8.40**	1.72^{ns}	0.51^{ns}	0.05^{ns}	6.34*	0.18^{ns}	2.80^{ns}
(Fc) Rates	8.77***	12.83***	10.17***	23.91***	2.36^{ns}	11.61***	2.08^{ns}	2.15^{ns}	0.97^{ns}	3.45*	10.09***	7.59***
(Fc) Spli x Rate	1.06^{ns}	2.30^{ns}	1.87^{ns}	1.84^{ns}	0.67^{ns}	2.77*	0.71^{ns}	0.16^{ns}	0.20^{ns}	1.13^{ns}	2.40^{ns}	3.25*

Subtitle: H: plant height; RCC: relative chlorophyll content, LNC: leaf N content; PB: plant biomass; EIH: ear insertion height; D: stem diameter; RPC: rows per cob; GPE: grains per ear; GPR: grains per row; TSW: thousand seed weight; Y: yield, AEI: Agronomic efficiency index; Fc calculated F; Spli: Splitting; Rate: Rates, * P < 0.05; ** P < 0.01, *** P < 0.001, and ns: non-significant.

mechanically and standardized to 13% moisture. In each plot were also removed five ears to determine grains per ear and rows per ear, grains per row and thousand seed weight. From the yield data, the efficiency in the use of N was determined by using the following formula:

(AEI) = (PGcf - PGsf) / (QIn) in kg kg^{-1}

Where: AEI = agronomic efficiency index; PGcf = grain yield with N fertilizer; PGsf = grain yield without N fertilizer and QIn = amount of the nutrient applied (kg).

The data were at first subjected to analyses. And then, the variables were analyzed by simple descriptive statistics (mean, standard deviation and coefficient of variation) for further analysis of variance (ANOVA). In the ANOVA, the effects of treatments and blocks on the variables (P <0.05) were analyzed. For comparison of the means of the different levels of the treatments, the Scott and Knott test (1974) at 5% probability was applied using the statistical program SISVAR 4.2 (Ferreira, 2011). To perform the Pearson correlation analysis, the SAS software (Statistics Analyst System) was used for Windows-NT, version 8.0 (Sas, 1999), by means of the PROC CORR command. The analyses of variance and Scott Knott test were conducted using the statistical program SISVAR 4.2 (Ferreira, 2011). For the effect of rates and splitting strategies, the data were subjected to regression analysis using also SISVAR 4.2 (Ferreira, 2011).

RESULTS AND DISCUSSION

The result of the analysis of variance of the different variables (Table 3) showed significant differences among the nine treatments for most traits evaluated except for ear insertion height (EIH), rows per cob (RPC), grains per ear (GPE) and grains per row (GPR). Such traits are mainly influenced by the genotype and followed by nutrient availability during the grain filling stage (Ohland et al., 2005).

There were significant effects of treatments (rates and splitting strategies of N) on plant height (H), relative chlorophyll content (RCC), leaf N content (LNC), plant biomass (PB), stem diameter (D), thousand seed weight (TSW), yield (Y) and agronomic efficiency index (AEI) (Table 3). There was an interaction between the rates and splitting strategies N for the variables D and AEI (Table 4). The distinct strategies of N splitting showed similar efficiency in H, RCC, LNC, PB, EIH, RPC, GPR, GPE and Y. According to Duete et al. (2008), possibly the little difference between the times of topdressing application of N is due to the coincidence between the time of N application and the stage of plant development in which linear absorption of nutrients occurs.

Rates of 0 and 60 kg ha^{-1} of N split into three times (T1 and T2, respectively) provided lower H. However, the rate of 60 kg ha^{-1} N applied at twice, 20 at planting and 40 at the 4 leaf stage showed behavior similar to the other rates (Table 5). According to Silva et al. (2005a), it should be taken into account that H is a characteristic of genetic heritability and less dependent on environment, unless the plant goes through a very severe nutrient deficiency, which may have occurred in treatments T1 and T2. The regression analysis stood out for H a quadratic PB (Figure 2a) on the basis of the rates of N applied. There was an increase in H with increased rate of N, reaching

Table 5. Means of agronomic **c**haracteristics, yield components and grain yield in response to different rates and splits of N in dystrophic Yellow Latosol of the Piauí. Cerrado.

