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# VARIATION IN BROOD- AND SEED-SIZE AND SEED PACKAGING COST IN *LEUCAENA LEUCOCEPHALA* (LAM.) DE WIT FROM KARACHI

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**ABSTRACT**

The brood- and seed –size and seed packaging cost have been investigated in *Leucaena leucocephala* (Lam.) de Wit. From 300 pods belonging to three mother plants of *L. leucocephala*, a total of 5583 seeds were recovered. Of these seeds, 355 seeds were in waste class (4% in mother tree-A, and 7% in mother tree- B and C each). The largest class (51.0 ± 2.09%) of the seeds was that of the large seeds (> 40mg in weight. This class varied from 46 to 56% in the mother trees. The seeds lesser than 20mg in weight were 3.46 ± 0.33% of the total number of seeds. Pod based inter-tree similarity calculated according to Czekanowski (1913), on the basis of seed types, was not lesser than 91% and not more than 96%. The mean brood size for all pods from three mother plants (N = 300) averaged to 17.33 ± 0.203 varying from 6 to 25 (CV: 20.28%). Brood size distribution was negatively skewed and mesokurtic. The brood size in 90 healthy pods of *Leucaena* (pods with no waste seeds) varied around 19.80% and averaged to 18.37 ± 0.3869. The single seed weight averaged to 39.86 ± 0.188, 38.53 ± 0.197 and 39.17 ± 0.194 mg in three mother plants, respectively. The composite sample (N = 5228, excluding waste seeds) exhibited negatively-skewed and leptokurtic distribution with a mean seed weight of 39.21 ± 0.112mg (CV: 20.6%). The weight of seeds within pods varied with position of the seed in the pod. The first (proximal) seed of *L. leucocephala* pods averaged to 33.30 ± 0.0009mg and was lighter than the subsequent seeds (number II to 25) by a quantum of c 4 to 10.8mg. In the composite sample of ninety healthy pods, seed packaging costs, SPC1 = g.pericarp.$g^{-1}$ seeds and SPC2 = g.pericarp.$seed^{-1}$ averaged to 0.8265 ± 0.0366 and 0.0321 ± 0.0011g, respectively. SPC1 didn't appear to follow normal distribution (positively skewed and highly leptokurtic, KS-z: 1.834, $p < 0.002$) where as SPC2 tended to be marginally away from the normalcy (KS-z: 1.258, $p < 0.084$). The results are discussed in view of available literature.

**Key Words:** *Leucaena leucocephala*, Brood size, Seed size variation, Position effect on seed weight, seed packaging cost

## INTRODUCTION

Within- fruit allocation of phytomass, brood size (*sensu* Uma Shaanker, 1988), seed size and seed packaging cost vary from species to species, individual within species and even amongst fruits of an individual plant due to various reasons as genetic load manifestation, resource depletion, predation, pathogen infection or infestation, maternal regulation, sibling rivalry etc. (Janzen, 1977; Stanton, 1984; Bawa and Webb, 1984; Nakamura, 1986; Temme, 1986; Mendez, 1997; Ganashaiah *et al.*, 1986; Ganashaiah and Uma Shaanker, 1988; Uma Shaanker *et al.*, 1988; Willson *et al.*, 1990; Busso and Perryman, 2005; Chan *et al.*, 2010; Khan and Zaki, 2012; Khan and Sahito, 2013a and b; Afsar uddin and Khan, 2015). Within-fruit reproductive allocation parameters in our local flora have been reported under local environment only in few publications e.g. *Cassia fistula* (Khan and Zaki (2012), *Delonix regia* (Khan and Sahito, 2013) and *Acacia nilotica* (Afsar uddin and Khan, 2015). The objectives of the present work were to study pod and seed characteristics of *Leucaena leucocephala* (Lam.) De Wit with reference to the pod and seed sizes and their variation. Besides investigations on brood size, effects of within-pod position of seeds on seed size and the seed packaging cost are also undertaken.

