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**African Journal of Agricultural Research**

*Full Length Research Paper*

# An evaluation of mango (*Mangifera indica* L.) germplasm for future breeding programme

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**Evaluation of physical and chemical characteristics of fruit crops has been successfully used for selection of improved cultivars for breeding programs. The study was conducted at typical subtropical conditions in North-West India for evaluating the variability of mango germplasm to conserve the elite ones and identify the superior genotypes based on fruit quality for multiplication and for future crop improvement. Fourteen genotypes were tested for their physiological and chemical characteristics. Randomized complete block design was used and critical difference was used to compare quality characteristics of fourteen mango genotypes. Maximum fruit weight was found in Chausa whereas maximum reducing and total sugars were observed in Malda. Dashehari ranked first in respect of yield per tree, that is, 148.90 kg/tree. Local Selection-I was the earliest to mature. Among all genotypes Chausa, Kala Gola, Hundel and Gola showed tendency towards regular bearing. The Alphonso, Malda and Chausa was identified for superior traits like total soluble solids/acid ratio (TSS/acid ratio); Chausa and Langra Banarasi for high pulp percentage and pulp stone ratio; Rattaul for excellent flavour; 'Local Selection-I' for regular bearing and Dashehari for higher yield. These identified genotypes may be good donor in future hybridization programme to evolve the superior varieties.**

**Key words:** Mango, breeding, germplasm, genotype.

## INTRODUCTION

Mango (*Mangifera indica* L.) is one of the ancient fruits of India and its cultivation appeared to have begin 4000 years ago (Condole, 1984). It was originated as alloploid and its home was suggested as eastern India extending from Assam to Burma or possibly further in Malay region (Popenoe, 1920). Based on recent findings (Mukherjee, 1997) the centre of origin and diversity of genus *Mangifera* is now firmly established in South East Asia.

The genus *Mangifera* consists of 41 species and all the edible cultivars of mango belong to single species *M. indica* L. A large variability exists in mango germplasm throughout the country (Gupta et al., 1996). Being an ancestral home for mango germplasm, India is having more than thousand verities, which are widely distributed in different agro climatic zones (Yadav and Rajan, 1993). India is the largest mango producing country, with an annual production of 16.2 mt and occupies an area of 2.38 m ha, which accounts for 65% of total world production. There are bright prospects of building up a flourishing trade for the export of this fruit. Although, mangoes are exported to nearly 20 countries, whereas its products are export to over 40 countries (Negi et al.,

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2000), still there are many doors pending to be opened to flourish trading for export of this fruit.

Punjab Govt. Progeny Orchard and Nursery, Attari, District Amritsar was established in an area of 101 acres before independence. A large number of varieties of mango, citrus and other minor fruits were collected with the objective to assess their performance and thereby multiplying the superior varieties for farmers of undivided Punjab. The old plantation of mango varieties in this orchard includes many commercial and local varieties.

In India, improvement of mango through breeding was initiated as early as 1911. However, systematic hybridization work was started during forties. As a result, four hybrids namely, Swarna Jehangir (Chinnswarnarekha × Jehangir), Neelogoa (Neelum × Mulgoa), Neeluddin (Neelum × Himayuddin) and Neelshan (Neelum × Baneshan) were released at Fruit Research Station, Kodur, Karnataka (India). Mango varieties Neelphonso (Neelum × Alphonso), Neeleshan (Neelum × Baneshan), Neeleshwari (Neelum × Dashehari) were developed at Gujarat Agricultural University. New hybrids Mallika (Neelum × Dashehari), Amarpali (Dashehari × Neelum) were developed at IARI, New Delhi. Ratna (Neelum × Alphonso) Sindhu (Ratna × Alphonso) which was referred to as seedless were developed at Konkan Krishi Vidyapeet, Dapoli, Maharashtra (India). At APAU, Hyderabad, AuRumani (Rumani × Mulgoa) and Manjeera (Rumani × Neelum) hybrids were developed after systematic hybridization work. At IIHR, Bangalore, Arka Aruna (Banganappalli × Alphonso), Arka Puneet (Alphonso × Banganappalli), Arka Anmol (Alphonso × JanardhanPasand) were developed which were regular bearer.

After the independence with the establishment of Punjab Agricultural University in 1962, the research activities undertaken by state Agriculture Department were shifted to the University. Thus, the planning of continuing the project of evaluation of germplasm was stopped in the Punjab Government Orchard. At present, the orchard is under the control of state department of Horticulture and main thrust is being given on commercial crop production and sale of grafted plants but not on evaluating and conserving the collected genetic diversity. Thus, the genetic base is eroding at very fast rate. The present studies were therefore undertaken for evaluating the variability of the mango in the orchard to conserve the elite ones for multiplication and crop improvement. The objective of the study was to evaluate morphological characteristics of the mango germplasm and isolate to identify the superior genotype based on fruit quality for future breeding programs.

## MATERIALS AND METHODS

The present study was conducted to study the performance of mango germplasm available in Punjab Government Progeny Orchards and Nursery, Attari District Amritsar. Amritsar represents typical subtropical conditions prevailing in North West India with an elevation of 231.52 m above sea level. Temperature reaches to 45°C in summer and winters are cold with occasional ground frost. The evaluations of mango germplasm for tree and fruit quality characters were carried out. The fruits from the identified germplasm of mango were harvested at maturity and were placed for ripening at room temperature for 2 to 3 days. Tree characters were measured with usual method. Thereafter, the fruit samples were analysed for physico-chemical evaluations in the P. G. Laboratory of Horticulture Department, Khalsa College, Amritsar.

### Physical characteristics

Tree height of different mango varieties were measured with the help of calibrated bamboo stick and expressed in meters, whereas tree spread and trunk girth were measured with the help of measuring tape. To get a mean canopy diameter, two observations on each of east west and north south sides of selected tree were recorded. The trunk girth was recorded at a height of 15 cm above the graft union on each selected tree. The weight of fruit sample of different mango varieties under testing was taken with the help of simple pan balance. Ten fruits of mango were randomly taken as sample from each tree. Average fruit weight was calculated in grams/fruit. Fruit size, length and breadth were recorded with the help of Vernier Caliper and their average was calculated in centimeters. The colour of fruit was assessed on the basis of Royal Colour Chart. These observations were taken at the optimum maturity of the fruit.

Pulp weight was calculated by subtracting the peel and stone weight from total weight of fruit. Pulp content was expressed in percentage. To calculate the Pulp/Stone Ratio, the stone weight was subtracted from the total weight of fruit and the value obtained was divided by stone weight.

### Chemical characteristics

#### *Total soluble solids and acidity*

The content of total soluble solids was determined with the help of digital Refractometer and the values were corrected at 20°C with the temperature correction chart and expressed as percent. The total titrable acidity was determined by titrating a known volume of finely blended juice with 0.1 N NaOH solution using phenolphthalein as an indicator. The end point was marked by appearance of pink colour which persisted for few seconds. The results were expressed as percent titrable acidity:

$$\%\ Acidity = \frac{0.067 \times 0.1N\ \ NaOH\, used\,(ml)}{Juice\, taken\,(ml)}$$

Total soluble solids/ Acidity ratio (TSS/Acidity ratio) was calculated by dividing the value of TSS with that of corresponding titrable acidity.

#### *Sugars*

Ten gram fruit pulp was taken in 100 ml beaker and volume made with distilled water. One gram of lead acetate was added for precipitating the extraneous matter. The solution was allowed to stand for half an hour. Then Potassium oxalate (1 g) was added to remove excess of lead. The filtered solution called as aliquot was ready for estimation of reducing total sugars.

### *Reducing sugars*

Five millilitres each of Fehling solution (A and B) were taken in a flask. The above prepared aliquot was taken in burette and four drops of methylene blue indicator were added. Then, it was titrated against Fehling solution (A and B) mixed over a hot plate. The blue colour of the solution started changing to red. The titration was continued till the end point was noted as appearance of permanent brick red colour. The volume of aliquot used was noted as ‘A’:

$$\text{Reducing Sugars \%} = \text{titrate value against Fehling solution} \times \frac{\text{Stock Solution}}{\text{Weight of Sample Sol. Used}} \times 100$$

$$ss = 0.05 \times \frac{100}{10 \times 'A'} \times 100$$

### *Total sugars*

Twenty-five millilitres of above aliquot was taken into 100 ml measuring flasks and to this 25 ml distilled water was added and thereafter, 5 ml of HCl (60% by Vol.) was added. The solution was left over night at room temperature for acid hydrolysis. The centigrade thermometer was placed in the flask and it was heated on water bath in such a way that the temperature rose to 68°C in 10 min. The flask was still kept at 68°C for another 5 min. Then, a piece of litmus paper was put into the flask and neutralized the inverted sugars with 10% NaOH in the initial stage and with 0.1 NaOH near the neutralization point. The volume was made 100 ml by adding distilled water and titrated this against boiling Fehling solution in case of reducing sugars:

$$Total\ Sugars(\%) = 0.05 \times \frac{Stock\ Sol.}{Wt.of\ sample} \times \frac{Second\ stock\ sol.}{Sol.of\ Aliquot\ used \times Vol.of\ sol.used} \times 100$$

### *Yield per tree*

The yield of mango varies with the variety, periodicity of flowering, growing conditions, influencing the size of plants and productivity varies also with locality. The yield/tree was recorded at optimum maturity in kg.

### *Time of optimum maturity*

The time of maturity depends upon various external characters like appearance of waxy coating, dots on the fruits and relative size of the fruit. Some ripened fruits when start dropping, it is said that maturity of the particular variety is reached. The fruit samples for physico-chemical analysis were taken at optimum maturity and maturity period was recorded.

### *Regularity of bearing*

Alternate bearing has been one of the major problem in north Indian mango cultivars. The bearing behaviour was depending upon various environmental conditions and genetical make up. The regularity of bearing was assessed during the research period. However, the knowledge about bearing in the past was also taken from the employees of the orchard. The data recorded for various parameters were subjected to statistical analysis by using Randomised Block Design (Singh et al., 1998). Analysis of variance was conducted for various characters by using computer programme CPCS1 (Cheema and Singh, 1990).

## RESULTS

### *Physical characteristics*

A wide range of variability in respect of various tree characters, viz. tree height, tree spread and trunk girth was observed. Maximum tree height was recorded in the genotype Kala Gola to the tune of 13.76 m, which was closely followed by Gola with the tree height of 13.60 m (Table 1). Whereas, the plants of Amarpali registered the minimum height (4.24 m).

Mango genotypes under study differed in their growth habit. Tree spread (North-South and East-West) ranged from 4.43-16.43 and 5.10-17.50 m, respectively (Figure 1). Significantly maximum tree spread of 16.16 m NS and 17.50 m EW was measured in Chausa followed by 14.30 m NS and 13.16 m EW, in Malda,14.63 m NS and 12.13 m EW ‘Local Selection-II’, whereas Amarpali had minimum tree spread (4.47 m NS and 5.10 m EW). In respect of trunk girth genotype Kala Gola recorded maximum trunk girth (2.87 m) followed by Chausa, Local Selection-I, Alphonso and Gola having trunk girth of 2.78, 2.63, 2.46 and 2.44 m, respectively, whereas Amarpali registered the lowest trunk girth (Figure 2).

The fruits of Chausa exhibited biggest fruit having fruit weight of 301.33 gm, whereas the genotype, Local Selection-II exhibited the smallest fruits with the average fruit weight of 80.63 gm. The average fruit weight of the evaluated germplasm ranged from 80.53 to 301.33 gm (Table 1). The selection Chausa recorded the maximum fruit weight to the tune of 301.33 gm showing the significant superiority over all evaluated genotypes. It was followed by Langra Banarasi and Gola having the fruit weight 293.60 and 236.42 gm, respectively (Table 1).

The data collected regarding fruit size (length and breadth) experienced a wide variation. The maximum fruit length of 11.70 cm was recorded in Hundel which was closely followed by 11.06 cm in Chausa and the minimum fruit length of 6.35 cm was observed in local Selection-I which was significantly less (Table 1). The fruits of Langra Banarasi were found to be having maximum fruit breadth (7.70 cm), which was followed by Hundel, Chausa, Kala Gola, where it was recorded as 7.60, 6.53, 6.10 cm, respectively. However, the fruits of Langra, Dashehari, Alphonso, Amarpali, and Dharbhanga recorded quite low fruit breadth to the tune of 5.80, 5.76, 5.70, 5.53 and 5.50 cm, respectively. The minimum fruit breadth of 4.60 cm was recorded by cultivar Local Selection-II (Table 1).

Dashehari, Langra Banarasi, Langra, Kala Gola and Dharbhanga were grouped together having different shades of yellowish green 144A, 144A, 144B, 144C, 144B, respectively (Table 3). On the other hand, the Alphonso, Amarpali and Local Selection-II were placed in second group having yellowish green with variable shades 153B, 153D and153A presented in the colour chart, respectively. The peel colour of Rattaul has given the different appearance greenish yellow 163C and thus

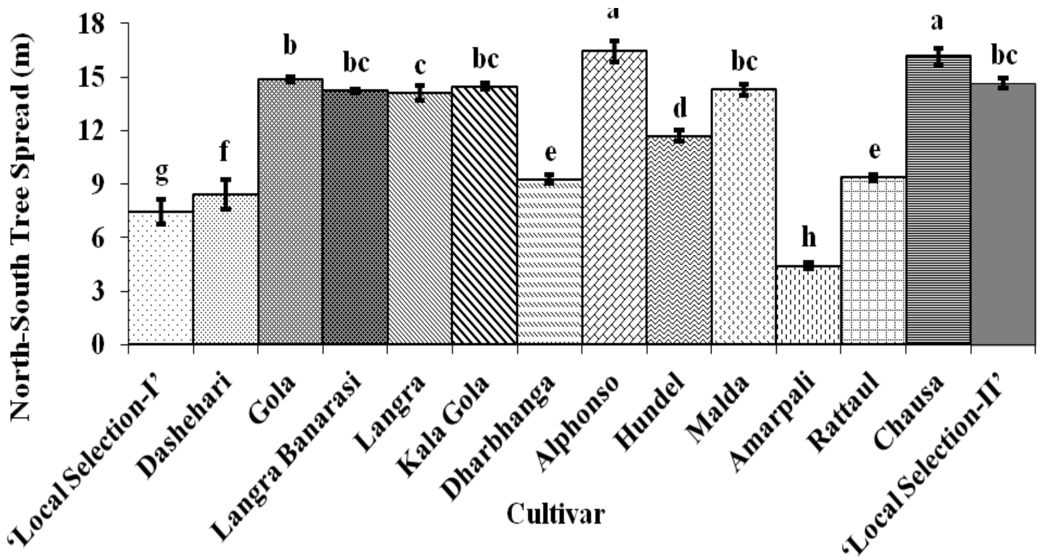


**Figure 1.** Variations in tree spread (TS) in North-South (NS) direction in mango cultivars.

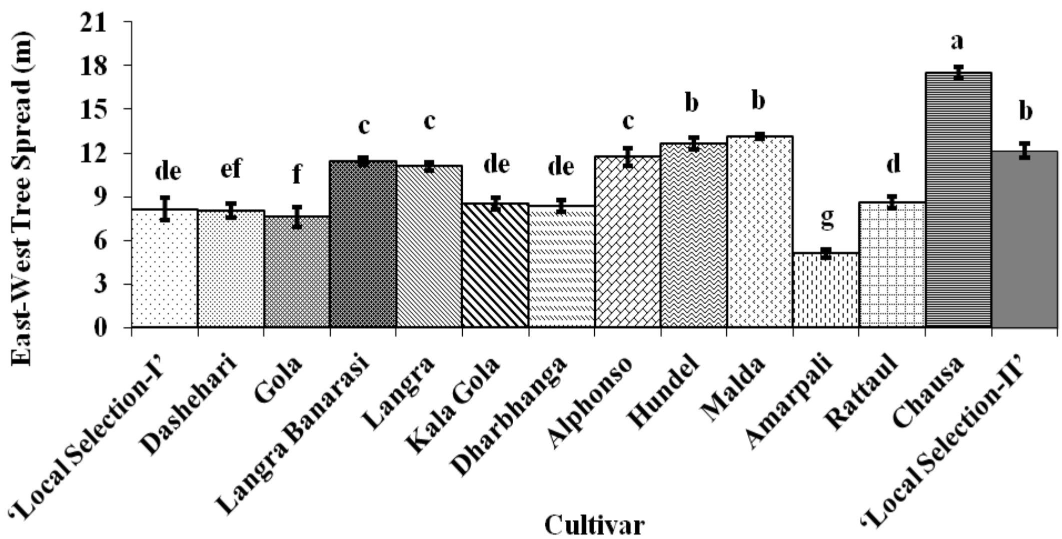


**Figure 2.** Variations in tree spread (TS) in East-West (EW) direction in mango cultivars.

showed marked variability for fruit colour. The pulp content (Table 2) of the different genotypes under evaluation experienced significant variability. The maximum pulp content of 89.78% was recorded in Chausa which was closely followed by 87.74% in Langra Banarasi and 84.34% in Local Selection-II. It was followed by Langra, Dashehari, Kala Gola, Dharbhanga and Alphonso with average pulp percentage of 79.23, 78.56, 74.00, 73.66, 73.16 and 72.1, respectively. The minimum average pulp content to the tune of (41.73%) was registered by Hundel.

The pulp stone ratio accrued in the present study experienced a highly striking variability (1.80-8.80) amongst the evaluated mango genotypes. The lowest pulp/stone ratio (1.80) was recorded in Local Selection-I and the highest (8.50) in Chausa. The genotypes Langra Banarasi, ranked at the next place with pulp stone ratio of 7.29. Amarpali was 3$^{rd}$ higher in pulp stone ratio (5.82). The genotypes, Local Selection-II, Rattual, Dharbhanga and Malda showed statistical equivalence with each other. As the pulp stone, ratio is most ideal parameter for judging the fruit quality on the part of consumer and processing industry. The pulp stone ratio of Dashehari, Gola and Hundel were 3.36, 3.03 and 2.98, respectively which is very low and thus can be rated as inferior. The minimum pulp stone ratio of 1.80 was recorded in Desi.

**Table 1.** Average tree height, and trunk girth of different genetic resources of Mango.

| S/N | Genetic resources | TH (m) | TG (m) | FW | FL | FB |
|---|---|---|---|---|---|---|
| $T_1$ | 'Local Selection-I' | 11.83 | 2.63 | 91.26 | 6.35 | 4.26 |
| $T_2$ | Dashehari | 6.66 | 1.53 | 171.36 | 10.56 | 5.76 |
| $T_3$ | Gola | 13.60 | 2.44 | 236.42 | 8.13 | 7.03 |
| $T_4$ | Langra Banarasi | 12.53 | 1.89 | 293.60 | 9.63 | 7.70 |
| $T_5$ | Langra | 8.50 | 1.84 | 121.33 | 8.83 | 5.80 |
| $T_6$ | Kala Gola | 13.76 | 2.87 | 124.33 | 7.03 | 6.10 |
| $T_7$ | Dharbhanga | 8.20 | 1.82 | 109.46 | 8.33 | 5.50 |
| $T_8$ | Alphonso | 12.43 | 2.46 | 131.00 | 8.46 | 5.70 |
| $T_9$ | Hundel | 9.66 | 1.76 | 190.00 | 11.70 | 7.60 |
| $T_{10}$ | Malda | 8.47 | 1.92 | 161.00 | 9.50 | 6.60 |
| $T_{11}$ | Amarpali | 4.24 | .54 | 199.66 | 9.23 | 5.53 |
| $T_{12}$ | Rattaul | 7.50 | 1.74 | 117.66 | 6.73 | 4.73 |
| $T_{13}$ | Chausa | 12.33 | 2.78 | 301.33 | 11.06 | 6.53 |
| $T_{14}$ | 'Local Selection-II' | 10.43 | 1.75 | 80.63 | 8.50 | 4.60 |
| | Mean | 10.2 | 2.014 | 176.32 | 8.86 | 5.96 |
| | C.D | 2.68 | .17 | 7.43 | 0.99 | 0.75 |
| | Range | 4.24-13.76 | .54-2.87 | 80.6-301.33 | 6.35-11.70 | 4.26-7.70 |

TH-Tree Height, TG- trunk Girth, TS- Tree Spread, NS- North South, EW- East West, FW-Fruit, Weight, FL- Fruit Length, FB- Fruit Breadth.

**Table 2.** Pulp percentage and Pulp/stone ratio of different genetic resources of Mango.

| S/N | Genetic resources | PP % | P/S ratio | TSS (°brix) | Acidity (%) | TSS/Acid ratio | Yield (kg) |
|---|---|---|---|---|---|---|---|
| $T_1$ | 'Local Selection-I' | 54.16 | 1.80 | 13.25 | 1.33 | 9.96 | 112.70 |
| $T_2$ | Dashehari | 78.56 | 3.36 | 17.40 | 0.30 | 58.00 | 148.90 |
| $T_3$ | Gola | 62.36 | 3.03 | 16.85 | 4.81 | 3.50 | 107.84 |
| $T_4$ | Langra Banarasi | 87.74 | 7.29 | 19.95 | 0.34 | 58.67 | 97.30 |
| $T_5$ | Langra | 79.23 | 4.54 | 21.68 | 1.57 | 13.81 | 104.56 |
| $T_6$ | Kala Gola | 73.66 | 2.52 | 16.95 | 7.86 | 2.16 | 97.35 |
| $T_7$ | Dharbhanga | 73.16 | 4.56 | 12.06 | 0.22 | 54.82 | 93.71 |
| $T_8$ | Alphonso | 72.1 | 3.11 | 26.84 | 0.33 | 81.33 | 93.33 |
| $T_9$ | Hundel | 41.73 | 2.98 | 15.88 | 0.20 | 79.42 | 113.31 |
| $T_{10}$ | Malda | 65.8 | 4.03 | 28.95 | 0.56 | 51.70 | 108.74 |
| $T_{11}$ | Amarpali | 63.59 | 5.82 | 23.25 | 0.40 | 58.12 | 44.03 |
| $T_{12}$ | Rattaul | 64.53 | 4.58 | 24.34 | 0.95 | 25.62 | 126.73 |
| $T_{13}$ | Chausa | 89.78 | 8.80 | 27.08 | 0.34 | 79.64 | 114.00 |
| $T_{14}$ | 'Local Selection-II' | 84.34 | 5.43 | 11.35 | 0.34 | 33.82 | 93.60 |
| | Mean | 64.09 | 4.81 | 19.70 | 1.15 | 46.36 | 107.46 |
| | C.D | 2.87 | .93 | .94 | 0.77 | 0.59 | 2.64 |
| | Range | 41.7-89.78 | 1.80-8.80 | 11.35-28.95 | 0.20-7.86 | 2.16-81.33 | 44.03-148.90 |

PP- Pulp Percentage, P/S- Pulp Stone Ratio, TSS- Total Soluble Solids, RS- Reducing Sugar, TS- Total Sugar.

***Chemical characteristics***

A high range of variability for TSS content (11.35-28.95° Brix) was recorded in evaluated mango germplasm (Table 2). Malda ($T_{10}$) ranked at the top with TSS content of 28.95° Brix, which was followed by Chausa (27.08), Alphonso (26.84) and Rataul (24.34). Minimum TSS of 11.35° Brix was recorded in Local Selection-II. Different mango varieties possessed varying level of acidity in the fruit pulp. The acidity ranged from 0.20 to 7.86% in different evaluated genotypes of mango. The minimum acidity of 0.20% was observed in Hundel that was closely followed by 0.22% in Dharbhanga and 0.33% in testing. Dashehari amongst the different genetic resources under

**Table 3.** Organoleptic rating of different genetic resources of mango.

| S/N | Genetic resources | Colour of fruit | OR | TM |
|---|---|---|---|---|
| $T_1$ | 'Local Selection-I' | Yellowish Green 151[A] | 3.0 | I[st] week of July |
| $T_2$ | Dashehari | Yellowish Green 144[A] | 6.6 | II[nd] week of July |
| $T_3$ | Gola | Yellowish Green 152[B] | 0.2 | IV[th] week of July |
| $T_4$ | Langra Banarasi | Yellowish Green 144[A] | 7.5 | II[nd] week of July |
| $T_5$ | Langra | Yellowish Green 144[B] | 6.8 | II[nd] week of July |
| $T_6$ | Kala Gola | Yellowish Green 144[C] | 1.5 | IV[th] week of July |
| $T_7$ | Dharbhanga | Yellowish Green 144[B] | 0.5 | II[nd] week of July |
| $T_8$ | Alphonso | Yellowish Green 153[B] | 8.0 | II[nd] week of July |
| $T_9$ | Hundel | Yellowish Green 151[A] | 7.0 | II[nd] week of July |
| $T_{10}$ | Malda | Yellowish Green 152[A] | 8.0 | III[rd] week of July |
| $T_{11}$ | Amarpali | Yellowish Green 153[D] | 7.6 | IV[th] week of July |
| $T_{12}$ | Rattaul | Greenish Yellowish 163[C] | 6.8 | IV[th] week of July |
| $T_{13}$ | Chausa | Yellowish Green 151[A] | 8.8 | IV[th] week of July |
| $T_{14}$ | 'Local Selection-II' | Yellowish Green 153[A] | 6.0 | III[rd] week of July |

OR- Organoleptic Rating at 10 Point Scale, TM- Time of Maturity.

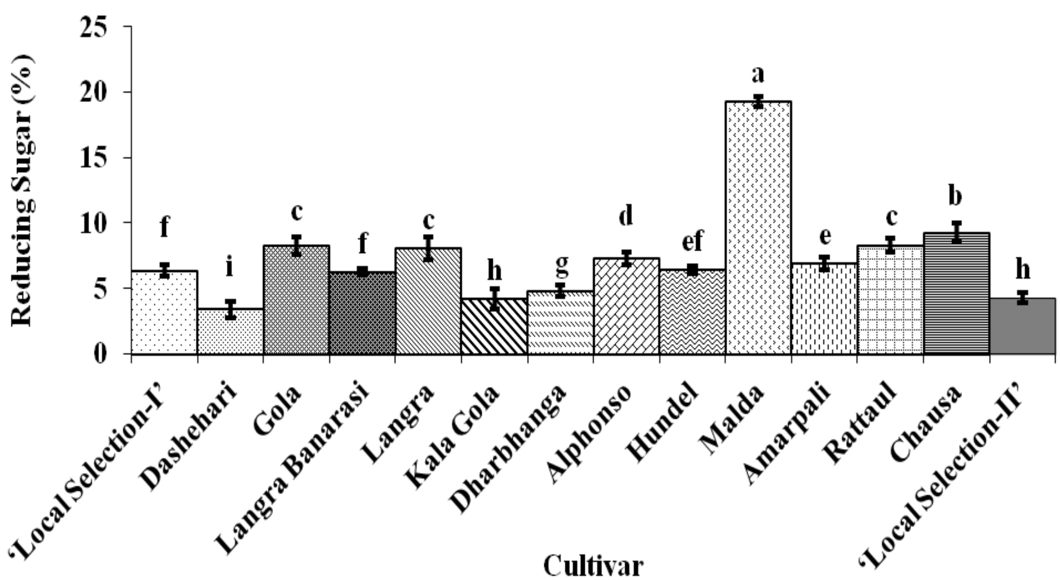


**Figure 3.** Variability of reducing Sugar (RS) content in different mango cultivars.

Among all the evaluated genotypes of mango, Malda exhibited significantly maximum total and reducing sugar (Figures 3 and 4), followed by Chausa, Rattaual and Alphonsowith total sugar content of 21.66, 19.36 and 19.23%, respectively (Figure 4). The minimum Total sugar content was recorded in, that is, Dharbhanga, (8.26%). The genotypes Rattaul, Alphonso and Amarpali showed total sugar content above (19%). The next best genotypes accrued in the study were Hundel, Dashehari, Langra Banarasi and Langra having total sugar percentage 11.53, 11.50, 14.2, 15.26, 16.13 and 18.16, respectively.

The yield of the mango varieties varied with the growing condition and size of the plant. The varieties Dashehari (148.90 kg/ tree) and Rattaul (126.73 kg/ tree) were high yielding varieties followed by Chausa, Hundel and Local Selection-I having yield 114, 113.31, 112.70 kg yield/ tree, respectively (Table 2). The varieties Malda, Gola and Langra recorded yield of 108.74, 107.84 and 104.56 kg per tree, respectively. Minimum yield/ tree to the tune of 44.03 kg/ tree was recorded with Amarpali. All the Mango varieties in the progeny orchard of State Department of Horticulture, Attari (Amritsar) were irregular bearers except Hundel Dharbhanga, Kala Gola

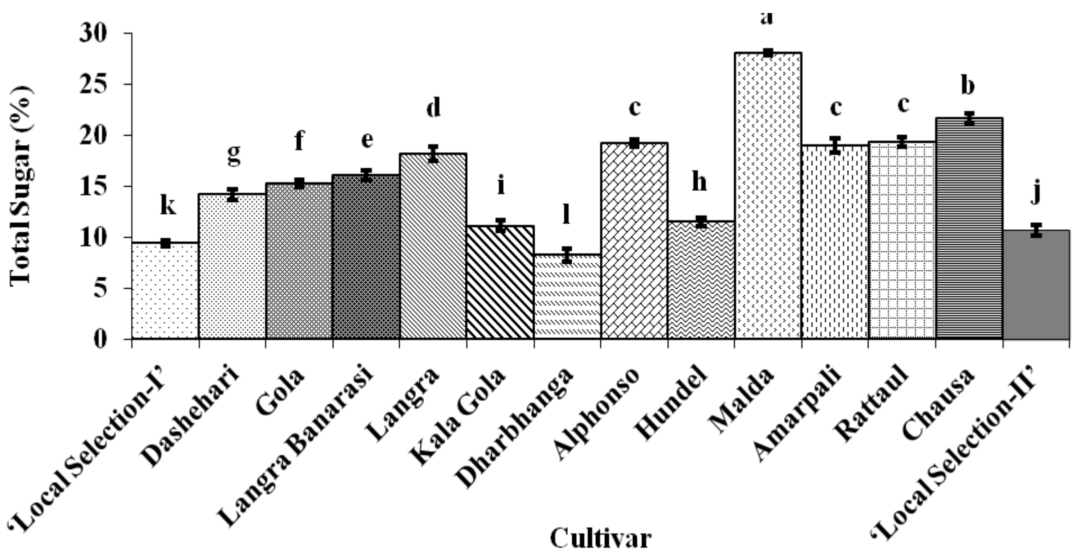


**Figure 4.** Variability of total sugar (TS) content in different mango cultivars.

and Local Selection-I which showed regularity in bearing (Table 2).

## DISCUSSION

### Physical characteristics

The tree height and tree girth of Kala Gola has been found better than any other mango cultivar in previous studies of (Bakshi et al., 2012; Barhate et al., 2012; Desai and Dhandor, 2000; Singh et al., 2000) who have found similar results in the different agro climatic regions of India. Likewise, Sharma et al. (1999), who evaluated mango cultivars for tree vigour in arid-irrigated region of Punjab and found that mango cv. Langra was the most vigorous followed by Mallika, Sandhuri and Amarpali. (Shivanandam and Shashidhara 2007) also conducted the similar studies in the eastern region of Karnataka to evaluate six varieties and nine hybrids of mango for their growth and recorded the higher values for growth parameters.

Amongst the evaluated genotypes, Local Selection-I and II retained the fruit weight less than 100 gm and thus may be rated as sucking type mango. The fruits of Chausa were having fruit weight > 300 g and were placed in Group-I, whereas Langra Banarasi and Gola were placed in Group-2 with medium fruit weight of 200 to 300 g. Hundel, Amarpali, Dashehari Malda and Rattaul were placed in Group-3 having fruit weight 100 to 200 g. The class of mango having fruit weight ranging about 200 g is universally accepted as best quality mango. According to the observations of the present study Amarpali and Hundel recorded the fruit weight as 191.6 and 190.00 g. Thus, these two may be rated as superior most according to the universal acceptance norms of top quality. The findings of present studies are in accordance with the findings of (Singh and Maurya, 1986; Syed, 2009; Uddini et al., 2007; Kumar, 2004), who conducted research on evaluation of mango germplasm in different parts of country and abroad. However, the difference in weight of fruit might be due to difference in climatic conditions and genetic behaviour of genotype.

Fruit size major quality indices play a major role for the success of any fruit variety. Results of fruit size found in agreement with the previous research findings of (Kumar, 2004; Gurmani, 1989), who reported the range for fruit length from 6.93 to 12.00 cm and average fruit breadth from 5.30 to 7.90 cm. The fruit size in terms of length and breadth varied singly and not collectively. This might be due to genetic makeup of individual genotypes.

Various mango genotypes under observation, in general, attained yellowish green colour at maturity. However, the different colour tones of yellowish green were evidenced in different genotypes. The colour rating on the basis of comparison with colour chart of Royal Horticulture Society, London, was of yellow green having different shades 144, 151 and 153. Present findings are in conformity with the previous research work of Singh et al. (1988) who also noted variation in the colour in different mango varieties. The fruits of Amarpali presented the attractive appeal. Hence, this variety can be concerned most attractive amongst different evaluated genotypes.

Variability in pulp content in all the mango cultivar did not find any trend but random and similar results were reported in the past (Syed, 2009; Zaied et al., 2007). The high variability in pulp content ranging from 67.56 to 83.21% of Indian mango varieties have been reported by Desai et al. (2000) amongst 77 varieties under Goa conditions. High range of variability in pulp/stone ratio has also been well documented by Kumar (2004), Zaied et al. (2007), Rajan et al. (2009) in different genotypes of mango.

## Chemical characteristics

High variability with respect to TSS content in different genetic resources collected from Punjab subtropics is in agreement with previous work done on mango germplasm evaluations in the country and abroad. Range for variability is in agreement with the earlier reports of Teaotia and Singh (1963) who reported variation in TSS from 13.8 to 22.0% in some important sucking mangoes of Uttar Pradesh, however, Bhuyan and Guha (1995) and Uddini et al. (2007) observed 16.22 to 24.14% in mango varieties of Bangladesh. Kumar (2004) reported variation in TSS from 18.40 to 21.54° Brix in selection of superior clones in cultivars Dashehari in Himachal Pradesh (Sirmor, Mandi and Kangra Districts). The study of Sharma et al. (1999) on four mango cultivars in arid irrigated region of Punjab is in close proximity with the findings of present study. The higher level of TSS observed in 'Dashehari' collected at government orchard Attari differ from the previous work of Yadav et al. (1982) on 'Dashehari' mango at Uttar Pradesh, also can be referred to the genotype variation in the same variety, may be the prevailing climatic condition. Gurmani (1989) also observed total soluble solids range from 16.25 to 18.75% in different mango genotypes.

The fruit acidity highly depends upon the genotype and climatic condition where genotype generally play larger in defining the quality characters. The variations in fruit acidity were also reported in several studies in past for example Kumar (2004), who observed the range for acidity to the level of 0.14 to 0.34% in different mango varieties. Variations in acidity were also shown by Singh, 1998). However, the different range of variability in acidity has been observed by Singh and Maurya (1986) and Chaudhari et al. (1997) which can be owned to the genetic and climate differences. In the present study, varieties like Kala Gola and Gola exhibited higher acidity and thus their unripe fruits can be imminently suitable for dehydration and pickling. The variety with higher acidity level is not conducive for table purpose but most ideal for pickling. The genotype Kala Gola and Gola are thus suitable for culinary maturity. Selection called as Local Selection-I, when attain full maturity, is attacked by serious pest making it unfit for human consumption but it remains good at culinary maturity, hence it is also utilized for pickles.

Significant difference has been found in reducing sugar (3.40 to 19.27%) content of different mango genotypes evaluated in the present study. Rathore et al. (2009) recorded (3.8%) reducing sugar in Dashehari. Uddini et al. (2007) also reported lowest results regarding reducing sugars content of range from 2.82 to 7.35%. Chaudhari et al. (1997) reported 2.6 to 7.1% reducing sugar in 19 south Indian mango genotypes. Yadav et al. (1982) reported maximum reducing sugar to the tune of 6.86% in Dashehari. The varieties having reducing sugars > 5.0% will be considered suitable for table purposes.

Total sugars have been found variable within the cultivar. Lodh et al. (1974) obtained 7.35 to 13.20% total sugars in eight varieties of mango. Similarly, Singh (1968) and Uddini et al. (2007) recorded the variability for total sugar to the tune of 11.5 to 25 and 12.71 to 20.34%, which might be due to genetic difference as well as agro climatic conditions.

Data regarding TSS/acid ratio showed wide variation. The results are contrary to the findings of Lodh et al. (1974) who recorded TSS/ acid ratio ranged from 5.50 to 109.20. Variation in fruit may be due to inherent genetic variation. Local Selection-I, matured during Ist week of July considered to be as early maturing variety. Dashehari, Langra Banarasi, Langra, Dharbhanga, Alphonso and Hundel were considered to be mid-season varieties that matured during 2$^{nd}$ week of July. While the late maturing varieties Gola, Kala Gola, Amarpali and Chausa matured during 4$^{th}$ week of July. These differences could be due to variation in the genotype evaluated. Uddini et al. (2007) also showed wide variation in TSS/acidity ratio which ranged from 24.19 to 81.57.

## Conclusion

Different genotypes can be used for different purposes. Tree height was maximum in Kala Gola, however, tree spread was recorded maximum in Chausa. Maximum stem girth was registered in Kala Gola and minimum in Amarpali. Maximum fruit weight was exhibited by Chausa. Amongst the evaluated germplasm, Chausa showed the highest pulp/ stone ratio. TSS content ranged from 11.35 to 28.95%. In the identified germplasm of mango 'Malda' contained the highest reducing and total sugars percent. The evaluated genotypes 'Dashehari' ranked first in respect of yield per tree, that is, 148.90 kg/tree.

Local Selection-I was the earliest to mature, therefore it has an advantages as it can easily escape the rainfall. Amongst the genetic resources, Chausa, Kala Gola, Hundel and Gola showed tendency towards regular bearing. Alphonso, Malda, Dharbhanga, Rattaul and Local Selection-II were typical alternate bearer. Local Selection-I and Amarpali were regular bearer. Critical evaluation of mango genotypes grown in Punjab States government orchard Attari has the worth to be isolated for superior traits. Alphonso, Malda and Chausa for TSS/acid ratio; Chausa and Langra Banarasi for high pulp percentage and pulp stone ratio; Rattaul for excellent flavour; Local Selection-I for regular bearing and

Dashehari for higher yield. These identified genotypes can be good donor in hybridization programme to evolve the superior varieties under Punjab conditions.

## Conflict of Interests

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# Effects of soaking on moisture: Dependent mechanical properties of some selected grains essential to design of grain drinks processing machine

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**The effects of soaking on moisture - dependent mechanical properties of some selected grains essential to design of an automatic continuous grain drinks processing machine was studied. Three grain types were used for the study, they are white maize, white sorghum and large seeded soya beans. The white maize and white guinea corn were soaked for 36 h, while large seeded soya beans was soaked for 12 h in accordance to standard procedure (Gaffa et al., 2003; Gbabo et al., 2012). The result obtained showed that moisture content level of the grains increased with increased in soaking time from 12.4 to 20.5% M.C. for corn when soaked for 36 h, 11.3 to 20.6% M.C for sorghum when soaked for 36 h and 10.9 to 20.5% M.C for soya beans when soaked for 12 h. Soya beans had the highest decreases in rupture force with increase in moisture content level (10.9 to 20.5%) from 197.47 to 89.47 N (54.69% decrease), while corn had the least decreases with increase in moisture content level (12.4 to 20.5%) from 266.67 to 170.23 N (36.17% decrease). Also soya beans had the highest increases in rupture energy with increase in moisture content level (10.9 to 20.5%) from 27.79 to 81.95 N mm (194.89% increase), while corn had the least increase with increase in moisture level (12.4 to 20.5%) from 42.626 to 49.40 N mm (15.89% increase). In addition the expected quantity of drink to be produce from the machine under 8 h operational hours per day were 274.4 L from 34.3 kg of soya beans, 520 L from 65 kg of sorghum and 411.2 L from 51.4 kg of corn . The result thus, obtained will assist in designing of the machine and also ensured its maximum performance.**

**Key words:** Soaking, moisture, mechanical, properties, rapture, force, energy and grain drink.

## INTRODUCTION

### Background of the study

Food processing is the transformation of raw ingredients into food, or food into their edible forms. Food processing increases shelf life, digestibility, flavour, nutritive value among other benefits (Mbah et al., 2012). According to Rachel (2010) food processing has a lot of benefits which include toxic removal, enhanced preservation, marketing,

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distribution, increased food consistency and increases seasonal availability of many foods.

Food processing sometimes involves mechanical processes that utilize large mixing, grinding, chopping and emulsifying equipment in the production process. For instance wet milling and wet sieving are unit operations in processing grains into drinks. The wet milling separates the seeds into its various components: germ, protein, fiber, and starch (Jasper, 2005; Gana et al., 2013), while the wet sieving separates the filtrate (milk) which is use for various industrial product including drinks from the residue which is useful as animal feed and can be used for other applications (Gaffa et al., 2003). In order to achieve this objective, various types of milling and sieving equipment have been developed. Earle and Earle (1983) enumerated some of this equipment which includes crusher, plate mills, rollers, ball mills, blenders, plate and frame filter press, rotary filters and centrifugal filters. Simonya et al. (2007) reported that the performance of these machines depends on the machine variables and crop parameters. According to Rachel (2010) the performance parameters for food are essential factors that are considered in designing process for food industry.