TRT	H	RCC	LNC	PB	EIH	D	RPC	GPE	GPR	TSW	Y	AEI
	(cm)		(g kg^{-1})	(g)	(cm)	(mm)				(g)	(kg ha^{-1})	(%)
T1	194.25^{b}	60.76^{c}	22.94^{b}	140.67^{c}	82.99^{a}	17.30^{b}	14.40^{a}	394.05^{a}	27.30^{a}	252.99^{b}	4,504.33^{b}	-
T2	201.92^{b}	57.94^{c}	18.89^{c}	176.10^{b}	81.41^{a}	18.42^{b}	14.65^{a}	384.05^{a}	26.02^{a}	261.23^{b}	5,121.33^{b}	10.28^{b}
T3	220.50^{a}	63.71^{b}	22.87^{b}	159.90^{c}	93.16^{a}	20.89^{a}	14.60^{a}	386.45^{a}	26.57^{a}	258.28^{b}	6,394.50^{a}	31.50^{a}
T4	216.25^{a}	63.34^{b}	25.58^{a}	188.42^{b}	93.83^{a}	18.83^{b}	14.90^{a}	419.85^{a}	28.25^{a}	269.18^{a}	5,793.42^{b}	10.74^{b}
T5	222.92^{a}	63.92^{b}	24.35^{a}	204.60^{a}	93.75^{a}	21.77^{a}	15.20^{a}	453.40^{a}	29.80^{a}	258.27^{b}	6,507.08^{a}	16.69^{b}
T6	222.42^{a}	66.64^{a}	25.79^{a}	239.03^{a}	89.91^{a}	21.08^{a}	14.80^{a}	440.85^{a}	29.75^{a}	284.09^{a}	7,251.66^{a}	15.26^{b}
T7	231.08^{a}	66.20^{a}	26.30^{a}	213.60^{a}	97.75^{a}	23.19^{a}	15.75^{a}	445.75^{a}	28.34^{a}	260.62^{b}	7,249.66^{a}	15.25^{b}
T8	220.50^{a}	67.78^{a}	25.93^{a}	211.85^{a}	91.08^{a}	20.13^{a}	14.75^{a}	403.55^{a}	27.50^{a}	276.07^{a}	7,197.44^{a}	11.22^{b}
T9	216.75^{a}	67.35^{a}	26.40^{a}	233.83^{a}	94.58^{a}	19.19^{b}	14.95^{a}	418.30^{a}	27.97^{a}	265.19^{b}	5,825.58^{b}	5.50^{b}

Means followed by the same letter in the column do not differ from each other at (P <0.05) of probability by the Scott-Knott test. TRT: Treatment, H: plant height; RCC: relative chlorophyll content, LNC: leaf N content; PB: plant biomass; EIH: ear insertion height; D: stem diameter; RPC: rows per cob; GPE: grains per ear; GPR: grains per row; TSW: thousand seed weight; Y: yield, AEI: Agronomic efficiency index; T1 = 0 kg ha^{-1} (= 0 kg planting, 4 leaves = 0 kg , 8 leaves = 0 kg), T2 = 60 kg ha^{-1} (planting = 20 kg, 4 leaves = 20 kg, 8 leaves = 20 kg), T3 = 60 kg ha^{-1} (planting = 20 kg, 4 leaves = 40 kg), T4 = 120 kg ha^{-1} (planting = 20 kg, 4 leaves = 50 kg, 8 leaves = 50 kg), T5 = 120 kg ha^{-1} (planting = 20 kg, 4 leaves = 100 kg); T6 = 180 kg ha^{-1} (planting = 20 kg, 4 leaves = 80 kg, 8 leaves = 80 kg), T7 = 180 kg ha^{-1} (planting = 20 kg, 4 leaves = 160 kg), T8 = 240 kg ha^{-1} (planting = 20 kg, 4 leaves = 110 kg, 8 leaves = 110 kg); T9 = 240 kg ha^{-1} (planting = 20 kg, 4 leaves = 220 kg).

its peak (2.25 m) at a rate of 176.05 kg ha^{-1} of N. This rate is close to the one observed by Silva et al. (2012) who found the highest H at rate of 147.9 kg ha^{-1} N with application of different sources of slow release urea in corn crop, and Silva et al. (2005a) obtained the highest H with 171 kg ha^{-1} N at no-tillage on dystrophic Red Latosol (oxisol).