*L. leucocephala* (Lam.) de Wit (commonly known as leucaena, Jumpy bean, wild tamarind, lead tree, horse tamarind, Ipil Ipil, Kadam - so many names the world over) is a large shrub or small, unarmed evergreen tree. Probably, it is native of Central America or South America now cultivated pantropically. It is generally cultivated in gardens of Punjab and Sindh (Ali, 1973). *L. leucocephala* is a legume of long history of diversified uses (Harris *et al*., 1994) e.g., forage for ruminants, fuel wood, charcoal and timber (Brewbaker, 1985). It is one of the most productive and fast growing forage legumes; beneficial in the recovery of degraded soils (Vanlauwe et al., 1998; Goel and Behl. 2002). It is useful in Biorefinery – in processing of biomass into spectrum of marketable products and energy (Feria et al., 2012b). *Leucaena* has remarkable re-sprouting capacity (Lopez et al., 2010; Feria et al., 2011). It helps to enrich soil and aids neighboring plants because its foliage is rich in nitrogen content and natural leaf drop returns this to the soil beneath the shrubs (Vietmeyer *et al.*, 1977). The genus *Leucaena* has been reported suitable for production of bioethanol and also studied for pulping and paper-making (Feria et al., 2012a and b) and described for the expanding the range of xylooligosaccharides applications including products for the food industry as novel sweeteners and probiotics (Playne and Crittenden, 2004). It has antioxidant and antimicrobial flavonoids glycoside (Mohammed *et al*., 2015).

## MATERIALS AND METHODS

Three mature plants of *L. leucocephala* were selected in Karachi University Campus for the collection of pods. The trees abounded with non-saline (ECe: 0.9-3.6 $dS.m^{-1}$) basic (pH: 8-9.02) soils of loamy sand to sandy loam texture in 0-30cm horizon.

**Collection of pods:** One hundred mature pods of current year growth were collected from each individual plant in February 2012. The sampling of pods was random – selecting them from all four sides of the canopy and only apparently undamaged pods were sampled. The pods were air dried in the laboratory for around sixty days.

**Measurements of pods and seeds:** The shape of each pod was drawn on the graph paper and seeds positions were located in each pod on graphs so that the inter-seed linear distance may be measured accurately. The seeds were weighed individually according to their position from proximal to distal end of the pod and noted on the graph besides the pod's shape diagram on the graph. An electrical balance with an accuracy of 0.1mg was used to weigh the seeds. After recovery of seeds, residual pod mass (Pericarp) was also weighed.

The pattern of distribution of seed size within pods was determined in 30 normal pods of each mother tree with the criterion that none of the pods had deformed shriveled, fungal infected or insect-eaten seeds. Mother plant-A had 55% of such pods and mother plant-B 36% and mother plant C had 34% healthier pods. From the available healthy pods, thirty representative sample pods were selected and studied for such parameters as distribution of seed-size in pods, brood size, weight of individual seed, inter-seed distance in pods, seed size variation with pods and the mother tree, and the seed packaging cost on individual pod basis.

**Seed classification: T**he seeds were classified into various sizes as per criterion given below. The deformed and shriveled seeds or those damaged by fungi or eaten by the insects were pooled as waste seeds. Seed size criterion was:

Small seeds: < 20mg; Medium seeds: 20.1- 40mg; large seeds: > 40mg.

**Within-pod-position of seed and seed size:** To investigate the seed size relations with the position of the seed within normal healthy pods, four approaches were employed following Afsar uddin and Khan (2015). In the first approach, a pod was divided into two parts- above and below the middle seed (s) and the middle seed weight was compared with the mean weight of the seeds falling above the middle seed (s) or below the middle seed (s). In the second approach, the weight of the first proximal seed was compared with the mean of the rest seeds falling below the proximal seed until the last distal seed. The third approach was converse to the second one. The weight of the most distal seed was compared with the mean of the seeds present above the distal seeds until the most proximal seed. In the fourth approach, average weight of $i^{th}$ seed starting from the first (proximal) seed to the ultimate (distal) seed was determined in healthy pods (usually 30) of each mother plant. To detect a pattern of the seed size distribution within pods, average weight of seeds (sequentially numbered from proximal to distal end) were plotted against the seed number for each mother plant.

**Estimation of seed packaging cost:** To determine the within-pod biomass allocation, the ratio of the mass of the pericarp to the seeds was calculated. The two parameters, pericarp $mass.seed^{-1}$ and pericarp $mass.g^{-1}$ seeds, were considered to represent the seeds packaging cost (Mehlman, 1993; Chen *et al.,* 2010, Khan and Sahito, 2013a and b; Afsar uddin and Khan, 2015) in the healthy pods of the three mother plants.

**Statistical analysis:** The location and dispersion parameters of data were calculated and the frequency distributions were characterized with skewness (G1) and kurtosis (G2) and their errors (SG1 and SG2, respectively) following Shaukat and Khan (1979). Kolmogorov-Smirnov z test (KS-z test) was performed to detect normal distribution (Sokal and Rholf, 1995). KS-z test assesses whether the observations could reasonably have come from the normal distribution. The ANOVA analysis was performed to distinguish main and interactive effects within and between the factors and compare the means. The data was analyzed on canned statistical packages such as costat and SPSS version 12.