Knowledge of mechanical properties (properties that have to do with the behaviours of agricultural product under applied forces) such as stress, strain, hardness and compressive strength is vital to engineers handling agricultural product (Chukwu and Orhevba, 2011). Tavakoli et al. (2009) reported that in order to design equipment in handling, processing and oil extraction from grains, there is need to know their various engineering properties as function of moisture content. These properties are important in designing of separating, sizing, grinding and oil extracting machine. The objective of this study was to study the effect of grain soaking on their moisture - dependent mechanical properties that are essential to design of an automatic continuous grain drinks processing machine

## MATERIALS AND METHODS

### Material preparations

The materials used are of maize (white maize), sorghum (white guinea corn) and soya bean (large seeded). The grains were obtained from Bida central market in Niger state, Nigeria. The grain samples were cleaned, sorted and soaked at recommended duration of 12 h for soya beans and 36 h for sorghum and maize (Gaffa et al., 2003; Gbabo et al., 2012). The soaking softens the grain kernels for milling operation. The selected mechanical properties include rupture force and rupture energy. Also the expected output of the machine was determined.

### Determination of some mechanical properties of the selected grains

The mechanical properties of the selected grains were computed using the formula below and the results are presented in Table 1.

### Determination of grains moisture content

The grains moisture content were determine using a microprocessor digital moisture meter (MC7821), as reported by Simonyan et al. (2007) and Sobukola et al. (2013), and the results are presented in Table 1.

### Mechanical properties of the selected grains

Mechanical properties of the selected grains were evaluated using California Bearing Ratio (CBR) equipment. The equipment was adapted for the test with some of its parts modified to suit the purpose. The CBR is a machine for determining the strength of the soil. It has a loading with a dial gauge attached for measuring the load and a second dial gauge that measure the deformation.

### Test procedure

The compressive tests were carried out following the American Society of Agricultural Engineers Standard 368.1 (ASAE, 2004). The device was equipped with a load cell of 50 KN, the accuracy of measurement for force and deformation was ±0.001 N and ±0.001 mm, respectively. The seed was loaded between the machine plunger heads, compressed at the pre-set condition until rupture occurred. Deformation of the material samples and the force causing the deformation were monitored on the dial gauges attached to the loading. The loading was discontinuous after observing a continuous reduction in the load with increase in deformation which signifies that rupture has occurred. The rupture force, deformation and energy used for rupture were measured at a fixed crosshead speed of 2 mm/min.

### Force deformation

The grain samples were placed between the plunger head of the machine and the base supporting unit, and force-deformation curves were obtained (ASAE, 2004). The amount of force and deformation at rupture point for various grain samples were directly obtained and the result is presented in Table 1.

### Absorbed (rupture energy)

Absorbed (rupture) energy by the grains samples at rupture point was determined by calculating the area under the force-deformation curves from the follow equation given by Ardebili et al. (2012)

$$E_a = FD/2 \quad (1)$$

Where $E_a$ = the rupture energy (Joules), F = the rupture force (N), D = the deformation at rupture point (mm)

### Expected output of the machine

The expected output of the machine is critical in planning for a business and it will also help in quantifying the amount of drink to be produce in a day. It can be determined using the following equation:

$$L_E = L_K M_T \frac{T_{DB}}{T_{TP}} (2) \quad (2)$$

**Table 1.** The effects of change in moisture content levels of soya beans, sorghum and corn on their mechanical properties.

| Parameter | Soya beans (10.9 M.C) | Soya beans (20.5 M.C) | Sorghum (11.3 M.C) | Sorghum (20.6 M.C) | Corn (12.4 M.C) | Corn (20.5 M.C) |
|---|---|---|---|---|---|---|
| Rupture force (N) | 197.56±3.6 | 89.47 ±7.46 | 189.23 ±7.56 | 114.44 ±3.38 | 266.67 ±5.58 | 170.23 ±4.684 |
| Deformation (mm) | 0.281±0.038 | 1.83 ±0.108 | 0.231 ±0.017 | 0.689 ±0.107 | 0.32 ±0.0223 | 0.579 ±0.1499 |
| Rupture energy (N mm) | 27.79 ±4.208 | 81.95 ±9.74 | 21.91 ±2.441 | 39.46 ±5.891 | 42.626 ±2.13 | 49.403 ±13.309 |

**Table 2.** The expected quantity of drinks to be produced by the machine when processing soya beans, sorghum and corn.

| Type of grain | Soya beans | Sorghum | Maize |
|---|---|---|---|
| Initial moisture content (%) | 10.9 | 11.3 | 12.4 |
| Steeping duration (hours.) | 12 | 36 | 36 |
| Final moisture content (%) | 20.5 | 20.6 | 20.5 |
| Volume of grains before soaking ($m^3$) | $8.72\times10^{-4}$ | $9.03\times10^{-4}$ | $8.75\times10^{-4}$ |
| Volume of grains after soaking ($m^3$) | $2.24\times10^{-3}$ | $1.22\times10^{-3}$ | $1.43\times10^{-3}$ |
| Initial mass of grain that after soaking will expand to 0.0016 $m^3$ | 0.5 | 0.94 | 0.75 |
| Expected litres of drink (time) (litres) | 4 | 7.52 | 6 |
| Mass of grain to be process (day) (kg) | 34.3 | 65 | 51.4 |
| Expected litres of drink (day) (litres) | 274.4 | 520 | 411.2 |

$$\textit{where},\quad M_T = \frac{\frac{V_C}{6}R_g}{V_f} M_i (3) \qquad (3)$$

Where, $L_E$ is expected litres of drinks to be produce in a day (8 h/day), $L_K$ is litres of drink that can be produce from 1 kg of grains (before steeping), $M_T$ is mass of grains (before steeping) to be process at a time, $T_{DP}$ is the operating time in a day (480 min), $T_{TP}$ is the processing time at a time (7 min), $V_C$ is the volume of the retaining cylinder ($m^3$), $R_g$is the ratio of grains to water to use for blending, $M_i$is the initial mass of the grain before soaking, $V_f$is the volume of the grain at the highest moisture level.

## RESULTS AND DISCUSSION

### Effect of increase in moisture level on rupture force of the grains

The results obtained from effect of increased in moisture level on rupture force of the grains is presented in Tables 1 and 2, corn with 12.4 M.C had the highest rupture force of 266.67 N while soya beans with 20.5 M.C had the least rupture force of 87.47 N.

From Figure 1, the values of rupture force of soya beans decreases with increase in moisture level (10.9 to 20.5%) from 197.47 to 89.47 N (54.69% decrease), and that of corn also decreases with increase in moisture level (12.4 to 20.5%) from 266.67 to 170.23 N (36.17% decrease), and sorghum had a decrease with increase in moisture level (11.3 to 20.6%) from 187.23 to 114.44 N (39.52% decrease). It was noted that the lower rupturing force were obtained at higher moisture contents for all the grains. This might have resulted from the fact that the grains might have soft texture at high moisture content. Mohammed (2010) and Altunas and Yildiz (2005) reported similar report for corn (*Sc. 704*) and faba been. The lower the moisture content of the grains the more force is required to rupture the grain seeds and this indicates higher cost of operation. On the other hand the more the moisture content (longer duration of soaking) the less force required to rupture the grain seeds. This indicated that soaking of the grains which resulted in increase in moisture content of the grains will reduce the cost of operation in terms of selection of power requirement of the machine. This is in line with similar results obtained by Karaj and Muller (2010).

### Effect of increase in moisture level on rupture energy of the grains

The results obtained from effect of increased in moisture level on rupture energy of the grains is presented in Table 1, corn had the least increased in rupture energy of 6.8 N mm with increased in moisture content level while soya beans had the highest increased in rupture energy of 54.16 N mm with increased in moisture content level.

From Figure 2, the values of rupture energy of soya beans increases with increase in moisture level (10.9 to 20.5%) from 27.79 to 81.95 N mm (194.89% increase), and that of corn also increase with increase in moisture level (12.4 to 20.5%) from 42.626 to 49.40 N mm (15.89% increase), and sorghum had an increase with increase in moisture level (11.3 to 20.6%) from 21.91 to

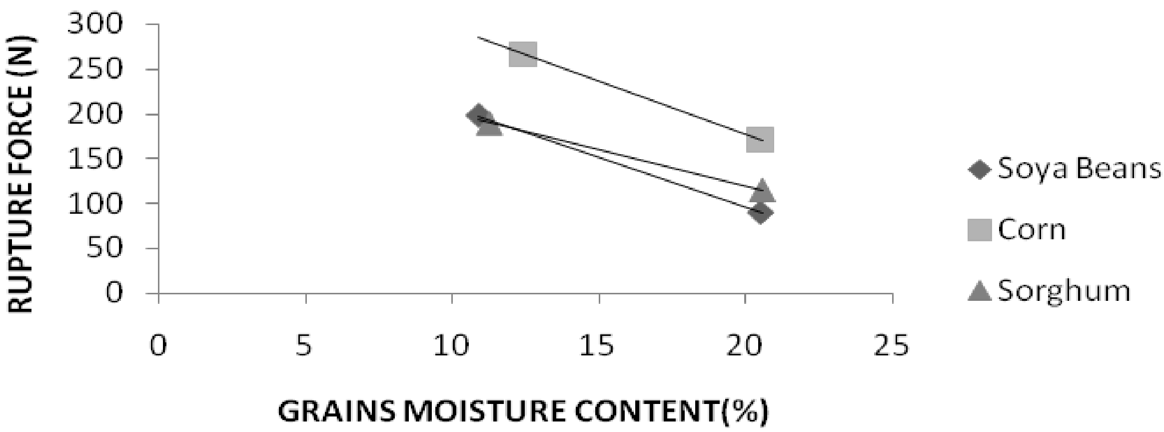


**Figure 1.** Relationship between increased in moisture content and rupture force of the grains.

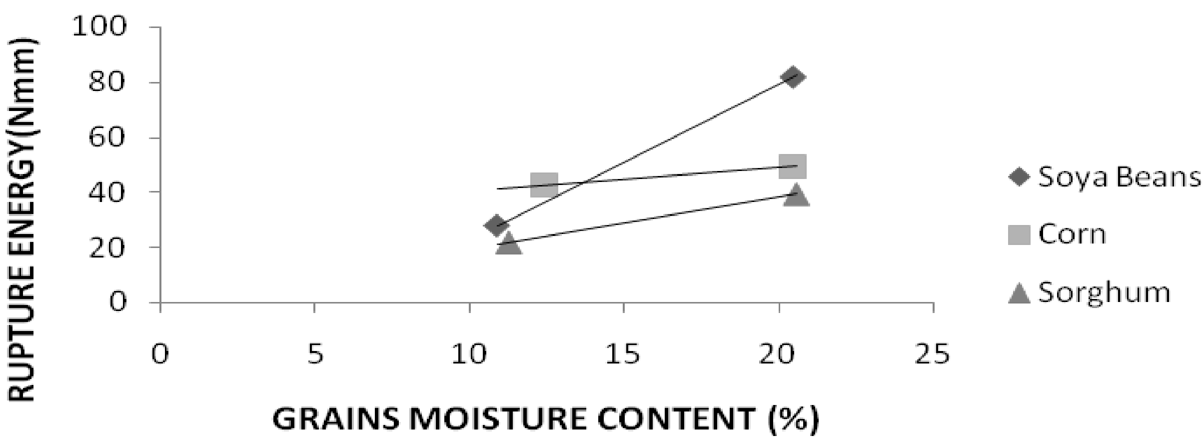


**Figure 2.** Relationship between increased in moisture content and rupture energy of the grains.

39.46 N mm (80.40% increase). It was noted that the lower rupturing energy were obtained at lower moisture contents for all the grains and vice versa. The rupture or absorbed energy was a function of both rupture force and deformation up to rupture point. It was observed that at low levels of moisture content, the grain seeds required high force to be ruptured and the deformation of the seed at that point was observed to be low. While when the grains were soaked which resulted to increase in their moisture content levels, the rupture force required by the grains was observed to be low and the deformation at that point was high. This results are similar to those reported by Guner et al. (2003) for hazelnut and Altuntas and Yildiz (2005) for faba bean.

**Expected output of the machine]**

The quantity of drinks to be produced depends on initial moisture content and volume of the grains. The expected quantity of beverages to be produced from the selected grains when processed using the machine is presented in Table 2. The expected quantity of drink to be produced is evaluated based on the volume of the blending chamber 0.0032 $m^3$, volume of the blending chamber to be occupied by the grains 0.0016 $m^3$. This is $\frac{3}{6}$ of the volume of the chamber, as water will occupy $\frac{2}{6}$ and the remaining $\frac{1}{6}$ of the volume will be left as safety volume. The litres of beverages to be produced from 1 kg of dry grains are approximately 8 L (Africa Do Business Limited, (ADBL) (2012)). Based on this the quantity of drinks to be produce in one phase of operation of the machine is 4 L from 0.5 kg of dry soya beans (bulk density of 780.44 $kg/m^3$), and total of 274.4 L from 34.3 kg processed in 8 h operational hours per day. For sorghum (bulk density of 787.17 $kg/m^3$) is 7.52 L of drink from 0.94 kg of dry sorghum, and total of 520 L from 65 kg processed in 8 h operational hours per day. Also 6 L can be obtain from

0.75 kg of dry corn (bulk density of 768.55 kg/m$^3$), and total of 411.2 L from 51.4 kg processed in 8 h operational hours per day. The least quantity of soya milk to be produced could be as result of high rate of increase in volume of soya beans seed compared to other grains under the study. It was also observed that soya beans when soaked for 12 h expanded 1.5 and 1.88 times more than corn and sorghum respectively when soaked for 36 h.

## Conclusions

The test on the mechanical properties of the grains was concluded. The results obtained were discussed and the following conclusions were made.

(1) Soya beans had the highest decreases in rupture force with increase in moisture content level (10.9 to 20.5%) from 197.47 to 89.47 N (54.69% decrease), while corn had the least decreases with increase in moisture level (12.4 to 20.5%) from 266.67 to 170.23 N (36.17% decrease). In terms of power required the more the soaking duration of the grains the more their moisture content increases and the less the power is required for their rupture.

(2) Rupture energy of soya beans had the highest increases in rupture energy with increase in moisture level (10.9 to 20.5%) from 27.79 to 81.95 N mm (194.89% increase), while corn had the least also increase with increase in moisture level (12.4 to 20.5%) from 42.626 to 49.40 N mm (15.89% increase). The rupture energy of the grains increases with increase in moisture content while rupture force decreases with increase in moisture content (soaking duration) of the grains.

(3) The expected quantity of drink to be produce in 8 h operational hours per day are 274.4 L from 34.3 kg of soya beans ( bulk density of 780.44 kg/m$^3$), 520 L from 65 kg from of sorghum (bulk density of 787.17 kg/m$^3$), and 411.2 L from 51.4 kg of corn (bulk density of 768.55 kg/m$^3$).

(4) These are important factor to be considered when designing as they will provide information on the quantity of grains the machine can process at a time in order to avoid over loading and underutilization of the machine due to change in volume of the grains. They are also essential in determination of expected power require by the machine in order to avoid machine failure and to achieve maximum machine performance.

## Conflict of Interests

The authors have not declared any conflict of interests.

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# Response of tomato (Lycopersicium lycopersicun, CV UC82B) to drip irrigation and planting conditions

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**Studies were conducted in 2007/2008 cropping seasons under a tropical greenhouse (GH) and open field (OF) of the National Horticultural Research Institute (NIHORT) experimental farm. Seeds were sown into the nursery in October, 2007 and January, 2008 for the GH and OF after soil sterilization. Soil samples were analyzed for micro and macro nutrients. Organic manure: Poultry (PM) and horse (HM) wastes and irrigation water were also analyzed using standard laboratory procedures. Drip irrigation and fertilization structures were installed into the GH and OF in 2007 and 2008, respectively. Five week old tomato seedlings were transplanted into the GH in December, 2007 and OF in February, 2008. Organic manure in solution (OMS) was prepared at the rate of 4.2 KgL$^{-1}$ and NPK at 0.6 kgL$^{-1}$. Three levels of drip irrigation consisting of water and three levels of fertigation with two separate tanks containing mineral fertilizer (NPK) and poultry manure were investigated for the GH with corresponding water, NPK and HM for OF. Applications were carried out once ($W_1$), twice ($W_2$) and thrice per week in both GH and OF conditions using split-plot arrangement fitted into randomized complete block design with three replicates. Plants under PM applied at $W_2$ gave the highest yield (7.4 ha$^{-1}$) with significantly higher lycopene formation. Vitamin C was however best under drip irrigation in the GH. B- Carotene was highest under HM for OF. Overall result showed that plants under PM+$W_2$ in the GH had better yield than others. Likewise, lycopene formation was significantly best under PM in the GH which suggests preference of GH tomato production over OF.**

**Key words:** Greenhouse, open field condition, tomatoes, drip irrigation, fertigation.

## INTRODUCTION

Tomato (Lycopersicum lycopersicum, CV UC82B) exhibits medicinal qualities as evident in its flavonoid, carotenoids, Vit. A, Xanthins, lutein, Vit. C and potassium contents (GURA, 1995; AVRDC, 1996; Wener, 2000).

The present world fresh tomato fruit production stands at 100 million metric tons. However, Nigeria ranked the 13$^{th}$ among the fifteen top countries accounting for 1.7% of the world index (FAO, 2010; Shankara et al., 2005) compared with China of production capacity rated 33.9% despite Nigerian's good weather that accommodates and support vegetables cultivation especially tomato. Generally, tomato production in Africa was 300,000 ha, about 7.7% of the world total and rated the 8$^{th}$ largest exporter of the 10 world leading continents. Strikingly, tomato production in Nigeria appears very low in terms of yield per hectare (20 t/ha) (Balarabe, 2012) compared with Brazil (60.7 t/ha), China (48.1 t/ha), USA (81.0 t/ha) and Italy (50.7 t/ha).

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This trend in Nigeria could possibly be due to unavailability of inputs like improved seeds, fertilizer, irrigation and greenhouse facilities.

George et al. (2009) reported that greenhouse production was an intensive investment, but can be very satisfying and rewarding in view of the relatively small area required compared with field grown crops. Basically, greenhouse structures vary from simple homestead to sophisticated prefabricated structures depending on the objective of the structure coupled with financial resource available. Comparatively, greenhouse structures minimize insect and disease incidence as opposed to susceptibility of open field cultivation to myriads of incidences (Wilson, 1993).

Jagdish et al. (2005) estimated that 50% of human populations depend on open field production resulting into about 83 million metric tons of N. About 50% of this applied N is not assimilated by plants (Tilman et al., 2002; Doberman and Cassman, 2004) thereby contributing to groundwater contamination, eutrophication, acid rain, ammonia deposition, global warming and stratospheric zone depletion with acute environmental pollution (Howarth et al., 1996, Van Drecht et al., 2001). Problems with over fertilization (Jagdish et al., 2005; Fageria and Balligar, 2005) necessitate research in the proper amounts of fertilizer for sustainable production. Organic manure is generally believed to be characterized with slow release of N allowing more effective uptake by plants and reduction of N leaching or denitrification losses (Fageria and Balligar, 2005).

Shoji et al. (2001) and Shoji and Kano (1994) confirmed that slow release of N facilitates reduction in N fertilization rates that contributes to conservation of air and water quality. Studies compared soils of organically and conventional inorganically managed farming systems and confirmed that higher soil organic matter and total N were obtained from organically farming systems with more improved nitrogen used efficiency (NUE) (Irshad et al., 2002; Clark et al., 1998; Chang et al., 1993; Ma et al., 1999).

Result of analysis carried out (Afolayan et al., 2008) indicated that cow, horse, pig and poultry manures were capable of producing 11.54% $CH_4$, 5.32% $CO_2$, 0.06% $N_2O$; 11.42% $CH_4$, 16.42% $CO_2$, O.14% $N_2O$; 11.12% $CH_4$, 16.62% $CO_2$, 0.13% $N_20$ and 15.51% $CH_4$, 7.88% $CO_2$, O.14% $N_2O$, respectively.

These gases when allowed to diffuse into the air could constitute nuisance and aggravated global warming. However, conversion of these wastes into compost has been reported to prevent production of $CH_4$ and other noxious gases (USEPA, 2003). Similarly, organic manure solution as fertigation is capable of circumventing production of gases in sufficient quantity with reduced effects on global warming. Much work had been done on drip irrigation and fertigation for crop production in developed countries (Hartz, 2005; Sharma and Sharma, 2002; Garcia et al., 2000; Holmes and Schnitzler, 1997), and specifically on vegetables (Alexander et al., 2006; Eunmi et al., 2012) little is reported in Nigeria especially as related to tomato production. This study was undertaken to determine responses of tomatoes to drip irrigation and fertigation in the greenhouse and open field.

## MATERIALS AND METHODS

The experiment was conducted at the National Horticultural Research Institute (NIHORT) experimental field Ibadan. Ibadan lies between longitude 3° $56^1$ and 3° $52^1$ E and latitude 7° $23^1$ and $7^0$ $28^1$ N with an altitude of 168 m above mean sea level. Seeds of tomato, CVUC82B, an indeterminate type of tomato variety compatible with both greenhouse and open field productions were planted into the nursery on $27^{th}$ October, 2007 for the GH experiment and January 3, 2008 for the open field (OF) to ensure similar condition to the greenhouse thereby eliminating the possibility of interruption by rainfall, since cessation of rain effectively occurs between the last week of October and the first week of November at the experimental location. A rectangular shaped greenhouse with effective cropping area of 5.42 $m^2$ (58.3 ft2) with ventilated side wall and raised concrete platform was used for this study. The orientation of the greenhouse was skewed to north-east direction and covered with an ultraviolet stabilized medium-density glass film of 4 cm thickness. The cropping area of the greenhouse (GH) was filled with sandy-loamy soil to the depth of 28 cm at 22.4 $m^3$ soil volume and was allowed to stabilize before physical and chemical properties were conducted for nutrients status. The soil was sterilized by subjecting the surface to burning with plant residues for about 10 min and allowed to attain optimum temperature before planting to prevent microbial predators with potentials soil-borne pathogens. The action was repeated for the OF.Irrigation water samples, PM and HM were analyzed following standard laboratories at the university of Ibadan soil analytical laboratory. Drip irrigation and fertigation systems were installed on separate platforms independent of each other in the GH and OF on $14^{th}$ December, 2007 and 15 January, 2008, respectively. The gravity propelled drip irrigation and fertigation systems were mounted on 2 m elevated prefabricated slabs as head capable of injecting 20 mm depth of water. Drip lines were made of non-pressure compensating drippers and kept at low length and connected with 4 mm adaptors.

The arrangement was made to ensure slow release of water directly to the root zones of the seedlings thereby soaking the root zone before any noticeable evaporation or run off could take place. This method thus guarantees minimum water coverage within the cropping area thereby controlling weed infestation and excess moisture on the plants with resultant effect of disease incidence.

The drip lines were flushed and cleaned with water to ensure clog-free drippers. Emitter (drippers) discharge was determined by placing empty containers at discharge points operated at 45° throttling of water valve and run for 60 s at three replications of 1.72 and 2.7 t/ha for GH and OF, respectively. The PM used for fertigation in the GH and HM for OF fertigations were dissolved in water and sieved with B540 standard sieve size (150 cm) until a soluble solid content of 4.2 kg was achieved. One thousand litre plastic storage tanks were used in all cases in accordance with greenhouse specifications (Richards, 2010).

Tomato seedlings were transplanted into the GH on $17^{th}$. December, 2007 and OF in February, 2008, five weeks after sowing (WAS) arranged in split-plot fitted randomized complete block design (RCBD) with three applications. Plant spacing was 0.3 by 0.75 m plot size of 5.42 $m^2$ transplanting to twenty four plants, an average of four seedlings per plot. Drip irrigation and fertigation

**Table 1.** Meteorological elements of the experimental period (2007/2008).

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Total rainfall (mm) | 16.9 | 68.5 | 237.1 | 222.8 | 255.3 | 138.7 | 286.3 | 245.4 | 132 | 3.4 |
| Air temp (%) | 29 | 29.1 | 28 | 26.4 | 25.2 | 25.1 | 26 | 27 | 27.4 | 27 |
| Max temp (%) | 40 | 35 | 33 | 31 | 30 | 29 | 31 | 32 | 33 | 34 |
| R. humidity (%) | 86 | 89 | 90 | 91 | 91 | 92 | 91 | 91 | 91 | 87 |
| Evaporation (mm) | 6.3 | 6.1 | 5.2 | 5.3 | 4 | 4 | 4.3 | 5 | 4.1 | 4.7 |
| 2008 (rainfall) | 88.8 | 181.8 | 234.4 | 372.8 | 250.7 | 221.4 | 401.1 | 273 | 27.2 | 20 |
| Rainy days | 4 | 6 | 17 | 20 | 20 | 19 | 20 | 16 | 3 | 2 |

**Table 2.** Physiochemical properties of water and manure.

| | Chemical properties | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Treatment** | **PH** | **Ca** | **Mg** | **Na** | **K** | **C** | **N** | **P** | **Cu** | **Zn** | **Fe** | **Mn** | **$SO_4^{2-}$** | **$CL^-$** | **$HCO_3^-$** | **$CO_3^{2-}$** |
| Water 1 | 6.5 | 1.35 | 0.28 | 19.78 | 3.01 | Nd | Nd | 5.05 | | 0.09 | Nd | Nd | 1.05 | 35.27 | 164 | 0.5 |
| Poultry | 5.3 | 38.75 | 7.64 | 9.46 | 3.76 | 30.4 | 1.64 | 48.83 | 57.85 | 1.23 | 21.25 | 3.15 | 5.76 | Nd | nd | Nd |
| Water 2 | 7.0 | 11.33 | 5.99 | 21.93 | 10.75 | Nd | Nd | 3.44 | 6.0 | Nd | Nd | Nd | 17.55 | 69.16 | 208 | 0.4 |
| Horse | 7.5 | 12.4 | 8.99 | 16.77 | 16.13 | 33.57 | 1.68 | 16.38 | 0.75 | 0.75 | 21.40 | 1.56 | 3.56 | nd | nd | nd |

treatments using control (drip), NPK (20-10-10), PM, in semi-soluble solution at the rates of 1.7 LhL-$^{1}$ , 1.8 Lh-$^{1}$ and 1.86 Lhr$^{-1}$, respectively were applied under GH, control, NPK (20-10-10) and HM in semi-soluble solution at the rates of 1.7 Lhr-$^{1}$ , 1.8 Lhr-$^{1}$ and 2.7 Lhr-$^{1}$, respectively were also applied under OF following the same design as GH. The variation in the emission rate of PM and HM was attributed to the composition of the two wastes. While PM was a formulated waste, HM was more of plant and other material residues which constitute the ration of horses as free-range animals.

Applications of water and fertilizer through drip irrigation and fertigation under both GH and OF continued until the crop reached full maturity after at least three harvestings when the plants growth and further fruit set were terminated due to physiological maturity. Plant height (PHT), number of branches (NOB), stem diameter (SG), number of flowers (NOFL) and fruit yield (FYLD) were determined. Environmental parameters (weather elements) of the periods were recorded. Data were pooled together and analyzed with analysis of variance (ANOVA) after sufficient processing using statistical analytical software (Vol. SAS Inc. Cary. NC).

## RESULTS AND DISCUSSION

The weather conditions of 2007/2008 cropping seasons appeared similar. The months of January to March, the most active periods indicated dry spells with little or no significant effect of atmospheric moisture (rainfall) on growth and fruit yield of the crop (Table 1).

The physiochemical properties of the different planting media which include control, PM, and HM before and after investigation are presented on Tables 2 and 3. The PH values of the water samples changed from 6.5 to 6.3 and 7.0 to 6.9 for Water 1 and 2, respectively suggesting insignificant implications on crop performance since the variation was below the acceptable limit (George et al., 2007) typical of irrigation water samples containing a certain degree of dissolved salts. Similarly, the N-values (0.17 and 0.15%), of the mineral fertilized used was within the same range indicating the stability of water samples void of contamination sufficient enough to corrupt the results of the experiment.

However, there were observed sharp changes in the $P^H$ range of PM and HM (Table 2). While the $P^H$ value of PM increased at 20.8% of the initial, HM decreased at 17.3% (Tables 2 and 3). This trend was however expected, considering the amount of precipitates typical of HM ration of predominance plant materials compared to the formulated PM easily broken down by microbial activities (George et al., 2009). Likewise, the concentration of the mineral fertilizer (NPK) used tagged NPK1 and NPK2 was due to different sources of product acquisition and was with the

**Table 3.** Physiochemical properties (residual) of experimental soils.

| | Chemical properties | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Treatment** | **PH** | **Ca** | **Mg** | **Na** | **K+ (mel/100 g** | **H+AL** | **ECEC** | **Base Sat** | **C (%)** | **N (%)** | **AvP (ppm)** | **Cu** | **An** | **Fe (mg/kg)** | **Mn (Mg/kg)** | **$HCO_3^-$** | **$CO_3^{2-}$** |
| Water 1 | 6.3 | 16.35 | 0.94 | 1.42 | 6.62 | 0.08 | 19.41 | 99.59 | 2.27 | 0.17 | 25.0 | 0.34 | 23.24 | 21.05 | 78.47 | nd | nd |
| PM | 6.4 | 17.46 | 0.83 | 1.38 | 0.68 | 0.09 | 20.44 | 99.59 | 2.59 | 0.25 | 44.9 | 0.99 | 25.25 | 26.20 | 79.61 | nd | nd |
| NPK 1 | 5.6 | 17.95 | 0.69 | 1.62 | 0.93 | 0.1 | 21.3 | 99.48 | 1.95 | 0.4 | 47.35 | 0.49 | 20.89 | 39.20 | 7512 | nd | nd |
| NPK 2 | 5.5 | 4.68 | 1.04 | 0.56 | 0.25 | 0.08 | 6.61 | 98.79 | 0.49 | 0.16 | 31.15 | 0.53 | 3.77 | 5.80 | 15.77 | nd | nd |
| Water 2 | 6.9 | 5.08 | 1.43 | 0.56 | 0.21 | 0.06 | 7.34 | 99.18 | 0.51 | 0.15 | 32.50 | 1.21 | 4.88 | 8.25 | 15.15 | nd | nd |
| HM | 6.2 | 6.59 | 2.08 | 0.49 | 0.25 | 0.07 | 9.58 | 99.27 | 0.52 | 0.17 | 21.25 | 1.93 | 2.71 | 7.90 | 17.17 | nd | nd |

Water 1 = Irrigation water for the green house, Water 2=Irrigation water for the open field; NPK 1, NPK 2 – fertilizer in GH and OF respectively; nd= not detected.

**Table 4.** Proximate analysis of Tomato fruit.

| | Proximate properties | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Treatment** | **Ca (mg/kg) ($mg.kg^{-1}$)** | **Mg (mg/kg)** | **Cu (mg/kg)** | **Zn (mg/kg)** | **Fe (mg/kg)** | **P (mg/kg)** | **Na** | **K** | **N (%)** | **Vit.C** | **Lycopene** | **B-Carotene** |
| Water 1 | 15.57 | 42.71 | nd | 2.21 | 4.2 | 118.18 | nd | nd | 0.15 | 36.20 | 6.09 | nd |
| PM | 25.61 | 60.43 | nd | 2.27 | 5.06 | 249.64 | nd | nd | 0.14 | 24.50 | 8.84 | nd |
| NKP 1 | 32.18 | 28.29 | nd | 2.30 | 4.54 | 113.76 | nd | nd | 0.17 | 32.0 | 5.22 | nd |
| Water 2 | 18.20 | 32.0 | 54.45 | 50.20 | 400 | 310.9 | 19.30 | 45.56 | 2.11 | 28.40 | 12.60 | 0.68 |
| NPK 2 | 17.69 | 46.17 | 14.50 | 8.38 | 11..41 | 533.8 | 18.28 | 4.35 | 2.02 | 19.20 | 17.00 | 0.51 |
| HM | 13.78 | 36.99 | 8.30 | 6.28 | 11.53 | 5.34 | 11.28 | 38.48 | 2.11 | 17.50 | 14.80 | 0.60 |

some level of acidity to the solution used in fertigation.

Results of the macro and micro elements conducted before and after experimentation are presented on Tables 2 and 3. The primary macro nutrients comprising nitrogen (N), phosphorus (P) and potassium (K) concentrations indicated 1.64 meg/100 gN, 48.83 meg/100 P and 3.76 meg/100 g for PM, 1.68 meg/100 gN, 16.38 meg/100 gP and 16.13 meg/100 K for HM. The K and N values in HM were higher than PM suggesting the ability of HM capable of supplying higher nutrients essential for plant physiological growth (Bianw, 1995).

However, the available phosphorous in PM was more superior than HM thereby revealing the possibility of plants grown in PM medium exhibiting less susceptibility to restricted root development and stunted growth (Bianw, 1995). The secondary macro elements showed concentrations levels in PM and HM as 38.75 meg/100 gCa, 7.64 meg/100 gMg, 12.4 meg/100 Ca and 8.99 meg/100 gMg, respectively. The concentrations in both PM and HM media were however adjudged sufficient for tomato especially when applied in solid form about two weeks before planting. The micro-elements reflected a similar trend with the macro-elements (Table 2).

Table 3 showed the physiochemical properties of the fertilizers after experimentation. Evidences of effective breakdown of element were noticed in all the brands of fertilizers used which suggested uniform utilization of soluble solids (Table 3). Physiochemical compositions of the tomato products obtained from the effect of drip irrigation and fertigation are presented in Table 4. Mean

**Table 5.** Effect of GH and OF drip irrigation and fertigation on growth parameters and yield of tomato.

| TRT | PHT | SD | NB | NFL | YLD t/ha | BM t/ha |
|---|---|---|---|---|---|---|
| $G_1W_1$ | 39.5 | 2.3 | 14 | 3 | - | 50-5 |
| $G_1W_2$ | 94.4 | 4.9 | 36 | 5 | 6.81 | 83.4 |
| $G_1W_3$ | 119 | 6.2 | 44 | 6 | 4.31 | 88.8 |
| $G_2W_1$ | 96.3 | 5.3 | 44 | 5 | 3.34. | 83.4 |
| $G_2W_2$ | 91.6 | 4.8 | 28 | 5 | 7.40 | 74.0 |
| $G_2W_3$ | 118.3 | 7.0 | 37 | 6 | 3.75 | 95.7 |
| $G_3W_1$ | 78.5 | 4.5 | 27 | 5 | 1.35 | 70.0 |
| $G_3W_2$ | 66.5 | 4.0 | 24 | 2 | 2.98 | 65.7 |
| $G_3W_3$ | 98.8 | 5.4 | 32 | 3 | 4.94 | 98.6 |
| **LSD (5%)** | **21.5** | **1.0** | **9.9** | **3** | **2.92** | **43** |
| $F_1W_1$ | 25.9 | 7.4 | Ng | 6 | 3.3 | Ng |
| $F_1W_2$ | 32.5 | 8.2 | Ng | 2 | 3.1 | Ng |
| $F_1W_3$ | 45.3 | 10.2 | Ng | 21 | 2.8 | Ng |
| $F_2W_1$ | 44.0 | 10.4 | Ng | 8 | 3.5 | Ng |
| $F_2W_2$ | 30.0 | 8.1 | Ng | 7 | 0.03 | Ng |
| $F_2W_3$ | 330 | 7.6 | Ng | 9 | 1.67 | Ng |
| $F_3W_1$ | 29.6 | 6.0 | Ng | 4 | 0.67 | Ng |
| $F_3W_2$ | 26.1 | 6.0 | Ng | 9 | 3.1 | Ng |
| $F_3W_3$ | 27.9 | 5.1 | Ng | 7 | 1.0 | Ng |
| **LSD (5%)** | **8.0** | **1.4** | **Ng** | **5** | **3.0** | **Ng** |

$G_1W_1$ = Green house + watering once per week; $G_1W_2$ = green house + watering twice per week; $G_1W_3$ = green house + watering twice per week; $G_2W_3$ = Fertigation with NPK once per week in the green house; $G_2W_2$ = Fertigation with NPK twice per week in the green house; $G_2W_3$ = Fertigation with NPK three times per week; $G_3W_1$ = Fertigation with PM once per week in the green house; $G_3W_2$ = Fertigation with PM twice per week in the green house; $G_3W_3$ = Fertigation with PM three times per week; $F_1W_1$ = open field water application once per week; $F_1W_2$ = Open field water application twice per week; $F_1W_3$ = Open field water application three times per week; $F_2W_1$ - fertigation in the open field with NPK once per week; $F_2W_2$ - fertigation in the open field with NPK per week; $F_2W_3$ - fertigation in the open field with NPK three time per weeks; $F_3W_1$ - fertigation in the open field with horse manure once per week; $F_3W_2$ - fertigation in the open field with horse manure twice per week, $F_3W_3$ - fertigation in the open field with horse manure three times per week; Ng – Negligible.

same $P^H$ range of 5.6 and 5.5. The percent N. composition of 20% in the minerals fertilizers (20-10-10) was sufficient enough to introduce values of Lycopene, B-carotene and Vitamin C indicated 36.2% Vitamin C, 6.09% Lycopene, and B-carotene for PM; 32% Vitamin C, 5.22% Lycopene, and B-carotene for plants under Water 1; 28.4% Vitamin C, 12.60% Lycopene, 0.68% B-carotene for plants under Water 2; 19.2% Vitamin C, 17.0% Lycopene and 0.60% B-carotene and 17.5% Vitamin C, 14.8% Lycopene and 0.60% B-carotene confirming the work of Renata et al. (2013). Vitamin C concentrations were highest in water based drip irrigation with corresponding mean values in PM and HM. The variation in Vitamin C content might possibly depend on environmental conditions as reported by Martinez et al. (2002) that variation in light intensity prior to harvest has a great influence on Vitamin C content.

Obviously, the tomato plants in the GH have the tendency of greater concentration of light intensity compared to the diffused nature typical of OF. However, Lycopene content was highest in NPK2 based fertigation followed by HM based fertigation. This observed trend might not be unconnected with the molecular structure of Lycopene as a liposoluble micronutrient thereby suggesting more tendency to remain in semi-soluble form in NPK than in organic materials like PM and HM, thus affecting the concentrations (Djuric and Powell, 2001). Contrariwise, B-carotene was more superior under HM in OF than others, although confirming the studies of other researchers (Renata et al., 2013; Bourn, 2002; Clinton, 1998).

The superiority of B-carotene in HM might not be unconnected with the degree of exposure of the tomato fruits under HM based fertigation in OF to light, temperature and degree of fruit ripeness thereby stimulating accumulation in accordance with the report of Abuslita et al. (2000). Effects of GH and OF drip irrigation and fertigation on growth parameter and fruit of tomato are presented in Table 5. Plants under GH using drip and watering thrice per week ($G_1W_3$) recorded the highest mean plant height, although one of the least fruit yields. This might be due to the difficulty in reproductive ability of transforming its flowers to fruit resulting from shortage of nutrients typical of ordinary water sample. Strikingly, the highest fruit yield was obtained under NPK based fertigation in combination with watering twice per week ($G_2W_2$). This phenomenon is however, expected in view of high mobility of typical mineral fertilizer with little or no constraints in the release of the nutrients for plant growth and fruit set compared to extremely low mineralization and consequent release of active nutrients for plant growth except when applied at least two weeks before planting in confirmation of Montagu and Goh (2012). Overall results showed that mean values of Lycopene and B-carotene formation were superior under HM while fruit yield was best under mineral fertilizer.

## Conclusion

Discoveries in the nutrient status of tomato had ranked the crop as a very powerful anti-oxidant that fights against many forms of cancer. Tomatoes are susceptible to various diseases most of which are caused by high humidity and air pollutants. However, water and soil nutrients are regarded as the most two important critical inputs in tomato production. Therefore, the need for in-depth study on drip irrigation and fertigation to optimize its water use efficient. In addition, green house intervention becomes the best alternative to screen off diseases and air borne vectors typical of open field cultivation. In this study, GH production using poultry in solution for fertigation appears more superior to expose open field cultivation and the conventional practice in Nigeria.

## Conflict of Interests

The authors have not declared any conflict of interests.