Increased RCC was obtained when the applied rates of 180 and 240 kg N ha^{-1} (T6, T7, T8, T9), regardless of the splitting strategy (Table 5). Sunderman et al. (1997) in evaluating the variability at the concentration of chlorophyll in corn hybrids determined the critical value of RCC in the flowering stage of 57.9. In the present study no treatment was obtained below this value. RCC increased linearly with higher N, obtaining maximum mean of 67.78 with the rate of 240 kg N ha^{-1} (Figure 2b). Hurtado et al. (2009) found that the metabolic potential of chlorophyll Yuction (point of highest response) in relation to the supply of N was achieved with 242 kg ha^{-1} N at the flowering stage (R1), with a mean of 62.6, a value equal to that of the present study for the highest chlorophyll synthesis (242 kg ha^{-1}) at flowering. Studies using the RCC as an parameter, indicating N in corn during the growing season, are still few in Brazil, however, the studies conducted in the country and worldwide have shown that the method is efficient to separate deficient plants from those with an adequate N level (Rambo et al., 2004). According to these authors, the method allows making a fast diagnosis of the crop and immediate decision-making on the application of topdressing. For the LNC, increased value was obtained from 120 kg N ha^{-1}, independent of the splitting strategy (Table 5). It was also found that for most N levels, the LNCs were lower than that reported as suitable for the cultivation of corn, the range of which lies between 27.5 and 32.5 g kg^{-1} of N according to Malavolta et al. (1997). Silva et al. (2005a) reported that lower LNC can be a characteristic of the hybrid utilized, since in the treatment in which grain yield above 7,000 kg ha^{-1} was achieved, the LNC has not reached the value regarded as adequate. In the present study, the one of the rates of N on the LNC proved linear (Figure 2c). Silva et al. (2005a) found an increase in the LNC content up to 145 kg ha^{-1}, while Silva et al. (2012) found a maximum value of 34.7 g kg^{-1} rate 114.4 kg ha^{-1}of N. According to Rambo et al. (2004), the LNC in general are able to detect deficiency in plant, but also shows luxury consumption, wherein the content of N continues to increase and the yield is stable with high rates of this nutrient.

Increased Yuction of PB was also obtained from 120 kg ha^{-1} N, when splitting twice (T5) (Table 5). Growing linear effect of the PB in relation to the rates of N was also found (Figure 2d). The increase was of 30.43% in relation to the lowest rate (T1 = 0 kg N ha^{-1}). Thus, the N required for higher yield of PB corresponds to the half of that found by Araújo et al. (2004) who obtained higher