## RESULTS

### THE PODS

The pods of *L. leucocephala* are flat, thin, dark brown, lustrous, minutely pilose and acute at the apex with elongated tip (Fig. 1). The pericarp of air-dried pods is thin and brittle. The pods dehisce explosively by opening sutures. Thus the dispersal unit is seed. Seeds are aligned transversely in pod.

### Pod length

The mean length of pod of *L. leucocephala* plants A, B, and C (100 pods each) was 17.22 ± 0.2344, 15.91 ± 0.2360 and 15.91 ± 0.1432 cm, respectively. The pod length in pooled sample (N=300) ranged from 7.4 to 22.7 cm averaging to 16.51 ± 0.1245) (Table 1).

**Pod breadth**

The mean pod breadth of *L. leucocephala* plant A, B and C were 1.87 ± 0.0044, 1.80 ± 0.0295 and 1.64 ± 0.015 cm, respectively. The pooled sample averaged to 1.77 ± 0.01 cm (Table 1).

**Pod weight**

The pod weight varied from 0.0766g to 1.76g in mother plant A (mean: 1.2037 ± 0.0366g) and from 0.0794 to 1.7765g (mean = 1.2293 ± 0.0295g) in plant B and from 0.0766 to 1.9145g in plant C (mean = 1.2866 ± 0.02891g) (Table 1). The pod weight in composite sample varied from 0.0747 to 1.9145g and averaging to 1.2411 ± 0.0183g varying around 25%.

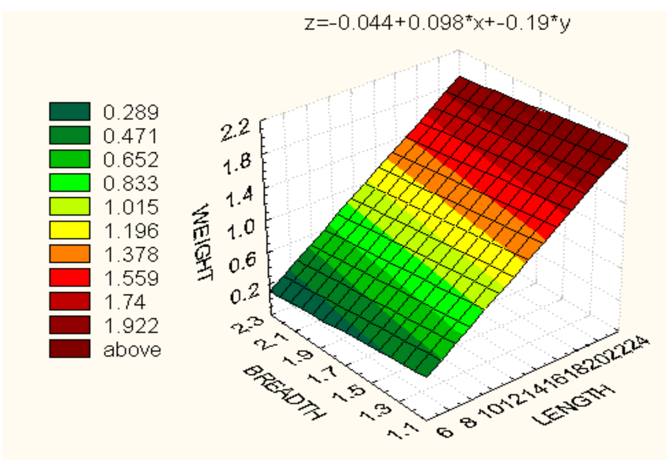
Fig. 1. The mature pods and seeds of *Leucaena leucocephala*.

**POD-SIZE COMPARISON**

The average pod length of mother plant A and B differed significantly from each other but plant B didn't differ significantly from plant C in pod length. All three plants, however, showed significant difference in average pod breadth (Table 2). There was no significant difference in average pod weight in three mother plants.

**INTERRELATIONSHIP OF POD WEIGHT, POD LENGTH AND POD BREADTH**

The pod weight (PW) related with pod length (PL) more closely than the pod breadth (PB) as is obvious from the zero order correlation between PW and PL and PW and PB. The predictive multiple regression equation pertaining to PW relationship with PL and PB is given in Fig. 2. PW was more dependent on PL which may be due to more variation in PL (CV= 13.06%) than in PB (5.66%). The explanatory powers of the multiple regression equation was, however, low (c 39%).




PW = - 0.44 + 0.098 PL – 0.190 PB ± 0.248065
t = -0.28 t = 12.97 t = -2.013
p , 0.778 p < 0.0001 p < 0.0045
R = 0.625, $R^2$ = 0.391, Adj. $R^2$ = 0.387
F = 95.44 (p < 0.001); N = 300

| | PL | PB |
|---|---|---|
| Zero order (r) = | 0.619 | 0.215 |
| Partial correlation (r) = | 0.610 | - 0.116 |

Fig, 2 Relationship of pod weight (PW, g) with pod length (PL, cm) and pod breadth (PB, cm).