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# Physiological and biochemical performance of *Pouteria ramiflora (*Mart.) Radlk. seeds harvested at different maturation stages and subjected to drying

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**This study aimed to evaluate alterations in the physiological and biochemical performance of *Pouteria ramiflora* (Mart.) Radlk. seeds at different maturation stages when subjected to drying. The seeds were harvested at two maturation stages (unripe and ripe). A sample of seeds at each maturation stage was not subjected to drying (control), and another sample was subjected to drying in a convection oven at 35°C until the seeds reached water content levels of 20 and 12% wet basis (w.b.). The drying of seeds up to 12% bu. negatively affected the physiological quality and vigor of the seeds, particularly in the unripe seeds. There was no detectable activity of catalase and peroxidase isoenzymes, and heat-resistant proteins were not observed on denaturing polyacrylamide gels. The α-amylase enzyme was most active in mature and moist seeds and showed the negative effect of drying, particularly with respect to unripe seeds. A greater intensity of esterase isoenzyme bands was detected in unripe seeds (regardless of water content) with little reduction of activity as the water content of mature seeds decreased. It can be concluded that the *P. ramiflora* (Mart.) Radlk. seeds show physiological and biochemical alterations when subjected to drying, they are intolerant to desiccation.**

**Key words:** Forest species, physiological maturity, desiccation tolerance, isoenzymes.

## INTRODUCTION

*Pouteria ramiflora* (Mart.) Radlk., locally known as ‘curriola’, ‘abiú-do-Cerrado’, ‘abiu-piloso’, ‘bacupari liso’, ‘fruta-de-veado’, ‘grão-de-galo’, ‘massaranduba’, ‘gunjara’, ‘mandapuca’ and ‘pitomba-de-leite’, is a woody fruit species that is widely distributed in the areas of Cerrado (Tropical Savanna); it is found in the Cerradão (Savanna Forest), Cerrado sensu stricto, Cerrado ralo (Open Cerrado), Borda de vereda (Palm-swamp border area) and mesophytic forest phytophysiognomies (Almeida et al., 1998). It has small hermaphrodite or

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pistillate flowers arranged in racemose inflorescences with short axes that are densely arranged in leaf axils along the branches. Flowering occurs from May to September, with open flowers peaking in August (during the dry season); the fruit ripens during the rainy season, which promotes seed dispersal (Gama et al., 2011). The fruit has a strong smell and pleasant taste; it is greenish in color with white pulp and is appreciated by local people as juice or *in natura* (Dalponte and Lima, 1999). Permanent preservation areas may be sought because it is a species suited for open environments (Lorenzi, 1992). There were no reports found in the literature about the physiological performance of its seeds in response to water loss. Thus, the physiological characteristics of seeds must be assessed because of their ecological relevance and economic potential with the aim of establishing conservation strategies and contributing to create protocols for the production of seedlings of this species. The physiological condition of the seed was initially studied by Roberts (1973), who classified the seeds as orthodox or recalcitrant according to their performance in storage. Following this, Ellis et al. (1990) introduced the concept of intermediate seeds. Because this physiological aspect is related to the degree of seed tolerance to dehydration, these should be classified as desiccation-tolerant or orthodox seeds, desiccation-intolerant or recalcitrant seeds, and intermediate seeds (whose performance during drying and storage is sometimes similar to orthodox seeds and sometimes similar to recalcitrant seeds).

The standard orthodox seed development is characterized by the following three main phases: embryogenesis (histodifferentiation), in which the fertilized zygote undergoes multiple cell division; maturation or seed filling, in which synthesis of storage reserves, rapid cell expansion and dry matter accumulation occur; and, finally, desiccation and quiescence, in which dehydration occurs until reaching equilibrium with the relative humidity of the air and showing relatively low moisture content by harvest (Jiang and Kermode, 1994; Angelovici et al., 2010). Typically, orthodox seeds tolerate desiccation and (most likely) depend on it to redirect the metabolic processes of development towards germination. With respect to drying, there are two distinct periods during seed development: desiccation-intolerant (first phase and part of the second) and desiccation-tolerant (part of the second phase and the entire third phase). Moreover, recalcitrant seeds lose little water during maturation (moving directly from development metabolism to germination), are susceptible to desiccation, and normally maintain moisture contents between 30 and 70% (Barbedo and Marcos-Filho, 1998; Marcos-Filho, 2005).

Plant cells require physiological, biochemical and morphological adaptations that are temporally coordinated by differential expressions of genes capable of preventing cell damage or death as water content is removed during seed development. The mechanisms of desiccation tolerance in angiosperms appear to have originated ancestrally from algae and endosymbiotic progenitors of mitochondria and chloroplasts in plants (Gaff and Oliver, 2013). The acquisition of desiccation tolerance is regulated and the possible mechanisms of tolerance are activated only at precise moments following fertilization; vegetative tissues must respond to environmental signals to activate protection mechanisms for the entire plant. Environmental signals of extreme water loss may conceivably activate an existing repertoire of genes that specifically protects seeds and vegetative tissues (Illing et al., 2005).

Estimates suggest that approximately 50% of tropical species produce seeds sensitive to desiccation (Tweddle et al., 2003). Desiccation tolerance in species able to withstand that process is acquired in the phase of reserve accumulation and may be induced artificially by slow drying at certain developmental stages. Abscisic acid (ABA) has a key role in desiccation tolerance in orthodox seeds and also controls the accumulation of reserve substances (Nambara et al., 2010). The concentration of ABA during development decreases near the end of this phase while desiccation tolerance increases (Pammenter et al., 1994). Seeds undergo changes at different stages of development and become more tolerant to higher temperatures of drying with gradual water loss after physiological maturity, which indicates that events occur concomitantly with the reduction of water content (Rosa et al., 2004).

Several biochemical changes occur in seed cells in response to desiccation. Stresses associated with extreme water loss include mechanical stress associated with the loss of turgor, free radical oxidative stress, and destabilization or loss of macromolecular integrity (Walters et al., 2002). In *Arabidopsis*, the transition between reserve accumulation in seeds and desiccation is associated with a significant change in their metabolism that results from the accumulation of a variety of sugars, organic acids, nitrogen-rich amino acids and shikimate-derived metabolites (Fait et al., 2006).

There is a change in seed gene expression with the onset of drying, in which genes encoding protective molecules play a key role in desiccation-tolerant tissues (Ramanjulu and Bartels, 2002). The acquisition of desiccation tolerance, the ability to tolerate very low water potentials is correlated with the accumulation of various protective compounds, including sugars and proteins. These compounds include heat shock proteins (HSP), free radical-scavenging enzymes and non-reduced sugars or low molecular-weight solutes, which typically accumulate during the late stages of embryogenesis and coincide with the acquisition of desiccation tolerance. A special group of proteins known as late embryogenesis accumulated (LEA) proteins become abundant in the final phase of seed maturation with the genetic control exerted by the phytohormone

ABA; these proteins most likely act as chaperones, protecting macromolecular structures from damage caused by desiccation (Hoekstra et al., 2001; Manfre et al., 2009). Formation of reactive oxygen species (ROS), occurs during seed desiccation, which can lead to oxidative stress and cell damage and may result in deterioration. However, cells express detoxifying enzymes and produce antioxidant compounds that remove the ROS. Thus, the detoxification mechanisms play a key role in the acquisition of desiccation tolerance in developing seeds (Bailly, 2004). Therefore, this study was performed to evaluate desiccation tolerance enabled through changes in the physiological and biochemical performance of *Pouteria ramiflora* (Mart.) Radlk. seeds at different maturation stages after being subjected to drying.

## MATERIALS AND METHODS

Fruits were collected at Fazenda Gameleira [Gameleira Farm] (16° 06' 20'' S – 51° 17' 11'' W, 592-m altitude), in the municipality of Montes Claros de Goiás, in December 2011. The research was performed in the Laboratory of Seed Analysis, Department of Agriculture, Federal University of Lavras, Minas Gerais (MG). The fruits were manually pulped and the seeds were washed in 2% sodium hypochlorite solution for one minute and then rinsed in distilled water. After washing, the seeds were placed in plastic trays covered with paper towels to remove excess water. The seeds were then divided into two groups according to the stage of their fruit ripening; the seeds from fruits picked from the ground under trees were considered mature, whereas seeds from fruits picked directly from the plant were considered unripe. The water content of seeds, unripe and ripe, was assessed using the air-oven method at 105±3°C for 24 h (Brasil, 2009) with four subsamples of 10 seeds. The calculation was performed on a wet basis (w.b.), and the result was expressed as a percentage. After assessing the initial water contents, some of the seeds were packed in paper bags and kept in a convection-drying oven at a temperature of 35±2°C. The sample mass was assessed using a precision scale every hour until values of water content of 20 and 12% w.b. were reached. Seed water loss was assessed using the formula recommended by the following rules for seed analysis (Brasil, 2009).

$$Wf = Wi \cdot \left(\frac{100 - WCi}{100 - WCf}\right)$$

where Wf: final sample weight (g); Wi: initial sample weight (g); WCi: initial seed water content (% w.b.); and WCf: desired seed water content (% w.b.).

Seeds at both maturation stages and with different water contents were evaluated with respect to the following parameters: germination (%); germination rate index; emergence (%); emergence rate index; X-ray test; electrical conductivity; respiration rate; quantification of reducing, non-reducing and total soluble sugars; the electrophoretic profile of enzymes (catalase, esterase and peroxidase) on a non-denaturing gel and of heat shock proteins by electrophoresis on a denaturing polyacrylamide gel; and the enzymatic activities of α-amylase and endo-β-mannanase. Seeds were subjected to manual scarification as a pre-germinative treatment, using sandpaper No. 50 (against the micropyle) to accelerate the process of soaking and, consequently, of germination.

### Germination test

Sowing was performed on germitest paper rolls with two sheets as the base and one for the cover; for the germination test, these sheets were moistened in distilled water at a ratio of 2.5 times the dry substrate weight (Brasil, 2009) with four replicates of 25 seeds. The rolls containing the seeds were kept in a germinator set at 30°C. Daily assessments were performed until complete stabilization to calculate the germination rate index (GRI), according to Maguire (1962), and the seeds with 1.0 cm radicle protrusion were considered to be germinated. Only normal seedlings in which all essential structures were developed (well-developed primary root, and developed hypocotyl and epicotyl) 45 days after sowing were analyzed to assess the germination percentage.

### Emergence test

A total of 100 seeds divided into four replicates of 25 each were used. Sowing was performed using a 40x30x8 cm plastic tray, containing a mixture of subsoil and sand at a ratio of 3:1 as a substrate. Sowing was performed at a depth of 3 cm; the substrate was moistened to 70% of its field capacity and subsequently irrigated when necessary. The trays were kept in a growth chamber (at 25°C) with an alternating light-dark cycle (12 h) for 30 days. Daily counts were conducted to assess the seedling emergence rate index (ERI), according to Maguire (1962), using as a criterion the emergence at a height of 1.5 cm (from the soil) for counting. After complete stabilization, the emergence percentage (E%) was also evaluated by analyzing only normal seedlings (well-developed primary and secondary roots, and expanded hypocotyl, epicotyl and foliage leaves).

### X-ray test

Images of the internal structures of seeds were recorded using an X-ray device to evaluate the structural changes during desiccation and for showing possible damage resulting from the drying process. The seeds from each treatment were arranged in clear acrylic plates on double-sided adhesive tape and subjected to radiation using a Faxitron HP X-ray device, Model 43855A X, at 45 kV for 25 s to record digital images of the internal structures of seeds.

### Electrical conductivity test

This test was performed with four subsamples of 10 seeds that were previously weighed on an electronic scale accurate to 0.001 g and then placed in plastic cups containing 75 ml of deionized water and kept in a biochemical oxygen demand (BOD)-type germination chamber set at 25°C for 24 h. After this period, the electrical conductivity of the soaking water was assessed using a benchtop digital conductivity meter, manufactured by Digimed, model CD 21A; the results were expressed as $\mu S.cm^{-1}.g^{-1}$.

### Respiration rate

The respiration rate was assessed using the titration method, according to the methodology reported by Crispim et al. (1994). A glass Erlenmeyer flask with a fine screen attached to the mouth as support for the seeds was used for each replicate. Two sheets of blotting paper were wrapped in the screen and moistened with distilled water at a ratio equivalent to 2.5 times the dry weight; the seeds were placed over the wrapped screen. Forty-milliliter of 0.1 N KOH were added to the bottom of each Erlenmeyer flask. A control without seeds was prepared for each sample. Subsequently, the

Erlenmeyer flasks containing the samples were sealed with plastic wrap to avoid gas exchange, and kept in a BOD-type growth chamber for 24 h at a constant temperature of 25°C. After this period, 25 ml of 0.1 N KOH solution were taken from each sample to which three drops of phenolphthalein were added for titration with 0.1 N HCl. The volume of HCl spent to neutralize the KOH solution in each of the test samples was recorded at the turning point. Four subsamples of 10 seeds were used for each treatment, which were previously weighed to assess their dry weight. After titration, the respiration rate was calculated using the following equation:

$$RR = \frac{(B - L) . C}{DW}$$

where B and L are the 0.1 N HCl volumes used in the blank and sample titrations, respectively; C is the constant termed correction factor with a value of 3.52; and DW is the dry mass of the analyzed seeds. The results were expressed as mg $CO_2$ per gram of dry seed.

## Determination of the content of reducing, non-reducing and total soluble sugars

Four hundred milligram of seeds were macerated in a crucible with liquid nitrogen for quantification of reducing (RS), non-reducing (sucrose) and total soluble sugars (TSS). Thereafter, 10 ml of potassium phosphate buffer (100 mM pH 7.0) were added and the mixture was kept at 40°C for 30 min. The mash was then centrifuged at 10,000 rpm for 10 min, and the supernatant stored in the freezer at -20°C until analysis. The levels of reducing sugars (RS) were quantified adding the reagent 3,5-dinitrosalicylic acid (DNS) to 400 µl seed extract and the glucose standard curve was used (Miller, 1959). The readings were performed on a spectrophotometer at 540 nm. The levels of TSS and sucrose were quantified according to Willis and Yemm (1954) and using the anthrone reagent. The seed extract was diluted in potassium phosphate buffer (100 mM pH 7.0) at a 1:2 ratio for the TSS using a 30 µl aliquot of that solution for quantification. For the quantification of sucrose, 800 µl of the extract prepared were added to 800 µl of 30% KOH. The solution was incubated in a water bath at 37°C for 15 min, and 10 µl were subsequently taken from the solution for sucrose quantification. The TSS and sucrose standard curves were plotted based on known glucose concentrations and the reading was performed on a spectrophotometer at 620 nm.

## Electrophoretic profile of enzymes

The enzymatic activities of α-amylase, catalase, esterase and peroxidase were assessed using non-denaturing polyacrylamide gel electrophoresis. Ten seeds from each treatment were ground in the presence of liquid nitrogen and PVP 40 (polyvinylpyrrolidone) antioxidant, and the extracts were immediately stored at -80±2°C until the analyses were performed. For the analysis of each enzyme, 100 mg aliquots were removed, Tris buffer (0.2 M Tris-HCl, pH 8 + 0.1% β-mercaptoethanol) was added at a ratio of 2.5 times its weight for catalase and esterase. A phosphate buffer (0.034 M disodium hydrogen phosphate, 0.2 M sucrose, 2.56% PVP; 0.003 M Dithiothreitol (DTT); 0.0057 M ascorbic acid; 0.0025 M sodium borate; 1% Polyethylene glycol (PEG) 6000; 0.2% β-mercaptoethanol) was used for the peroxidase extraction. Samples from the materials of each treatment were kept overnight in a refrigerator at 4°C and then centrifuged at 16,000 x g for 30 min at 4°C; 60 µl of supernatant from each sample were subsequently added to the discontinuous polyacrylamide gel system at 4.5% (stacking gel) and 7.5% (separating gel). The gel/electrode system was Tris-glycine pH 8.9 and the electrophoretic run performed at 4°C under constant 150-V voltage. The gels were stained after the electrophoretic run according to Alfenas (1998) for the esterase and catalase enzyme system and according to Tanksley and Orton (1983) for peroxidase.

The seeds were subjected to germination tests for 48 h for α-amylase analysis. Seedlings were removed after this period, and the reserve tissue was ground in a crucible (over ice) in the presence of liquid nitrogen and PVP; the samples were stored in an ultra-freezer at - 80±2°C until the time of analysis. Enzyme extraction was performed by adding 200 µl of 0.2 M Tris-HCl, pH 8.0 extraction buffer to 100 mg samples from each treatment. The homogenate was kept for 12 h in a refrigerator at a temperature of 5°C and centrifuged at 16,000 x g at 4°C for 30 min; 40 µl volumes of extract were then applied to a polyacrylamide gel at 4.5% (stacking gel) and 7.5% (separating gel – containing 5% soluble starch). The bands of α-amylase enzyme activity were stained according to Alfenas (1998), and the presence or absence of clear bands on a bluish background (negative staining) (resulting from the reaction with amylose) and the relative intensity of the isoforms were assessed following the treatment with iodine. The evaluation of gels was performed on transilluminator after staining, and the presence or absence of bands and relative intensity of the different isoforms were analyzed for each sample.

## Assessment of the electrophoretic profile of heat shock proteins

The proteins of interest were separated and visualized by electrophoresis on a denaturing polyacrylamide gel (Sodium Dodecyl Sulfate PolyAcrylamide Gel Electrophoresis, SDS-PAGE). The seeds (wet and dry) were ground using liquid nitrogen and the extraction buffer was subsequently added buffer, consistent with Alfenas (1998), at a ratio of 10 parts buffer to 1 part sample. The samples were centrifuged at 16,000 x g for 30 min at 4°C. The supernatant was removed and incubated in a water bath at 85°C for 10 min to assess the heat shock proteins. The samples were again centrifuged as above. Prior to application on a denaturing polyacrylamide gel, 40 µl sample buffer (2.5 ml glycerol; 0.46 g SDS; 20 mg bromophenol blue; Tris-HCl pH 7.5) were added to 70 µl of each extract and boiled for five minutes. Next, 50 µl of each sample were applied to an SDS-PAGE of polyacrylamide gel at 12.5% (separating gel) and 6% (stacking gel); the running buffer used was Tris-glycine + SDS pH 8.9, and the electrophoretic run was performed on a vertical system at room temperature and constant voltage of 150 V for four hours. A molecular weight marker ranging from 10 to 100 KDa (Invitrogen) was used to assess the molecular mass of the polypeptide chains. Gels were stained after the run in 0.05% Coomassie Brilliant Blue solution for 24 h and destained in a solution of ethanol, acetic acid, and water at the ratios of 0.5:1:8.5 (v:v), respectively, consistent with Alfenas (1991). The evaluation of gels was performed on a transilluminator, which analyzed the presence and absence of bands.

## Endo-β-mannanase enzymatic activity

The seeds from each treatment, pre-soaked in distilled water for 24 h and without the seed coat, were macerated in liquid nitrogen and PVP 40 for the extraction of endo-β-mannanase. Six hundred microlitre of extraction buffer [0.1 M Hepes; 0.5 M NaCl and 0.5% ascorbic acid, pH 8.0] were added to 200 mg of seed macerate from each treatment. Then, the microcentrifuge tubes containing the samples were stirred using a vortex-type stirrer for 1 min and centrifuged for 30 min at 10,000 x g at 4°C. Twenty microlitre of supernatant were run on a gel containing 6 ml of locust bean gum-Sigma nr 0753 (LBG), 0.24 g of agarose (Qbiogene) and 24 ml of buffer pH 5.0 (11 ml of 1 M citric acid, 50 ml of $Na_2HPO_4$ and 149

**Table 1.** Germination rate index (GRI); germination percentage (G (%)); emergence rate index (ERI) and seedling emergence percentage (E (%)) of *P. ramiflora* (Mart.) Radlk. seeds at two maturation stages and with different water contents.

| Variables | Maturation | Water content (% w.b.) | | | CV (%) |
|---|---|---|---|---|---|
| | | 38 | 20 | 12 | |
| GRI | Ripe | 2.70 bA* | 0.87 aB | 0.00 aC | 13.50 |
| | Unripe | 3.20 aA | 0.00 bB | 0.00 aB | |
| G (%) | Ripe | 74.20 aA | 36.81aB | 0.00 aC | 19.62 |
| | Unripe | 80.27 aA | 0.00 bB | 0.00 aB | |
| ERI | Ripe | 0.50 aA | 0.06 aB | 0.00 aB | 16.34 |
| | Unripe | 0.17 bA | 0.00 aB | 0.00 aB | |
| E (%) | Ripe | 54.98 aA | 15.67 aB | 0.00 aC | 22.32 |
| | Unripe | 33.91 bA | 0.00 bB | 0.00 aB | |

CV: coefficient of variation; *Means followed by the same letter (lowercase in columns and uppercase in rows) do not differ at a 5% significance level under Tukey's test.

ml of distilled water). The aliquots were applied on 2 mm holes made in the gel using a gel comb, and the gel containing the samples was then transferred to a germinator at 25°C for 21 h in a moist chamber in the dark. Thereafter, the gel was covered with the dye Congo red at 0.5% for 30 min and destained in ethanol for 10 min. The ethanol was removed with distilled water and a solution of 1 M NaCl was added until visual observation of the formation of white halos in the holes that will contain the samples. The halo diameters were then measured in two directions with a caliper to obtain an average value. A comparison with the standard curve generated by the commercial endo-β-mannanase of *Aspergillus niger* (Megazyme) was performed for the calculation of enzyme activity. The calculation of the endo-β-mannanase enzymatic activity was performed according to Downie et al. (1994). The assay was conducted with three replicates for each treatment.

#### Statistical design

The experimental design was entirely randomized in a 2x3 factorial scheme, with two maturation stages (ripe seeds and unripe seeds) and three water contents (38% - initial water content, 20% and 12% w.b.). The data were statistically interpreted by an analysis of variance and the means were compared using Tukey's test at 5% probability using the software Sisvar (Analysis of Variance System) for Windows (Ferreira, 2000). Four replicates were used to quantify the reducing sugars, total soluble sugars and sucrose, and the means were compared using the Scott-Knott test at 5% probability. The data expressed as percentages were transformed into arcsine $\sqrt{x/100}$, where x refers to the percentage and the numerical data were transformed into $\sqrt{x+0.5}$.

## RESULTS AND DISCUSSION

The physiological quality (Table 1) of seeds harvested at the unripe stage and subjected to drying was below those harvested at the ripe stage and not dried (initial water content). Seed drying up to 12% w.b. negatively affected the physiological quality, regardless of the stage of seed maturation, and reached null values for germination and seedling emergence. Lower values were found when comparing the physiological performance between wet and dry seeds in each maturation stage for all variables when the seeds were dried to 12% water content, particularly for unripe seeds. Thus, seed drying caused changes in physiological performance, and such effects were more pronounced in seeds that had not reached full physiological maturity. These results corroborate those found by Nakada et al. (2011), who also found lower physiological quality and vigor in dry seeds at early stages of development when studying cucumber seeds at different stages of maturation subjected to shade drying. Coffee (*C. arabica* L.) seeds harvested at the green-cane and cherry stages had better physiological condition than those harvested unripe; thus, seeds harvested at the unripe stage were considered intolerant to desiccation (Brandao Junior et al., 2002).

The evaluation of integrity and internal morphology of seeds using X-ray testing (Figure 1) showed morphological changes in the endosperm that resulted from the drying process. The X-ray images showed an increase in the free space between the embryo and the seed coat (embryo/embryonic coelom) and a reduction in embryonic area with the decrease in the water content of seeds, particularly for unripe seeds. These results directly affect the physiological performance of seeds, which shows that the damage resulting from the drying process caused losses in physiological quality (as assessed by the germination and seedling emergence tests) and that these losses were more pronounced when seeds were dried to 12% w.b., particularly for immature seeds.

Similar result was found in the study conducted by Goodman et al. (2005), who also noted the recalcitrant nature of northern red oak (*Quercus rubra* L.) seeds, assessing the separation between cotyledon-cotyledon and cotyledon-pericarp with advanced drying and

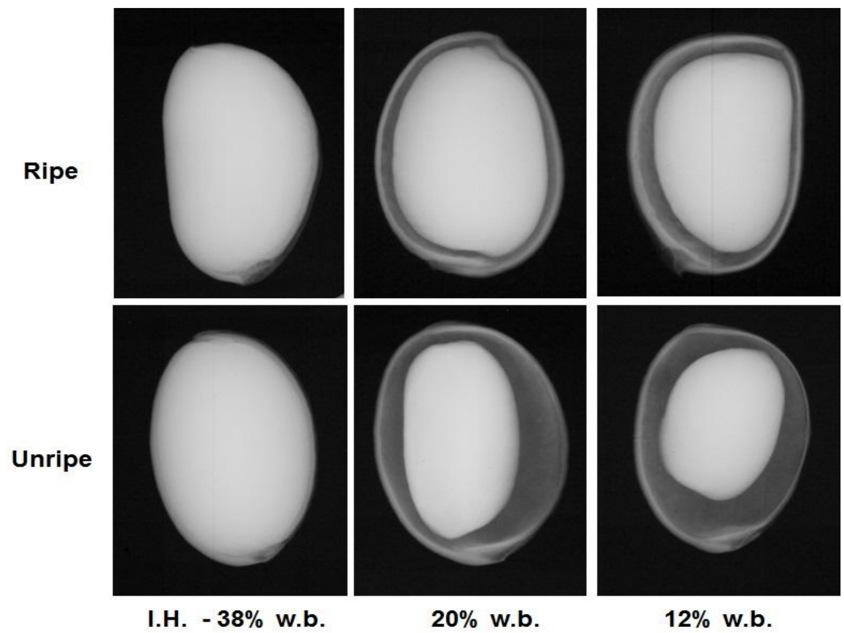


**Figure 1.** Radiographic images of *P. ramiflora* (Mart.) Radlk. seeds at two maturation stages and with different water contents. (I.H. – initial humidity - 37% w.b.; 20% w.b.; 12% w.b.). Federal University of Lavras (Universidade Federal de Lavras – UFLA), Lavras-MG, 2012.

establishing an important relationship between the damage assessed in X-ray images and the viability of seeds and seedlings. These authors emphasize that the morphological condition of seeds most likely reflects a cumulative result of all factors affecting viability loss; thus, the conditions observed in X-ray images can provide a more comprehensive representation of damages accumulated during desiccation, which confirms the potential of the analysis of X-ray images as a fast and non-destructive evaluation method of seed viability. According to Dell'Aquila (2007), the area of free space may be considered an indicator of germination potential and is closely related to the increase in abnormal seedlings because a gradual reduction in the formation of normal seedlings from radiographed pepper seeds (with a free area between the embryo and the endosperm exceeding 2.7%) was noted.

Regarding seed vigor, there was an effect of the maturation stage and water contents on electrical conductivity and respiration rate (Table 2).

Analysis of Table 2 shows that ripe seeds not subjected to drying had higher leachate electrical conductivity values than dried seeds. There was no difference in electrical conductivity between wet and dry seeds with respect to unripe seeds. Seeds that have not yet reached physiological maturity are more sensitive to desiccation and have lower vigor than ripe seeds because cell membrane repair may be ongoing in these seeds in response to damage after drying. Coffee (*Coffea arabica* L.) seeds harvested at the unripe stage are also more susceptible to damage from the drying process than seeds harvested at the green-cane and cherry stages, which indicates that the mechanisms of membrane protection occur between the unripe and cherry stages (Guimarães et al., 2002). The electrical conductivity of pepper (*Capsicum annuum* L.) seeds harvested from fruits picked 40 days after anthesis (immature) was high when compared to seeds derived from fruits picked at later periods (70 days after anthesis), which demonstrates that immature seeds have poor membrane organization and therefore greater solute leaching (Vidigal et al., 2009).

The respiration rate of ripe seeds was higher than that found in unripe seeds for all water contents, which indicates that ripe seeds were more efficient in reactivating their metabolism and respiration processes. Seeds with low water content had higher values of respiration rate in both stages of maturation. According to Wang et al. (2012), the survival of pea seeds after drying and soaking increases linearly with increased capacity to recover mitochondrial integrity, which indicates that the structural and functional recovery of mitochondria during germination has a key role in seed tolerance to desiccation.

The mean values regarding the concentrations of sugars are shown in Table 3. There was no difference between maturation stages and water contents with respect to the levels of reducing sugars. There were differences between water contents in both stages of maturation regarding total soluble sugars, with higher

**Table 2.** The electrical conductivity (EC) and respiration rate (RR) of *P. ramiflora* (Mart.) Radlk. seeds at two maturation stages and with different water contents.

| Variables | Maturation | Water contents (% w.b.) | | | CV (%) |
|---|---|---|---|---|---|
| | | 38 | 20 | 12 | |
| EC ($\mu S.cm^{-1}.g^{-1}$) | Ripe | 5.12 aA* | 3.39 bB | 3.43 bB | 11.35 |
| | Unripe | 3.61 bA | 4.36 aA | 4.27 aA | |
| RR (mg $CO_2$/ g D.M.) | Ripe | 5.63 aB | 7.19 aA | 7.64 aA | 13.13 |
| | Unripe | 4.36 bB | 5.92 bA | 5.78 bAB | |

CV: coefficient of variation; *Means followed by the same letter (lowercase in columns and uppercase in rows) do not differ at a 5% significance level under Tukey's test.

**Table 3.** Concentrations of reducing sugars (RS), total soluble sugars (TSS), and sucrose (S) in *P. ramiflora* (Mart.) Radlk. seeds at two maturation stages and with different water contents.

| Variables | Maturation | Water contents (% w.b.) | | | CV (%) |
|---|---|---|---|---|---|
| | | 38 | 20 | 12 | |
| RS ($mg/g^{-1}$) | Ripe | 0.15 aA* | 0.19 aA | 0.21 aA | 9.13 |
| | Unripe | 0.16 aA | 0.20 aA | 0.23 aA | |
| TSS ($mg/g^{-1}$) | Ripe | 2.81 aB | 3.81 bB | 7.67 bA | 16.58 |
| | Unripe | 2.97 aB | 8.20 aA | 9.19 aA | |
| S ($mg/g^{-1}$) | Ripe | 18.18 aC | 48.00 bB | 66.56 aA | 17.91 |
| | Unripe | 21.22 aB | 77.90 aA | 71.74 aA | |

CV: coefficient of variation; *Means followed by the same letter (lowercase in columns and uppercase in rows) do not differ at a 5% significance level under the Scott-Knott test.

concentrations in seeds with lower water contents, both for unripe and ripe seeds. There was an increase in the concentrations of sucrose with the decrease in the water content of seeds in both maturation stages, which demonstrates the effect of drying on the accumulation of these sugars in seeds. The levels of total soluble sugars in Brazilwood (*Caesalpinia echinata* Lam.) seeds corresponded to approximately 10% dry matter of ripe seeds; sucrose was detected at high ratios throughout the entire process of development, and the accumulation of soluble carbon reserves is thus related to the degree of seed maturation and the final quality of the seeds (Borges et al., 2006). In *Acer platanoides* L. seeds, the concentration of sucrose in wet seeds harvested at early stages of development was higher than in seeds harvested upon reaching maturity. Rapid drying of immature seeds promoted the increase of sucrose concentration, whereas drying had no effect on the concentration of sucrose in ripe seeds (Hong et al., 2000). Starch and sucrose stand out among the reserve carbohydrates of seeds and may affect membrane stability during desiccation and the resumption of embryonic growth in addition to their function as reserves (Hellmann et al., 2008).

No catalase or peroxidase enzymatic activity was found in the electrophoretic profile of isoenzymes, regardless of maturation stage or water content. The activity and structure of certain seed enzymes or proteins sensitive to desiccation may be permanently altered by drying, which results in loss of activity (Nkang et al., 2000). Another possibility is that the extraction buffer used in this study may not be adequate for *P. ramiflora* seeds because the protocol was designed for different seeds than the studied species.

The electrophoretic pattern of the α-amylase and esterase enzymes in *P. ramiflora* (Mart.) Radlk. seeds at two stages of maturation and with different water contents is shown in Figure 2. According to the methodology used, the results showed the activity of only one isoform of α-amylase and esterase in this species under the conditions evaluated. The analyzed variables (maturation and water content) did not induce the expression of new isoforms, although reducing the water content to 12% w.b. in unripe fruits resulted in the enzymatic inhibition of α-amylase, which is shown by the disappearance of its isoform (Figure 2A).

The esterase and α-amylase of *P. ramiflora* (Mart.) Radlk. seeds showed enzymatic activity with reverse behavior, particularly with respect to the degree of maturation of their seeds (Figure 2). Higher activities in

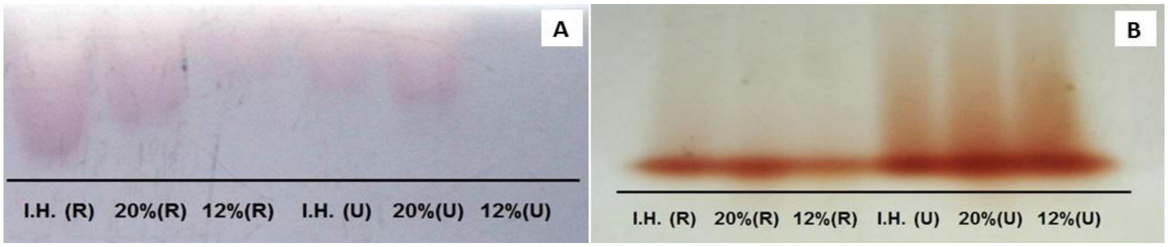


**Figure 2.** Electrophoretic pattern of α-amylase (A) and esterase (B) enzymes in *P. ramiflora* (Mart.) Radlk. seeds, at two maturation stages and with different water contents. (I.H. – Initial Humidity – 38%; 20% w.b.; 12% w.b.; R – ripe; U – unripe). Federal University of Lavras (Universidade Federal de Lavras – UFLA), Lavras-MG, 2012.

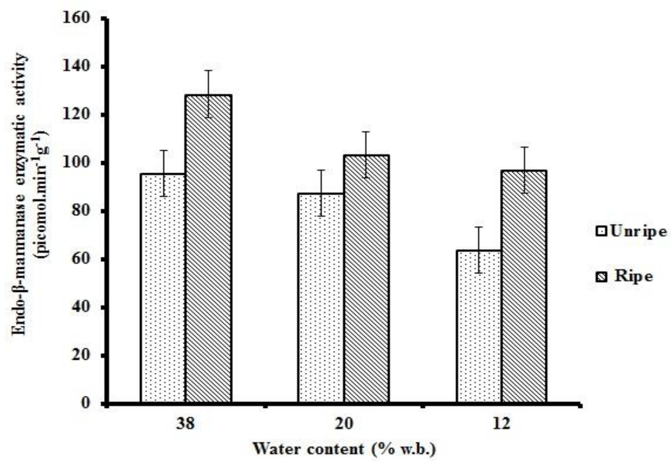


**Figure 3.** Endo-β-mannanase enzymatic activity in *Pouteria ramiflora* (Mart.) Radlk. seeds at two maturation stages and with different water contents. Federal University of Lavras (Universidade Federal de Lavras – UFLA), Lavras-MG, 2012.

α-amylase are found in ripe seeds than unripe seeds when fixing the water content in the seeds and varying their degree of maturation (Figure 2A); the opposite effect was observed in the activity in the esterase isoform, in which unripe seeds showed higher activity (Figure 2B). The reduction in seed moisture caused a reduction in α-amylase activity, and the effect was more drastic in unripe seeds with 12% w.b.; a slight decrease in esterase activity occurs as the water content is reduced in ripe seeds but the reducing water content apparently does not affect its activity in unripe seeds.

The α-amylase enzyme is essential for starch hydrolysis and its synthesis also occurs in response to the desiccation of cereal seeds (Rosa et al., 2004). Drying in the field and the artificial drying of corn seeds harvested at different stages of maturation led to increased α-amylase enzymatic activity, which favored seed germination (Faria et al., 2004). Esterase is a key enzyme that characterizes seed deterioration (Nakada et al., 2010) and presents increased activity during that physiological process (Walters, 1998; McDonald, 1999). The pattern observed for this enzyme corroborates the physiological and vigor results because its intense activity in unripe seeds may indicate cell membrane damage that culminates in reduced germination capacity and advanced deterioration. The esterase enzyme affects ester hydrolysis reactions and is directly linked to lipid metabolism, including total membrane phospholipids (Santos et al., 2005).

According to Tunes et al. (2011), esterase enzyme expression in barley seeds was barely affected by the harvest season. Nakada et al. (2011), when studying wet and dry Omega cucumber seeds ('caipira' type), found increased esterase activity in the first two and in the final stage of development (30, 35 and 55 days after anthesis, respectively), and that result correlated with the intensification of the deterioration process.

The endo-β-mannanase enzymatic activity in unripe and ripe seeds with different water contents is shown in Figure 3.

The endo-β-mannanase enzymatic activity was higher in ripe than unripe seeds, with a decrease in its activity in response to the decrease in seed water content at both stages of maturation. Thus, the increased endo-β-mannanase activity can be correlated to the results found in germination and seedling emergence tests, in which the best values were found in ripe seeds with high water contents. The increase in the endo-β-mannanase enzymatic activity of tomato seeds coincided with an increase in seedling ERI, which explains why the increase in its activity may facilitate radicle protrusion because this is a main degradative enzyme of reserves in seeds (Albuquerque et al., 2010). That enzymatic activity, which is key to the germination of pepper seeds from the Habanero Yellow variety (*Capsicum chinense* Jacquin), was higher in more advanced stages of development when seeds had reached physiological maturity with full development of the enzymatic mechanisms that are involved in germination (Queiroz et al., 2011). Furthermore, Nascimento and Cantliffe (2001) found that the endo-β-mannanase enzymatic activity of lettuce seeds was higher one hour before radicle protrusion and in seeds of thermotolerant-genotypes when compared to thermosensitive seeds.

There was no detection of heat shock proteins by electrophoretic analysis of extracts prepared for that purpose. The absence of bands is presumably not an artifact of the technique because this is well-established methodology in other biological systems. Thus, drying did not induce changes in the pattern of heat-shock polypeptides in the species analyzed. Several recalcitrant seeds do not produce LEA proteins and, in this case, the presence of soluble sugars may minimize the effects of drying. The lack of LEA protein synthesis is one of the main differences between desiccation-tolerant and non-tolerant seeds (Marcos-Filho, 2005). LEA proteins are usually able to protect other proteins or membranes, similar to sugars, by acting as water replacement molecules (Goyal et al., 2005). Another possibility is that these proteins have different functions under different moisture contents and may yet have antioxidant properties that minimize the damaging effects of ROS (Mowla et al., 2006). The absence of such proteins is presumably a sign of intolerance to desiccation in *P. ramiflora* (Mart.) Radlk. seeds considering the relationship between the functions of these proteins and the acquisition of desiccation tolerance.

## Conclusions

The physiological quality and vigor of *P. ramiflora* (Mart.) Radlk. seeds are affected by their maturation stage and drying activities. Ripe seeds performed better and were more tolerant to desiccation than unripe seeds. *P. ramiflora* (Mart.) Radlk. seeds showed only α-amylase and esterase isoforms; the first enzyme showed greater activity in ripe seeds, was sensitive to desiccation, and was not detected in seeds with 12% w.b.; conversely, the esterase isoform was much more active in unripe seeds, with little or no reduction in activity resulting from the reduction in water content. Catalase and peroxidase isoforms and heat shock proteins were not detected under the conditions evaluated. *P. ramiflora* (Mart.) Radlk. seeds are intolerant to desiccation, and this intolerance is maximal before seeds reach physiological maturity.

## Conflict of Interests

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

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# A concise scheme of vegetation boundary terms in subtropical high mountains

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**There are numerous terms used to describe the vegetation boundary in high mountains. It is essential to define and agree on a unified usage for these terms. The literature review method had been used in this paper to clarify the vegetation boundary terms and their scopes. The result revealed that these terms were much diversified and ambiguous, and more or less related and infringed with each other in ecological concept and visible landscape, even mutually used as synonyms in some cases. We proposed a concise scheme for clarifying these terms and illustrating their relationship, including timberline [instead of previously used economic (rational, generative) forest-line, economic timberline, limit of continuous forest], forestline [instead of physiognomic (empirical, vegetative, biologic) forest-line, actual timberline, physiognomic forest-limit], treeline (instead of tree limit and treeline), krummholzline (instead of tree-species line, tree species limit, krummholz limit), and historic treeline. The 5 boundary terms could facilitate the comprehension of spatial sequence of vegetation transformation in high mountains. However, all boundaries do not necessarily occur in all mountains of the world concurrently. In some papers, the term timberline or treeline once referred to the ecotone from continuous forest to tree less landscape, but the term forest–tundra ecotone should obtain more commendations when vegetation transition is much more gradual particularly around the subarctic. To avoid the confusion from using these boundary terms, we suggest that authors studying boundary related issues should interpret their scope of terminologies and provide the basic description about environment and vegetation outlines in the complex high mountains.**

**Key words:** Timberline, forestline, treeline, krummholzline, high mountains.

## INTRODUCTION

There are mainly four vegetation types in high mountains, including closed-canopy forest, open-canopy forest, krummholz, and treeless tundra. The boundaries among them, usually called treeline or timberline etc., are the most conspicuous vegetation boundaries (Holtmeier and Broll, 2010; Hoch and Körner, 2012). In recent years, the

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treeline related issues have been increasingly discussed due to concern over the effects of climatic change (Holtmeier, 1985, 2009; Holtmeier and Broll, 2005, 2007; Timoney et al., 1992), as the boundary between forest and tundra may be a sensitive indicator or bellwether of response to global warming (Kullman, 2001; Smith et al., 2009). Some authors considered that boundaries may serve as indicators only under certain circumstances (Risser, 1995; Slayter and Noble, 1992), but undoubtedly, it is an interesting and important issue in ecology and landscape.