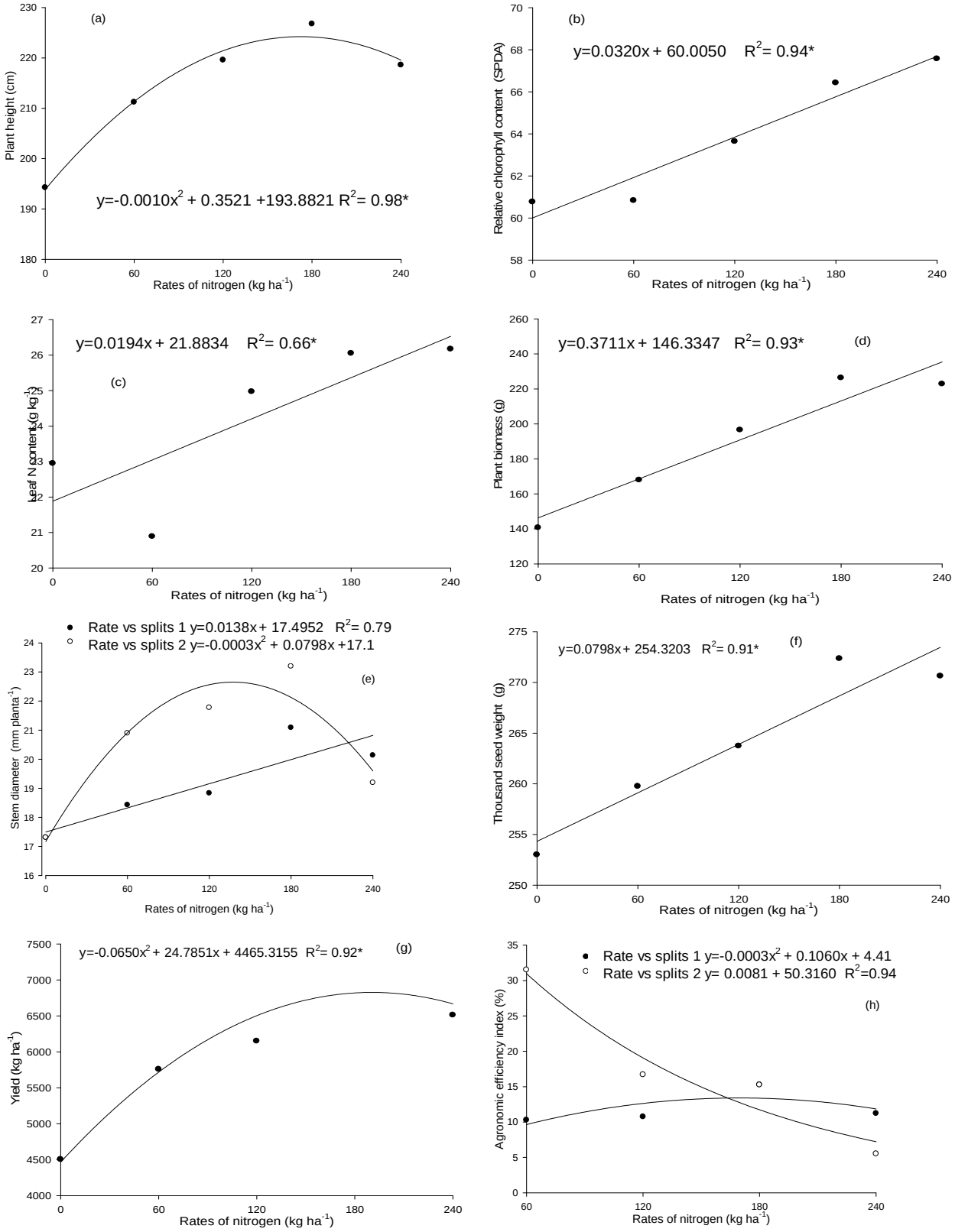

Figure 2. Plant height (a), relative chlorophyll content (b), leaf N content (c) plant biomass (d), stem diameter (e), thousand seed weight (f), yield (g) and agronomic efficiency index (h) in response to different rates and splits of nitrogen in corn in dystropheric Yellow Latosol of the Cerrado Piauí. Splits 1: 20 kg at planting and the rest divided into 50% at the four expanded leaf stage (V1) + 50% at the eight expanded leaf stage (V2); splits 2: 20 kg at planting and the rest at the four expanded leaf stage.

yield of shoot dry weight with 240 kg ha^{-1} N. The applications of 60 and 120 kg ha^{-1} N split in twice (T3 and T5, respectively), 180 kg ha^{-1} N, regardless of the split (T6 and T7) and 240 kg ha^{-1} N in splits in three times (T8), provided higher D (Table 5). According to Silva et al. (2005c), N acts on vegetative growth, directly influencing the cell division, expansion and photosynthetic process, combined with increase in D. The splitting strategy, 20 kg at planting and the remainder at 4 leaf stage enabled the obtaining of higher means (22.47) at the estimated rate of 136 kg ha^{-1} of N, but, the splitting of 20 kg at planting and the remainder divided in 50% at 4 expanded leaf stage + 50% at the 8 expanded leaf stage showed a growing linear effect in relation to rates N applied (Figure 2e). Cruz et al. (2008) studying the rates of N rates of the highest agronomic yield upon the morphological components of corn, found that rates above 85 kg ha^{-1} did not contribute to the increase of the D. Silva et al. (2012) also found, an increase in D with increasing rated, reaching higher value (17.39 mm) at the rate of 143.97 kg ha^{-1} of N.