**THE SEEDS**

The seeds of *Leucaena* are oval, glaucous, dark brown, with a prominent U-shaped mark on either side. Seeds are said to be coffee substitute. The seeds are attacked by the seed-boring insects. Food called "tempe lamtoro" is prepared of fermented seeds, in Indonesia (www.worldagroforestry.org/treedb/AFTPDFS/Leucaena-leucocephala.pdf). Seeds contain mimosine, an iminoacid, toxic to ruminant animals (Salem *et al.*, 2011). Mimosine in leaves may be reduced in concentration by using ethyl methanesulphonate (Zayed *et al.*, 2014). Seed oil is said to be active against Gram positive and negative bacteria (Aderibige *et al.*, 2011) and seed gum is good tablet binder in ibuprofen tablet (Verma and Balkishen, 2007).

Table 1. Pod and seed characteristics of *L. leucocephala.*

| Characteristics | Plant A | Plant B | Plant C | Pooled sample |
|---|---|---|---|---|
| | N = 100 | N = 100 | N = 100 | N = 300 |
| Pod Length (cm) | 17.22 ± 0.2344 | 15.91 ± 0.2360 | 15.91 ± 0.1432 | 16.51 ± 0.1245 |
| Pod breadth (cm) | 1.87 ± 0.0044 | 1.80 ± 0.0132 | 1.64 ± 0.0150 | 1.768 ± 0.0100 |
| Pod weight (g) | 1.2037 ± 0.0366 | 1.2293 ± 0.02953 | 1.2866 ± 0.02891 | 1.2411 ± 0.0183 |
| Brood size | 17.84 ± 0.391 | 16.04 ± 0.322 | 18.10 ± 0.294 | 17.33 ± 0.2029 |
| Single Seed weight (mg) | N = 1794<br>39.86 ± 0.1881 | N = 1642<br>38.53 ± 0.1970g | N = 1792<br>39.17 ± 0.194 | N = 5228<br>39.21 ± 0.1117 |
| Healthy pods ( N = 30 each) | | | | |
| Pod weight (g) | 1.321 ± 0.4589 | 1.2419 ± 0.1254 | 1.3497 ± 0.0399 | 1.3042 ± 0.0259 |
| Pericarp weight (g) | 0.5493 ± 0.0245 | 0.5752 ± 0.0366 | 0.5798 ± 0.02275 | 0.5688 ± 0.01641 |
| Seed yield per pod (g) | 0.7717 ± 0.0332 | 0.6648 ± 0.0330 | 0.7699 ± 0.0327 | 0.7355 ± 0.0198 |
| Single seed weight (mg) | N = 561<br>40.62 ± 0.2901 | N = 510<br>38.89 ± 0.2959 | N = 542<br>40.05 ± 0.2861 | N = 1610<br>39.88 ± 0.1689 |
| Brood per pod | 19.03 ± 0.846<br>CV: 24.35% | 17.00 ± 0.621<br>CV: 20.01% | 19.07 ± 0.423<br>CV: 12.15% | 18.37 ± 0.3867<br>CV: 11.53% |

Table 2. Paired Samples Test (pod dimensions compared in three mother plants of Leucaena).

| Pairs | Characters Compared | Paired Differences | | | | | t | df | p |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | SD | SE Mean | Lower | Upper | | | |
| Pair 1 | LEU1PL - LEU2PL * | 1.8190 | 3.2402 | 0.3240 | 1.1761 | 2.4619 | 5.614 | 99 | 0.0001 |
| Pair 2 | LEU1PL - LEU3PL | 1.8170 | 2.5035 | 0.2503 | 1.3203 | 2.3137 | 7.258 | 99 | 0.0001 |
| Pair 3 | LEU2PL - LEU3PL | -0.0020 | 2.5513 | 0.2551 | -0.5082 | 0.5042 | -0.008 | 99 | 0.994 |
| Pair 4 | LEU1PB - LEU2PB | 7.400E-02 | 0.1862 | 1.862E-02 | 3.705E-02 | 0.1109 | 3.974 | 99 | 0.0001 |
| Pair 5 | LEU1PB - LEU3PB | 0.2330 | 0.2198 | 2.198E-02 | 0.1894 | 0.2766 | 10.603 | 99 | 0.0001 |
| Pair 6 | LEU2PB - LEU3PB | 0.1590 | 0.1944 | 1.944E-02 | 0.1204 | 0.1976 | 8.178 | 99 | 0.0001 |
| Pair 7 | LEU1PWT - LEU2PWT | -0.115656 | 1.077640 | 0.107764 | -0.329483 | 0.09817 | -1.073 | 99 | 0.286 |
| Pair 8 | LEU1PWT - LEU3PWT | -0.082903 | 0.462844 | 0.046284 | -0.174741 | 0.00894 | -1.791 | 99 | 0.076 |
| Pair 9 | LEU2PWT - LEU3PWT | 0.032753 | 1.048918 | 0.104892 | -0.175375 | 0.24088 | 0.312 | 99 | 0.756 |

*Acronyms: LEU, *Leucaena leucocephala;* .PL, Pod Length (cm); PB, Pod Width (cm); PWT, Pod weight (g). The numeral associated with the plant acronym is the plant number (1, 2 or 3) of the species.