Terms and concepts crucial to understanding ecology have often been criticized for their tautological, equivocal or nonoperational nature (Colautti and MacIsaac, 2004). The tree line related terms are numerous and rather ambiguous due to the great ecological, physiognomic, and taxonomic varieties and the multidisciplinary and multilingual nature of the subject matter (Autio, 2006). It is often unclear or unstated, moreover, what criteria to define these terms. Differences in terminology and definition itself lead to miscommunication and may make direct comparisons difficult among areas studied by different authors (Timoney et al., 1992). When using the treeline as criterion for assessing effects of climate change, for example, we cannot validate whether the worldwide treeline in different reports (such as 10 different tree line positions in Walther et al., 2005) is consistent. Also, it is insecure to compare the positions of alpine vegetation boundaries indifferent papers whose references used several different boundary terms (Crausbay and Hotchkiss, 2010; Körner, 1998; Körner and Paulsen, 2004; Tuhkanen, 1993). Thus, clarifying the treeline, related terms is fundamental for studying high-mountain issues. The aims of this paper are to review the treeline related terms and their scopes, and to recommend several concise terms to integrate various and ambiguous terms regarding the boundaries and ecological zones, and to illustrate the relationship between different terms as fundamental work for future research.

## MATERIALS AND METHODS

The literature review method was used in this paper for clarifying the tree line related terms and their scopes. Thomson Institute for Scientific Information (ISI; http://apps.webofknowledge.com/) and Google Scholar (GS; http://scholar.google.com/) were used to search tree line related terms. ISI is today's premier research platform for information in the sciences and commonly used as source of bibliometric data; and GS database provides broader data not only from the strict ISI criteria, but also from conference proceedings, working papers, and books (Schiederig et al., 2012).

For the literature review and analysis, a bibliometric search from the scientific and popular literatures in ISI and GS had been performed (Harzing and Wal, 2008). Publications were collected using the search strings 'tree line', 'forest line', and 'timber line' as well as all their variants. Then, the content with respect to definition, scope, and scheme of these tree line related terms have been analyzed further.

## RESULTS AND DISCUSSION

### Historic usage of boundary terms

Table 1 is the number of publications of tree line related terms through search in ISI database and GS database. The total number of publications is 2,652 in ISI searching by topic, 1,394 in GS searching by topic, and 83,965 in GS searching by all. The result of "search by topic" in ISI reveals that 1,757 (66.63%) publications apply the notion 'tree line' and all its variants, 678 (25.56%) for 'timber line', 203 (7.65%) for 'forest-line', 2 (0.08%) for 'historical tree line', and 2 (0.08%) for 'tree-species line'. For "search by topic" in GS, a search for 'tree line' retrieves 860 (61.69%) items, for 'timber line' 426 (30.56%) items, for 'forest-line' 106 (4.61%) items, for 'historical tree line' 2 (0.14%) items, and for 'tree-species line' 0 (0.00%) items. These results reveal that treeline is the most common used term for representing the boundary line and timberline is the second common term. When the suggestion "search by topic" in GS by Webster and Watson (2002) adopted, the string 'tree-species line' or 'historical tree line' had no result. They are in frequently used terms.

Historically, many terms were used to name the boundaries between two adjacent plant communities in high mountains, mainly including 5 different kinds of lines (Tables 1 and 2). Many authors, such as Hustich (1979), Payette (1983), Tuhkanen (1993), Scott (1997), Körner and Paulsen (2004), Autio (2006), Holtmeier and Broll (2010) and Harsch and Bader (2011) once illustrated their schemes regarding these terms. An earlier scheme of boundary terms had been proposed by Hustich (1979). The schematic succession of boundary terms was similar reported by Scott (1997), as appeared in Table 2, he stated "Alpine environments are here described as those that exist above timberline, a rather rich concept in itself as five different kinds of timberline are listed: economic forest line (above which trees cannot be economically harvested), forest limit (physiognomic forest line), tree limit (some species reach tree size), tree species limit (tree species are stunted but present, that is, krummholz, elfinwood or krupelkiefer), and historic tree line (indicating earlier climatic regimes)."

These boundary terms sometimes had their substitute spelling or variant and occasionally used interchangeably or as synonyms in appropriately (Tables 2 and 3). A veritable "Babel of nomenclatures" of the boundary related terminology did exist (Hare and Ritchie, 1972). In fact, these terms can complement or conflict with each other, more or less related and infringed in the ecological concept and in the visible landscape (Armand, 1992; Hustich, 1979; Körner and Paulsen, 2004). Although, there have been many attempts for clarifying these terms (Holtmeier, 2009; Hustich, 1983), none have proved satisfactory, and the generally accepted classification scheme is far from complete (Autio, 2006).

**Table 1.** Total number of items in Institute for Scientific Information (ISI) and Google Scholar (GS) database for five boundary terms and their synonyms and variants.

| Term | Items in ISI (search by topic) | Items in GS (search by topic) | Items in GS (search by all) |
|---|---|---|---|
| Historical tree-line* | 2 | 0 | 14 |
| Historical treeline | 0 | 2 | 17 |
| Historical tree-limit | 0 | 0 | 2 |
| Historical treelimit | 0 | 0 | 0 |
| Historic tree-line | 0 | 0 | 6 |
| Historic treeline | 0 | 0 | 5 |
| Post-glacial tree line | 0 | 0 | 1 |
| Subtotal | 2 | 2 | 45 |
| Tree-species line | 1 | 0 | 60 |
| Tree-species limit | 0 | 0 | 52 |
| Krummholz-line | 0 | 0 | 14 |
| Krummholzline | 0 | 0 | 0 |
| Krummholz-limit | 1 | 0 | 32 |
| Krummholzlimit | 0 | 0 | 0 |
| Subtotal | 2 | 0 | 158 |
| Tree-line | 755 | 251 | 29,400 |
| Treeline | 893 | 542 | 19,100 |
| Tree-limit | 119 | 67 | 3,270 |
| Treelimit | 0 | 0 | 54 |
| Subtotal | 1,767 | 860 | 51,824 |
| Forest-line | 87 | 40 | 2,780 |
| Forestline | 3 | 3 | 94 |
| Forest-limit | 113 | 63 | 2,550 |
| Forestlimit | 0 | 0 | 2 |
| Subtotal | 203 | 106 | 5,426 |
| Timber-line | 39 | 35 | 6,700 |
| Timberline | 639 | 391 | 19,600 |
| Timber-limit | 0 | 0 | 211 |
| Timberlimit | 0 | 0 | 1 |
| Subtotal | 678 | 426 | 26,512 |
| Forest–tundra ecotone | 106 | 119 | 1,650 |
| Tree-line ecotone | 26 | 9 | 227 |
| Treeline ecotone | 84 | 60 | 1,010 |
| Forest-line ecotone | 3 | 1 | 33 |
| Forestline ecotone | 0 | 0 | 1 |
| Timber-line ecotone | 0 | 2 | 52 |
| Timberline ecotone | 35 | 56 | 586 |
| Subtotal | 254 | 247 | 3,559 |

* The search string 'tree-line' and 'treeline', also as other terms, are synonyms (dash = space).

## Concepts of the transitions of high-mountain environment and vegetation

Environmental conditions become progressively harsher with an increase in altitude due to a decrease in temperature as well as increases in wind-speed, snow cover and other severe factors related to the survival, growth, and regeneration of plant (Körner, 2003; Schickhoff, 2005; Sveinbjörnsson, 2000). The increasingly severe environment gradually affects the transition of flora and physiognomy (Bader et al., 2007; Holtmeier, 2009). The transition from the closed mountain forests to treeless alpine vegetation is commonly a gradient of increasing stand fragmentation and stuntedness (Körner and Paulsen, 2004), as gradually opening canopy and declining tree size.

**Table 2.** Examples of different definition or expression regarding high-mountain boundary terms.

| Terms | Definition or expression of boundary terms (their synonyms and variants marked in italic)* |
|---|---|
| Historic treeline | Beyond and above the tree species line there are to be found numerous remnants of trees that grew at those sites when the climate was warmer than nowadays, and the outermost occurrences of these constitute the historical tree line, generally understood as the highest post-glacial tree line (Heikkinen, 2005). |
| | Determined by the location of subfossil trees, that is, ancient, dead trees that remain well preserved (Kharuk et al., 2009) |
| Krummholzline | A subalpine transitional zone or krummholz line characterized by increasingly stunted and dwarfed trees is the norm in circumpolar and northern temperate zone mountains (Sarmiento and Frolich, 2002). |
| | Tree species limit or krummholz limit: the uppermost limit of isolated and small individuals (Finsinger and Tinner, 2007) |
| Treeline | I will use the term treeline in a general sense to refer to the transition from forests to treeless vegetation (Tuhkanen, 1993). |
| | We use the term treeline to denote the actual upper boundary of contiguous closed-canopy forest, irrespective of whether or not this represents the potential climatic upper limit for tree growth (Bader et al., 2007). |
| Forestline | physiognomic forest line: limit of vegetatively reproducing trees (Johnson and Miyanishi, 1999) |
| | Actual timberline is a generic term denoting to continuous, altitudinal mountain timberline. This line has also been called an empirical forest line/timberline, and physiognomic forest line. Areas above the actual timberline are characterised by smaller trees, which grow in groups or alone (Autio and Eolpaert, 2005). |
| Timberline | The term timberline has been variously used. It may refer to the economic forest line or even to treeline proper (Tuhkanen, 1993). |
| | Timberline or forest limit: maximum elevation of forest with cover of at least 30~40% given by arboreal individuals >5 m high (Batllori et al., 2009). |

* There is more or less disagreement in the connotation and domain of these terms.

At a global scale, natural vegetation transitions along altitude are controlled primarily by climate (Tuhkanen, 1993) mainly caused by heat deficiency (Holtmeier and Broll, 2010), that is, insufficient temperature and growing season length (Holtmeier, 2009; Hustich, 1979; Schickhoff, 2005). The altitudinal and thermal gradient directs the pattern of vegetation transition, but local climate, topography, site history, ecology of plant species, current biotic and anthropogenic influences (Bader, 2007; Holtmeier, 1985, 2009; Schickhoff, 2005; Wardle, 1971) greatly modify this pattern in different ways (Camarero and Gutiérrez, 2001). Thus, the patterns of transitional vegetation in high mountains include wide ecotone, mosaic of patch, and abrupt boundary (Arno and Hammerly, 1984; Bader et al., 2007; Holtmeier, 1985, 2009; Körner, 2003).

Also, Norton and Schönenberger (1984) once illustrated four transitional types how forest is replaced by alpine vegetation, including: (1) abrupt forest limit bordering alpine vegetation, (2) transition zone (ecotone), (3) true krummholz belt above the upright growing forest, and (4) gradual transition from high-stemmed forest to crippled trees of the same species bordering alpine vegetation. Harsch and Bader (2011) illustrated conceptual diagram of four tree line forms, diffuse, abrupt, islands, and krummholz showed the zone between the upper and lower end varied greatly in width and character. Generally speaking, the vegetation transition is sharper near a boundary or more gradual close to an ecotone (Holtmeier, 2009; Körner, 2003; Payette, 1983; Schickhoff, 2005).

Is the transition between two adjacent communities a line or an ecotone? It is difficult to answer this question conclusively. An explanation had once been made by Armand (1992): “Any natural boundary is in reality a transition zone, which has its own two boundaries. They are, in turn, also transition zones with their own boundaries, and so on endlessly. So localization of a natural border is in principle inexact and therefore determined by convention.” Moreover, the transitional gradient and spread of vegetation are dissimilar in different parts of the world. For example, the actual transition for Nothofagus in the Southern Hemisphere often forms a sharp boundary at its upper limit (Fajardo and McIntire, 2012; Wardle, 1971, 1998); whereas around the subarctic, the breadth of the forest-tundra transition zone is rather wide and indistinct, often exceeding 40 km and even over 200 km (Virtanen et al., 2004).

In some mountains, the transition zone is not abrupt, such as Pinus albicaulis in Montana alpine, which is called as diffuse tree line (Fajardo and McIntire, 2012; Harsch and Bader, 2011). Consequently, sometimes the vegetation boundary was seen as a line or sometimes as a synonym of an ecotone despite the zonal width (Young and León, 2007).

A natural boundary receives discordant identifications in relationship to observation scale and transition zone width (Bader et al., 2007). Most people would intuitively agree about the position of the boundary when it is

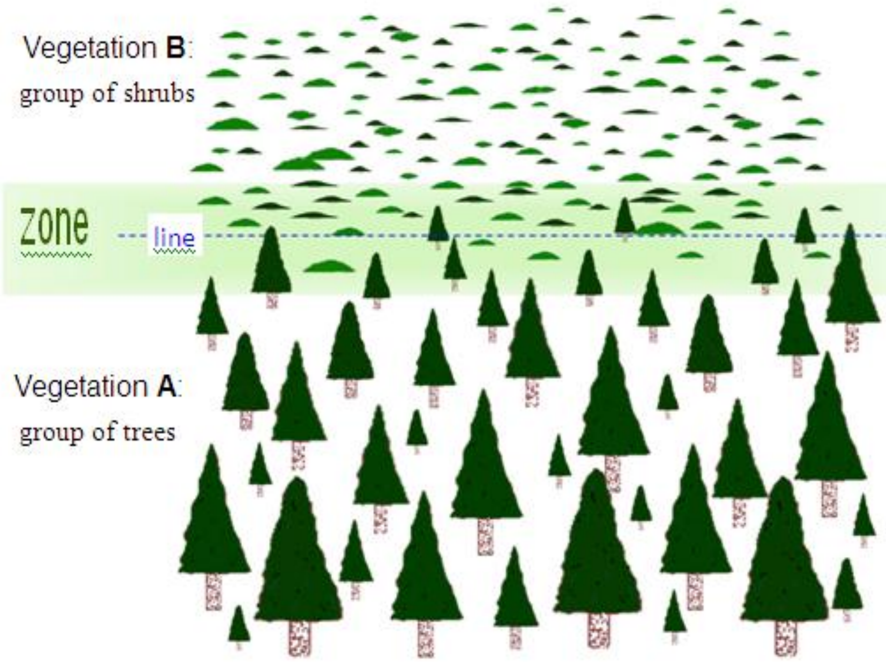


**Figure 1.** The presentation of vegetation or life-form transition from A (group of trees) to B (group of shrubs) could be considered as two representative types, a sharp line or a gradual zone of varying width, depending on the observation scale, the width of transition zone, and the characteristic of species (genetically-predetermined vs. environmentally-induced forms).

viewed from an airplane at great distance, but would strongly disagree when faced with the local situation on the ground (Körner, 1998). Holtmeier (2009) once said that “Timberline is a biological boundary, a more or less wide ecotone.” to metaphorically contain both lineal and zonal concepts within tree-line related term itself. As Figure 1 illustrates, we could consider vegetation below the boundary to be A (group of trees) whereas vegetation above the boundary is B (group of shrubs), that is, the boundary is where A ends and B begins. Figure 2 shows some cases in Mt. Shei (its peak 3884 m asl) of Taiwan: Figure 2a and b as vegetation A; Figure 2c and d as vegetation B; and Figure 2e and f show the boundary between vegetation A and B. When the transition from A to B is narrow and sharp, the vegetation boundary is considered as a line. In contrast with this line, the vegetation boundary is considered as a transition zone or an ecotone when the transition from A to B is wide and gradual. Thus, in essence, the high-mountain vegetation boundary between closed forest and treeless area is a line or a transition zone (Holtmeier and Broll, 2005, 2010) often determined by convention (Armand, 1992; Körner and Paulsen, 2004). At coarse scales, the terms alpine tree-line ecotone and forest–tundra ecotone were often used to represent the transition from forest to tundra. On the other hand, when we considered that the transition zone or ecotone was a boundary, the vegetation zones separated by the boundary will form more uniform physiognomy, such as forest and krummholz (or tundra).

## Concise terms and scheme of boundaries

In this paper, the concise terms regarding these boundaries from lower to higher elevation and their general definitions had been suggested as follows and illustrated in Figure 3. The reasons of this concise scheme and terms to replace others had been interpreted in the following subsection:

1) Timberline is the uppermost elevational limit of continuous closed forest. It represents the topmost boundary of forest with larger trees and more closed canopy.
2) Forestline is the uppermost elevational limit of open forest. It represents the topmost boundary of forest with smaller trees and opener canopy.
3) Treeline is the uppermost elevational limit of individual trees. It represents the topmost boundary of at least 2 m height and scattered trees.
4) Krummholzline is the uppermost elevational limit of shrubby, stunted or mat-form woody plants. The krummholz can be divided into genetically-predetermined krummholz (genotype) and environmentally-induced

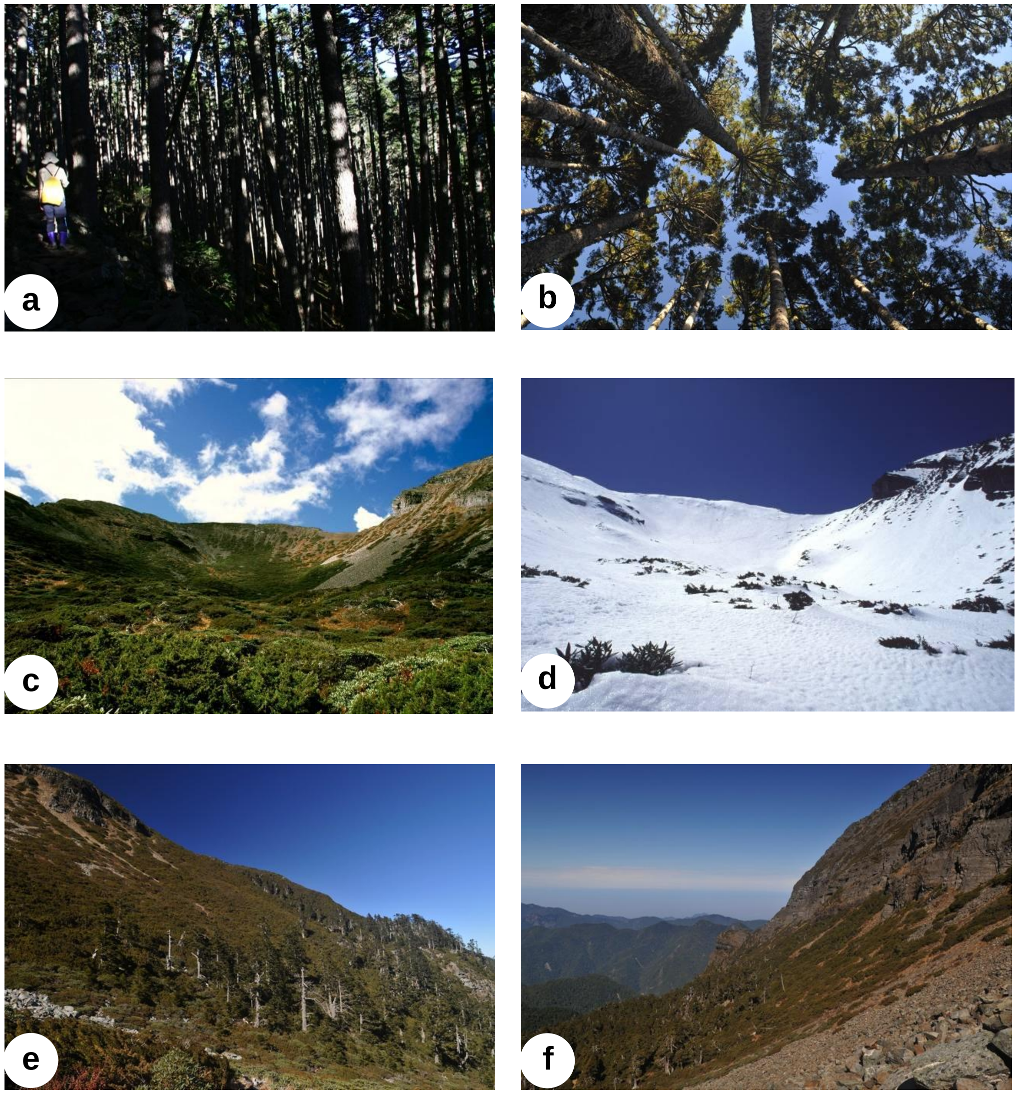

**Figure 2.** (a)*Abies kawakamii*, its growth form only upright tree, comprises the closed-forest (at the east slope of Mt. Shei, ca. 3,375 m asl). (b) The canopy of closed-forest is more than 60% where tree crowns usually interlocking. (c) The summer scenery at the semi-circular glacial cirque formed during the last glaciation, the *krummholz* (at the east slope of Mt.Shei, above ca. 3,650 m asl) is mainly composed of the shrubs *Juniperus squamata* var. *morrisonicola* and *Rhododendron pseudochrysanthum*. (d) The winter scenery as the same view of photo c, the *krummholz* is shaped principally from strong wind, gravel, winter snowpack, and insufficient air and soil temperatures. (e) At the east slope of Mt. Shei, there is no significant tree line. The fingered forest line where *Abies* forest ends and *krummholz* begins occurs at ca. 3,600 m asl. (f) At the west slope of Mt. Shei, the transition from forest to scrub is sharper than on photo e. The forest-line where *Juniperus* forest ends and *krummholz* begins occurs at ca. 3,700 m asl. (Photos a and b by Chun-Min Wang in 2010; c and d by Ching-Chi Hsu in 2004, 2005; e and f by Ching-An Chiu in 2008 and 2010).

krummholz (phenotype) (Holtmeier, 1981, 2009).

5) Historic treeline is the uppermost elevational limit of the trees during Hypsithermal period, identified from paleoecological subfossil evidence. The subfossil trees exist, that is, the ancient and dead trees remain well preserved.

Of various criteria used to delimit the boundaries in high mountains, emphases have been placed on the height, stem density, and growth form of woody plants (Holtmeier, 2009; Timoney et al., 1992). For clarifying the difference among these terms, two questions must be answered: What is a tree? What is a forest? As Lund (2009) had shown, there were 199 definitions of the term tree in use throughout the world. A tree is defined as an upright woody plant with a dominant above-ground stem

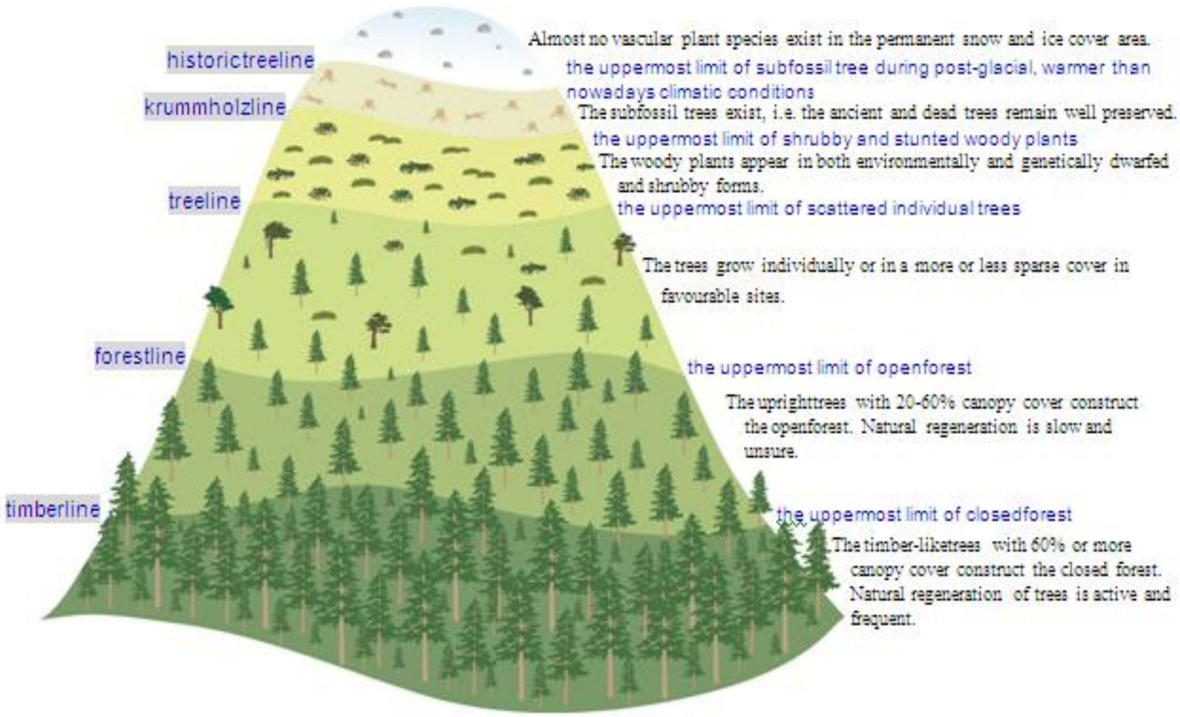


**Figure 3.** A concise schematic diagram designed to explain the transitional gradients and their boundaries among high-mountain ecosystems: ideas and concepts adopted from Hustich (1953, 1979), Payette (1983), Tuhkanen (1993), Sveinbjörnsson (2000), Körner and Paulsen (2004), Autio (2006), Holtmeier (2009), and Harsch and Bader (2011), but more concise and more intuitive. These concise terms timber line, forest line, tree line, krummholz line, and historic tree line (in blue words) are ordered in sequence by increasing altitude ideally.

with a minimum height from 1 to 8 m (Autio, 2006; Holtmeier, 2009; Hustich, 1979). Trees are also described as timber-sized (Wardle, 1965), or as any individual with one or more stems 10 cm diameter at 50 cm above the forest floor (Cullen et al., 2001). In this paper, we prefer that a tree is defined as an upright woody plant with an erect stem which reaches a height of at least 2 m, independently of whether reproduction occurs or not (Körner, 1998) and multi-stemmed or not. The definition using a critical trunk height makes it rather straight forward to distinguish trees from shrubs.

Furthermore, a forest is loosely understood as an area with a high density of trees or a biological community dominated by trees. There are more than 950 definitions of the term forest (Helms, 2002; Lund, 2009), based on the various criteria. We recommend that a forest is an ecosystem dominated by trees with a total canopy cover of 20% or more. Based on the critical canopy cover by referring to international vegetation classification (Grossman et al., 1998), the area is considered as openforest if the tree cover is between 20 and 60% and as closedforest if the tree cover is more than 60% where tree crowns usually interlocking.

First and foremost, we had better make sure the difference between timberline and forestline. The term timberline seems clear enough in semantics and is “the upper limit of tall, erect timber-sized trees (Wardle, 1965)” to represent the uppermost elevational limit of closedforest. As revealed in Table 2, the more brief term timberline can replace the limit of continuous forest (Payette, 1983), the economic limit of forest (Hustich, 1953), or the economical, rational, and generative forest-line (Hustich, 1979; Scott, 1997; Tuhkanen, 1993). It can also replace the economic timber-line (Autio, 2006; Holtmeier, 2009). Tree regeneration is active and timber harvesting is possible below the timberline, whereas natural regeneration of tree is slower and uncertain above the timberline. In contrast with timberline, we propose to use the term forestline to represent the uppermost elevational limit of open forest. As revealed in Table 2, the more brief term forestline can replace the physiognomic forest-limit (Payette, 1983), biological limit of forest (Hustich, 1953), physiognomic (biologic) forest line (Tuhkanen, 1993), forest limit (Scott, 1997), empirical timberline (Dahl, 1998), actual timberline (Autio and Colpaert, 2005; Autio, 2006; Holtmeier, 2009), or physiognomic (empirical, vegetative) forest-line (Hustich, 1979).

Although, treeline was defined as the connection between the highest elevation groups of trees, and Körner and Paulsen (2004) proposed that such a definition was a convention for communication and did not deserve a major scientific debate. We suggested that it is necessary to separate treeline from other terms such as timberline and forestline or from treeline ecotone (Körner, 1998, also discussed in the next section). Here we defined the term treeline as the uppermost elevational limit of scattered individual trees, giving up the different writing forms such as treeline and tree limit (Autio, 2006; Scott, 1997) for the sake of uniformity in our scheme. Note that this definition ignored certain circumstances, such as isolated tree outposts or tree islands [trees occurring above the treeline in the more preferable microhabitat as described by Holtmeier and Broll (1992).

Above the treeline, woody plants still occur and are often much shorter and more crooked than those at lower elevations. Their twisted and deformed physiognomies are genetically predetermined or shaped by climatic influences (Holtmeier, 1981). These shrubs are often known as krummholz, and both are genetically and phenotypically determined (Holtmeier, 2009); Such krummholz scrubs may be composed of both living and dead stems, and branches several hundred years old (Payette et al., 2008) but still be a stunted dwarf (Holtmeier, 1981, 2009). We defined the term krummholz line as the uppermost elevational limit of shrubby, stunted or mat-form woody plants, that is, the beginning of the treeless alpine zone (Körner, 1998). It is often referred to as the tree species limit (Payette, 1983; Scott, 1997), tree-species line (Autio, 2006; Hustich, 1979; Tuhkanen, 1993), tree species line (Körner and Paulsen, 2004), species line (Sveinbjörnsson, 2000), or krummholz limit (Finsinger and Tinner, 2007) (also see Table 2). But their positions of tree-species line and krummholz line are different, particularly in some alpine beyond the krummholz line where creeping willow and woody cushion plants occur. Sometimes, the term historic treeline was identified beyond the krummholz line based on macrofossils and other evidence (Holtmeier, 2009; Hustich, 1983) that existed during post-glacial, warmer than nowadays climatic conditions, but it had never been reported in tropical high mountains.

Tables 2 and 3 list some examples of boundary terms. They reveal that there are spelling differences or synonyms in custom, usage, or opinion by different authors. A bibliometric search using these various terms (including all their synonyms and variants, Table 3) in GS shows their counts of scholarly publications and citations (Harzing and Wal, 2008). The term treeline, a total of 51,824 items in GS is the most familiar usage, and timberline is the secondary one (26,512 items in GS); indeed, they are often rather ambiguous and may be inter-invasive or interchangeable in different papers. Thus, we proposed to clear the 5 different boundaries and give the more concise terms and the identical writing form. Figure 3 illustrated a schematic diagram designed to concisely explain the vegetation transition and their boundaries in high mountains physically. These concise terms timberline, forestline, treeline, krummholzline, and historic treeline are ordered in sequence by increasing altitude. The primary ideas and concepts are adopted from Hustich (1953, 1979), Payette (1983), Tuhkanen (1993), Sveinbjörnsson (2000), Körner and Paulsen (2004), Autio (2006), Holtmeier (2009), Harsch and Bader (2011), but more concise and more intuitive.

**Termsto replace “ecotone”**

Various biotic and abiotic factors and processes result in the manifold patterns of vegetation transition in different regions. The boundaries mentioned above are sometimes regarded as a transition zone (Armand, 1992), an ecotone (Holtmeier, 2009), or as an area of ecological tension over which one type of vegetation is gradually replaced by another (Walter, 1985). In other words, the upper elevational limit of forest and tree growth and survival on high mountains, defined as a line is included within this ecotone (Batllori et al., 2009; Holtmeier, 2009) (Figure 1).

The terms timberline and treeline were often used to refer to ecotone from closed continuous forest to treeless alpine, such as: (1) Leuschner (1996) noted “The term timberline as used here includes forest line and tree line.” Wieser and Tausz (2007) noted “The timberline ecotone stretches from the forest line or upper limit of a continuous forest canopy to the tree limit which is the extreme upper limit of the occurrence of tree species.” (2) Holtmeier and Broll (2005) noted “The term treeline is applied to the transition zone extending from closed subalpine or northern forests to the uppermost or northernmost usually scattered and stunted individuals of the forest-forming tree species – regardless of their height. The upper or northern limit of the treeline ecotone is called the tree-limit.”

Besides, Finsinger and Tinner (2007) noted “The treeline ecotone spans the timberline and the tree species limit or krummholz limit.” In fact, more examples revealed that the ecotone terminology and its scopes are as individual as the persons (cf. Autio and Colpaert, 2005; Camarero et al., 2006; Holtmeier, 2009; Liu et al., 2002), also as the confused boundaries mentioned previously.

The concept of timberline or treeline ecotone is close to the forest–tundra ecotone (254 items in ISI; 247 items in GS by topic; 3,559 items in GS by all) or alpine timber-line/tree-line ecotone. Zeng et al. (2007) described the alpine forest–tundra ecotone as this transition zone from contiguous forest cover to open alpine tundra, being the elevational limit to the growth of tree forms and tree species and encompassing the range from upright trees to small patches of prostrate krummholz. Hence, the treeline or timberline ecotone is the transitional belt from timberline to krummholzline as proposed by Holtmeier (1981), Körner and Paulsen (2004), and Holtmeier and Broll (2007). In contrast, the forest–tundra ecotone may be more suitable and more intuitive than the treeline ecotone or timberline ecotone for describing an area where the vegetation transition from the forested zone to the treeless zone is much more gradual (Löve, 1970). The usage of forest–tundra ecotone should be restricted to the subarctic region, because there is no significant differentiation between timberline, treeline, krummholzline, and forest–tundra ecotone at the continental scale or within the subtropics (Li and Chou, 1984; Schickhoff, 2005). Thus, we do not recommend the term forest–tundra ecotone to represent the sudden shift of vegetation in tropical or subtropical mountain ranges. If only a boundary must be used within/near the subtropical area, we prefer using the forestline, in contrast to treeline or timberline, to represent the forest edge when the vegetation shows a considerably abrupt transition.

## Conclusion

Generally, low temperature limits tree growth and recruitment, particularly in high mountains (Fajardo and McIntire, 2012). The upper limit of woody plant forms the boundary from mountain closedforest to alpine treeless tundra. The width and form of boundary have been affected by internal driver (e.g. species) and external driver (e.g. environment). Because of the hedge between the diverse natural phenomena and different human languages in the world (Wilhelm, 2002), these boundary terms have various expressions and writing forms in different literatures, and are thus likely to result in ambiguities and difficulties during comparison.

In this paper, the concise terms and illustrations have been tried to serve for the human understanding intuitively. The concise scheme has been suggested, including timberline, forestline, treeline, krummholzline, and historic treeline, to replace some verbose and ambiguous terminologies. The 5 different boundary terms could facilitate the comprehension of spatial sequence of vegetation transformation in high mountain idealistically, however, all boundaries do not necessarily occur in all mountains of the world concurrently. We propose to use the term forest line, instead of timber line or tree line once recommended by others, to represent the boundary between forest and scrub or grassland when the vegetation transition is abrupt and conspicuous, particularly in the subtropical or tropical alpine. Besides, we propose to use the term forest–tundra ecotone to represent the transitional vegetation belt where the closedforest gradually transforms into the treeless tundra and spans a broad area, particularly around the subarctic. The use of simple terms to articulate ecological concepts can confuse ideological debates and undermine management efforts (Colautti and MacIsaac, 2004). To avoid the confusion from using these boundary terms, we suggest that authors studying boundary related issues should interpret their scope of terminologies and provide the basic description about environment and vegetation outlines in the complex high mountains.

## Conflict of Interests

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# Evaluation of organic and inorganic amendments on nutrient uptake, phosphorus use efficiency and yield of maize in Kisii County, Kenya

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**Soil phosphorous and nitrogen are the major constraints to maize production in Nyanza Province of Kenya. The yields are typical of low input systems ranging below 1.0 t ha$^{-1}$ against a potential of 5.0 t ha$^{-1}$ per season. This study was conducted at Bototo, Kisii County in Nyanza Province, Kenya, during the long and short rains seasons in 2007. The aim was to determine the effects of phosphatic fertilizers and manure on nutrient uptake, nutrient use efficiency, maize yields and soil nutrients content at harvest. A Randomized Complete Block Design (RCBD) was used with the farms as blocks. Maize H614 hybrid was sown at a spacing of 0.75 × 0.60 m. The plot sizes were 3.75 m by 4.8 m. All plots were top dressed with Calcium ammonium nitrate (CAN) fertilizer at a uniform rate of 30 kg N ha$^{-1}$. Diammonium Phosphate (DAP), Minjingu Rock Phosphate (MRP) and Triple Super Phosphate (TSP) fertilizers were applied at a rate of 60 kg ha$^{-1}$ $P_2O_5$ (P) and farmyard manure (FYM) at 10 t ha$^{-1}$. One rate of P at 60 kg ha$^{-1}$ was applied on all the phosphorus fertilizers and a no P treatment (check) plus lime only treatment was included in determining the effects due to the applied P in the acidic soils. Complete soil chemical analysis was done in all the plots at the planting time. There was significant ($p \leq 0.01$) crop growth vigour, grain yield, total dry matter, harvest index, nutrient uptake and removal by the crop, available soil P, agronomic phosphorus use efficiency (APUE), physiological P use efficiency (PPUE) due to fertilizers and manure application, with a corresponding reduction in the total soil N, P, K, Ca and Mg. Phosphate fertilizers and manure applications are essential to improve maize yields and nutrient P use efficiency.**

**Key words:** Soils, fertilizer use efficiency, nutrient uptake, lime, maize yield.

## INTRODUCTION

Appropriate fertilizer use leads to increased crop yields and high crop recovery of applied nutrients. Some elements may be hazardous to the environment if not used in various forms such as nitrates and phosphates (Buresh et al., 1997). Efficient fertilization is therefore important in ensuring crops attain maturity within specific

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growing seasons (Lelei et al., 2006).

Soil reaction products have made agronomic evaluation of P fertilizers complicated due to range in characteristics of those materials coupled with the complex nature of fertilizers. Effectiveness of P sources therefore depends on the chemical and physical properties, rate and method of application, soil and climatic conditions and the crop species grown (Mokwunye and Bationo, 2002).

Over the recent years, there has been increased use of fertilizers mainly to improve crop production thus increase the economic returns, for example, Diammonium Phosphate (DAP), Triple Super Phosphate (TSP) and Minjingu Rock Phosphate (MRP) fertilizers (Negassa, et al., 2005). Several drawbacks have been reported while using Diammonium Phosphate where young crops have shown injury due to wrong application (Okalebo, 1997). Diammonium Phosphate has also been shown to lower the availability of the soil magnesium, calcium and potassium ions forming insoluble compounds (Opalla et al., 2013). This study was done to establish the influence of phosphorus application rate in different types of fertilizers and manure on growth and yields of maize, in order to allocate appropriate fertilizers and manure to suit varying agricultural conditions in the long and short rains planting seasons.

Maize nutrient (nitrogen, phosphorus, potassium, calcium and magnesium) uptake, use efficiency and yields in Nyanza Province of Kenya have been on the decline (Opalla et al., 2013; Negassa et al., 2005). This may be associated with lack of suitable fertilizer application rates and soil acidity since the current research recommendations, to specific areas in Kisii, Kenya were developed more than two decades ago (FURP, 1994). The extent of nutrient depletion is unknown and phosphate fertilizer and manure application by farmers is not commensurate with the plant requirements and / or nutrient levels in the soil. Twenty years have elapsed since the last country wide fertilizer use recommendation was carried out including Kisii County; during this period a decline of maize yields have been realized (Smalling et al., 1997). Maize is a major food crop for the community within Kisii County of Kenya. This has raised concerns about food insecurity. One of thecauses of food insecurity in the study area is soil acidity which affects crop production, especially maize. There was therefore need to determine the nutrient use efficiencies of phosphate fertilizers and manure and their effects in acidic soils of smallholder mixed farms. There was necessity to develop appropriate recommendations on application rates of fertilizer and manure that can be used by smallholder farmers in acidic soils in Bototo, Kisii County in Kenya. The study was conducted during the long and short rains planting seasons in 2007.

## MATERIALS AND METHODS

Field experiment was conducted at an on–farm site in Bototo at an altitude of 1590 masl, 34°44'E and 0° 39'S and 1200 to 2100 mm rainfall. The site is located in upper midland zones ($UM_1$) with agro climatic conditions suitable for maize, tea, coffee and sweet potatoes production (Jaetzold et al., 2007). Maize is the leading food crop in the region and was planted twice a year, during the long rains March to July 2007 and short rains August to December 2007. The upper midlands ($UM_1$) lie between 1500 and 1900 m above sea level. It is warm and humid with annual mean temperature of 18 to 25.5°C and a mean minimum temperature of 11 to 14°C. The annual average precipitation is greater than 80% of the potential evaporation ($E_0$).

The soils are mollic Nitisols or sandy loam Nitisols with moderately high fertility (Jaetzold et al., 2007). Maize is planted twice annually (March to July) and (August to December). The experimental design was Randomized Complete Block Design (RCBD) with selected farms as blocks (ten men and ten women). The sites were chosen randomly based on being dominated by acidic soils and having agro-ecological similarities. The previous cropping history and management showed that the performance was poor. The treatments were:

i). No phosphorus fertilizer used at planting -Control
ii). Lime only- 250 kg ha$^{-1}$ $CaCO_3$
iii). Diammonium Phosphate (DAP)- 60 kg ha$^{-1}$ $P_2$ $O_5$
iv). Minjingu Rock Phosphate (MRP)- 60 kg ha$^{-1}$ $P_2$ $O_5$
v). Triple Super Phosphate (TSP) - 60 kg ha$^{-1}$ $P_2$ $O_5P_2$
vi). Farm Yard Manure (FYM) -10 t ha$^{-1}$
vii). ½ FYM + ½ DAP - 5 t ha$^{-1}$ FYM + 30 kg ha$^{-1}$ $P_2$ $O_5$

The nutritional status of FYM was analysed and found to be relatively the same for all the farms.