Increased TSW was obtained when 120, 180 and 240 kg ha^{-1} N (T4, T6 and T8, respectively) were applied, these ones being split in twice, that is, 20 kg, at planting and the rest at four expanded leaf stage (Table 5). Linear effect was also found in relation to the rate tested for TSW, that is, as the rate of N was increased, there was a corresponding increase in grain weight (Figure 2f). A similar result was observed by Silva et al. (2005a), found the linear effect of TSW on different rates of nitrogem fertilizer in corn crop. Increased TSW due to the increase in the rates of N may have been determined mainly by the differences in the effective grain-filling period. Ferreira et al. (2001) observed that in the treatments without N and in those with lower rates, the lower leaves and ear straws presented themselves very dry, while in the treatments with higher N dosage, the plants were much more green, extending the period of sugar and N retranslocation to the grains, thereby increasing the final weight, a fact which was also observed in the present study.

For Y, the application of 60 kg ha^{-1} N split in twice (T3), 120 kg ha N split in twice (T5), 180 kg ha^{-1} of N independent of the splitting (T6 and T7) and 240 kg ha^{-1} N split three times (T8) provided greater means (Table 5). The difference between the Y provided by the application of 180 kg N ha^{-1}, split in three times and the control treatment was 2747.33 kg ha^{-1}, representing an increase of 60.99%. Duete et al. (2008) also obtained a 47% increase in Y in raising the rate of N from 0 to 135 kg ha^{-1} of N. Araújo et al. (2004) found that application of 240 kg ha^{-1} of N resulted in higher Y compared to the control, that is, 2,448 kg ha^{-1} (28%).

Higher Y with application of 60, 120 and 180 kg ha^{-1} of N split in twice, possibly, happened due to all N being applied till 40 days after sowing (DAS), demonstrating increased N supply in the early stage of crop growth, supported grain yield. In addition, the increase in Y provided by the application of N (Table 5) took place owing to this nutrient having supported an increase in the plant biomass (Figure 2d) and possibly leaf area, thus conditioning increased synthesis of assimilates, since N is a constituent of the chlorophyll molecule, acting in the processes of cell division and expansion (Ferreira et al., 2001). This assumption is confirmed by the lower amount of plant biomass of the control treatment and by its linear increase due to the increase in rate of N (Figure 2d). That took place also by the possible N mobilization and the high N requirement of corn, even in early stages, in which the full use of N contributes in yield in an advanced manner.

Data from Y (Figure 2g) responded quadratically to N rates. With the increase of the rate, one has an increase in the corn Y up to the estimated rate 190.65 kg ha^{-1}, that is, 6828 kg ha^{-1}. The quadratic effect of the rates of N on corn kernel yield is in agreement with the results obtained by Silva et al. (2005a) and Bastos et al. (2008). All those results allow stating that the N dynamics in the soil-plant system and, consequently, the efficiency of the use of this element by corn, is dependent on the amount and time of fertilizer application.

Highest AEI was obtained with application of 60 kg ha^{-1} N split twice (T3) (Table 5). The splitting strategy, 20 kg at planting and the rest at the 4 leaf stage yielded the highest AEI (23.29) at the estimated rate 114.43 kg ha^{-1} of N. but the split of 20 kg at planting and the rest divided into 50 % at the four expanded leaf stage +50% at the eight expanded leaf stage presented no significant effect (Figure 2h). Fernandes et al. (2005) studying the efficiency of N use in corn plants, using rates from 0 to 180 kg ha^{-1} N, reported that the use of N decreased with increase of rates applied, due to the supply of N exceeding the needs of the corn crop. For Farinelli and Lemos (2010), this reduction is coming from likely losses of ammonia and nitrate by leaching after the nitrification process, which increased with applied rate and this increase may be either linear or exponential.