Table 3. Classification of seeds recovered from 100 pods each from three *L. leucocephala* plants into various types.

| Seed Types | Plant A (N = 100) | Plant B (N = 100) | Plant C (N = 100) | Pooled (N = 300) |
|---|---|---|---|---|
| Waste seeds | 84 (4 ) * | 127 (7) | 144 (7) | 355 (6.0 ± 1.0) |
| Small seeds | 60 (3) | 63 (4) | 70 (4) | 193 (3.46 ± 0.33) |
| Medium seeds | 694 (37) | 752 (43) | 737 (38) | 2183 (39.13 ± 1.86) |
| Large seeds | 1040 (56) | 627 (46) | 985 (51) | 2852 (51.08 ± 2.89) |
| Total | 1878 | 1769 | 1936 | 5583 |

Inter-tree similarity amongst plants on the basis of seed types as per Czekanowski (1913) – Plant A to Plant B = 91.31 %; Plant A to Plant C = 95.59 % and Plant B to Plant C = 94.68

**Seed Classification**

From 300 pods belonging to three mother plants of *L. leucocephala*, a total of 5583 seeds were recovered. Of these seeds, 355 seeds were in waste class (4% in mother tree-A, and 7% in mother tree- B and C each). The largest class (51.0 ± 2.089%) of the seeds was that of the large seeds. It varied from 46 to 56% in the mother trees. The seeds lesser than 20mg in weight were 3.46 ± 0.33% of the total number of seeds. Pod based inter-tree similarity calculated according to Czekanowski (1913), on the basis of various seed types was not lesser than 91% and not more than 96% (Table 3).

**SEEDS / SEED CHAMBERS RATIO**

The seeds / seed chambers ratio for normal seeds recovered, as calculated in the composite samples of pods, was 0.9363 (N = 300) which indicated high seed setting in this species.

Table 4. Two-way ANOVA of seed mass data collected from three *L. leucocephala* plants (30 pods from each).

| Source | SS | df | MS | F | p |
|---|---|---|---|---|---|
| Mother plant | 0.0069917 | 2 | 0.003495843 | 9.87 | 0.0001 |
| Pods | 0.0271869 | 29 | 0.0009374798 | 2.64 | 0.0001 |
| Mother plant x pods | 0.0553514 | 29 | 0.0006543352 | 2.69 | 0.0001 |
| Error | 0.7650983 | 2160 | 0.0003542124 | - | |
| Total | 0.8546288 | 2249 | | | |

**BROOD SIZE**

Following Uma Shaanker *et al.,* (1988), brood size was represented as the number of healthy seeds recovered from a pod. The mean brood size in all pods (N = 300) of *Leucaena* averaged to 17.33 ± 0.203 varying from 6 to 25 (CV: 20.28%). Brood size distribution was negatively skewed and mesokurtic (Fig. 3). The brood size in 90 healthy pods of *Leucaena* (pods with no waste seed) varied around 11.53% and averaged to 18.37 ± 0.3869 (Table 1). The brood size of healthy pods (N = 90) was higher than that in all types of pods (N = 300) studied (t = 2.38, p < 0.0001).