Land preparation was done prior to the start of the rains (the long rains March to July 2007 and short rains August to December 2007), ploughed and harrowed twice using oxen to obtain a fine tilth seedbed. There were seven plots per block each measuring 3.75 m wide by 4.80 m long giving plot area of 18 m$^2$. Lime was applied in one of the seven plots per block at the rate of 250 kg ha$^{-1}$ $CaCO_3$ two weeks before sowing. Each plot consisted of 5 rows each with 8 hills. Maize hybrid H614 was the test crop in Bototo, chosen on the basis of being a suitable variety for the study area (Jaetzold et al., 2006).

At the onset of rains (the long rains March to July 2007 and short rains August to December 2007), three seeds were sown per hill. Fertilizers were applied at time of planting along the furrows and mixed with soil to avoid direct contact with the seeds to avoid scotching. All plots were uniformly top dressed with CAN fertilizer at a rate of 30 kg N ha$^{-1}$ as recommended (FURP, 1994).

Furadan was applied in each planting hole at the rate of 10 kg ha$^{-1}$ to protect the seeds and seedlings against soil borne pests after which the seeds were placed and covered with a small quantity of soil. The crop was protected against maize stalk borer *(Busseola fusca)* by application of Kombat (Carbaryl), a commercial insecticide, applied to the maize funnels at 4 weeks after planting at the rate of 4 kg ha$^{-1}$. Weeding was done twice after crop germination, one month after planting, then two months respectively.

Gladiator was applied in each trial site to prevent termite damage. Whenever a termite attack was visible, insecticide (Gladiator 4TC, liquid, active ingredient 480 gl À1 chlorpyrifos) was applied at the base of the maize plants to control this damage. The crop was harvested at physiological maturity from a net harvest area of 18 m$^2$ and threshed by hand. All ears affected by pests or rotten were excluded in yield measurements. All the consumable grains per plot were weighed and then sub–sampled. The maize stover was cut at about 5 cm above the ground level, weighed and sub-sampled for dry matter determination.

Data collected included planting date, emergence date at principal growth stage (0) (germination), code 00 (dry seed), code

09 (emergence), stand count at 21 days after emergence (DAE), scores for crop growth vigor on a scale of 1 to 7 at 21 DAE according to BBCH scale at principal growth stage 3 (stem elongation), code 33 (3 nodes detectable) on a rating scale of 1-7 (1- least vigorous, 7- most vigorous) based on the length of the maize plant, plant stand count at harvest (Lancashire et al., 1991). Common diseases (bacterial diseases, fungal diseases, nematodes, parasitic and viral diseases), flowering date, harvesting date, and yield per plot converted to t ha $^{-1}$. Data was collected in a net plot of 18 $m^2$.

Crop harvest data included field grain, cob and stover weights recorded. Field grain moisture content was recorded using a grain moisture tester (model DjGMTS. N. 0528.).

Sub-samples of grain, cobs and stover were then taken for oven drying and subsequent dry matter yield determination. The grain yield (adjusted to 15% moisture content), total above ground dry matter yield, harvest index, and total nutrient uptake and phosphorus use efficiency was calculated using the following formulae:

(i) Grain yield (at 15% moisture) = GW × (100 – MCA) / 100 – MCD
(ii) Total dry matter yield (kg ha $^{-1}$) = GY + SY +CY
(iii) Harvest index = GY / Total dry matter yield
(iv) Total nutrient uptake = (NCG X GY) + (NCS X SY) + (NCC X CY)

Where: *GY, SY* and *CY* are grain, stover, and cob dry matter yields respectively; *GW, MCA* and *MCD* are fresh grain weight, moisture content of fresh grain and moisture content of grains at 15% moisture respectively; *NCG, NCS* and *NCC* are nutrient (N, P, K, Ca, and Mg) concentrations in grain, stover and cob respectively.

Nutrient phosphorus use efficiency was calculated using the following formulae:

(v) Agronomic P use efficiency = $\frac{Y_f - Y_O}{P}$

(vi) Physiological P use efficiency = $\frac{Y_f - Y_O}{P_{uf} - P_{uo}}$

Where: $Y_f$, and $Y_o$ are yields of fertilized and unfertilized crops respectively; $P$ is the rate of fertilizer P applied, $P_{uf}$ and $P_{uo}$ are P uptake in fertilized and unfertilized crops respectively.

The changes in soil nutrient contents at harvest were determined by difference method:

(vii) Change in soil nutrient content = $P_x - P_o$.

Where: *Px* is the nutrient content for a given fertilizer application rate.*Po* is the nutrient content for the check (zero) fertilizer treatment (Sigunga et al., 2002).

At the start of the experiment, soil samples were randomly collected from 5 spots in a zigzag pattern at a depth of 0 to 30 cm at each experimental farm using a 5-cm diameter auger. The samples were used to assess initial soil fertility status. The soil samples were mixed to obtain a composite sample. About 3 kg sub–samples were obtained from the composite sample, air–dried in a well-ventilated room for three days and ground to pass through 2-mm sieve. The soil samples were analyzed for pH 1:2.5 soil: solution ($H_2O$ and 0.01 M $CaCl_2$), extractable P, %P, %N, texture, organic carbon, exchangeable acidity, cation exchange capacity (CEC), and exchangeable bases. At crop harvesting, composite soil samples were collected per plot at 3 spots per row, to assess changes in soil chemical properties with respect to the fertilizer treatments applied. The samples were analyzed for extractable P, %N, %P, %K, and %Ca contents (Okalebo et al., 2002).

Plant samples were separated into stover, cob, and grain. The stover was chopped using a chaff cutter. The stovers, cobs, and grains were sub-sampled, weighed, and oven–dried to a constant weight at 70°C for 48 h for determination of the above ground dry matter yield. The dried plant material was ground using Cromption Willey mill to pass through a 2 mm sieve and sub-sampled for total N, P, K, Ca and Mg determination.

### Data analyses

Data on growth vigor, grain yield, total dry matter yield, harvest index, nutrient (N, P, K, Ca, and Mg) uptake, phosphorus use efficiency, soil nutrient contents (extractable P, %P, %N, %K, % Ca at harvest, were subjected to the General Linear Model (GLM). Twenty farms serving as blocks/replicates in the GLM and mean at $p = 0.001$ using Tukeys. Correlation analysis was carried out to estimate the relationship between the variables such as grain yield, total dry matter yield, harvest index and nitrogen uptake, phosphorus uptake, calcium uptake, magnesium uptake, phosphorus PAUE, PPUE, extractable phosphorus, total soil phosphorus, total soil potassium, total soil calcium, and total soil magnesium. SAS statistical package was used for analysis.

## RESULTS

### Maize yields

Initial soil analysis indicated that the soils at the site were low in fertility, acidic, with low amounts of total N, organic carbon, and total and extractable phosphorus and exchangeable bases (Table 1).

Visual observations revealed P deficiency symptoms (over-all stunted growth) in the Control plots at early crop growth stages (4 weeks after crop emergence) at the site (Table 2). There were significant difference ($p \leq 0.0001$) crop growth vigour response to the fertilizers and manure. Plants that received fertilizer and manure were more vigorous in growth than those in the control plots. Maize growth vigour varied from 1.15 in the Control plots to 6.95 in the ½ DAP + ½ FYM treatment with a mean of 3.76. The maize growth vigour scores were 5.0, 4.75, 3.95, 2.95 and 1.15 in TSP, FYM, DAP, MRP and lime respectively (Table 2). There were P deficiency symptoms in plants that did not receive P treatments which indicated that P limited crop growth. There was significant difference ($p \leq 0.0001$) effect of fertilizer on grain yield. Grain yield varied from 1722 kg ha$^{-1}$ in the Control plots to 6244 kg ha$^{-1}$ in the ½ DAP + ½ FYM treatment with a mean of 3932 kg ha$^{-1}$. The grain yields were 4961, 4274, 3995, 3760 and 2569 kg ha$^{-1}$ in TSP, FYM, DAP, MRP and lime respectively. The significant grain yield response to fertilizers and manure application at the site (Table 2) is attributed to the low soil P status of these soils (Table 1). There was significant difference ($p \leq 0.0001$) effect of fertilizer and manure on total dry matter yield. Total dry matter yield varied from 6.49 t ha$^{-1}$ in the control plot to 16.33 t ha$^{-1}$ in the ½ DAP + ½ FYM treatment with a mean of 11.47 t ha$^{-1}$. The total dry matter yields were 13.61, 12.32, 11.69, 11.2 and 8.68 in TSP,

FYM, DAP, MRP and lime respectively (Table 2). There

**Table 1.** The initial pH, extractable P, phosphorus, nitrogen, potassium, calcium and magnesium at the start of the experiment at the study site (0 to 30 cm).

| Treatment | pH | Extractable P (mg P /kg) | Phosphorus (ppm) | Nitrogen (%) | Potassium (%) | Calcium (%) | Magnesium (%) |
|---|---|---|---|---|---|---|---|
| ½ DAP + ½ FYM | 4.3 | 3.0 | 16.6 | 0.11 | 4.9 | 1.10 | 0.85 |
| TSP | 4.4 | 3.1 | 16.4 | 0.10 | 4.8 | 1.20 | 0.70 |
| FYM | 4.0 | 3.2 | 16.4 | 0.20 | 5.0 | 1.10 | 0.90 |
| DAP | 3.9 | 2.9 | 16.6 | 0.10 | 4.8 | 1.20 | 0.90 |
| MRP | 4.2 | 2.9 | 16.4 | 0.20 | 4.7 | 1.10 | 0.80 |
| Lime | 4.1 | 3.3 | 16.8 | 0.12 | 5.1 | 1.12 | 1.0 |
| Control | 4.4 | 3.0 | 16.7 | 0.11 | 4.9 | 1.10 | 0.8 |
| Mean | 4.2 | 3.1 | 16.5 | 0.14 | 4.9 | 1.13 | 0.85 |
| COV % | 3.3 | 2.7 | 8.3 | 7.3 | 2.3 | 6.93 | 4.2 |
| SE +/ - | 0.031 | 0.019 | 0.315 | 0 | 0.025 | 0.017 | 0.008 |
| MSD | 0.131 | 0.079 | 1.339 | 0.009 | 0.105 | 0.074 | 0.034 |

**Table 2.** Effects of treatments on mean maize growth vigour scores, maize grain yield, total dry matter yield and harvest index at the study site.

| Treatment | Maize growth vigour score* | Grain yield (kg/ha) | Total dry matter yield (t/ha) | Harvest Index (HI) |
|---|---|---|---|---|
| ½ DAP + ½ FYM | 6.95 | 6244 | 16.33 | 0.37 |
| TSP | 5.0 | 4961 | 13.61 | 0.35 |
| FYM | 4.75 | 4274 | 12.32 | 0.33 |
| DAP | 3.95 | 3995 | 11.69 | 0.33 |
| MRP | 2.95 | 3760 | 11.2 | 0.32 |
| Lime | 1.55 | 2569 | 8.68 | 0.30 |
| Control | 1.15 | 1722 | 6.49 | 0.26 |
| Mean | 3.757 | 3932 | 11.47 | 0.321 |
| COV % | 20.57 | 32.52 | 22.24 | 10.79 |
| SE + / - | 0.1728 | 285.92 | 0.571 | 0.0078 |
| MSD | 0.4841 | 801.02 | 1.598 | 0.0217 |

*Growth vigour ranked on a scale of 1-7 (1-least vigorous, 7 –most vigorous.) according to BBCH scale at principal growth stage 3 (stem elongation), code 33 (3 nodes detectable).

was significant effect of fertilizer and manure on harvest index ($p \leq 0.0001$). The harvest indexes varied from 0.26 in the Control plots to 0.37 in the ½ DAP + ½ FYM treatment with a mean of 0.321. The harvest indexes were 0.35, 0.33, 0.33, 0.32 and 0.30 in TSP, FYM, DAP, MRP and lime respectively (Table 2).

## Nutrient uptake

Fertilizer and manure application had significant ($p \leq 0.0001$) effect on nutrient uptake. Nitrogen uptake varied from 21.1 kg $ha^{-1}$ N in the Control plot to 67.8 kg $ha^{-1}$ N in the ½ DAP + ½ FYM plot with a mean of 49.83 kg $ha^{-1}$ N. Nitrogen uptake correlated positively ($p \leq 0.0001$, r = 0.84) with total dry matter yield. There was significant P uptake response to the fertilizers and manure (Table 3). The P uptake varied with the fertilizers and manure from 18.3 kg $ha^{-1}$ P in the control plots to 63.5 kg $ha^{-1}$ P in the ½ DAP + ½ FYM plots with a mean of 47.4 kg $ha^{-1}$ P. Phosphorus uptake correlated positively ($p \leq 0.0001$, r = 0.72) with grain yield and total dry matter yield. Potassium uptake increased significantly due to the fertilizers and manure (Table 3). K uptake varied from 46.7 kg $ha^{-1}$ K in the control plots to 105.1 kg $ha^{-1}$ K in the ½ DAP + ½ FYM plots with a mean of 77.3 kg $ha^{-1}$ K. Calcium uptake increased significantly due to the fertilizers and manure (Table 3). Ca uptake varied from 3.34 kg $ha^{-1}$ Ca in the control plots to 8.25 kg $ha^{-1}$ Ca in the ½ DAP + ½ FYM plots with a mean of 5.78kg $ha^{-1}$ Ca. Magnesium uptake increased significantly ($p \leq 0.0001$, r = 0.77) due to fertilizers and manure (Table 3).

**Table 3.** Nutrient uptake by the maize plants at physiological maturity.

| Treatment | Nitrogen uptake (kg ha$^{-1}$ N) | Phosphorus uptake kg ha$^{-1}$ P | Potassium uptake (kg ha$^{-1}$ K) | Calcium uptake (kg ha$^{-1}$ Ca) | Magnesium uptake (kg ha$^{-1}$ Mg) |
|---|---|---|---|---|---|
| ½ DAP + ½ FYM | 67.8 | 63.5 | 105.1 | 8.25 | 4.12 |
| TSP | 47.8 | 43.3 | 82.9 | 5.06 | 2.99 |
| FYM | 67.3 | 59.6 | 80.6 | 8.17 | 3.49 |
| DAP | 56.3 | 51.6 | 86.0 | 6.74 | 3.98 |
| MRP | 50.8 | 56.8 | 80.2 | 6.33 | 3.44 |
| Lime | 37.7 | 38.4 | 59.5 | 3.56 | 2.69 |
| Control | 21.1 | 18.3 | 46.7 | 3.34 | 1.9 |
| Mean | 49.83 | 47.35 | 77.27 | 5.78 | 3.23 |
| COV % | 7.68 | 11.35 | 8.85 | 8.36 | 12.89 |
| SE +/ - | 0.856 | 1.202 | 1.530 | 0.108 | 0.093 |
| MSD | 2.398 | 3.367 | 4.287 | 0.303 | 0.261 |

**Table 4.** Phosphorus agronomic use efficiency and physiological use efficiency.

| Treatment | Phosphorus agronomic use efficiency (kg grain/kg P applied) | Phosphorus physiological use efficiency (kg grain/kg P taken up) |
|---|---|---|
| ½ DAP + ½ FYM | 29 | 39 |
| TSP | 24 | 42 |
| FYM | 42 | 36 |
| DAP | 43 | 38 |
| MRP | 21 | 29 |
| Lime | - | 35 |
| Control | - | 29 |
| Mean | 31.85 | 35.53 |
| COV % | 6.80 | 6.02 |
| SE +/ - | 0.485 | 0.478 |
| MSD | 1.365 | 1.74 |

Mg uptake varied from 1.9 kg ha$^{-1}$ Mg in the control plots to 4.12 kg ha$^{-1}$ Mg in the ½ DAP + ½ FYM plots with a mean of 3.23 kg ha$^{-1}$ Mg. There were significant ($P \leq 0.0001$) treatment effects on nutrient uptake which indicates significant N responses to fertilizers and manure (Table 3). Nitrogen uptake was highly correlated ($p \leq 0.0001$, $r = 0.84$) with total dry matter yield while phosphorus uptake correlated positively ($p \leq 0.0001$, $r = 0.78$) with grain and total dry matter yields. Lack of significant difference in N content between the control and lime treatments in all the plots was due to the blanket application of the recommended N rate.

## Nutrient use efficiency

Fertilizer and manure application had significant difference ($p \leq 0.0001$, $r = 0.61$) effect on PUE. P agronomic use efficiency varied from 21 kg grain per kg P applied in the Minjingu Rock Phosphate plots to 43 kg grain per kg P applied in the DAP plots with a mean of 32 kg grain per kg P applied (Table 4). Control and lime treatments had no applied P and thus no data. Physiological P use efficiency responded significantly to fertilizers and manure (Table 4). Physiological P use efficiency varied from 29 kg grain per kg P taken up in the control and Minjingu Rock Phosphate plots to 42 kg grain per kg P applied in the TSP plots with a mean of 36 kg grain per kg P applied (Table 4).

## Effects of treatments on soil nutrient contents

Fertilizer and manure application had significant difference ($p \leq 0.0001$) effect on soil nutrient contents. Fertilizers and manure application significantly increased the extractable soil P content above the control. Extractable P varied from 3.5 mg P/kg in the farmyard manure plots to 7.6 mg P/kg in the Minjingu Rock Phosphate plots with a mean of 5.43 mg P/kg (Table 5).

**Table 5.** Final pH, extractable P, phosphorus, nitrogen, potassium, calcium and magnesium.

| Treatment | pH | Extractable P (mg P /kg) | Phosphorus (ppm) | Nitrogen (%) | Potassium (%) | Calcium (%) | Magnesium (%) |
|---|---|---|---|---|---|---|---|
| ½ DAP + ½ FYM | 5.1 | 6.8 | 2.2 | 0.01 | 2.02 | 0.0023 | 0.035 |
| TSP | 5.0 | 5.9 | 2.6 | 0.01 | 2.15 | 0.0027 | 0.024 |
| FYM | 5.1 | 3.5 | 2.4 | 0.01 | 2.96 | 0.0020 | 0.027 |
| DAP | 5.0 | 4.7 | 2.3 | 0.01 | 2.27 | 0.0024 | 0.024 |
| MRP | 5.4 | 7.6 | 2.3 | 0.01 | 2.87 | 0.0028 | 0.029 |
| Lime | 5.4 | 5.6 | 2.3 | 0.01 | 2.88 | 0.0029 | 0.022 |
| Control | 4.5 | 3.6 | 2.4 | 0.01 | 2.88 | 0.0022 | 0.020 |
| Mean | 5.1 | 5.4 | 2.35 | 0.01 | 2.56 | 0.0025 | 0.026 |
| COV % | 3.5 | 5.4 | 3.59 | 0 | 6.53 | 5.92 | 4.93 |
| SE +/ - | 0.039 | 0.065 | 0.019 | 0 | 0.037 | 0.00003 | 0.0003 |
| MSD | 0.167 | 0.277 | 0.080 | 0 | 0.159 | 0.0001 | 0.001 |

Total soil P contents were significantly ($p \leq 0.0001$) different. The total soil P content varied from 2.1% in the control plots to 2.5% in the TSP plots with a mean of 2.2% (Table 5). The fertilizers and manure had no significant effect on total N. The total N % was approximately 0.01. The fertilizers and manure had no effect on the total N % in the soils because a blanket application of 30 kg $ha^{-1}$ N was done so that it was not limiting to the maize germination, growth and yield (Table 5). Total soil potassium (K) significantly ($p \leq 0.0001$) changed with the different fertilizers and manure applied. The total soil K varied from 2.02% K in the ½ DAP + ½ FYM plots to 12.27 % K in the DAP plots with a mean of 4.09% K (Table 5). Total soil calcium significantly ($p \leq 0.0001$) differed in the different fertilizers and manure treatments. The total soil Ca varied from 0.002% Ca in the farmyard manure plots to 0.0029% Ca in the lime plots with a mean of 0.0025% Ca (Table 5). Total soil magnesium differed ($p \leq 0.0001$) in the different fertilizers and manure treatments. The total soil Mg varied from 0.2% Mg in the control plots to 0.035% Mg in the ½ DAP + ½ FYM plots with a mean of 0.026% Mg (Table 5). Fertilizer and manure application had significant difference ($p \leq 0.0001$) effect on soil nutrient content. Fertilizers and manure application significantly increased the extractable soil P content above the control. The mean values for extractable soil P content was 5.43 mg P/kg. Minjingu Rock Phosphate and the ½ DAP + ½ FYM treatments significantly increased extractable P than the control. The application of lime significantly increased extractable P as compared to the control (Table 5). Total soil P contents, total soil potassium, total soil calcium and total soil magnesium were significantly different. The mean values for total soil P was 2.2% P, total soil potassium 2.63% K, total soil calcium 0.003% Ca and total soil magnesium 0.03% Mg. The fertilizers and manure had no significant effect on total N (Table 5).

The mean value for pH in farmyard manure at Bototo was 8.6. The mean values for N, P, Ca, Mg, K and C in farmyard manure were 11.6, 2.2, 8.8, 2.6, 7.8 and 116 g $kg^{-1}$. Nutrient analysis of the manures show that for example 5 t $ha^{-1}$ cattle manure can supply approximately 58 kg N, 11 kg P, 39 kg K, 44 kg Ca, and 13 kg Mg $ha^{-1}$, however these potential values, particularly for N, K, Ca and Mg vary across farms.

## DISCUSSION

The study conducted during the long and short rains seasons in 2007 confirmed that the low soil fertility could be attributed to the continuous cropping of land with little or no nutrient returns, thus resulting into low nutrient content and therefore decline in soil fertility (Gudu et al., 2005). The crop response to P fertilizers and manure application in these soils was therefore expected from the initial soil analysis results. The increase in yield is therefore, attributed to the increased available P due to fertilizers and manure application. The significant difference in growth vigour response to fertilizers and manure (Table 2) could be attributed to the fact that maize depends on fertilizer P at its early stages of growth and this might have stimulated root proliferation and acquisition of nutrients for growth.

Acidic soils render P and N unavailable through P fixation and slowing down of nitrification rates, respectively (Gudu et al., 2005). Therefore the control gave lowest yields, probably because of reduced N and P. Combinations involving manure and DAP gave high grain yields. This underlines the importance of FYM and DAP in crop performance and more so for these acidic soils (pH 3 to 6). High yields of maize were observed in TSP plots because it contained Ca (12 to 14%) and hence large doses of P are similar to liming.

The relative difference in nutrient (N, P, K Ca, and Mg) uptake was related to differences in dry matter yield production (Mengel and Kirkby, 2001). The higher nutrient uptake with combined N and P than the sole P application could be attributed to the synergistic N enhancement of P uptake (Negassa et al., 2005).

Mengel and Kirkby (2001) proposed that the decreased efficiency in P uptake following P application was a result of conversion of fertilizer P to relatively insoluble forms. The reductions in total soil P content with application of fertilizers and manures could be attributed to the increased dry matter production and hence higher nutrient P removal by the crop following N application. This may be due to N effect in promoting dry matter production. It could also be attributed to the synergistic interaction between N and P (Brady and Weil, 1984), whereby the availability and P uptake was increased hence the reduction of P in the soil that was observed in this study. The lack of significant change in total soil N with application of fertilizers and manure could be attributed to the blanket application of the recommended rate of N. The reduction in total soil K, Mg and Ca contents with fertilizers and manure applications could be attributed to the increased crop nutrient removal following fertilizer N and P application. The results agree with Smalling et al. (1997) that increased fertilizer N and P application could result in deficiency of other nutrients (such as K, Ca, Mg and Zn) due to rapid crop removal. The low Ca and Mg uptake could be related to their relatively low levels in these soils: 0.003 and 0.03% respectively. The gradual net depletion of soil cations if not compensated by fertilizer and manure inputs, would eventually affect crop yields.

Crop responses to decomposed or non-decomposed manure application may be due to increases in soil pH, N, P, Cations such as Ca and Mg or to physical effects of addition soil organic matter on water filtration and retention. However the responses to cattle manure application are highly variable due to differences in the chemical composition of the manures. Poor storage conditions may result in ammonia losses through volatilization and leaching of nitrates. A survey in Bototo, Kisii County, Kenya to determine how livestock and manure management practices (stocking rate, feeding, collection, composition and storage) affect the quality of the manure for crop production indicated that collecting boma manure and just heaping it on the soil surface resulted in very low quality manure (KARI, 1991). The differences in organic C and N could be due to cattle diets, method of collection, storage, and degree of decomposition of the manure.

## CONCLUSIONS AND RECOMMENDATIONS

The study conducted at Bototo, Kisii County in Nyanza Province, Kenya, during the long and short rains seasons in 2007 showed that farmers should use phosphate fertilizers and manure when planting. Phosphate fertilizers and manure application significantly ($p \leq 0.0001$) increased maize grain and dry matter yields. ½ FYM + ½ DAP application makes N and P nutrients readily available from the fertilizers used and manure and effectively regulate the soil acidity. Farmers should apply ½ DAP + ½ FYM because this makes N and P available to the maize crop and regulates the soil acidity. Investigations should be done on the effectiveness of manure to binding Fe and Al ions in acid soils. Fertilizers and manure are essential to improve maize yields, nutrients uptake, nutrients use efficiency and soil nutrient contents. Soils in the site were low in fertility, acidic (pH 3 to 6), thus, low amounts of total N, organic carbon, total and extractable phosphorus and exchangeable bases.The soils require phosphate fertilizers and farmyard manure. Further long-term studies in these soils to investigate the effects of fertilizer use nutrient balance, as a basis for fertilizer formulations and recommendation is necessary.

## Conflict of Interests

The authors have not declared any conflict of interests.

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# Impact of differential storage conditions on seed germination and viability of some medicinal plants

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**The present study describes the impact of different storage comportments on germination and viability of seven plant species of ethno-medicinal importance in Chhattisgarh state of India. Seeds were subjected to various storage environments (room and low temperature); storage types (polythene bag, cloth bag, aluminum foil, paper bag) and storage durations (3, 6 and 9 months). Observation on germination percentage clearly indicated that at room temperature poly bag was found to be most suitable for storage compared with other materials whereas, under low temperature aluminum foil tend to be most ideal for seed storage. Seeds stored in cloth bag were found with least germination percentage among the tested plant species. Irrespective of plant species, germination percentages were significantly higher when seeds are stored in room temperature. Interaction between storage conditions, type of storage and plant species on the viability of seed was also found to be significant. It was interesting to note that seed germination percentage varied within seven plant species collected from different wild habitats. Further studies on important medicinal plant species would be useful to optimize the storage components. The data also exert implications for conservation of tested medicinal plant species in respect to proper seed storage.**

**Key words:** Germination, storage behavior, medicinal plant, ethno-medicinal, conservation.

## INTRODUCTION

The importance of seed storage has been recognized ever since humans began to domesticate plants. Since the dawn of commercial agriculture, farmers have had to sustain viable seeds from one growing season to the next (that is, short-term seed storage, typically 3 to 9 months but occasionally up to 18 months). The duration of successful storage depends upon both the objectives and the plant species concerned. Seeds of different plant species respond in varied degree to the ambient environment before and during storage. Three main categories of seed storage behavior are now been recognized: Orthodox and recalcitrant (Roberts, 1972) and a third category which is intermediate between the orthodox and recalcitrant (Ellis et al., 1991). Seeds of plant species with orthodox seed storage behavior can be maintained satisfactorily *ex situ* over a long term in appropriate environments. The maintenance of the viability of seeds with recalcitrant and intermediate seed storage behavior is difficult. However, seeds can be stored for medium-term with intermediate storage

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behavior provided the environment is well-defined. Seed is a living entity and is subjected to various environmental stresses which ultimately affect the quality. In storage, the viability and vigor of the seeds not only vary within different genotypes, but it also regulated by many physico-chemical factors like moisture content, atmospheric relative humidity, temperature, initial seed quality, physical and chemical composition of seed, gaseous exchange, storage structure, packaging materials etc. (Doijode, 1988). Among these, the major factors are temperature and relative humidity, which results in drastic deterioration of seed. Several attempts have been made on many crops to develop methods for maintaining the viability and vigor of seeds for longer period during storage (Ader, 1978; Agrawal, 1980; Arati, 2000; Caneppele et al., 1995; Doijode, 1997; Padma and Reddy, 2002). It is now a well-established fact that the choice of materials for seed coating/treatment, storing containers and storage environment exert a positive effect on the viability and vigor of seeds. Roberts (1972) reported that seed deterioration during storage is due to the damage to seed membrane, enzyme, proteins and nucleic acid. Accumulation with time such degenerative changes result in complete disorganization of membranes and cell organelles and ultimately causing death of the seed and loss of germination.

The demand for medicinal plants has increased globally due to the resurgence of interest and acceptance of herbal medicine. Most of the demand is being met through collection of large quantities of medicinal plants or the useful parts from wild habitats. As a consequence, the rate of these ethno-medicinal plant species exploitation exceeding those of local natural regeneration. In recent years a depletion in the natural habitats has been observed (Pushpangadan and Nair, 2011; Rao and Rajasekharan, 2002). There is thus, an urgent need to develop and implement conservation strategies for exploited medicinal plant species. In other way, commercial cultivation of these plant species may mitigate the depletion. This largely depends on the availability of viable seeds to the cultivator. In the present effort, research was conducted on effect of different storage conditions on seed germination and viability of ethno-botanically important medicinal plant species.

## MATERIALS AND METHODS

The entire study was conducted in departmental laboratory, T.C.B. College of Agriculture and Research Station, Bilaspur (C.G.) during 2009 to 2011. The temperature, rainfall and humidity variation throughout the study is given in Table 1 which was obtained from meteorological departments of the college.

### Seed source

Seven plants of significant ethno-medicinal use and which is found in abundant locally was selected for the study (Table 2). The fresh harvested seeds were obtained from medicinal plant nursery established inside the college campus. Before storage, the cleaned seeds were visually graded for uniform size and moisture content was calibrated to 10% to annul the microbial infection.

### Treatments

The experiment constructed with three factors *viz*. (i) two storage temperatures (room and low [4°C]); (ii) four storage materials (poly bag, cloth bag, aluminum foil, paper bag) and (iii) storage durations (3, 6 and 9 months).

### Method of storage

A standard quantity of 50 g seed was maintained for all the treatments. Polythene bags were sealed by using commercial polythene heat sealer where as aluminum foils are sealed using resin hardener and paper bags were closed with stapled pin after double folding. Seeds in respective storage materials were kept on sterilized plastic trays at room temperature and at low temperature (4°C) inside laboratory refrigerator.

### Experimental design, data parameter and analysis

Each treatment was replicated thrice and was laid out in complete randomized design. Observations were recorded on germination or viability percentage for each treatment. One hundred seeds were taken from each replication per each treatments and the germination test was conducted using ‘between paper method’ as per ISTA Rules (Anon., 1996). In brief, the rolled paper towels were placed in the germinator in slanting position at a constant temperature of 25±1 and 95±1% relative humidity. The number of normal seedlings was counted at the end of $10^{th}$ day and the mean count/ replication/ treatment was expressed in percentage. The data recorded was subjected to statistical analysis as described by Gomez and Gomez (1984). The analysis of variance was done by the method described by Fisher (1935).

## RESULTS AND DISCUSSION

The purpose of this study was to assess the tolerance level of different medicinal plant seeds under the effect of storage materials, durations and temperatures respectively. Under the influence of aforesaid treatments the seed germination percentage varied by a wide spectrum (Table 3). For convenience, detailed review effect of different storage conditions are explained separately.

### Effect of storage materials

Data presented in Table 3 revealed an inconsistency in germination percentage when seeds were kept in different storage materials. To summarize the effect of storage materials on seed germination with different storage durations and temperatures, mean data were calculated irrespective of studied plant species (Figure 1). It was observed that, one month after storage, seeds in aluminum foil were found with highest germination at

**Table 1.** Mean monthly weather data of Bilaspur, Chhattisgarh, India (Year -2006-08) at the time of experiment.

| Parameter | Year | Month | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
| MXT | 2009 | 28.2 | 33.2 | 33.0 | 38.6 | 40.1 | 38.5 | 30.2 | 30.1 | 42.2 | 32.4 | 29.8 | 28.0 |
| | 2010 | 27.9 | 29.4 | 32.9 | 39.0 | 40.4 | 37.5 | 30.1 | 31.3 | 31.5 | 31.1 | 29.2 | 27.5 |
| | 2011 | 27.5 | 27.4 | 34.8 | 39.1 | 41.7 | 34.3 | 32.2 | 31.7 | 32.3 | 32.1 | 30.1 | 29.3 |
| MNT | 2009 | 10.2 | 13.8 | 17.9 | 21.7 | 25.9 | 26.4 | 24.8 | 24.4 | 24.3 | 19.9 | 16.4 | 12.1 |
| | 2010 | 11.1 | 14.7 | 17.6 | 22.1 | 25.0 | 26.4 | 24.6 | 24.3 | 24.4 | 18.9 | 14.2 | 11.6 |
| | 2011 | 11.8 | 11.6 | 18.2 | 21.0 | 25.4 | 25.1 | 25.0 | 25.1 | 24.1 | 19.4 | 15.3 | 12.0 |
| RH-I | 2009 | 87.0 | 83.0 | 82.0 | 67.0 | 63.0 | 75.0 | 91.0 | 92.0 | 91.0 | 92.0 | 91.0 | 92.0 |
| | 2010 | 85.0 | 87.0 | 75.0 | 66.0 | 61.0 | 75.0 | 93.0 | 95.0 | 93.0 | 90.0 | 92.0 | 91.0 |
| | 2011 | 91.0 | 84.0 | 74.0 | 68.0 | 64.0 | 84.0 | 92.0 | 93.0 | 92.0 | 91.0 | 91.0 | 89.0 |
| RH-II | 2009 | 36.0 | 27.0 | 38.0 | 26.0 | 32.0 | 42.0 | 78.0 | 78.0 | 69.0 | 49.0 | 44.0 | 38.0 |
| | 2010 | 39.0 | 38.0 | 35.0 | 25.0 | 30.0 | 52.0 | 76.0 | 77.0 | 73.0 | 47.0 | 38.0 | 37.0 |
| | 2011 | 41.0 | 44.0 | 30.0 | 27.0 | 29.0 | 67.0 | 78.0 | 76.0 | 68.0 | 54.0 | 41.0 | 32.0 |
| SS | 2009 | 9.7 | 10.1 | 9.1 | 9.2 | 8.5 | 6.6 | 1.9 | 3.5 | 7.2 | 9.3 | 8.2 | 9.0 |
| | 2010 | 8.9 | 9.0 | 9.4 | 9.5 | 9.3 | 5.8 | 4.2 | 3.8 | 5.0 | 8.8 | 11.7 | 8.6 |
| | 2011 | 8.2 | 8.1 | 8.6 | 10.2 | 9.3 | 3.2 | 3.9 | 4.1 | 7.0 | 8.8 | 7.7 | 8.5 |
| EVP | 2009 | 3.0 | 4.1 | 4.8 | 6.5 | 7.1 | 6.6 | 3.0 | 3.0 | 3.6 | 3.6 | 2.6 | 2.5 |
| | 2010 | 2.8 | 3.7 | 4.9 | 6.4 | 6.9 | 6.5 | 3.2 | 2.9 | 3.1 | 3.3 | 2.8 | 2.4 |
| | 2011 | 2.6 | 3.1 | 4.7 | 6.4 | 8.3 | 4.6 | 3.9 | 3.4 | 3.8 | 3.3 | 2.7 | 2.4 |
| RF | 2009 | 0.0 | 0.0 | 45.8 | 12.8 | 67.0 | 67.6 | 353.6 | 299.8 | 130.2 | 11.6 | 1.4 | 0.0 |
| | 2010 | 0.0 | 77.4 | 48.6 | 10.2 | 22.4 | 218.2 | 341.8 | 461.8 | 118.4 | 12.0 | 0.0 | 0.0 |
| | 2011 | 1.4 | 14.6 | 12.6 | 29.6 | 4.4 | 280.2 | 165.4 | 241.0 | 213.8 | 15.4 | 6.4 | 0.0 |

Source: Agro-meteorology Observatory of TCB College of Agriculture and Research Station (IGKV), Bilaspur, Chhattisgarh, Inida. MXT, Maximum temperature (°C); MNT, minimum temperature (°C); RH, relative humidity (%); SS, sunshine hour (h/day); EVP, evaporation; RF, rainfall (mm).

**Table 2.** List of medicinal plant species tested for long term seed storage.

| Medicinal plant species | Local name* | Status* | Usage of seed |
|---|---|---|---|
| *A. precatorius* | Ratti / Gunja | Threatened | Much valued in native jewelry as for bright color, abortifacient, anodyne, aphrodisiac, antimicrobial and diuretic |
| *C. speciosus* | Keokand | Endangered | Additional source of diosgenin yields fatty oil, rich in linoleic acid and lowers cholesterol level in blood |
| *D. motorium* | Telegraph plant | Endangered | Greenhouse, conservatory or house plant |
| *C. forskohlii* | Pasandbhed | Threatened | Alkaloid Forskohlin is extracted, drug for hypertension, glaucoma, asthma, congestive heart failures |
| *P. zelaynica* | Chitrak | Endangered | Used medicinally as a stimulant, caustic, digestion |
| *A. moschatus* | Kasturi Bhindi | Endangered | Inhalation, when suffering from hoarseness used as a flavouring for liqueurs or to scent coffee |
| *C. anthelminticum* | Vanjeera | Endangered | Used as purgative, for asthma, kidney troubles |

*, In India.

room (43.9%) as well as in low temperature (17.1%). In a similar trend, two months later seeds from poly bags found with maximum seed germination at room (28.6%) and low temperature (8.4%). Whereas, after three

**Table 3.** Effect of storage types and storage conditions on seed viability of medicinal plant species expressed in per cent germination.

| Months | Conditions | Storage materials | Medicinal plant species | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | ***A. precatorius*** | ***C. speciosus*** | ***D. motorium*** | ***C. forskohlii*** | ***P. zeylanica*** | ***A. moshchatus*** | ***C. anthelminticum*** |
| **Three** | Room temperature | Poly bags | 60 | 53.3 | 15 | 84.7 | 50 | 18.3 | 6.7 |
| | | Cloth bags | 60 | 36.7 | 13.3 | 90 | 6.7 | 21.7 | 2 |
| | | Aluminum foil | 73.3 | 26.7 | 15 | 94 | 73.3 | 23.3 | 2 |
| | | Paper bags | 80 | 50 | 6.7 | 76.7 | 56.7 | 16.7 | 6.3 |
| | Low temperature | Poly bags | 10 | 23.3 | 10.7 | 2 | 33.3 | 0 | 0 |
| | | Cloth bags | 0 | 20 | 2 | 2 | 36.7 | 1.7 | 0 |
| | | Aluminum foil | 3.3 | 13.3 | 15 | 6.7 | 80 | 1.7 | 0 |
| | | Paper bags | 6.7 | 28.3 | 6.7 | 0 | 40 | 3.3 | 0 |
| (A): 0.89; (B): 1.02; (C): AB: 2.00; 1.87; AC: 2.65; BC: 3.75; ABC: 5.31 ; Coefficient of variation: 7.32%, C. D. at 5% | | | | | | | | | |
| **Six** | Room temperature | Poly bags | 56.7 | 3.3 | 33.3 | 47.3 | 41.7 | 11.7 | 6 |
| | | Cloth bags | 60 | 3.3 | 23.3 | 10 | 0 | 18.3 | 4.3 |
| | | Aluminum foil | 53.3 | 6.7 | 16.7 | 10.7 | 40.7 | 18.3 | 0 |
| | | Paper bags | 73.3 | 10 | 20 | 8 | 50 | 18.3 | 0 |
| | Low temperature | Poly bags | 3.3 | 0 | 8.3 | 0 | 36.7 | 7.3 | 3.3 |
| | | Cloth bags | 0 | 0 | 3.3 | 4 | 20 | 5 | 0 |
| | | Aluminum foil | 0 | 0 | 6.7 | 0 | 13.3 | 3.3 | 0 |
| | | Paper bags | 0 | 0 | 0 | 2.7 | 36.7 | 5 | 0 |
| (A): 1.56; (B): 1.20; (C): 1.58; AB: 1.69; AC: 2.24; BC: 3.16; ABC: 4.48; Coefficient of variation: 11.31%, C. D. at 5% | | | | | | | | | |
| **Nine** | Room temperature | Poly bags | 63.3 | 31.7 | 35 | 52 | 73.3 | 73.3 | 63.3 |
| | | Cloth bags | 60 | 23.3 | 30 | 17 | 3.3 | 18.3 | 60 |
| | | Aluminum foil | 53.3 | 23.3 | 23.3 | 18 | 80 | 18.3 | 53.3 |
| | | Paper bags | 83.3 | 40 | 20 | 16 | 80 | 20 | 83.3 |
| | Low temperature | Poly bags | 6.7 | 16.7 | 11.7 | 2.7 | 70 | 10 | 6.7 |
| | | Cloth bags | 3.3 | 20 | 23.3 | 10.7 | 80 | 8.3 | 3.3 |
| | | Aluminum foil | 10 | 10 | 29.3 | 4.7 | 73.3 | 3.3 | 10 |
| | | Paper bags | 6.7 | 20 | 11.7 | 8 | 80 | 10 | 6.7 |
| (A): 2.66; (B): 1.52; (C): 2.01; AB: 2.15; AC: 2.84; BC: 4.02; ABC: 5.69; Coefficient of variation: 7.48, C. D. at 5% | | | | | | | | | |

(A), Storage types; (B), storage conditions; (C), medicinal plant species.