The Pearson correlations among the characteristics were to a great extent significant, positive and low to moderate magnitude (Table 6). Among the variables which correlated with the Y were: H, RCC, EIH, D and GPE (Table 6), confirming that these components may be important in the Y of crop. Concerning H, these results corroborate with those observed by Silva et al. (2006). According to these authors, H is a parameter that determines the degree of development of the crop, having a positive correlation (0.50) with Y, therefore, for the same hybrid, larger plants tend to be more yielding, probably because they suffer less stress during their development and accumulate higher amounts of reserves in stem. Silva et al. (2005c), in another study on times and modes of N application also observed a positive correlation between H and Y of corn, with value of r of (0.87). Cruz et al. (2008) found a significant and positive

Table 6. Pearson correlation coefficients in response to different rates and splits of N on corn in dystrophic Yellow Latosol of the Piauí Cerrado.

Variable	H	RCC	LNC	PB	EIH	D	RPC	GPE	GPR	TSW	Y	AEI
H	1.00											
RCC	0.71***	1.00										
LNC	0.48**	0.71***	1.00									
PB	0.47**	0.52***	0.44**	1.00								
EIH	0.77***	0.60***	0.54***	0.36*	1.00							
D	0.66***	0.57***	0.21^{ns}	0.35*	0.46**	1.00						
RPC	0.44**	0.32^{ns}	0.39*	0.21^{ns}	0.33*	0.27^{ns}	1.00					
GPE	0.45**	0.44**	0.33^{ns}	0.17^{ns}	0.33*	0.46**	0.41*	1.00				
GPR	0.33*	0.36*	0.22^{ns}	0.10^{ns}	0.24^{ns}	0.39*	0.04^{ns}	0.92***	1.00			
TSW	0.34*	0.46**	0.32^{ns}	0.37*	0.25^{ns}	0.20^{ns}	0.04^{ns}	0.20^{ns}	0.22^{ns}	1.00		
Y	0.56***	0.42**	0.11^{ns}	0.25^{ns}	0.53***	0.54***	0.34*	0.54***	0.44***	0.31^{ns}	1.00	
AEI	0.47**	0.22^{ns}	0.01^{ns}	-0.05^{ns}	0.38*	0.53**	0.13^{ns}	0.27^{ns}	0.24^{ns}	0.06^{ns}	0.66***	1.00

H: plant height; RCC: relative chlorophyll content, LNC: leaf N content; PB: plant biomass; EIH: ear insertion height; D: stem diameter; RPC: rows per cob; GPE: grains per ear; GPR: grains per row; TSW: thousand seed weight; Y: yield, AEI: Agronomic efficiency index. * P <0.05, ** P <0.01, *** P <0.001, and ns: non-significant.

correlation between D and Y in Alagoas, Brazil. According to these authors, normally the D presents a correlation with Y due to being a storage organ of the plant. According to Fanceli and Dourado Neto (2000), the stem acts as the storage structure of soluble solids that are used later in the grain formation. But, the correlation between GPE and Y is explained by the fact GPE to be an explicative trait of the Y, corroborating with the value obtained by Silva et al. (2006) which was of 0.73.

Studies have also reported a correlation between the RCC and Y (Argenta et al., 2002, 2004; Hurtado et al., 2009). According to Argenta et al. (2002) the RCC in corn leaf is highly associated with Y, its being able this way to replace the determination of the N content in the leaf for diagnosis of this nutrient in plant.

Conclusions

Nitrogen significantly increased agronomic characteristics of corn and the highest grain yield was obtained with 190.65 kg ha^{-1} N. Plant height, relative chlorophyll content, leaf N content, plant biomass, stem diameter, thousand seed weight, yield and agronomic efficiency index were positively influenced by the rates of nitrogen. The split of topdressing in twice was not suitable in the dystrophic Yellow Latosol of the Piauí Cerrado. Plant height, relative chlorophyll content, ear insertion height, stem diameter, grains per ear and rows per ear, grains per row and agronomic efficiency index positively correlated with grain yield.

Conflict of Interests

The authors have not declared any conflict of interests.

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