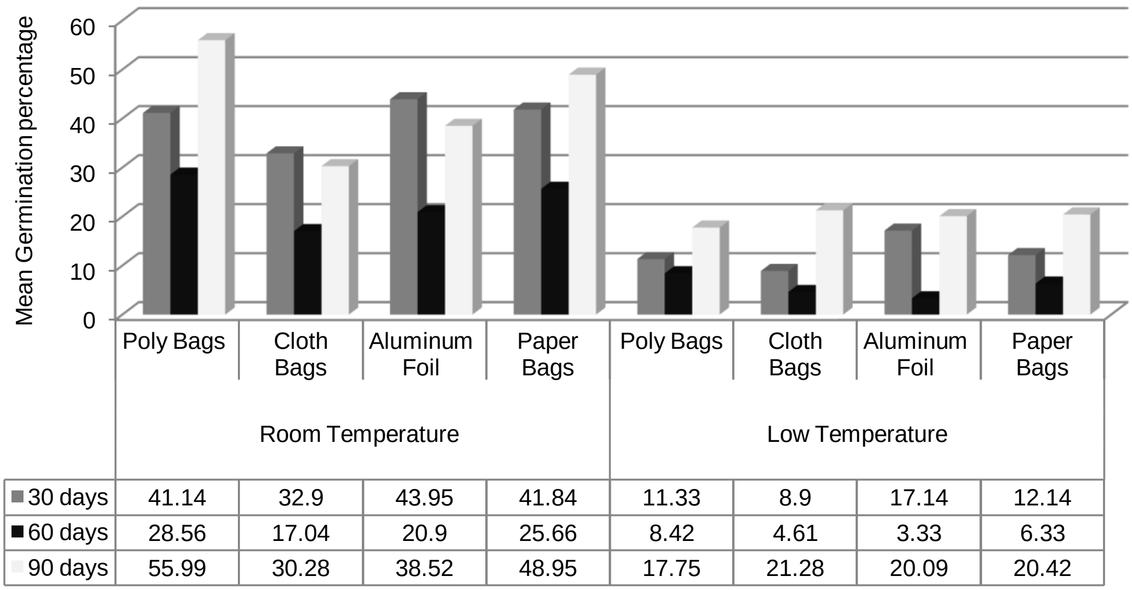


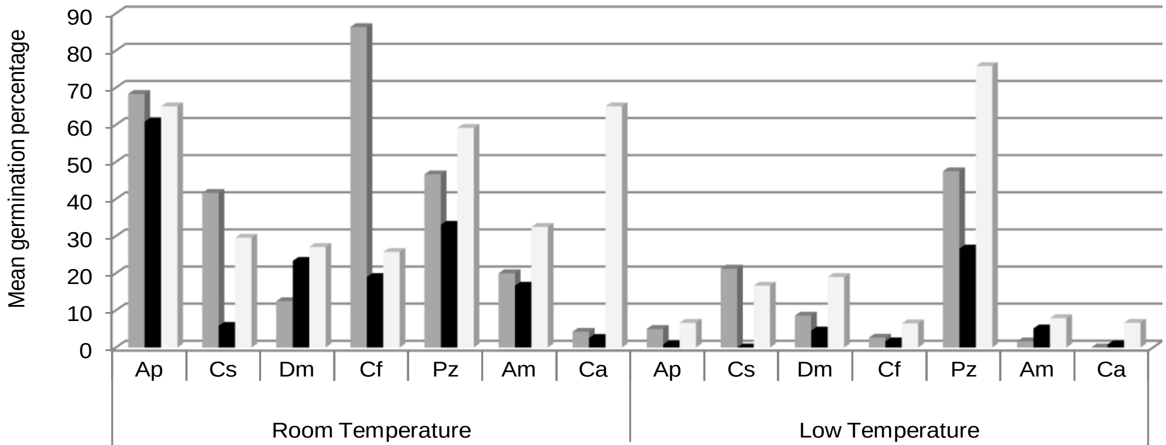

**Figure 1.** Mean seed germination percentage under room and low temperature at three storage durations (30, 60 and 90 months) with different storage conditions irrespective of medicinal plant.

months germination of seeds was found best from poly bags under room temperature (55.9) and from cloth bags (21.3%) under low temperature. It was clearly observed that at room temperature poly bags were found to be most suitable compared with other materials in terms of seed storage and its subsequent viability. Under low temperature aluminum foil tend to be most preferable than other materials for the retrieval of viable seeds. In general, seeds stored in cloth bag found with least germination percentage among the plant species (Figure 1). As early as in 1973, Harrington found that a package which is moisture proof or moisture resistant would be more valuable in sustaining vigor and germination frequency. Silva et al. (1997) revealed that the viability and vigor of chili seeds can be best maintained in triple laminated aluminum foil and the seeds also exhibited less variation in the moisture content. Doijode (1995) reported that when stored for seven years with 6.5% moisture content, seed viability was higher in a glass container and aluminium foil laminated pouches compared to polythene bag (200 gauge) and craft paper.

Polythene bag and aluminum foil generally acts as vapor proof barriers in maintaining lower moisture content in the seeds. Harrington (1973) found that a seed package which is moisture proof or moisture resistant would be more valuable in prolonging germination and vigor. In our study, the seed germination and viability percentage of all the medicinal plant species were found maximum in polythene bags under room temperature while in low temperature, aluminum foil was found most effective irrespective of duration time. This probably is due to keeping of proper moisture content and exchange of gases in the seeds which also helps in maintaining proper level of sugars and starches etc. which are required for good germination of seeds. These results are in line with Ahmed et al. (1992) with *Albizia chinensis* and Chand (1994) in *Toona ciliata*. Low seed viability with cloth bags storage is due to the change in the seed moisture level during storage which reduced the seed longevity. The low germination ability and viability of seeds stored in cloth bags can also be to changes in the physiochemical state particularly seed metabolism due to the reduction in moisture content. The changes in seed metabolism are earlier reported as one of the major factors for low seed germination and viability (Abdul-Baki and Anderson, 1980).

### Effect of storage temperature

Mean data undoubtedly depicts that seeds stored in room temperature came up with high germination percentage than in low temperature irrespective of plant species, storage materials and duration. It has been also observed that percentage germination of stored seed was significantly ($P<0.05$) higher in room temperature than low temperature (Figures 1 and 2). Seeds of some of the plant species *viz. C. anthelminticum*, *C. speciosus* showed no germination when stored in low temperatures (Table 2). Mean seed germination percentage of *P. zeylanica* was found superior at low temperature storage for one and three months respectively (47.5 and 75.8%) than at room temperature (46.7 and 59.2%) irrespective of storing materials (Figure 2). A striking result was observed when germination percentage of *P. zeylanica* seeds was found higher in low temperature irrespective




| | Ap | Cs | Dm | Cf | Pz | Am | Ca | Ap | Cs | Dm | Cf | Pz | Am | Ca |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 30 days | 68.3 | 41.7 | 12.5 | 86.3 | 46.7 | 20.0 | 4.3 | 5.0 | 21.3 | 8.6 | 2.7 | 47.5 | 1.7 | 0.0 |
| 60 days | 60.8 | 5.8 | 23.3 | 19.0 | 33.1 | 16.7 | 2.6 | 0.8 | 0.0 | 4.6 | 1.7 | 26.7 | 5.2 | 0.8 |
| 90 days | 65.0 | 29.6 | 27.1 | 25.8 | 59.2 | 32.5 | 65.0 | 6.7 | 16.7 | 19.0 | 6.5 | 75.8 | 7.9 | 6.7 |

**Figure 2.** Mean seed germination percentage of different medicinal plant species under room and low temperature at three storage durations (30, 60 and 90 months) irrespective of different storage conditions.

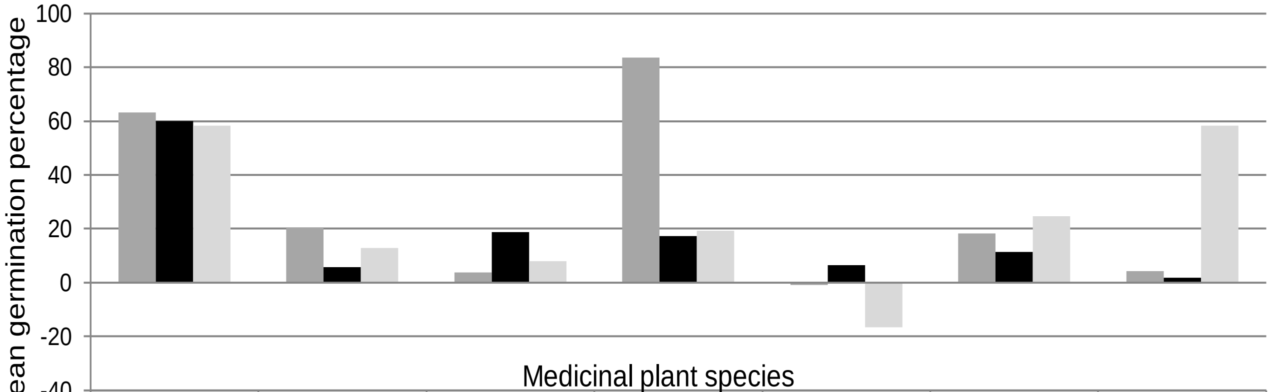
of storing materials. *A. precatorius* and *C. forskohlii* experienced maximum difference in mean percentage seed germination between room and low temperature (Figure 3). Earlier Harrison and Carpenter (1977) revealed that onion seeds can be stored for more than three years at a low temperature of -19°C and -20°C without loss of germination factor, nuclear damage and cytological damage. Also, Garica and Perez (1985) recorded higher seed germination values *viz*., 80, 77, 83 and 78% at 100°C, while lower germination values 78, 75, 35 and 0% at 25°C when stored for nine months under 50, 60, 70 and 80% relative humidity levels respectively in storage environment. Our results contradicts that of Egharveba and Uwadiae (1994), who reported that among two varieties of *Chrysophylium albidum*, second variety was statistically significant for interaction between variety and storage treatment (refrigerator). The scarified seeds and seeds stored in refrigerator (3 to 100°C) recorded significantly better germination and growth.

## Effect of storage duration

A wide variability was observed in seed germination frequency for three storage durations (30, 60 and 90 months) on seed germination of seven medicinal plant species (Table 2), though the data revealed an inconsistency pattern. Amongst the tested plant species only seeds of *A. precatorius* showed a gradual reduction in germination percentage (63.3, 60.0 and 58.3%) as the storage duration increases (30, 60 and 90 days) (Figure 3). *C. speciosus* and *C. forskohlii* experienced most reduction in mean germination percentage (20.5 and 83.7%) after 30 days than 90 days (12.9 and 19.2%) than 60 days (5.8 and 17.3%). Similarly, *D. motorium, P. zeylanica* experienced most reduction in mean germination percentage (3.9 and -0.8%) after 30 days than 90 days (8.1 and -16.7%) than 60 days (18.8 and 6.4%). However, for *A. moschatus* reduction in mean germination percentage was higher after 90 days of storage (24.6%) followed by 30 days (18.3%) than 60 days (11.5%). *C. anthelminticum* showed a tremendous reduction in seed germination percentage after 90 days of storage (58.3%) compared with 60 days (1.8%) and 30 days (4.3%) (Figure 3). The variable trend pattern in mean data is due to cofounding effect of other factors, that is, storage materials, temperatures and plant species.

## Response of seed germination of different plant species

Seeds of seven medicinal plant species germinated in varied frequency as influenced by the different storage components. It was also observed that seeds stored in room temperature ensured high viability than in low temperature irrespective of the storage materials. However, there is minimum effect on the germination of *P. zeylanica* seed by temperature. At room temperature freshly harvested seed of *C. speciosus* has the highest germination percentage (53.67%) whereas, *C. anthelminticum, C. forskohlii* and *A. precatorius* recorded least (1.0%) (Table 1). However, after storing in different materials *A. precatorius* seed maintained its viability as




| | *Abrus precatorius* | *Costus speciosus* | *Desmodium motorium* | *Coleus forskohlii* | *Plumbago zelaynica* | *Abelmoschus moschatus* | *Centratherum anthelminticum* |
|---|---|---|---|---|---|---|---|
| 30 | 63.3 | 20.5 | 3.9 | 83.7 | -0.8 | 18.3 | 4.3 |
| 60 | 60.0 | 5.8 | 18.8 | 17.3 | 6.4 | 11.5 | 1.8 |
| 90 | 58.3 | 12.9 | 8.1 | 19.2 | -16.7 | 24.6 | 58.3 |

**Figure 3.** Relative difference in mean percentage seed germination calculated from room and low temperature irrespective of storage materials for studied medicinal plant species at three storage durations (30, 60 and 90 months).

seen by higher germination percentage compared with other species (Figure 2).

## Conclusion

Proper storage of seed  an important sequel after collection from wild or cultivation. The successful cultivation of any crop is depend large extent on the viability of the seed during storage and the ability to germinate.

The limited amount of wild medicinal plant seeds that are usually collected by gene-banks does not sufficiently allow in maintaining constant viability and subsequent germination for long durations. The interaction of moisture, temperature, initial seed quality and even specific genotype background results in a large variability on the viability of seeds even though following standard protocols. Present study evidently conclude that using appropriate storage materials with proper environmental exposure can sustain the viability of seeds following better germination even after long time. The results presented here suggest that it is problematic to establish a general optimal long term storage conditions for individual entire plant species with native wild habitat.

Therefore, it is of great significance to take up the research work on seed storage to identify best material and optimum conditions for short as well as long term storage of medicinal plant species. Further research in this avenue will fetch results that can be deployed as conservation strategies for threatened wild medicinal plant species as well as an important tool to cultivators engaged in formulation of herbal drugs.

## Conflict of Interests

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

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# Less oil but more money! Artisanal palm oil milling in Cameroon

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**The present study was carried out in four of the seven oil palm production basins generated during the Fonader-sponsored smallholder development scheme in the late Seventies and Eighties. The four basins include: Eseka, Dibombari, Muyuka, and Lobe. The objective of our study was to understand why oil palm smallholders prefer to mill their fresh fruit bunches (FFB) despite the low extraction rates of the artisanal mills and the remarkable presence of industrial mills where they could sell bunches. Our study included the submission of 200 semi-structured questionnaires to different categories of palm oil processors from 131 artisanal mills. Categories included both millers (mill owners and mill managers) and users (smallholders and intermediaries). Our results showed that the processing of FFB in artisanal mills was able to generate a better income to all categories of processors especially during the low production season. Smallholders in Dibombari and Muyuka were found to get the highest additional profit reaching 65.2 and 74%, respectively at low season, when compared to income generated by the selling of FFB at 48,000 FCFA and 50,000 FCFA /ton to Socapalm and CDC mills, respectively. The artisanal milling activity also provided temporary employment opportunities to young men, with an impact on juvenile delinquency and rural exodus. The present study also revealed that the cost of FFB processing the extraction rates of the mills and the demand for red palm oil were amongst the factors which greatly affected the decision making of oil palm processors.**

**Key words:** Artisanal milling, crude palm oil, *Elæis guineensis*, extraction, vegetable oil.

## INTRODUCTION

Artisanal milling is quite common to the oil palm belt of West and Central Africa, which is the cradle of the oil palm (*Elæis guineensis* Jacq.). Fresh fruit bunches (FFB) from natural stands of oil palm (Dura variety) are known to be harvested for immemorial times for the production of red palm oil, which is used in the preparation of numerous traditional dishes. Ngando et al. (2011) estimated that artisanal mills in Cameroon contribute to 30% of the total palm oil produced in the country. Historically, the most common method of artisanal milling was the trampling method, for which boiled nuts were taken to the side of a nearby stream and placed on a concave rock, trampled by foot in order to extract the crude oil which was later skimmed-off to produce red palm oil. Another common method was the pounding of boiled nuts with the use of a mortar and pestle. These

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two forms of artisanal milling are nowadays replaced byimproved mechanical artisanal mills with better milling efficiency.

In Cameroon, the first industrial plantation (Ferme Suisse) was developed in 1907/1909. By 1928 Pamol Plantations was created followed by the Cameroon Development Corporation (CDC) in 1947/1948, then Safacam in 1959 and lastly Socapalm in 1968 (Carrère, 2010). Such agro-industrial plantations exploit their own palms whose bunches are supplied to industrial mills for processing. In the late 1970s the government of Cameroon began to develop the smallholders' oil palm sector with funding assistance from the World Bank under the control of FONADER (National Fund for Agriculture and Rural Development) (Bakoumé et al., 2002). This scheme was in charge of supplying the necessary funds to agro-industries which were in turn responsible for the supply of the necessary in-kind resources and technical expertise to the settlers who were eligible to join the nucleus estate and smallholder scheme (NESS). Smallholders involved in the scheme were supposed to supply all their harvested FFB to the company's mill in order to pay back their credit. The size of holdings ranged from 2 to 5 ha. By then, artisanal milling was virtually unknown in the surroundings of these companies. With the collapse of Fonader in the early 1990s, smallholders started facing problems for the supply of FFB to agro-industrial companies such as Pamol, CDC or Socapalm.

First, smallholders who supplied their FFB to the companies were not paid regularly. Sometimes, they had to wait for 3 months or more to get paid. In the case of CDC, the Smallholders' Department that used to exist as a separate entity was merged with Estate Management under the control of the Estate Manager and this became a major obstacle to the smooth functioning of the Smallholders' Department. The transportation of harvested FFB from the smallholder's farm to the company's mill also experienced delays as the priority was given to the company's fruits. Smallholders at times had to wait for 2 to 3 days or even a week to ship their palm fruits, which were then downgraded at the mill. Even when smallholders tried to transport their FFB by their own means, they often had to face high transportation cost due to the poor state of the roads especially during the rainy season. Smallholders also complained about low FFB prices and they based their argument on the fact that the companies made use not only of the CPO, but also of the other by-products like kernel, fiber, empty fruit bunches and kernel oil. Stringent quality control measures put in place for FFB delivered at the mill were at the origin of supplementary discounts. After the collapse of Fonader in the 1990s, the supply of inputs, technical advice, and quality planting material came to an end. Such services were considered as the bond between smallholders and agro-industrial companies. Smallholders became increasingly reticent to pay back their loans through the supply of their FFB to the Company. This situation often generated conflicts between company officials and smallholders. Some smallholders considered the Project as a governmental subsidy to the poor farmers' population, something which did not need to be reimbursed. Such conflicts strained the existing relationship between smallholders and the major companies.

The fall in the market prices for cocoa and coffee, the economic crisis of the late 1980s and early 1990s and the devaluation of the Franc CFA fueled the diversion of farmers from main cash crops to the cultivation of oil palm (Ngando et al., 2011). This situation also gave rise to the development of independent smallholders, especially the "elites", who were richer newcomers able to develop large areas of plantations (Levang and Nkongho, 2012). The liberalization policy followed by the Government of Cameroon also meant that in due time, public companies were supposed to be privatized and as such the subsidies they used to tap from the Government, which enabled them to cater for smallholders were drying out.

The new generation of small- and medium-holders which appeared in the 1990s and which was not always located in the vicinity of industrial mills had to look for means of processing their own FFB production. The first generation of dependent oil palm smallholders was also fed up with the management system put in place by the companies, so they decided to process their own FFB given that the demand of red palm oil in the local market was rapidly increasing. Within two decades, the number of artisanal palm oil mills grew tremendously and the supply of FFB to the major companies decreased accordingly.

Officials in the Ministry of Agriculture and Rural Development consider artisanal milling as a huge waste because of its low extraction efficiency compared to industrial mills. Agro-industrial companies must temporarily close down their mills during the low production season due to the absence of FFB from smallholders, which usually complement FFB from the estate. Last but not least, such companies consider that a large proportion of the FFB processed in artisanal mills is stolen from their estates. Thus, plantation companies regularly ask the Government to close down artisanal mills, at least those which are close to estates. This did not happen, as the number of artisanal mills is still on the rise.

The overall objective of the present study is to assess the profitability of the processing and marketing of red palm oil. Our specific objectives were basically:

i. To identify the different types of people involved in artisanal milling; the production efficiency of the various types of artisanal mills, the quality control measures put in place during processing of FFB;

**Table 1.** Distribution of respondents by type of service provider and users.

| Type of service provider/users | Frequency of service providers/users in: | | | | Total |
|---|---|---|---|---|---|
| | Eseka | Dibombari | Muyuka | Lobe | |
| Millers | 33 | 41 | 23 | 34 | 131 |
| Smallholders | 11 | 05 | 19 | 13 | 48 |
| Intermediaries | 06 | 04 | 08 | 03 | 21 |
| Total | 50 | 50 | 50 | 50 | 200 |

* Note: 95% of millers also own oil palm plantations.

ii. To assess the return to labor, milling charges and contribution to income for each type of palm oil processor;
iii. To identify processors involved in the sale of red palm oil in the domestic markets; describe the market chains - artisanal or informal (wholesalers and retailers);
iv. To describe fluctuations in the price of red palm oil in the local market over the years;
v. To assess the financial contribution from the sale of red palm oil to household livelihood as well as problems hindering the smooth functioning of the sector.

The major underlying question was: Why do oil palm smallholders prefer to mill their FFB irrespective of the low extraction rates of these artisanal mills?

## METHODOLOGY

A preliminary survey resulted in the selection of four oil palm production basins. This choice was based on the long-standing relationship between oil palm producers and agro-industrial Companies in basins such as Eseka, Dibombari, Muyuka and Lobe. Each basin is located close to an industrial mill belonging to one of the following companies: Eseka- Socapalm; Dibombari- Socapalm; Muyuka- CDC and Lobe- Pamol (Table 1).

As a first step, information about the distribution and abundance of the different types of artisanal palm oil mills was obtained from the Department of Agriculture at the local level, oil palm smallholders and employees of the nearby agro-industries. Then a randomized sample of the different types of artisanal mills in each zone was selected. A total of 131 artisanal mills were sampled with the submission of 200 semi-structured questionnaires during the peak season of oil palm production, which falls within the months of February to June.

Three types of service providers were identified in the course of the survey, namely: mill owners, mill managers and mill workers. Information concerning the functioning of the mill was obtained from either the mill owner who personally supervised the mill or the mill manager when the owner was absent. Out of the 131 sampled millers, 125 were processing FFB from either their own farms or through the purchase of FFB from other smallholders in addition to the utilization of their mill for commercial purpose.

The survey also identified two types of mill users, namely oil palm smallholders and middlemen. A total of 48 of the sampled users were oil palm smallholders who did not own artisanal mills but brought their FFB to the mill for processing. A total of 21 users were middlemen who did not own oil palm plantations but were buying FFB from smallholders for processing in an artisanal mill.

## RESULTS

### Type of service providers (millers) and users

The respondents under study were categorized into oil palm processors, namely mill owners and mill managers; and users, that is, smallholders and middlemen. Mill owners own and manage their own palm oil mill, while mill managers are employed by the proprietor of the mill to manage and supervise the various activities in the mill. Smallholder users are those who carry their own FFB to the mill, pay for milling and labor charges and return with the palm oil. Middlemen do not own oil palm plantations, they buy FFB from smallholders, organize transportation to the mill, pay for the milling and labor charges, and return with the palm oil.

Our survey revealed that 95% of mill owners and mill managers do own plantations. The primary reason for them to buy a mill was basically to mill the FFB from their plantation in order to get more income from the sale of red palm oil, before using the mill for commercial purpose. Smallholders who did not own artisanal mills harvested and processed FFB in a commercial artisanal mill. Women and young people constitute the major part of the middlemen: they buy FFB and process it in an artisanal mill. If compared to oil palm smallholders, middlemen were relatively scarce in the sampled mills because most smallholders preferred to mill their FFB because of added value.

We found that artisanal milling was a major source of income for the different service providers/processors. Other sources of income for the sampled respondents included farming of other cash crops/food crops, as well as off-farm activities.

### Identification of service providers and mill users

The personal information for the service providers and users is shown in Table 2. With regards to gender repartition, more men were involved in artisanal milling than women and young people, probably because men have customary rights to land and are able to plant and harvest directly from their farms during peak and low season while women and young people must purchase

**Table 2.** Personal information from respondents in the four zones under study.

| Personal information | Mill owner | Mill manager | Smallholder (user) | Intermediary (user) | Total |
|---|---|---|---|---|---|
| Household head | 52 | 47 | 33 | 09 | 141 |
| Non-household head | 11 | 21 | 15 | 12 | 59 |
| **Gender** | | | | | |
| Male | 55 | 60 | 41 | 13 | 169 |
| Female | 08 | 08 | 07 | 08 | 31 |
| Average age | 47 | 41 | 39 | 34 | 40 |
| **Ethnic group** | | | | | |
| Native | 26 | 21 | 24 | 07 | 78 |
| Non-native | 37 | 47 | 24 | 14 | 122 |
| **Level of education** | | | | | |
| Primary | 25 | 25 | 18 | 09 | 77 |
| Junior high school | 25 | 20 | 17 | 05 | 67 |
| High school | 10 | 12 | 09 | 04 | 35 |
| University | 03 | 11 | 04 | 03 | 21 |
| **Marital status** | | | | | |
| Married | 52 | 47 | 34 | 11 | 144 |
| Single | 08 | 20 | 11 | 08 | 47 |
| Widow(er) | 02 | 01 | 02 | 02 | 07 |
| Divorced | 01 | 0 | 01 | 0 | 02 |

FFB. Mill owners are older on average, while middlemen are younger, thus revealing that more young people and young women are involved in the activity.

More non-natives were involved in the activity, thus indicating that they need to stabilize their income levels especially when they are still to get fully integrated in their new community. The survey also reveals that more mill managers as compared to the other categories were university graduates and they were able to use this activity to generate income to register for public examinations or to continue their studies. More married persons were involved in artisanal milling as compared to singles. While the husband was involved in the processing of FFB, the wife was involved in the marketing of the palm oil.

## Types of artisanal palm oil mills in the sampled zones

The study identified six different types of artisanal palm oil mills in the selected zones. These are: i) manual vertical press; ii) digester with separate manual metallic cage press (hand-operated screw press); iii) motorized horizontal screw press; iv) digester with separate hydraulic press; v) combined motorized digester/hydraulic press system (digester screw press) and vi) semi-automated press.

The Manual press is locally called tournée tournée (manual vertical press): This press adopts the wet process during which the sterilized fruits are poured into the digester and the fruits are macerated by manually turning the vertical shaft to extract a mixture of oil and water which is collected at the base. The resulting mixture of water and oil is poured into larger drums. This mixture is then clarified to extract red palm oil.

Manual or hand press with a digester adapted to a car engine [digester with separate metallic cage press (hand operated screw press)]; this press follows the dry process. A vertical digester adapted to a car engine is used in the maceration process. A mixture of oil, moisture, fibers and nuts is collected at the base and this mixture is hand-pressed in a metallic cage to extract the oil.

Motorized press (motorized horizontal screw press); this press follows the wet process during which sterilized fruits are poured into the digester and the fruits are macerated. Hot water is continuously poured into the digester at a regular rate in order to wash off the released oil. The resultant mixture of water and oil is poured into larger drums. This mixture is then clarified to extract red palm oil.

Digester with separate hydraulic press; this press adopts the dry process technique where by sterilized fruits are poured into the digester and the fruits are macerated. A mixture of oil, moisture, fibers and nuts is collected at the base and this mixture is pressed using a hydraulic press to extract the oil.

Combined motorized digester/hydraulic press system

**Table 3.** Type of artisanal mills utilized by sampled respondent.

| Type of processing equipment | Frequency of equipment used by respondents | | | | Distribution of types of presses in the four study areas |
|---|---|---|---|---|---|
| | Eseka | Dibombari | Muyuka | Lobe | |
| Manual vertical press | 26 | 20 | 3 | 0 | 49 |
| Digester with separate manual cage press | 0 | 2 | 0 | 26 | 28 |
| Motorised horizontal screw press | 7 | 16 | 15 | 0 | 38 |
| Digester with separate hydraulic press | 0 | 0 | 3 | 5 | 8 |
| Combined motorised digester/ hydraulic press | 0 | 1 | 0 | 4 | 5 |
| Semi-automated press | 0 | 1 | 1 | 1 | 3 |
| Total | 33 | 40 | 22 | 36 | 131 |

(digester screw press); this press also adopts the dry process technique. In this press, the digester is linked to the press through an operating table. Here digestion and pressing take place simultaneously powered with an engine. Semi-automated press; this press also adopts the dry press technique. Here little or no human labor is needed as most of the processing stages (boiling, digestion, and clarification) are mechanized. This system is the most efficient and the most expensive one which makes it unaffordable to the majority of small-scale millers.

Depending on the zone, it was common to find respondents who were linked to specific artisanal mills for reasons partly linked to availability and production efficiency. Table 3 shows the utilization of the different artisanal palm oil mills by sampled respondents in the four areas under study.

Motorized horizontal screw presses were preferred in all four zones, followed by manual vertical press while semi-automated palm oil press was rarely used. As opposed to the other artisanal palm oil presses, which were mostly used for commercial purpose, the manual vertical press was mostly bought for home use. The cost of a given type of artisanal mill depends on the complexity and extraction efficiency of the mill. Taking the exchange rate at 1 USD = 500 FCFA, the manual vertical mill was the cheapest one in terms of cost with an average of 150,000-250,000 FCFA (300 to 500 USD), the most expensive artisanal mill was the semi-automated with an average price of 15 to 20 million FCFA (30,000 to 40,000 USD), while the others ranged in price from 2 to 5 million FCFA (4000 to 10,000 USD). The durability of the palm oil press depends on whether it was purchased as new or second hand. The maximum intake capacity of the sampled mills was 1 ton FFB per hour in the semi-automated mills.

## Various steps of FFB processing

Pickup cars are used to transport FFB from the plantation to the mill, while motorbikes equipped with special bags carry loose fruits, or even FFB where roads are not passable by cars. When bunches arrive at the artisanal mill, they are stored in separated piles for each farmer and are covered with jute bags or palm leaves and left to ferment for almost one week. Depending on the work plan of the mill manager, FFB could either be chopped/splitted into halves with a machete before being allowed to ferment. After 7 days, the fruits from fermented bunches can easily get detached from spikelets. The next operation is the stripping of whole or chopped bunches with the use of the blunt edge of a cutlass. The sieving of loose nuts separates the nuts from the dirt with the use of a locally fabricated wire mesh. As compared to the chopping/splitting, stripping operations which are considered as operations for men only, the selection of loose nuts is mostly done by women. The loose fruits are then poured into 200 L (or bigger) metallic drums for boiling. The required amount of water is put in the drum, and a fire is lighted underneath.

The mixture is allowed to boil for around 4 to 6 h. Boiling the loose nuts before digestion plays an important role to soften the nuts, inactivate lipase enzymes and coagulate proteins (Babatunde et al., 2003; Chow and Ma, 2007). When the boiled nuts are ready, digestion can start. Digestion is the process by which the boiled or sterilized fruits are macerated for easy separation of oil from fibers. This operation utilizes the palm oil press from the manual mill to the more sophisticated semi-automated press. Jannot (2000) distinguished between two types of digestion. Indeed, in the continuous type, oil mixed with water and sludge are collected at one outlet while the chaff comes out through another. In the discontinuous type, after digesting the boiled fruits, the mash can further be pressed using the same machine or a separate one in order to extract red palm oil. The last operation is clarification, which involves the boiling of a mixture of sludge and effluent with the addition of the required quantity of water for a period of 2 to 3 h. At the end of this operation, the red palm oil is left suspending

**Table 4.** Extraction efficiency of the different types of palm oil presses.

| Efficiency parameter (in peak season) | Type of presses (ranked from least to most efficient) | | | | | |
|---|---|---|---|---|---|---|
| | Manual vertical press | Digester with manual metallic cage press | Motorised horizontal screw press | Digester with separate hydraulic press | Combined motorised digester/ hydraulic press | Semi-automated press |
| Average quantity (in L) of CPO/ton of FFB | 148.86 | 156.94 | 163.55 | 166.22 | 167.77 | 200.00 |
| Average quantity (in kg) per ton of FFB | 133.98 | 141.25 | 147.2 | 149.6 | 151 | 180.00 |
| Extraction efficiency (%) | 13.3% | 14.1% | 14.7% | 14.9% | 15.1% | 18.0% |

on the top of a mixture of water and effluent. The red palm oil is then skimmed off and placed into gallons of various sizes and allowed to cool before being corked.

## Estimation of extraction rates from the different types of palm oil presses

Extraction efficiency refers to the time it takes to press a given quantity of loose nuts, as well as the quantity of red palm oil produced at the end of the milling process. Based on the different types of palm oil mills identified during the present study, the semi-automated mill was ranked first with the highest extraction efficiency reaching an average 18%, while the manual vertical press had the lowest extraction efficiency with an average 13.3% during peak season as shown on Table 4. On average there was a 0.7% reduction in the quantity of palm oil produced at low season when compared to the peak one. Reasons for this reduction could be linked to poor fruit set during low season of oil palm production. The peak season falls within the months February to May. The low season falls within the months June to September, it is a period during which oil palm registers a drop in production. The mid-season falls within the months October to January, with a production in between the two former ones. These production seasons are directly linked to the extended periods of drought observed in the course of the year.

## Type of labor utilized and comparative duration of milling operations for peak and low season

In Dibombari, Muyuka and Lobe, majority of the sampled respondents utilized hired labor to process FFB, while few respondents used both hired and family labor. Only in Eseka, the sampled respondents employed either family or hired labor, depending on the quantity of FFB to process and the availability of labor, or in most instances a combination of both family and hired labor. This can be due to the dominance of manual mills for household use in Eseka. Similarly to the case of smallholder oil palm plantations, labor in the artisanal mills is dominated by non-natives (migrant workers). The type of operations performed in the mills (from chopping/splitting, stripping, sieving/selection, boiling, to digestion and clarification) requires physical strength and is often provided by young men aged 18 to 45. It is rare to find women working in the artisanal mills, and even when they do so; their work is limited to the sieving/selection of loose nuts or to office work in larger mills.

The labor time and working days are stepped up during peak production season due to an increase in the number of bunches entering the mill as compared to during the low production season. The peak season for oil palm production in Cameroon falls within the months of February to May, the low season falls within the months of June to September, while the mid-season falls within the months of October to January. These seasonal differences in the production of oil palm also have an effect on the quantity of work available in these artisanal mills at each given season, and this is one of the reasons why artisanal mills tend to recruit temporary workers, with payment based on the quantity of FFB processed (Nchanji et al., 2013).

## Profitability of FFB processing to millers and users

The processing of FFB into red palm oil provides

**Table 5.** Seasonal changes in average cost/net income (in FCFA) for processing one ton of FFB.

| Variables | Eseka | | Dibombari | | Muyuka | | Lobe | |
|---|---|---|---|---|---|---|---|---|
| | Peak | Low | Peak | Low | Peak | Low | Peak | Low |
| Cost of 1 ton FFB | 40,000 | 45,000 | 42,000 | 50,000 | 41,310 | 47,405 | 36,875 | 40,800 |
| Transportation | 11,347 | 11,347 | 11,624 | 11,624 | 8,735 | 8,735 | 12,237 | 12,237 |
| Labour charge | 8,845 | 8,435 | 9,723 | 9,723 | 6,392 | 6,392 | 7,868 | 7,868 |
| Milling charge | 7,139 | 7,139 | 5,376 | 5,376 | 5,592 | 5,592 | 3,726 | 3,726 |
| Total expenditure | 67,331 | 70,923 | 68,723 | 76,723 | 62,029 | 68,124 | 60,706 | 64,631 |
| Palm oil price FCFA/L | 400.2 | 543.8 | 498.2 | 669.7 | 513.6 | 658.8 | 399.2 | 505.7 |
| Red oil produced in L /ton FFB | 145.3 | 140 | 172.8 | 158.3 | 186 | 163.6 | 165.9 | 153.6 |
| Gross income | 58,150 | 76,131 | 86,090 | 106,018 | 95,526 | 107,787 | 66,225 | 77,675 |
| Net income | -9,181 | +5,200 | +17,367 | +29,295 | +33,497 | +39,663 | +5,519 | +13,044 |

income to the mill owner through the payment of milling charge, and to the mill workers through the payment of labor charge. In all four zones under study, artisanal milling was a major source of income for the respondents especially during the peak season because of an upsurge in the number of bunches to process. During the peak season when the supply of FFB is high, it is easy for a middleman to buy bunches and process, as compared to during the mid-peak and low season when there is a drop in production. This drop in production is inversely proportional to an increase in the demand of red palm oil. Table 5 provides data on costs and benefits incurred in the processing of one ton of FFB according to the season.

The purchase and milling of FFB gives a net positive income to middlemen in all studied zones except for Eseka in which a negative balance can occur during peak season. In all other areas, even women and youths who do not own oil palm plantations can make a positive turn over when they buy and process FFB. One ton of FFB is able to produce slightly more palm oil after milling during the peak production season as compared to during the low production season. However, this increase in production is counterbalanced by the lower price of palm oil during peak production. The four factors which impact the financial output of artisanal palm oil milling are: i) the cost of production, ii) the price paid for one liter of red palm oil (depending on the season), iii) the cost of transportation to major markets and iv) the extraction efficiency of the artisanal mill.

## Why do smallholders prefer to mill FFB by themselves?

Even though the milling efficiency of artisanal mills is lower, smallholders make more money through self-milling than selling FFB to the agro-industrial companies. Oil palm smallholders also say that they make good use on some of the by-products released in the course of processing. For example, kernels and fibers are utilized as fuel for the boiling of loose nuts and during clarification, as such very little is spent for the purchase of fuel wood.

The purchase price of a ton of FFB by the three agro-industrial companies in Cameroon: Socapalm, CDC and Pamol is 48,000 FCFA, 50,000 FCFA and 42,000 FCFA respectively and this price is constant during peak and low season. Any change in the price of crude palm oil in the global market has no incidence on the price paid to smallholders for FFB. From Table 6, it is clear that artisanal milling is most profitable to oil palm smallholders

in the Dibombari and Muyuka zones during both low and peak season. The situation is quite different for oil palm smallholders in the Eseka and Lobe zones where artisanal milling is only profitable during the low season. As compared to Dibombari and Muyuka areas which benefit from the presence of a large local market for the sale of red palm oil, Eseka and Lobe are more isolated and buyers have to travel for longer distances from the towns and cities. Poor state of roads has also a negative impact on the price of red palm oil especially during the peak season when supply outweighs demand. Results from Table 6 also show that artisanal milling is profitable to oil palm smallholders without mills in Eseka and Lobe only during the low season. When there is a fall in the

**Table 6.** Comparative net income of a smallholder from the sale of red palm oil or FFB.

| For 1 ton of FFB | Eseka | | Dibombari | | Muyuka | | Lobe | |
|---|---|---|---|---|---|---|---|---|
| | Peak | Low | Peak | Low | Peak | Low | Peak | Low |
| Sold to an intermediary | 40,000 | 45,000 | 42,000 | 50,000 | 41,310 | 47,405 | 36,875 | 40,800 |
| Sold to an agro-industry | 48,000 | 48,000 | 48,000 | 48,000 | 50,000 | 50,000 | 42,000 | 42,000 |
| Processed in an artisanal mill | 30,819 | 50,200 | 59,367 | 79,295 | 74,807 | 87,068 | 42,394 | 53,844 |
| Processed in own mill | 37,958 | 57,339 | 64,743 | 84,671 | 80,399 | 92,660 | 46,120 | 57,570 |

**Table 7.** Average wholesale price of palm oil sold in artisanal mills in FCFA/Liter.

| Zone | Size of container | 2009 | | 2010 | | 2011 | | 2012 | |
|---|---|---|---|---|---|---|---|---|---|
| | | Peak | Low | Peak | Low | Peak | Low | Peak | Low |
| Eseka | 22 L | 242 | 281 | 281 | 343 | 356 | 474 | 400 | 544 |
| Dibombari | 20 L | 488 | 564 | 523 | 585 | 541 | 614 | 498 | 670 |
| Muyuka | 77.4 L | 384 | 521 | 506 | 674 | 522 | 658 | 514 | 659 |
| Lobe | 118 L | 295 | 338 | 329 | 372 | 369 | 420 | 399 | 506 |

price of red palm oil during peak production season, especially in zones where they cannot break even, it is advisable for oil palm smallholders to supply their FFB to the agro-industrial mills. When there is an increase in the price of red palm oil, it is advisable for oil palm smallholders to mill their FFB in artisanal mills.

### Marketing of red palm oil

The wholesale and retailing of red palm oil is a major source of income especially to the female population. Red palm oil is sold right at the premises of the palm oil mill, where it is common to find men and women alike coming to buy this product. When palm oil is purchased at the doorstep of artisanal mills; it is then transported to the villages, nearby towns or city markets where it can either be wholesaled or retailed. Soap production and palm oil refining companies like AZUR and MAYOR, based in Douala have their agents in the field who buy palm oil directly from the artisanal mills in large quantities and transport it to factories using big tankers. Red palm oil is also purchased and transported to the northern part of Cameroon where oil palm is not cultivated due to unfavorable climatic conditions. It is also sold to neighboring countries like Nigeria, Equatorial Guinea, Gabon, Congo, Central African Rep. and Chad.

There has also been a steady increase in the wholesale and retail price for red palm oil over the years, and this can be attributed to both the increase in population and the increase in the utilization of palm oil especially by downstream industries as shown in Table 7.

Palm oil is sold in wholesale at artisanal mills while in villages, towns and city markets, it is sold either in wholesale or at retail prices. There are periods in the year (especially during low production season) when the price of a liter of red palm oil can reach 1,000 to 1,300 FCFA especially in large cities like Yaoundé and Douala. In towns like Buea, Limbe, and Kumba; the retail price for a liter of palm oil during low season can reach 800 to 900 FCFA. Meanwhile, during peak production season, the price for a liter of red palm oil can fall down to 400 to 500 FCFA.

## DISCUSSION

### The profitability of artisanal milling

Artisanal milling generates income to people from all social classes, age groups and it is not gender biased in the sense that even women who do not have customary rights to own land (with the exception of Eseka) can buy fresh fruit bunches, process them in the artisanal mills, sell the resulting red palm oil and make a meaningful profit (Ibeckwe, 2008; Olagunju, 2008). According to Soyebo et al. (2005) and Ezealaji (2011), women make more profit when they buy FFB, process and sell the resulting palm oil than if they just buy and sell red palm oil alone. Women are disadvantaged by the lack of necessary capital, limited access to extension services and lack of land ownership. Since most women fall in the category of middlemen, they often have to face the problem of the unavailability of FFB during the low season, since most smallholders will not want to sell their FFB because of price increase in the processing and sale of red palm oil.

Indeed, one of the ways to adapt to this situation is to process and store red palm oil during the peak season and wait for the low season, in order to sell it when the

**Table 8.** Additional profit for smallholders when FFB is processed in artisanal mills.

| Fate of FFB | Eseka | | Dibombari | | Muyuka | | Lobe | |
|---|---|---|---|---|---|---|---|---|
| | Peak | Low | Peak | Low | Peak | Low | Peak | Low |
| Processed in an artisanal mill | 30,819 | 50,200 | 59,367 | 79,295 | 74,807 | 87,068 | 42,394 | 53,844 |
| Sold to agro-industry | 48,000 | 48,000 | 48,000 | 48,000 | 50,000 | 50,000 | 42,000 | 42,000 |
| Additional profit from artisanal milling | -35.8% | 4.6% | 23.7% | 65.2% | 49.6% | 74% | 0.9% | 28.2% |

price is better. Some middlemen also rent mature oil palm plantations for the purpose of harvesting and processing FFB. The young men who work in these artisanal mills also benefit from the milling activity and this is an efficient way to reduce rural exodus and juvenile delinquency. Taiwo et al. (1999) and Ekine et al. (2006) also describe the involvement of men in high-energy demanding jobs, while more women accept less strenuous jobs, while Solomon and Okolo (2008) refer to the oil palm as a "male crop". The smallholders are also able to make additional profit from the processing of FFB, compared to the sale of bunches directly to the agro-industrial mills, especially during the low season when the price of red palm oil is on the rise as shown in Table 8.

Artisanal milling was quite profitable in three of the four zones under study, for both peak and low season with the exception of Eseka which recorded a deficit at peak season. Dibombari and Muyuka zones recorded the highest profit from artisanal milling at low season. Such variation in profit between different zones could be linked to the cost of production, the existing market for palm oil, the extraction efficiency of the mill and the cost of transportation. When oil palm smallholders cannot make a profit from the processing of their FFB, it is advisable for them to supply their FFB to the agro-industry.

The mill owners are not left behind as they also benefit from milling charges paid by smallholders and middlemen alike who come to mill their FFB. All these advantages of artisanal milling are linked to the presence of a domestic and sub-regional market for red palm oil, despite the fluctuation in price depending on the season of production.

## The shortcomings of artisanal milling

The quality of red palm oil processed in these artisanal mills has often raised a lot of questions. Previous studies reveal an increase in free fatty acid, moisture content and dirt on red palm oil when stringent quality control procedures are not put in place and this reduces the "shelf life" of red palm oil which can easily get rancid. Corley and Tinker l. (2003), Owolarafe et al. (2008) and Ngando et al. (2011) reported on the increase of free fatty acid concentration during the fermentation of fresh fruit bunches and Poku (2002) recommends the processing of FFB at 48 h after harvesting at the latest. The capacity and extraction rates of artisanal mills are much lower with an average of 14% (Cheyns and Rafflegeau, 2005) and thus they cannot compete with industrial mills, which are providing extraction rates of 23% and more and have higher milling capacity.

The dilapidated nature of some of the mills makes milling not sustainable because of frequent breakdowns, coupled with the recruitment of untrained personnel to manage the mills as reported by Akangbe et al. (2011) and Ibitoye et al. (2011). Okonkwo (2011) highlighted the need for mill personnel to receive training in order to ensure food safety and product quality. Another concern is the unhygienic conditions in which the milling operations are performed and the uncontrolled disposal of waste generated by such mills. Finally, from the marketers' point of view, the red oil is subject to price fluctuation and high cost of transportation depending on the season of production (Ekine et al., 2006).

Different types of by-products are produced by the processing of fresh fruit bunches into red palm oil. These by-products are: empty fruit bunches, empty kernels, kernel cake, fibers and sludge. Such by-products are now increasingly recycled in industrial mills and they pose a threat to the environment when disposed of in an uncontrolled manner. For example, empty fruit bunches could be recycled to the field as a source of organic mulch or composted. Palm kernel can be used as a source of energy and the kernel cake could be a source of animal feed. Fibers are another important source of energy when used as fuel and palm oil mill effluent could also be an important source of organic manure, but when disposed of carelessly into nearby streams, it could be a source of pollution, a breeding ground for mosquitoes and unpleasant smell.

## Agro-industrial companies want to get rid of artisanal mills

Plantation companies consider artisanal milling as detrimental to their business. Indeed, in many occasions they have asked the Government to forbid artisanal milling because they suspect the rampant theft of fresh fruit bunches in their plantations to be the result of artisanal milling. Companies consider that peripheral plantations are the most affected because of theft. The theft of fresh fruit bunches from agro-industrial plantations occurs mostly at night and results not only in the loss of bunches from affected palm trees but also to unsustainable harvesting, as live fronds are often destroyed in order to easily cut the peduncle of the bunch with a machete.

The local population complains that the company does not provide jobs and that they were expelled from land belonging to their fore-fathers. Thus, stealing and processing from the company is merely considered as compensation. The price of red palm oil produced by artisanal mills is not submitted to any regulation by the government, while the price of crude palm oil produced by agro-industries is presently fixed at 450 FCFA/ Liter to avoid the closure of downstream industries. When asked to increase FFB buying prices, company managers respond that because of the fixed CPO price they would go bankrupt. As an adaptive response to the increase of theft of FFB in agro-industrial plantations, the companies have hired the services of security officials/guards to carry out regular patrols on their plantation with eminent court action on defaulters.

The transportation of palm oil in areas where these agro-industries are located is subject to strict control by security officials/guards in order to ascertain the source of the palm oil. In the worst cases, the company decides the replacement of oil palm with other crops like banana and rubber in peripheral plantations which face high levels of theft as noticed in CDC Mondoni palms and Socapalm Nkappa.

## Which way forward?

One of the possible ways forward would be for oil palm smallholders/artisanal millers to group themselves into cooperatives in order to increase their bargaining power (Jelsma et al., 2009; Ibitoye et al., 2011). At present, smallholders process and sell the red palm oil individually without a common price at a particular season of production. They are not informed about improved sustainable processing methods, market strategies or market information systems. There is also a need to improve on the extraction rates and quality control procedures of artisanal mills (Badmus, 1991). Partnerships between smallholders and agro-industrial companies should be facilitated by the Government, especially with the recent interest shown by foreign oil palm companies to develop plantations in Cameroon (Levang and Nkongho, 2012; Hoyle and Levang, 2012; Nkongho et al., 2014).

The round table on sustainable palm oil (RSPO) emphasizes the need for the certification of independent oil palm smallholders under group certification and the certification of scheme oil palm smallholders through the agro-industries in which they are in partnership. Group certification of independent oil palm smallholders and their respective artisanal mills will involve the grouping of these smallholders/artisanal millers into cooperatives and the training of both smallholders and artisanal millers on the principles and criteria of RSPO. Smallholders would gain knowledge on the identification and delimitation of high conservation value forest (HCVF), primary forest and peat land, etc. Techniques that minimize soil erosion would be adopted where appropriate. These include practices such as ground cover management, biomass recycling, terracing, and natural regeneration or restoration; knowledge on best management practices such as integrated pest management (IPM) (incorporating cultural, biological, mechanical and physical methods to minimize the use of chemicals), the health and safety of plantation workers, updated and efficient fertilization, as well as farm record keeping.

At the level of artisanal mills, workers should receive training on safety and product quality, as well as ways of disposing solid and liquid waste with little effect on the soil and water ways, amongst others. A water management plan should be put in place and this should take account of the efficiency of use and renewability of sources, ensuring that the use and management of water during milling operations does not result in adverse impacts on other users within the catchment area. The treatment of palm oil mill effluent (POME) to required levels and regular monitoring of discharge quality especially biochemical oxygen demand (BOD), should be in compliance with national regulation. The adherence to RSPO certification and others would also open new doors for funding and market opportunities (RSPO, 2013).

## Conclusion

Our study identified six different types of artisanal mills commonly used in the four study zones. In terms of milling efficiency semi-automated mills showed the best milling efficiency, with the poorest performance coming from manual presses. Different categories of millers and users were identified, namely mill owners, mill managers, oil palm smallholders, and middlemen. All categories were able to make a sizeable profit from their activity in the processing and marketing of red palm oil. Oil palm smallholders were found to mill part of their FFB and sell part of it to agro-industrial companies depending on the size of their farms and the season of production, while middlemen were able to mill and store red palm oil until the selling price became more profitable.

Nevertheless, artisanal milling has its own setbacks. These include low extraction efficiency, old, unsafe and dilapidated mills, the absence of quality control procedures, fluctuations in the price of red palm oil for those involved in marketing and the worrying increase in theft of bunches from agro-industrial plantations. Our study finally highlights a need for improvement in the extraction efficiency and quality control measures in these mills, the need for millers and marketers to group themselves into cooperatives, and the need for developing partnerships between oil palm smallholders and agro-industries.

## Conflict of Interests

The authors have not declared any conflict of interests.

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# Occurrence of green lacewings (Neuroptera: Chrysopidae) in two coffee cropping systems

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**The aim of this study was to explore the diversity of lacewings in two coffee (*Coffea arabica* L.) cropping systems, located in the southwestern region of Bahia State, Brazil, and to study the structure of their communities and the seasonal occurrence of the species. These studies were conducted from December 2003 to November 2004, with coffee plants that were either shaded or unshaded by silky oak trees (*Grevillea robusta* A. Cunn.) at a property located in the municipality of Barra do Choça, Bahia State. Monthly collections were carried out with insect nets and traps baited with mango juice and hydrolyzed protein. The characterization of the Chrysopidae community was calculated by faunal indices, relative frequency, constancy, dominance, richness and diversity. The study on the species seasonal occurrence was conducted with the total number of specimens within each collection time and sampling. In order to assess the effects of climatic factors, only species classified as constant were considered. The occurrence of the following 15 species, in association with coffee plants, was recorded: *Ceraeochrysa cubana*, *Ceraeochrysa cincta*, *Ceraeochrysa dislepis*, *Ceraeochrysa everes*, *Chrysoperla externa*, *Chrysopodes spinella*, *Leucochrysa guataparensis*, *Leucochrysa cruentata*, *Leucochrysa intermedia*, *Leucochrysa rodriguezi*, *Leucochrysa santini*, *Leucochrysa scomparini*, *Leucochrysa cidae*, *Leucochrysa annae* and *Leucochrysa* sp.4. There was a considerable difference in community structures regarding the occurrence of different species and abundance of individuals. The species *C. cincta*, *L. guataparensis*, *L. cidae* and *L.* sp.4 occurred exclusively on shaded coffee plants, while *L. cruentata*, *L. intermedia*, *L. scomparini* and *L. annae* were found only on unshaded plants. On the other hand, *C. externa* and *L. rodriguezi* stood out in both environments. *C. externa* was the most frequent and dominant species in both cropping systems, as it was considered constant on unshaded coffee plants and accessory on shaded plants. *C. everes*, *C. externa* and *C. spinella* occur during the day; *C. cincta*, *C. dislepis*, *L. guataparensis*, *L. rodriguezi*, *L. cidae*, *L. cruentata*, *L. intermedia*, *L. santini*, *L. scomparini* and *L. annae* occur during the night; and only the species *C. cuban* was collected during both the day and night.**

**Key words:** Faunal analysis, biodiversity, *Coffea arabica*, predator.

## INTRODUCTION

The globalization of the world trade has been determining changes in agricultural production systems, requiring more quality, food safety and sustainability of agroecosystems in order to reach ecological, economic and social stability. In this sense, there is the integrated production, whose principles take into consideration the use of integrated management based on decision-making for crop protection. The integrated production aims at the minimization of pesticide use and prioritization of cultural, biological and biotechnological methods to control pests and diseases, therefore preserving natural enemies and supporting the introduction of predator species and parasitoids.

Among several biological control agents that act on the population control of arthropods, lacewings are highlighted (Insecta: Neuroptera: Chrysopidae). Lacewings are considered key natural enemies in several integrated management programs due to their predatory activity during larval stage, wide geographic distribution, easy mass rearing and adaptive potential on different crops (Núñez, 1989). Characterized as generalist predators, lacewings generally prey on small-size arthropods of thin cuticle such as aphids, scale insects, thrips, whiteflies, eggs and larvae of moths, mites and small spiders (Freitas and Fernandes, 1996). They assume, though, an important role in the biological control in some economically important crops, like fruits, vegetables, cotton and coffee (Freitas, 2001).

Bioecological studies on lacewings have been carried out in several parts of the world, showing significant advances in recent decades. In Brazil, there is still much to be learned about these predators in order to widely use them in agriculture. Different studies have presented new species (Cardoso et al., 2003; Scomparin, 1997; Souza et al., 2004) and some recently described ones (Freitas and Penny, 2001; Freitas, 2003, 2007), which certainly magnifies the richness of this group under Brazilian conditions.

Regarding the ecological, climate and soil conditions in Bahia State, Brazil, few studies have been carried out to enhance the knowledge about predators. From these, the reports on the occurrence of *Chrysopodes divisa* (Walker, 1853) (Adams and Penny, 1985). In southwestern Bahia, consolidated crops, such as irrigated fruits and coffee, have been presenting pest problems. In coffee, for instance, the leaf miner (*Leucoptera cofeella* Guérin-Méneville, 1842), which is a key pest of systematic occurrence, is a potential prey for lacewings (Ecole et al., 2002).

Coffee plants are cultivated in several Brazilian regions that often do not present the ideal climatic conditions for a good crop development. Locations characterized by water deficit determine fruit malformation and drop, which causes low coffee yields. In Brazil, arboreal components are used at coffee plantations as windbreaks, with effectiveness that has been proven by the crop management (Baggio et al., 1997). However, coffee afforestation, with the aim to reduce the effects of unfavorable climatic conditions at certain times of the year, is an old and common practice in tropical countries (Jaramillo-Botero et al., 2006). Coffee plants cultivated under agroforestry systems have been found in southwestern Bahia as a way to reduce the effects of long periods of drought. The main cultivated trees, in association with coffee, are silky oak (*Grevillea robusta* (Cunn.)), avocado (*Persea americana* Mill) and inga (*Inga* sp.). Coffee afforestation determines changes in yield (Beer et al., 1998; Miranda et al., 1999), agroecosystem microclimate and leaf morphophysiology (Gomes et al., 2008). In this sense, it is possible to hypothesize that the occurrence of pests and their natural enemies on coffee plants may be influenced by the agroecosystem management, as some authors have already described (Campanha et al., 2004; Resende et al., 2007). The agroforestry system affects the main coffee pest (*L. coffeella* (Guérin-Mèneville, 1842) and, also, the predation indices by wasps as demonstrated by Lima (2010) and Souza (2012).

It is believed that studies on lacewings may be useful to plan and implement programs of integrated production in local agroecosystems, which include strategies of either natural biological control (conservation) or applied biological control (inundative releases). Also, it could contribute to agricultural development according to the

global production trends and knowledge enhancement concerning lacewings in Brazil. Hence, this work aimed to understand the diversity of lacewings in two different coffee (*Coffea arabica* L.) cropping systems – shaded and unshaded, located in southwestern Bahia as well as to study the community structure and seasonal occurrence of the species.

## MATERIAL AND METHODS

The area of study is located in Barra do Choça, southwestern Bahia, Planalto da Conquista, latitude 14°52' S and longitude 40°34' W, 892 m above sea level, with an annual mean temperature of 21°C and annual mean rainfall of 1,300 mm.

The area for the study was selected due to the presence of crops which either had or had not received insecticide treatment, as well as the proximity of other crops and native bushlands. In a property, we delimited two 10-year old plots cultivated with the *Catuaí Amarelo* coffee variety, spaced 2 m between plants and 4 m between rows. The plots were approximately 350 m far from each other; one was exposed directly to the sun while the other was forested with silky oak trees (*G. robusta* A. Cunn.). The silky oak tiers were arranged with 11 m between plants and rows. The surroundings presented other coffee crops and native bushlands (characterized as Mata-de-Cipó – deciduous forest – a transitional biome between the Atlantic Rainforest and Caatinga).

The collection with traps was performed between December 2003 and November 2004, comprising 12 collections for each

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cropping system. McPhail traps were used, with two kinds of food baits: 7% BioAnastrepha® hydrolysed protein based, and mango juice from the homogenization of 500 g of *in natura* Haden mango pulp; plus 250 g of crystal sugar and 1,500 mL of water. The hydrolysed protein was selected for this study as it is an attractive feed used for the official monitoring of the Program of Fruit Flies of Bahia State. An area of 1 ha was delimited at each experimental plot, where eight plants were marked at the distance, between them, of approximately 50 m and 10 m from the border. The traps were then installed at 1.5 m height in the plant canopies. Four traps, out of eight, were baited with mango juice and, the other four, with 250 mL of hydrolysed protein. The differently baited traps were distributed alternatively in the area from 6 am to 6 pm on pre-established days during the month. After 12 h in daylight, the biological material was collected, baits exchanged, and traps replaced at the same sampling points. After nightfall (from 6 pm to 6 am), the capture material was collected again and traps removed to be reinstalled 30 days later. The trap contents were placed in plastic flasks and spilled on a sieve, washed with water and put on white trays. The lacewing individuals found on the tray were carefully removed with a paintbrush and deposited into a becker filled with water to be washed once more. Subsequently, the individuals were laid on filter paper to remove the excess of humidity and then transferred to petri dishes identified according to time, type of crop, date and trap code for conservation under freezing temperatures.

Collections using insect nets were carried out between March and November 2004 after verifying that traps were really helping to collect a unique genus of lacewings during the first three months (from December 2003 to February 2004). Thus, nine collections were performed with insect nets. This method was used only during daylight – from 7:30 to 8 am, and from 4 to 4:30 pm, at the same places and dates as the bait trap collections – to collect adults by hitting the net against both coffee plants and vegetation at the inter-rows. The captured individuals were taken to the laboratory, transferred to petri dishes, labelled and sacrificed by freezing them in freezers, where they were stored.

The characterization of the lacewing community was done using faunal indices, such as relative frequency, constancy, dominance, richness, diversity and similarity, calculated for each sampling method. Frequency was calculated by the formula f=n/Nx100, where f = frequency percentage, n = number of individuals of the same species, and N = total number of individuals in the sample (Silveira Neto et al., 1976). Constancy was calculated by the occurrence percentage of the collected species, using the formula C=p/Nx100, where C = constancy percentage, p = number of collections containing the species, and N = total number of collections. The species were then separated into categories: constant species (W) – present in more than 50% of the collections; accessory species (Y) – present in 25-50% of the collections; and accidental species (Z) – present in less than 25% of the collections (Silveira Neto et al., 1976). Richness (S) was obtained by the total number of species observed in the community (Silveira Neto et al., 1976). One species is considered dominant when it presents a frequency superior to 1/S, where S is the total number of species in the community (Uramoto, 2002). Diversity was measured by the Shannon index proposed by Krebs (1985) – H'=-∑(pi)(log2.pi), where H = diversity index, S = number of species, and $p_i$ = proportion of individuals belonging to that certain species. Quotient of similarity (QS) was based on Sorensen (Silveira Neto et al., 1976) – QS=2j/a+b, where a = number of species in area A, b = number of species in area B, and j = number of collections containing both species.

The study concerning the seasonal occurrence of lacewing species was carried out from the total number of specimens in each collection time according to the sampling method.

Information regarding the crop phenological stage (vegetative, flowering, fructification, postharvest) was registered for each system as well as observations about the presence or absence of weeds in the inter-row area for each sampling.

## RESULTS AND DISCUSSION

The lacewing survey in both areas (shaded and unshaded coffee plants) resulted in 148 individuals of 14 different species. In the shaded area, 16 individuals (35.5%) of seven species (43.7%) of lacewings were collected using traps while 29 individuals (64.4%) of four species (13.8%) were captured by the insect net, summarizing 6.6% of all captured individuals and 78.6% of the species. In the unshaded coffee area, the use of traps with mango juice captured 13 individuals (12.6%) of seven species (50.0%) while 90 individuals (87.4%) of three species (21.4%) were captured by the insect net. No lacewing was captured using traps with hydrolysed protein in any area (Table 1).

In shaded coffee, captures using the net (64.44%) indicated a richness consisting of four species while the specific richness provided by traps was seven, resulting in a total number of ten species on the crop. Only *Ceraeochrysa cubana* was common in both cultivation systems. From all captured species, *Leucochrysa rodriguezi* and *Chrysoperla externa* were the most frequent and the only species classified as accessory and dominant in the collection with net and traps, respectively. No species was predominant on this crop. The diversity indices were 1.91 for traps and 1.03 for the insect net (Table 2).

In unshaded coffee, ten species were sampled, from which seven (the highest richness) were obtained by traps. Regarding the other three species collected by the net, *C. externa* was predominant, presenting a relative frequency of 64.44% and occurring in more than 50% of the collections. Also, *L. rodriguezi* was the most frequent accessory and dominant species, followed by *Ceraeochrysa dislepis* and *Leucochrysa scomparini*, with a frequency of 15.40% in the trap collections. Diversity was also higher for traps (2.48) in relation to nets (1.13) (Table 3).

Comparing the lacewing communities of the two cropping systems, we observed that, despite the proximity among communities, a significant difference was found in their structures regarding the occurrence of different species and abundance of individuals. The species *Ceraeochrysa cincta*, *Leucochrysa guataparensis*, *L. cidae* and *L.* sp.4 occurred exclusively on shaded coffee plants while *L. cruentata*, *Leucochrysa intermedia*, *L. scomparini* and *Leucochrysa annae* were found only on unshaded coffee plants. On the other hand, *C. externa* and *L. rodriguezi* occurred in both environments.

In general, considering both collection methods, we observed that richness was equal for both coffee cropping systems when using traps (seven), and higher for shaded coffee when using insect nets (four). Three

**Table 1.** Number (Nº) and percentage (%) of individuals and species of lacewings collected on shaded and unshaded coffee plants according to the type of collection, between December 2003 and November 2004 (traps), and March and November 2004 (insect net). Barra do Choça, Bahia State, Brazil.

| Type of collection | Shaded coffee plants | | | | Unshaded coffee plants | | | |
|---|---|---|---|---|---|---|---|---|
| | Individuals | | Species | | Individuals | | Species | |
| | Nº | % | Nº | % | Nº | % | Nº | % |
| Trap with mango extract | 16 | 35.5 | 7 | 43.7 | 13 | 12.6 | 7 | 50.0 |
| Trap with hydrolysed protein | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Insect net | 29 | 64.4 | 4 | 13.8 | 90 | 87.4 | 3 | 21.4 |

**Table 2.** Lacewing faunal analysis according to the sampling method in the shaded coffee agroecosystem, between December 2003 and November 2004 (traps), and March and November 2004 (insect net). Barra do Choça, Bahia State, Brazil.

| Species | Trap | | | | Insect net | | | |
|---|---|---|---|---|---|---|---|---|
| | N | F | C | D | N | F | C | D |
| *Ceraeochrysa cincta* (Schneider, 1851) | 1 | 6.25 | Z | nd | - | - | - | - |
| *Ceraeochrysa cubana* (Hagen, 1861) | 1 | 6.25 | Z | nd | 3 | 10.34 | Z | Nd |
| *Ceraeochrysa dislepis* (Freitas and Penny) | 1 | 6.25 | Z | nd | - | - | - | - |
| *Ceraeochrysa everes* (Banks, 1920) | - | - | - | - | 2 | 6.90 | Z | Nd |
| *Chrysoperla externa* (Hagen, 1861) | - | - | - | - | 23 | 79.31 | Y | D |
| *Chrysopodes spinella* (Adams e Penny nova espécie) | - | - | - | - | 1 | 3.45 | Z | Nd |
| *Leucochrysa guataparensis* (Freitas and Penny, 2001) | 1 | 6.25 | Z | nd | - | - | - | - |
| *Leucochrysa rodriguezi* (Navás,1913) | 10 | 62.5 | Y | d | - | - | - | - |
| *Leucochrysa cidae* (Freitas, 2007) | 1 | 6.25 | Z | nd | - | - | - | - |
| *Leucochrysa* sp.4 | 1 | 6.25 | Z | nd | - | - | - | - |
| **Total** | 16 | 10.00 | - | - | 29 | 100.00 | - | - |
| **Percentage** | 35.55 | | | | 64.44 | | | |
| **S** | 7 | | | | 4 | | | |
| **H'** | 1.91 | | | | 1.03 | | | |

N: total number of individuals; F: relative frequency; C: constancy, constant (W), accessory (Y) and accidental (Z);
S: richness; D: dominance, dominant (d) and non-dominant (nd); H': diversity index.

species out of 14 were predominant in both cropping systems: *C. externa* (on shaded and unshaded coffee plants), *Chrysopodes spinella* and *L. rodriguezi* (on unshaded and shaded coffee plants). Outstanding species in frequency and dominance were: *C. externa* and *L. rodriguezi* on shaded coffee plants; *C. externa*, *C. dislepis* and *L. rodriguezi* on unshaded coffee plants (Tables 2 and 3).

Five species were common to both cropping systems: *C. cubana, C. dislepis, C. externa, C. spinella* and *L. rodriguezi.* From these, *C. externa* and *L. rodriguezi* stood out as either predominant or dominant while *C. cubana* and *C. spinella* were accidental.

Studies on the analysis of lacewing communities are rare, especially in Brazil. One of them that should be highlighted was conducted by Scomparin (1997) in rubber tree plantations in Mato Grosso State, Brazil. From 39 species captured by Scomparin, 25 (64%) had never been recorded. Among the species that had already been described, nine were also found in southwestern Bahia as a result of this survey. According to Scomparin (1997), ten species were constant and ten dominant; *C. externa* was dominant and *L. rodriguezi* predominant (constant and dominant). Such results corroborate the studies concerning the diversity of lacewings carried out in coffee plantations, pastures and forest fragments in the region of Alto do Rio Grande, Minas Gerais State, Brazil, which evidenced the occurrence of *C. externa* in the three sampled areas (Souza et al., 2004). Cardoso et al. (2003) also observed that *C. externa* was the most common species in *Pinus* crops. Souza et al. (2004) observed the presence of *C. externa, C. tucumana* (Navás, 1919) and *Ceraeochrysa* sp.2 on coffee plants in Minas Gerais State.

With respect to diversity, in terms of the highest absolute values, unshaded coffee plants presented a value of 2.48 against 1.91 from the shaded ones. Scomparin (1997), adopting the same model to estimate

**Table 3.** Lacewing faunal analysis according to the sampling method in the unshaded coffee agroecosystem, between December 2003 and November 2004 (traps), and March and November 2004 (insect net). Barra do Choça, Bahia State, Brazil.

| Species | Trap | | | | Insect net | | | |
|---|---|---|---|---|---|---|---|---|
| | N | F | C | D | N | F | C | D |
| *Ceraeochrysa cubana* | - | - | - | - | 4 | 4.44 | Z | Nd |
| *Ceraeochrysa dislepis* | 2 | 15.40 | Z | d | - | - | - | - |
| *Chrysoperla externa* | - | - | - | - | 58 | 64.44 | W | D |
| *Chrysopodes spinella* | - | - | - | - | 28 | 31.11 | Z | Nd |
| *Leucochrysa cruentata* (Schneider, 1851) | 1 | 7.70 | Z | nd | - | - | - | - |
| *Leucochrysa intermedia* (Schneider, 1851) | 1 | 7.70 | Z | nd | - | - | - | - |
| *Leucochrysa rodriguezi* | 5 | 36.46 | Y | d | - | - | - | - |
| *Leucochrysa santini* (Freitas and Penny, 2001) | 1 | 7.70 | Z | nd | - | - | - | - |
| *Leucochrysa scomparini* (Freitas and Penny, 2001) | 2 | 15.40 | Z | nd | - | - | - | - |
| *Leucochrysa annae* (Freitas, 2007) | 1 | 7.70 | Z | nd | - | - | - | - |
| **Total** | 13 | 100.00 | - | - | 90 | 100.00 | - | - |
| **Percentage** | 12.62 | | | | 87.37 | | | |
| **S** | 7 | | | | 3 | | | |
| **H'** | 2.48 | | | | 1.13 | | | |

N: total number of individuals; F: relative frequency; C: constancy, constant (W), accessory (Y) and accidental (Z); S: richness; D: dominance, dominant (d) and non-dominant (nd); H': diversity index.

lacewings' diversity, and presented an index of 4.73 in rubber tree plantations – greater than that obtained in this work. According to Krebs (1985), in Shannon's model, the information about diversity depends not only on the number of individuals and total of species but on the proportion of the number of individuals of each species, increasing therefore the difficulty to establish comparisons among values obtained from distinct sampling universes.

In this work, coffee crops were classified as environments with little or no similarities. Afforestation can be one of the factors responsible for such conditions as it eases the temperature range, decreases evapotranspiration during dry periods and reduces the wind action (Matsumoto et al., 2003). The change of abiotic ecological factors, such as temperature, humidity and wind, can affect insect development, distribution and abundance (Silveira Neto et al., 1976).

In this study, the highest number of lacewings was obtained on unshaded coffee plants. The proximity of this crop to an area of native forest may be another relevant factor in the differences of lacewing abundance found between both coffee cultivation areas. Only *C. externa* (on unshaded coffee plants) was classified as constant while the other 13 species were either accessory or accidental with sporadic occurrence throughout the experimental period. All species captured using traps, with the exception of *L. rodriguezi,* occurred at low population density and only in December (*L.* sp.4 and *L. cidae*), July (*C. dislepis*), September (*C. cincta*) and October (*C. cubana*). *L. rodriguezi*, the most abundant species, occurred in February, April and July. Regarding those four species captured with nets, all occurred in August; *C. externa* also occurred in July, October and November, and *Ceraeochrysa everes*, in November.

In general, no species were captured in January, March or June. The highest number of species was observed during the partial flowering and fructification periods.

*C. cubana* and *C. spinella* were collected using nets in, May, and June and October, respectively, on unshaded coffee plants. The other species occurred sparsely throughout the year indicating the presence of at least one individual in each sample.

The results for *C. externa* were quite similar to those obtained by Scomparin (1997) and Souza and Carvalho (2002) in Mato Grosso and Minas Gerais states, respectively, where they observed that the main population peaks occurred between June and September – a period of lower temperatures for the studied regions.

Minimum temperature has been mentioned as one of the factors which change the occurrence of *C. externa*. Citrus orchards in Minas Gerais State also showed a significant increase in the number of captured adults according to the decrease of the minimum temperature (Souza and Carvalho, 2002). Such authors highlight that the temperature is the climatic factor that most influences the population dynamics of *C. externa*, since cool and dry periods imply an increase in population density. However, *C. externa* occurred throughout the entire year of study, evidencing that, in certain times, the climatic conditions allow the population to survive but may hinder the species in their growth, development and reproduction.

During the collection with insect nets, a greater concentration of lacewings of the species *C. externa* was found on the grass at the inter-rows, what was reduced when the grass was dry – an observation that corroborates the results of collections carried out in pastures of *Brachiaria decumbens* Stapf in the Alto de Rio Grande region, Minas Gerais State, Brazil, where only *C. externa* was collected (Souza et al., 2004).

The absence or reduction of vegetation at the coffee inter-rows from January to June, as a consequence of herbicide application, and fruit predominance, may be the cause of the decrease of species richness and number of individuals. On the other hand, on the unshaded coffee plants, the same management was applied to the inter-row vegetation and the phenology was the same; however, the species occurred during the entire year of study. An explanation for this fact would be the presence of native forest at the surroundings. Several works in the literature support the idea that landscape heterogeneity in the surroundings of agroecosystems increases diversity and may attract and shelter organisms (Santos et al., 2002; Santos and Perez-Maluf, 2012). The presence of these environments allows the dispersion of natural enemies so that the forest may act as a reserve or ecological corridor, allowing the flow and distribution of these insects, therefore enhancing the diversity of the species. Such justification is supported by Altieri (2004) who clearly present this hypothesis and mention that natural enemies tend to have bigger populations in diversified habitats where they can regulate their hosts by greater availability of pollen and nectar as well as alternative hosts for their adults.

The change in the microclimate may also be considered a beneficial effect for the crop. Shade may either increase or decrease the initial pest infestation; some authors have shown that insects present specific microclimatic preferences (Altieri et al., 1977). A study performed in Jaboticabal County, São Paulo State, Brazil, allowed us to relate some species of natural enemies of pest insects to the presence of some of the main weeds in different phonological stages. In respect to the neuropterans, the authors verified the association of lacewings and brown lacewings with peppercress (*Lepidium virginicum* L.) and natal grass (*Rhynchelytrum repens* (Willd.)) during, the flowering and maturation stages respectively (Martinelli et al., 1988).

Regarding the use of herbicides, Scomparin and Freitas (1996) verified the negative influence on lacewing population, implying in the predator population reduction not only on remaining plants but in citrus canopies.

“Weeds” play an active role in the constant changes in agroecosystems. Such plants, which are more frequent at the inter-rows, are an alternative food source (pollen and nectar) for natural enemies. The cover vegetation also promotes a friendly microclimate for natural enemies during extreme climatic conditions and may shelter alternative preys for predators (Landis et al., 2000; Oliveira et al., 2010, 2012; Venzon et al., 2006).

Mango extract was more attractive than hydrolysed protein and, for this kind of study, traps should remain in the field at least 24 h for the collection of both diurnal and nocturnal species. Moreover, two sampling methods must be used concomitantly in lacewing surveys as they are complementary; in the current study, for instance, some species were collected only with insect nets.

The conservation of plant diversity in agroecosystems and surroundings makes the agroecosystem more heterogeneous – apparently, stimulating the maintenance of the population of natural enemies in the area. In this study, it is evidenced that there is a diversity of lacewing species in the studied agroecosystem, which may be associated with plant management at the inter-rows. The management of both lacewings and plants could then be adopted in integrated pest management programs for coffee crops.

## Conclusions

For the first time, we registered the occurrence of *C. cubana, C. cincta, C. dislepis, C. everes, C. externa, C. spinella, L. guataparensis, L. cruentata, L. intermedia, L. rodriguezi, Leucochrysa santini, L. scomparini, L. cidae, L. annae*, and *Leucochrysa* sp.4 in the municipality of Barra do Choça, Bahia State, Brazil, associated with coffee plants (*C. arabica* L.).

There was a considerable difference in their community structures regarding the occurrence of different species and abundance of individuals. The species *C. cincta*, *L. guataparensis*, *L. cidae*, and *L.* sp.4 occurred solely on shaded coffee plants while *L. cruentata*, *L. intermedia*, *L. scomparini* and *L. annae* were found only on unshaded plants. On the other hand, *C. externa* and *L. rodriguezi* stood out in both environments. The species *C. externa* is predominant in unshaded coffee crops.

The species *C. everes*, *C. externa*, and *C spinella* occur during the day; *C. cincta*, *C. dislepis*, *L. guataparensis*, *L. rodriguezi*, *L. cidae*, *L. cruentata*, *L. intermedia*, *L. santini*, *L. scomparini*, and *L. annae*, during the night; only *C. cubana* occurs during both the day and night.

## Conflict of Interests

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

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# Impact of entrepreneurship training on rural poultry farmers adoption of improved management practices in Enugu State, Nigeria

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**The study examined the impact of entrepreneurship training on rural poultry farmers' adoption of improved management practices in Enugu State, Nigeria. Data were collected from two hundreds randomly selected rural poultry farmers that were trained on entrepreneurship by use of questionnaire. Data were analyzed with both descriptive and inferential statistics. Results showed that before the training, majority (70%) of the rural poultry farmers were unaware of some of the improved management practices like record-keeping, consulting veterinary doctors, vaccination, debeaking, etc. However, after the training, all the farmers (100%) were aware of the management practices and majority (85%) of poultry farmers adopted these practices. Educational level (t=3.3501), farming experience (t=2.9511), income level (t=2.6188) as well as farm size (t=2.8183) were found highly significant and positively related to farmers adoption of the improved poultry management practices. Constraints identified were high cost of inputs (80%), low capital outlay (95%), difficulty in obtaining loan (80%) and poor extension (75%) visits. Government should organize more entrepreneurial training and provide soft loan to farmers as to facilitate adoption of the improved management practices thereby improving poultry meat supply and ensuring food security.**

**Key words:** Entrepreneurial training, rural poultry farmers, improved management practices, adoption, meat supply and food security.

## INTRODUCTION

Poultry production is an important part of farming in Nigeria agriculture. People depend on poultry for food and it serves as an additional occupation to supplement the income of small and marginal farm families. The poultry sector has developed such that large scale production is being practiced and a lot of people derive their means of livelihood from poultry and its associated industries. Oluyemi and Robert (2000) stressed the importance of poultry industry in Nigeria as producing two main products, egg and meat to meet up with the protein demand of the populace. Poultry occupies an essential position because of its vast potential to bring about rapid

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economic growth especially to the weaker section. Further, it needs low capital investment and yet assumes quick returns within weeks and months in case of broilers and layers respectively (Ojo, 2002).

In spite of the increased productivity of the industry in recent years, the industry has been faced with challenges which results in the loss of major market. Oladiro et al. (2006) reported that the downward trend of this industry has been attributed to two factors namely, high cost of feed, and poor knowledge of or experience in the management of the enterprise. These have resulted to risks such as low productivity and heavy disease infestation. These result in dwindling profits to producers, hence many farmers have been forced to fold up.

Cheeke (2002) stressed the need for improved poultry technologies that are capable of raising the farmers' agricultural production. According to him, poultry technology development is necessitated by population growth, changing climate, markets and needs. He further opined that these technologies are innovations and skills in selection of strains, brooding techniques, vaccination, handling, feed and feed techniques. There remains a wide discrepancy between what research findings show to be feasible or available and what the farmers know and use to increase their poultry production in Nigeria (Herbert, 2002). In this regard, Sherief (2005) opined that entrepreneurship training/education that exposes farmers to life applicable issues is capable of helping the farmers in adoption of new management practices and strengthen their confidence and ability to risk and accept a new technology. To buttress more on this, Badi and Badi (2006) ascertains that entrepreneurship education/training provides cultural, social and technological awareness. Responding to this, Kuratko (2005) opined that farmers will venture into new technologies if they are taught the likely

pitfall they are probable to face and the possible strategy to curb them. This makes entrepreneurship training of great importance in adoption of new technologies. The Centre for Entrepreneurship and Development Research, University of Nigeria, Nsukka is currently carrying out a project where rural farmers in Enugu State are receiving training in Animal Husbandry which includes poultry production. This has encouraged innovative and new development initiatives and ideas among the rural farmers of the state. Through the training expositions, a better understanding of their challenges and more imaginative strategies for resolving these challenges has been achieved.

Therefore, it becomes necessary to assess the impact of entrepreneurship training on rural poultry farmers level of adoption of improved poultry management practices in Enugu State Nigeria, with a view to making appropriate policy recommendations that will promote agricultural development in the state and the nation. The study sought to examine the socio-economic characteristics of small scale poultry farmers; ascertain the level of adoption of improved poultry management practices before and after the training; identify constraints; and determine the influence of socio-economic characteristics on adoption of improved management practices.

## Hypothesis

1) There is no significant difference on the level of adoption of improved poultry management practiced before and after the training.
2) The socio-economic characteristics of the farmers have no significant relationship with their level of adoption of the management practices.

## METHODOLOGY

The present study was conducted in Enugu State Nigeria. The selection of study area was based on the fact that the entrepreneurship training is going on in Centre for Entrepreneurship and Development Research (CEDR), (2003) University of Nigeria, Nsukka which involved rural farmers from Enugu State. The training was imparted in two phases with an interval of six months. The data were collected at the end of the second training. The sample frame comprised of a list of rural poultry farmers in Enugu State supplied by CEDR. From the list 200 rural poultry farmers were selected and used for the study, using simple random sampling technique.

Questionnaire was used for data collection. To ascertain the adoption level of management practices by farmers before and after the training, adoption scale was provided with a list of technologies for farmers to tick on, thereby indicating the level they were in the adoption scale. To identify constraints militating against adoption of improve management practices by farmers a list of constraints was provided for farmers to tick the constraints affecting adoption level adversely. Data were analyzed with both descriptive and inferential statistics. Hypothesis 1 was analysed using students' t-test while objective 2 was analyzed using regression analysis. All analysis was done at 5% probability level.

$Y = f(X_1, X_2, X_3, X_4, X_5, X_6, X_7, e)$

Where Y = Level of adoption of improved poultry management practices

$X_1$ = Age (years)

$X_2$ = Sex (dummy variable, 1 for Male, 0 for female.

$X_3$ = Level of Education (years spent in school).

$X_4$ = Farming experience (years)

$X_5$ = Source of finance (3 point scale of personal savings - I, money lenders - 2, and financial institution - 3).

$X_6$ = Level of income (naira)

$X_7$ = Farm size (No. of birds reared)

E = error term.

Four functional forms namely: the linear, semi-log, double-log and exponential were fitted and the linear functional form was selected on the basis of having the highest values of $R^2$, F-ratio and significant variables.

## RESULTS AND DISCUSSION

Majority of the respondents were male (79%) with an average of 12 years farming experience (Table 1). The mean age of the farmers was 42.5 years showing that

**Table 1.** Distribution of respondent based on socio-economic characteristics of the poultry farmers.

| Characteristics | Frequency | Percentage | Mean |
|---|---|---|---|
| **Age (years)** | | | |
| 20 – 30 | 35 | 17.5 | |
| 31 - 40 | 40 | 20.0 | |
| 41 – 50 | 90 | 45.0 | 42.5 |
| >50 | 35 | 17.5 | |
| | | | |
| **Sex** | | | |
| Male | 158 | 79.0 | |
| Female | 42 | 21.0 | |
| | | | |
| **Educational level** | | | |
| No formal education | 5 | 2.5 | |
| Primary | 75 | 37.5 | |
| Secondary | 95 | 47.5 | |
| Tertiary | 25 | 12.5 | |
| | | | |
| **Farm experience (years)** | | | |
| 1 – 10 | 55 | 27.5 | |
| 11 – 20 | 75 | 37.5 | |
| 21 – 30 | 40 | 20.0 | |
| >30 | 30 | 15.0 | |
| | | | |
| **Source of finance** | | | |
| Personal savings | 75 | 87.5 | |
| Money lender | 25 | 12.5 | |
| Financial institution | - | - | |
| | | | |
| **Income level per annum** | | | |
| Less than ₦200,000 | 20 | 10.0 | |
| ₦200,000 – ₦400,000 | 143 | 71.5 | |
| ₦400,001 – ₦600,000 | 25 | 12.5 | |
| >₦600,000 | 12 | 6.0 | |
| | | | |
| **Farm size** | | | |
| Less than 50 birds | 9 | 4.5 | |
| 51 – 100 birds | 83 | 41.5 | |
| 101 – 150 birds | 62 | 31.0 | |
| 151 – 200 | 31 | 15.5 | |
| >200 | 15 | 7.5 | |

Source: Field Data (2011).

they were in their productive age. About 98% had some form of formal education. This shows that the occupation is taken up by people who can read and write and more so read manuscript and labels on poultry feeds, poultry drugs and medication. If more educated farmers would continue to take up poultry production as a business in the future, then the production of meat and eggs will sustain the provision of meat protein to the teeming population in the third world in general and Nigeria in particular. Their major source of finance was from personal savings (87.5%). The average annual income was ₦301,665. It was also observed that majority (41.5%) had stock size ranging from 51 to 100 birds. The small stock size may be attributed to lack of fund, sales level or even high mortality rate due to low adoption of improve management poultry technologies by farmers

**Table 2.** Distribution of farmers by level of adoption of improved management practices before and after the training (n = 200).

| Management practices | Aware | | Interest | | Evaluation | | Trail | | Adoption | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Before | After | Before | After | Before | After | Before | After | Before | After |
| Vaccination | 100 | 100 | 100 | 100 | 80 | 100 | 65 | 100 | 60 | 100 |
| Brooding | 100 | 100 | 100 | 100 | 80 | 100 | 75 | 100 | 50 | 80 |
| Culling | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Debeaking | 60 | 100 | 45 | 100 | 35 | 100 | 20 | 10 | 10 | 60 |
| Record keeping | 50 | 100 | 40 | 100 | 35 | 100 | 30 | 100 | 20 | 100 |
| Use of disinfectants | 45 | 100 | 35 | 100 | 30 | 90 | 25 | 85 | 15 | 80 |
| Consulting Vet doctors | 50 | 100 | 35 | 100 | 30 | 80 | 20 | 75 | 10 | 70 |
| Use of drugs | 100 | 100 | 100 | 100 | 80 | 85 | 75 | 80 | 55 | 75 |

Source: Field Survey (2011).

**Table 3.** Difference of poultry farmer's level of adoption of improved management practices before and after the entrepreneurship training

| Variable | X | N | SD | SE | t-value | Sign 2 Tailed | Critical value | Df | SL |
|---|---|---|---|---|---|---|---|---|---|
| Adoption level before the training | 30 | 200 | 1081 | 67.284 | | | | | |
| Adoption level after the training | 70 | 200 | 2984 | 204 | 22.00 | 000.0 | 1.96 | 199 | 0.05 |

Source: Field Survey (2011).

before the training. However, with the training this may improve in future. Udoh (2010) noted that poultry farmers in Akwa Ibom State expanded their flock size due to adoption of improved poultry techniques.

About 72% of the respondents obtained an annual income of ₦200,000 to ₦400,000 in the poultry enterprise while 12.5% obtained ₦400,001 to ₦600,000 in their poultry business. However, 6% of the farmers obtained income of more than ₦600,000 from their business per annum. If farmers could increase production by adopting new technologies, they will increase their profit and income per year. The level of income realized from the venture even at small scale, may be the reason why farmers attended the entrepreneurship training in other to learn new and more techniques in poultry production that may enhance productivity.

## Adoption of improved management practices by farmers before entrepreneurship training

All the farmers were aware of vaccination, brooding, culling of birds and use of drugs (Table 2); while 60% were aware of debeaking, followed by record keeping (50%), consulting of veterinary doctors (50%) and use of disinfectant (45%). However, low adoption was recorded in debeaking (10%), records keeping (20%), use of disinfectant (15%) and consulting of veterinary doctors (10%). The low adoption of improved management practices by the farmers before the entrepreneurship training was observed in the study area. However, culling was adopted by all the respondents. The poultry owners sold their birds presuming that once the animals / birds get sick, there will be heavy loss to the farmers.

## Adoption of improved management practices by farmers after the entrepreneurship training

All the farmers adopted culling, record keeping and vaccination of birds (Table 3). However, majority (75%) of farmers adopted use of drugs, use of disinfectants (80%) and consulting veterinary doctors (70%). These findings are in line with Iguisi (2002) who opined that entrepreneurship training/education that exposes farmers to life applicable issues is capable of helping the farmers in adoption of new improved management practices. The high adoption level of management practices after the training will definitely lead to improved and increase poultry production among the farmers.

## Difference in adoption level before and after the entrepreneurship training

The t-test result reveals values for items 1 and 2 as well as t-calculated which is higher than actual t-value of 1.96 (Table 4). This indicates a significant ($p<0.05$) difference in the adoption level before and after the training. This shows that farmers adopted improved poultry technologies more after the training. It is therefore important for farmers to be trained frequently on new poultry technologies as these will definitely increase production. Consequently, this may go a long way to

**Table 4.** Distribution of respondents by problems militating against increased poultry production in the area (n = 200).

| Problems* | Frequency | Percentages |
|---|---|---|
| High cost of inputs | 160 | 80 |
| Scarcity of inputs | 70 | 35 |
| Sourcing of inputs | 60 | 30 |
| Poor extension visit | 150 | 75 |
| Poor market for products | 80 | 40 |
| Low capital outlay | 190 | 95 |
| Difficulty in obtaining loan | 160 | 80 |

* Multiple responses recorded. Source: Field Survey (2011).

**Table 5.** Regression estimates for socio-economic factors influencing level of adoption of improved poultry management practices.

| Explanatory variables | Coefficient | t-ratio |
|---|---|---|
| Age ($X_1$) | -8.3287 | -1.0832 |
| Sex ($X_2$) | 4.7025 | 1.2369 |
| Educational level ($X_3$) | 2.6818 | 3.3501** |
| Farm experience ($X_4$) | 3.8768 | 2.9511** |
| Sources of finance ($X_5$) | 4.3636 | 1.0511 |
| Income level ($X_6$) | 4.3449 | 2.6188** |
| Farm size ($X_7$) | 5.3329 | 2.8183** |

Constant = 10.2108; Std. Error = 0.9043; $R^2$ = 0.7108; F-ratio = 18.7099. ** Significant at 5% level. Source: Field Survey data (2011).

improve the socioeconomic status of farmers and protein intake of Nigerians.

## Constraints militating against poultry production among farmers

Results in Table 5 show that 95% rural poultry farmers perceived low capital outlay as a constraint which affects the rate of adoption of poultry management practices adversely which in turn affects poultry production. This is because low capital will not allow the farmers to buy vaccines or pay veterinary doctors when consulted. This is in line with Krueger (2005) who reported that low capital affects expansion and improvement of small business. Other constraints include high cost of inputs (80%), difficulty in obtaining loan (80%) and poor extension visit (75%), scarcity of inputs (30%) and poor market for products (40%).

Small producers and processing firms are frequently eliminated from markets for failure to understand market dynamics or, because of their inability to meet new production, sanitary and quality standards. Because poultry production is strongly dependent upon knowledge and adoption of improved technologies, technical inputs must be provided if interventions and growth are to be sustainable (Levie, 1999). Producers in developing regions often lack access to appropriate inputs and the necessary technical production skills due to inadequate input and credit markets as well as weak extension systems (USAID, 2005). Improving access to appropriate inputs and information resources can help farmers raise productivity and contribute to sound natural resource management (Ojo, 2002).

The absence of an effective, well-trained extension network is a significant constraint to the development of the poultry industry and the capacity of small producers in particular. Ineffective and inaccessible extension and education networks have resulted in inadequate human technical capacity and expertise throughout the poultry industry. Access to credit is of core importance to all aspects of the poultry industry as these may hinder expansion (Oluyemi et al., 2000).

## Socio-economic factors influencing level of adoption of improved poultry management practices

The result of multiple regression analysis of socio-economic factors influencing level of adoption of improved poultry management practices is presented in Table 6. The socio-economic features were treated as independent variables such as age, sex, educational level and farm size of the farmers. The results indicates that educational level, farming experience, income level as well as farm size are highly significant at 5% levels and positively related to the farmers' adoption of the improved poultry management practices. The implication is that any increase or improvement in any of these variables will result to increase or higher adoption of the management practices and vice versa. The result agrees with Adeyemi (1998) who reported that education can predispose an individual the liberation of the mind towards acceptance of change especially as they consider it to be same pathway to the liberation of the mind and basis for the improvement of other socio-economic statuses of the individual. The effect of income cannot be over stressed as it guarantees affordability thereby making adoption a pleasurable venture. Therefore the hypothesis which stated that socio-economic characteristics of the farmers have no significant relationship with their level of adoption of the management practices is rejected.

The coefficient of multiple determination ($R^2$) value (0.7158) indicates that about 71% of the sample variation was jointly explained by these seven variables towards adoption of improved poultry management practices.

The implication of the study is that the entrepreneurship training has a positive impact on the rural poultry farmers. It helped them to adopt improved poultry management practices thereby improving poultry meat supply and ensuring food security.

## Conclusion

The study has shown that before the entrepreneurship training, only some of the improved management practices namely culling of birds, vaccination and brooding were adopted by the poultry farmers. However, after the training, practices such as record keeping, use of disinfectants, consulting veterinary doctors, and debeaking were adopted in addition to the previous practices. This indicates that the entrepreneurship training had a positive effect on the adoption level of improved management practices of the farmers. Educational level, farming experience, income level as well as farm sizes are important determinants of farmer's adoption of improved poultry management practices. The farmers were constrained by high cost of inputs, low capital outlay, difficulty in obtaining loan and poor extension visits.

The following recommendations are made:

(1) Farmers should organize themselves into co-operative groups so as to facilitate loan procurement.
(2) Government should provide soft loans to these farmers, so as to facilitate adoption of the improved management practices thereby improving poultry meat supply and ensuring food security.
(3) Government should help organize more entrepreneurial trainings to farmers in other states of the nation.

## Conflict of Interests

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

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# Productivity of maize-legume intercropping systems under rainfed situation

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**The present experiment was carried out to study the intercropping effect of green gram, black gram, soybean, groundnut and red gram with maize during *kharif* season of 2009 and 2010. Treatments consisted of sole crop of maize [rows spaced 60 cm apart ($M_1$) and paired rows (30 cm apart) spaced at 90 cm apart ($M_2$)], green gram (GG), black gram (BG), soybean (SB), groundnut (GN), and red gram (RG); intercropping of $M_1$+GG, $M_1$+BG, $M_1$+SB, $M_1$+GN and $M_1$+RG with 1:1 and $M_2$+2GG, $M_2$+2BG, $M_2$+2SB, $M_2$+2GN and $M_2$+2RG with 2:2 row proportions. Maize equivalent yield was always higher in all the intercropping situations as compared to pure stand yield of maize. The highest maize grain yield was obtained with maize + green gram intercropping (2783.11 kg ha$^{-1}$) in 1:1. The highest maize equivalent yield was observed with maize + red gram (5270.46 kg ha$^{-1}$) in 2:2 intercropping system. Values of land equivalent ratio, relative value total, relative net return and area time equivalent ratio were greater than unity and differed significantly in between both the groups of 1:1 and 2:2 proportions of intercropping. Highest gross and net return of Rs.55191.60 ha$^{-1}$ and Rs.39950.30 ha$^{-1}$ respectively was recorded in maize+red gram (2:2) intercropping.**

**Key words:** Intercropping, maize, land equivalent ratio, area time equivalent ratio, sowing ratio, monetary advantage, net return, equivalent yield, rainfed.

## INTRODUCTION

Intercropping is gaining popularity day by day among small growers as it provides yield advantage as compared to mono cropping through yield stability and fulfilling diversified domestic needs. Cereal-legume intercropping facilitates to maintain and improve soil fertility (Andrews, 1979). Intercropping is advocated due to its benefits for yield increase (Chen et al., 2004), conserving soil, control of weeds, control legume root parasite infections and high quality fodder. Cereal-legume intercropping plays an important role in subsistence food production in developing countries, especially in situations of limited water resources (Tsubo et al., 2005). Maize based intercropping system with legume helps in improving soil health as well as yield of main crop (Beedy et al., 2010). Maize-legume intercrops yielded more and were associated with less risk than the maize-legume rotations (Kamanga et al., 2010). Maize in association with legumes gives higher total yield and net return (Patra et al., 2000). Hence, the present investigation was carried out to evaluate intercropping advantages over the respective sole crop of maize with different legumes in different sowing proportions.

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## MATERIALS AND METHODS

A field experiment was conducted in humid tropics of Nadia, West Bengal during the *Kharif* seasons of 2009 and 2010. The experimental site was situated at approximately 22º56´N, 88º32´E and at an altitude of 9.75 m above mean sea level. The soil was typical gangetic alluvium with sandy-loam in texture. The soil leads to almost neutral pH (6.8) and with 0.051% nitrogen, 18.79 kg $ha^{-1}$ available P and 90 kg $ha^{-1}$ K. The experiment was laid out in an Augmented Randomized Block Design (RBD) having 17 treatment combinations replicated thrice with the plot size of 5.4 x 4 m. Treatments consisted of sole crop of maize (rows spaced 60 cm apart ($M_1$) and paired rows (30 cm apart) spaced 90 cm apart ($M_2$)], green gram (GG), black gram (BG), soybean (SB), groundnut (GN), and red gram (RG), intercropping of $M_1$+GG, $M_1$+BG, $M_1$+SB, $M_1$+GN and $M_1$+RG with 1:1 and $M_2$+2GG, $M_2$+2BG, $M_2$+2SB, $M_2$+2GN and $M_2$+2RG with 2:2 row proportions. The recommended dose of fertilizer for sole maize (80:40:40 kg N, $P_2O_5$ and $K_2O$ $ha^{-1}$) and legumes (20:40:20 kg N, $P_2O_5$ and $K_2O$ $ha^{-1}$) were applied separately.

Aggressivity, competitive ratio (CR), land equivalent ratio (LER), land equivalent co-efficient (LEC), area-time equivalent ratio (ATER), relative value total (RVT), monetary advantage (MA) and relative net return (RNR) were calculated by using standard procedures. The prices of the inputs [seeds (Maize- Rs 61 $kg^{-1}$, Green gram- Rs 55 $kg^{-1}$, Black gram- Rs 45 $kg^{-1}$, Soybean- Rs 70 $kg^{-1}$ and Peanut- Rs 40 $kg^{-1}$), fertilizers (Urea- Rs 5.50 $kg^{-1}$, Single Super Phosphate- Rs 4.50 $kg^{-1}$ and Muriate of Potash- Rs 4.50 $kg^{-1}$), *Rhizobium* culture- Rs 100 $kg^{-1}$, Labour- Rs. 90 $day^{-1}$] that prevailed during experimentation were considered for working out of the cost of cultivation. Monetary return values were estimated on the basis of market price of the produce (maize grain- Rs 9 $kg^{-1}$, green gram- Rs 35 $kg^{-1}$, black gram- Rs 25 $kg^{-1}$, soybean- Rs 15 $kg^{-1}$ and peanut- Rs 30 $kg^{-1}$) during harvest period. Net return (Rs. $ha^{-1}$) was calculated by deducting the cost of cultivation (Rs. $ha^{-1}$) from the gross return. Benefit-cost ratio (B:C) and per day return (PDR) were calculated by using the following formula:

B:C ratio = [Gross return (Rs. $ha^{-1}$) / Cost of cultivation (Rs. $ha^{-1}$)]
PDR = [Net return (Rs. $ha^{-1}$ / Cropping period (days)]

## RESULTS AND DISCUSSION

### Yield attributes

An increasing trend was observed with respect to the number of cobs $plant^{-1}$, number of grains $cob^{-1}$ in intercropped maize due to the development of both temporal and spatial complementarity as a result of which there was no competition for nitrogen and there was a possibility of current transfer of fixed nitrogen to the cereal crop like maize, however, no significant change in 1000 grain weight was noticed (Table 1). There was decreasing trend of percentage of barren stalk in all the intercropping situations. Patra et al. (1999) reported increased number of cobs $plant^{-1}$ and grains $cob^{-1}$ due to maize legume intercropping, however, 1000 grain weight of maize was not significantly influenced but there was an increasing trend.

Number of pods $plant^{-1}$ and seeds $pod^{-1}$ in green gram, black gram, soybean and groundnut were significantly reduced due to intercropping. All the intercrops grown with maize were shorter in height and could utilize lower percentage of incoming solar radiation. Red gram was least affected due to its longer duration and taller stature (Table 2). Sole legumes always obtained higher 1000 seed/kernel weight than intercropped legumes though they were statistically at par.

### Maize grain yield

Maize when planted with 60 cm row spacing always recorded higher grain yield as compared to paired rows spaced 90 cm apart both as sole crop as well as intercrop (Table 3). Grain yield of maize was increased when intercropped with legumes like green gram, black gram and soybean. The yield advantage of maize in intercropping systems with legumes probably occurred from the difference in the timing of utilization of resources by the different crops from different soil layers, especially during peak vegetative and reproductive stages of growth, thus resulting in both temporal and spatial complementarities. Also, the increase in grain yield of maize might be resulted from maize-legume association due to symbiotic nitrogen fixation by legumes and current transfer of nitrogen to the associated maize plants. In addition, there was bonus yield from legume component, which corroborated the findings of Rana et al. (2001). A decreasing trend in maize grain yield was recorded when intercropped with red gram and groundnut in both the proportions of sowing.

### Intercrop yield

Yield of intercrops were reduced due to intercropping with maize (Table 2). Actual yield was slightly higher at 2:2 proportion of sowing than 1:1 proportion due to receipt of higher amount of solar radiation. Yield was mostly affected in the short statured under sown leguminous crops. Tall growing maize plants shaded the leguminous crops and the main reason for reduction in yield was probably due to the receipt of lower amount of incoming solar radiation which affected the rate of photosynthesis and thereby translocation of photosynthates from source to sink. Relatively tall growing crop like red gram was less affected with respect to receipt of incoming solar flux. Similar results were also obtained by Mandal and Mahapatra (1990), Patra et al. (1999) and Patra et al. (2000).

### Combined yield

Combined yield was always higher in intercropping situations both in 1:1 and 2:2 proportions of planting than mono cropping (Table 3). It might be attributed due to the inclusion of yield of maize with some yield of legumes. In maize legume association, maize was benefitted by nitrogen fixation of intercropped legumes.

**Table 1.** Effect of intercropping on cobs plant$^{-1}$, grains cob$^{-1}$, 1000 grain weight and barren stalk percentage of maize (pooled data of 2 years).

| Treatments | Cobs plant$^{-1}$ | Grains cob$^{-1}$ | 1000 grain weight (g) | Barren stalk (%) |
|---|---|---|---|---|
| $M_1$ | 1.43 | 314.33 | 215.43 | 17.90 |
| $M_2$ | 1.38 | 307.79 | 214.22 | 19.65 |
| SE m (±) | 0.07 | 7.00 | 10.37 | 1.03 |
| CD (P=0.05) | NS | NS | NS | NS |
| Maize (comb) | 1.40 | 311.06 | 214.82 | 18.77 |
| Legume (comb) | 1.36 | 308.26 | 216.05 | 16.95 |
| SE (diff.) | 0.05 | 5.42 | 8.04 | 0.80 |
| CD (P=0.05) | NS | NS | NS | NS |
| Group 1 (1:1) | 1.38 | 311.18 | 216.65 | 16.20 |
| Group 2 (2:2) | 1.33 | 305.33 | 215.45 | 17.70 |
| SE m (±) | 0.03 | 3.13 | 4.64 | 0.46 |
| CD (P=0.05) | NS | NS | NS | NS |
| **Group 1 (1:1)** | | | | |
| $M_1$ + GG | 1.50 | 320.79 | 216.59 | 15.83 |
| $M_1$ + BG | 1.48 | 316.88 | 216.70 | 15.35 |
| $M_1$ + SB | 1.45 | 315.48 | 216.25 | 14.82 |
| $M_1$ + GN | 1.25 | 304.66 | 217.21 | 17.20 |
| $M_1$ + RG | 1.22 | 298.12 | 216.49 | 17.81 |
| SE m (±) | 0.07 | 7.00 | 10.37 | 1.03 |
| CD (P=0.05) | 0.20 | 20.52 | NS | NS |
| **Group 2 (2:2)** | | | | |
| $M_2$ + 2GG | 1.42 | 312.84 | 215.38 | 17.99 |
| M2 + 2BG | 1.40 | 311.09 | 215.22 | 17.53 |
| $M_2$ + 2SB | 1.39 | 310.39 | 215.21 | 17.81 |
| $M_2$ + 2GN | 1.24 | 301.23 | 216.40 | 17.39 |
| $M_2$ + 2RG | 1.20 | 291.12 | 215.04 | 17.80 |
| SE m (±) | 0.07 | 7.00 | 10.37 | 1.03 |
| CD (P=0.05) | 0.20 | 20.52 | NS | NS |

Details of treatments are given in materials and methods; NS, Non Significant.

## Maize equivalent yield

Maize grain equivalent yield (Table 3) was recorded to be higher in all the cases of intercropping with respect to pure stand yield of maize which corroborated the findings of Patra et al. (1999, 2000). The highest maize grain equivalent yield was obtained in maize + red gram (2:2) intercropping (5270.46 kg ha$^{-1}$) due to higher yield and price of red gram.

## Competition functions

### *Aggressivity and Competitive ratio*

Aggressivity values were positive (+ve) in maize which obviously indicated that maize was the dominant crop, whereas the associated intercrops appeared to be the dominated ones having negative (-ve) values (Table 4). Between the two spatial arrangements, 1:1 proportion of intercropping resulted in higher values of aggressivity which denoted higher interspecific competition. Likewise, competitive ratio for maize was always higher as compared with the associated intercrops and higher competitive ratio of maize was observed at 1:1 proportion of intercropping than 2:2 proportions. Being a $C_4$ plant, maize appeared to be more competitive and the subsidiary intercrops were found to be less competitive with respect to utilization of available resources. Among the intercrops, red gram was more competitive and offered the highest competition to maize in both the proportions of sowing. Similarly, Sawargaonkar (2008) reported that the maize-based intercropping systems were more remunerative than sole maize; Maize + black gram and maize + green gram were superior to maize + soybean for grain yield and parameters related to

**Table 2.** Effect of intercropping on yield attributes and yields of associated legumes (pooled data of 2 years).

| Treatments | Pods plant$^{-1}$ | Seeds pod$^{-1}$ | 1000 grain weight (g) | Seed yield (kg ha$^{-1}$) | Harvest index (%) |
|---|---|---|---|---|---|
| Green gram | 23.88 | 9.78 | 25.16 | 915.92 | 19.73 |
| Black gram | 29.05 | 6.24 | 34.38 | 979.33 | 22.12 |
| Soybean | 60.73 | 2.40 | 132.26 | 2245.07 | 36.09 |
| Groundnut | 13.25 | 1.80 | 367.21 | 1814.15 | 33.15 |
| Red gram | 95.20 | 3.51 | 31.94 | 1553.23 | 24.00 |
| SE m (±) | 2.36 | 0.25 | 10.96 | 69.34 | 0.31 |
| CD (P=0.05) | 6.85 | 0.73 | 31.74 | 200.83 | 0.89 |
| Sole legume | 44.42 | 4.75 | 118.19 | 1501.54 | 27.02 |
| Intercrop legume | 37.67 | 4.62 | 114.85 | 601.29 | 25.45 |
| SE (diff.) | 1.30 | 0.14 | 6.00 | 37.98 | 0.17 |
| CD (P=0.05) | 2.65 | NS | NS | 77.78 | 0.35 |
| Group 1 (1:1) | 36.85 | 4.57 | 114.38 | 569.50 | 24.83 |
| Group 2 (2:2) | 38.49 | 4.67 | 115.32 | 633.08 | 26.07 |
| SE m (±) | 1.06 | 0.11 | 4.90 | 31.01 | 0.14 |
| CD (P=0.05) | NS | NS | NS | NS | 0.40 |
| **Group 1 (1:1)** | | | | | |
| $M_1$ + GG | 16.67 | 9.51 | 24.34 | 323.27 | 21.11 |
| $M_1$ + BG | 21.58 | 5.94 | 32.33 | 358.28 | 19.98 |
| $M_1$ + SB | 53.08 | 2.35 | 129.28 | 691.27 | 32.03 |
| $M_1$ + GN | 7.89 | 1.58 | 358.62 | 563.15 | 28.87 |
| $M_1$ + RG | 85.05 | 3.48 | 27.34 | 911.51 | 22.18 |
| SE m (±) | 2.36 | 0.25 | 10.96 | 69.34 | 0.31 |
| CD (P=0.05) | 6.85 | 0.73 | 31.74 | 200.83 | 0.89 |
| **Group 2 (2:2)** | | | | | |
| $M_2$ + 2GG | 18.55 | 9.68 | 24.55 | 360.79 | 22.49 |
| $M_2$ + 2BG | 24.86 | 6.09 | 33.15 | 388.45 | 20.94 |
| $M_2$ + 2SB | 56.58 | 2.37 | 130.26 | 755.16 | 33.22 |
| $M_2$ + 2GN | 8.80 | 1.69 | 361.54 | 635.64 | 30.20 |
| $M_2$ + 2RG | 83.65 | 3.50 | 27.09 | 1025.35 | 23.53 |
| SE m (±) | 2.36 | 0.25 | 10.96 | 69.34 | 0.31 |
| CD (P=0.05) | 6.85 | 0.73 | 31.74 | 200.83 | 0.89 |

Details of treatments are given in materials and methods; NS, Non significant.

competitive ability.

***Land equivalent ratio***

LER values were always recorded to be higher than unity signifying yield advantages of intercropping over monoculture (Table 4). Yield advantages occurred due to the development of both temporal and spatial complementarities. The highest value of LER (1.418) was obtained from maize + black gram (2:2) intercropping which was closely followed by maize + green gram (2:2) intercropping (1.417). Sharma and Behera (2009) reported that, land equivalent ratio and other competitive functions were favourably influenced with intercropped maize + green gram and maize +cowpea.

***Area time equivalent ratio***

ATER values were also greater than unity in all the cases of intercropping. ATER values, similar to LER, were higher in cases of maize-legume combinations and at 2:2 proportion of sowing. So, the intercropping system was found to be advantageous in comparison to monoculture. Maize + black gram (2:2) intercropping recorded the highest ATER value (1.362), which was achieved probably due to the development of temporal as well as spatial complementarity (Table 4). The area time equivalent ratio was higher in maize + legume in 1:2

**Table 3.** Effect of intercropping on grain yield, harvest index, combined yield and equivalent yield of maize (pooled data of 2 years).

| Treatments | Maize grain yield (kg ha$^{-1}$) | Harvest index (%) | Combined yield (kg ha$^{-1}$) | Maize equivalent yield (kg ha$^{-1}$) |
|---|---|---|---|---|
| $M_1$ | 2690.96 | 31.58 | 2690.96 | 2690.96 |
| $M_2$ | 2586.67 | 31.85 | 2586.67 | 2586.67 |
| SE m (±) | 219.00 | 0.25 | 122.21 | 355.35 |
| CD (P=0.05) | NS | NS | NS | NS |
| Maize (comb) | 2638.81 | 31.72 | 2638.81 | 2638.81 |
| Legume (comb) | 2521.56 | 31.11 | 3122.84 | 4281.29 |
| SE (diff.) | 169.64 | 0.19 | 94.67 | 275.25 |
| CD (P=0.05) | NS | 0.40 | 196.34 | 570.87 |
| Group 1 (1:1) | 2580.43 | 30.87 | 3149.92 | 4244.43 |
| Group 2 (2:2) | 2462.69 | 31.35 | 3095.76 | 4318.15 |
| SE m (±) | 97.94 | 0.11 | 54.66 | 158.92 |
| CD (P=0.05) | NS | 0.32 | NS | NS |
| **Group 1 (1:1)** | | | | |
| $M_1$ + GG | 2783.11 | 31.33 | 3106.38 | 4040.26 |
| $M_1$ + BG | 2764.84 | 31.31 | 3123.12 | 3760.07 |
| $M_1$ + SB | 2752.33 | 31.58 | 3443.61 | 3904.45 |
| $M_1$ + GN | 2570.69 | 31.96 | 3133.85 | 4447.87 |
| $M_1$ + RG | 2031.16 | 28.15 | 2942.67 | 5069.52 |
| SE m (±) | 219.00 | 0.25 | 122.21 | 355.35 |
| CD (P=0.05) | 642.35 | 0.72 | 358.46 | 1042.27 |
| **Group 2 (2:2)** | | | | |
| $M_2$ + 2GG | 2655.88 | 31.82 | 3016.67 | 4058.95 |
| M2 + 2BG | 2647.85 | 32.24 | 3036.29 | 3726.86 |
| $M_2$ + 2SB | 2635.21 | 32.20 | 3390.37 | 3893.81 |
| $M_2$ + 2GN | 2521.87 | 32.20 | 3157.51 | 4640.68 |
| $M_2$ + 2RG | 1852.63 | 28.31 | 2877.98 | 5270.46 |
| SE m (±) | 219.00 | 0.25 | 122.21 | 355.35 |
| CD (P=0.05) | 642.35 | 0.72 | 358.46 | 1042.27 |

Details of treatments are given in materials and methods; NS, Non significant.

proportion than in 1:1 proportion (Mohan et al. 2005).

### *Land equivalent coefficient*

Land equivalent co-efficient values were always recorded to be greater than 0.25 which indicated yield advantages in maize + legume intercropping situations in both the proportions of intercropping (1:1 and 2:2). The highest LEC (0.47) was recorded with maize + red gram (2:2) intercropping (Table 4).

### *Relative value total*

The values of RVT were always greater than unity. The highest value of RVT was obtained with maize + red gram (2:2) intercropping (2.033) due to higher market price of red gram (Table 4). Maize + legume intercropping brought about higher RVT value probably due to higher combined yield in maize-legume association, which was in agreement with the findings of Patra et al. (1999).

### *Monetary advantage*

Higher monetary advantages were always obtained when maize was intercropped with leguminous crops. Maize + red gram (2:2) intercropping gave rise to the highest monetary advantage (Rs. 13,011.22). The lowest monetary advantage (Rs. 8,424.99) was found in maize + soybean (1:1) intercropping (Table 4). Similar observation was also made by Refey and Prasad (1992).

### *Relative net return*

It was found that growing of legumes in between the

**Table 4.** Effect of intercropping on aggressivity value, competitive ratio, land equivalent ratio, area time equivalent ratio, monetary advantage, land equivalent coefficient, relative value total and relative net return of maize and associated legumes (pooled data of 2 years).

| Treatments | Aggressivity value | | Competitive ratio | | Land equivalent ratio | Area time equivalent ratio | Monetary advantage | Land equivalent co-efficient | Relative value total | Relative net return |
|---|---|---|---|---|---|---|---|---|---|---|
| | $A_{ab}$ | $A_{ba}$ | $CR_a$ | $CR_b$ | | | | | | |
| Group 1 (1:1) | 0.591 | -0.591 | 2.805 | 0.499 | 1.344 | 1.234 | 9708.63 | 0.36 | 1.578 | 1.726 |
| Group 2 (2:2) | 0.265 | -0.265 | 2.500 | 0.493 | 1.378 | 1.266 | 10644.23 | 0.39 | 1.667 | 1.844 |
| SE m (±) | 0.040 | 0.040 | 0.190 | 0.039 | 0.006 | 0.006 | 261.42 | 0.003 | 0.007 | 0.020 |
| CD (P=0.05) | 0.119 | 0.119 | NS | NS | 0.018 | 0.018 | 776.75 | 0.011 | 0.020 | 0.059 |
| **Group 1 (1:1)** | | | | | | | | | | |
| $M_1$ + GG | 0.701 | -0.701 | 3.015 | 0.348 | 1.389 | 1.255 | 10162.34 | 0.36 | 1.506 | 1.645 |
| $M_1$ + BG | 0.683 | -0.683 | 2.911 | 0.365 | 1.393 | 1.341 | 9559.79 | 0.37 | 1.399 | 1.417 |
| $M_1$ + SB | 0.730 | -0.730 | 3.594 | 0.663 | 1.333 | 1.286 | 8736.18 | 0.32 | 1.455 | 1.641 |
| $M_1$ + GN | 0.673 | -0.673 | 3.199 | 0.331 | 1.261 | 1.221 | 8424.99 | 0.29 | 1.651 | 1.879 |
| $M_1$ + RG | 0.168 | -0.168 | 1.307 | 0.787 | 1.341 | 1.069 | 11659.86 | 0.44 | 1.878 | 2.047 |
| SE m (±) | 0.089 | 0.089 | 0.426 | 0.087 | 0.013 | 0.013 | 584.56 | 0.01 | 0.015 | 0.044 |
| CD (P=0.05) | 0.265 | 0.265 | 1.265 | 0.258 | 0.040 | 0.040 | 1736.87 | 0.03 | 0.045 | 0.131 |
| **Group 2 (2:2)** | | | | | | | | | | |
| $M_2$ +2GG | 0.319 | -0.319 | 2.719 | 0.400 | 1.417 | 1.268 | 10825.50 | 0.40 | 1.567 | 1.711 |
| $M_2$ +2BG | 0.318 | -0.318 | 2.663 | 0.396 | 1.418 | 1.362 | 9929.30 | 0.40 | 1.440 | 1.580 |
| $M_2$ +2SB | 0.347 | -0.347 | 3.040 | 0.332 | 1.354 | 1.308 | 9184.98 | 0.34 | 1.505 | 1.698 |
| $M_2$ +2GN | 0.319 | -0.319 | 2.950 | 0.370 | 1.322 | 1.278 | 10270.14 | 0.34 | 1.789 | 2.026 |
| $M_2$ +2RG | 0.021 | -0.021 | 1.127 | 0.966 | 1.378 | 1.118 | 13011.22 | 0.47 | 2.033 | 2.208 |
| SE m (±) | 0.089 | 0.089 | 0.426 | 0.087 | 0.013 | 0.013 | 584.56 | 0.01 | 0.015 | 0.044 |
| CD (P=0.05) | 0.265 | 0.265 | 1.265 | 0.258 | 0.040 | 0.040 | 1736.87 | 0.03 | 0.045 | 0.131 |

Details of treatments are given in materials and methods; NS, Non significant.

maize rows at both the sowing ratios 1:1 and 2:2 were profitable in comparison to sole cropping of maize when differential cost of cultivation was taken into consideration (Table 4). Maize + red gram (2:2) intercropping gave the highest RNR values (2.208). This might be due to the spatial as well as temporal complementarity which resulted in substantial yield advantages from intercropping. Similar results were also obtained by Mandal et al. (1986b, 1990a and 1991a).

## Economic analysis

Intercropping in paired rows of maize spaced at 90 cm was advantageous than 60 cm row to row spacing of maize (Table 5). 2:2 proportion of intercropping brought about higher gross returns as compared to 1:1 proportion of intercropping. The highest gross and net return was obtained from maize + red gram (2:2) amounting to Rs. 55,191.60 and Rs. 39,950 $ha^{-1}$ respectively. From the pooled data, it was observed that 2:2 proportion of intercropping always fetched net

**Table 5.** Effect of intercropping on gross return, net return, B:C ratio and Per day return of maize and associated legumes (pooled data of 2 years).

| Treatments | Gross return (Rs. $ha^{-1}$) | Net return (Rs. $ha^{-1}$) | B : C ratio | Per day return (Rs.) |
|---|---|---|---|---|
| Group 1 (1:1) | 41448.54 | 25521.24 | 2.60 | 215.63 |
| Group 2 (2:2) | 42814.85 | 27438.93 | 2.80 | 229.32 |
| SE m (±) | 1006.12 | 960.78 | 0.18 | 9.06 |
| CD (P=0.05) | NS | NS | NS | NS |
| **Group 1 (1:1)** | | | | |
| $M_1$ + GG | 39362.31 | 24457.11 | 2.64 | 232.92 |
| $M_1$ + BG | 36840.60 | 22019.40 | 2.48 | 209.71 |
| $M_1$ + SB | 38140.08 | 21108.88 | 2.24 | 191.90 |
| $M_1$ + GN | 43030.84 | 26349.64 | 2.58 | 239.54 |
| $M_1$ + RG | 49868.88 | 33671.18 | 3.08 | 204.07 |
| SE m (±) | 2012.24 | 1921.56 | 0.35 | 18.12 |
| CD (P=0.05) | 5977.99 | 5708.60 | NS | NS |
| **Group 2 (2:2)** | | | | |
| $M_2$ + 2GG | 39530.54 | 25050.34 | 2.73 | 238.57 |
| $M_2$ + 2BG | 36541.73 | 22195.53 | 2.55 | 211.39 |
| $M_2$ + 2SB | 38044.28 | 21475.38 | 2.30 | 195.23 |
| $M_2$ + 2GN | 44766.08 | 28523.08 | 2.76 | 259.30 |
| $M_2$ + 2RG | 55191.60 | 39950.30 | 3.65 | 242.12 |
| SE m (±) | 2012.24 | 1921.56 | 0.35 | 18.12 |
| CD (P=0.05) | 5977.99 | 5708.60 | 1.04 | 53.83 |

Details of treatments are given in materials and methods; NS, Non significant.

return than 1:1 proportion. It was also noticed that 2:2 proportion of intercropping always recorded higher B: C ratio than 1:1 proportion and the highest (3.65) B:C ratio was recorded in maize + red gram (2:2). Maize + groundnut (2:2) gave the highest Per day return of Rs. 259.30. Bharati et al. (2007) reported that maize based intercropping generated higher net return than sole crop of maize. Kamanga et al. (2010) opined maize + legume intercropping was more productive and remunerative as compared to sole cropping which was in close agreement with the present findings.

## Conclusion

Maize when intercropped with legumes found to be beneficial and profitable. Maize- legume intercropping were found to be more advantageous (in additive series) with respect to maize grain equivalent yield and monetary returns in both the proportions of sowing (1:1 and 2:2) but 2:2 proportion was appeared to be more remunerative.

## Conflict of Interests

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

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