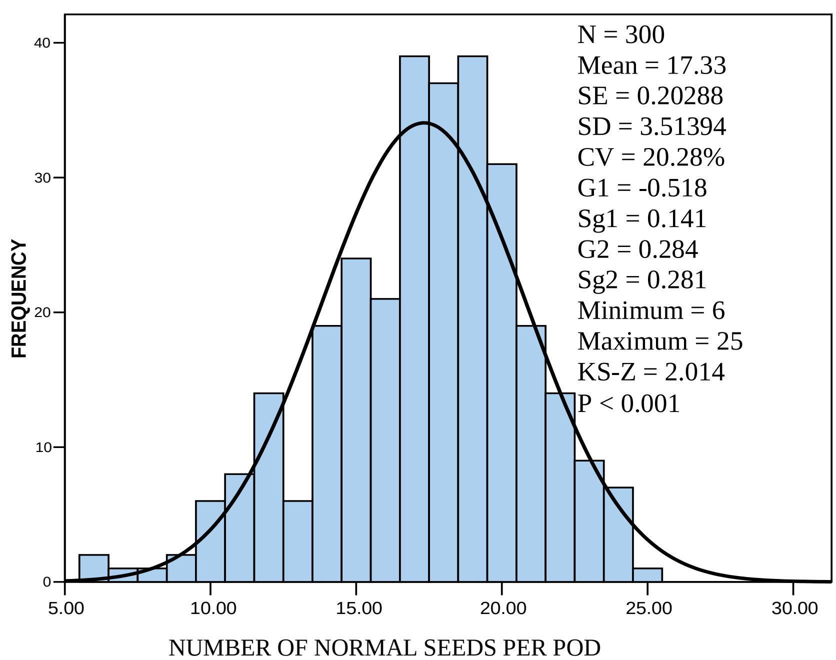



Figure 3. Distribution of number of normal seeds per pod amongst the pods (N = 300) of *Leucaena leucocephala.*

**SEED WEIGHT DISTRIBUTION**

The seeds of *Leucaena* were lighter in weight and averaged to 39.86 ± 0.1881, 38.53 ± 0.1971 and 39.17 ± 0.1942 mg for three mother plants, respectively (Table 1). The composite sample (N = 5228, excluding waste seeds) exhibited negatively-skewed and leptokurtic distribution with a mean seed weight of 39.21 ± 0.1117 mg with a variability of 20.6%. The seed weight varied around 25-fold in the composite sample (Fig. 4). Mostly seeds concentrated around the mean value. There were only 10 seeds weighing > 65 mg i.e. c 0.19% of the total seeds.

**MOTHER PLANT- AND POD-BASED VARIATION IN SEED WEIGHT**

Two-way ANOVA for seed weight data indicated that both, the mother tree ($F = 9.87$, $p < 0.0001$) and the pods ($F = 2.64$, $p < 0.0001$), influenced seed weight significantly. The mother tree and the pods interacted significantly ($F = 2.69$, $p < 0.0001$) in this respect (Table 4). The results highlighted the role and significance of genotypic peculiarities and the ecological history of the mother trees as well as the developmental and the environmental history of the pods.

**INTER-SEED DISTANCES**

Inter-seed distance in *Leucaena* pods averaged 0.751 ± 0.0048 cm. It ranged from 0.4 to 2.0 cm and distributed asymmetrically – skewed positively and leptokurtic (Fig. 5).

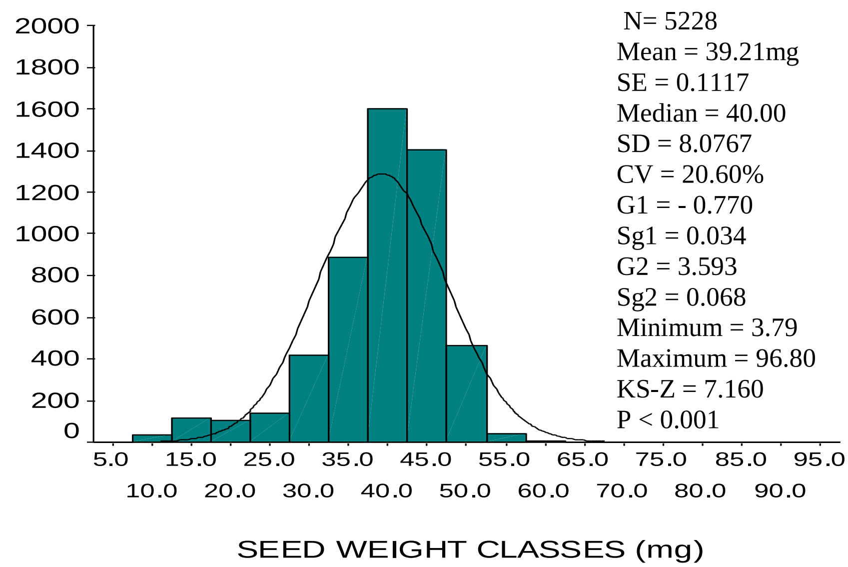



Fig. 4. Seed weight distribution in composite sample of seeds (N = 5228, excluding 355 waste seeds) recovered from 300 pods of *L. leucocephala* sample plants (A, B and C).

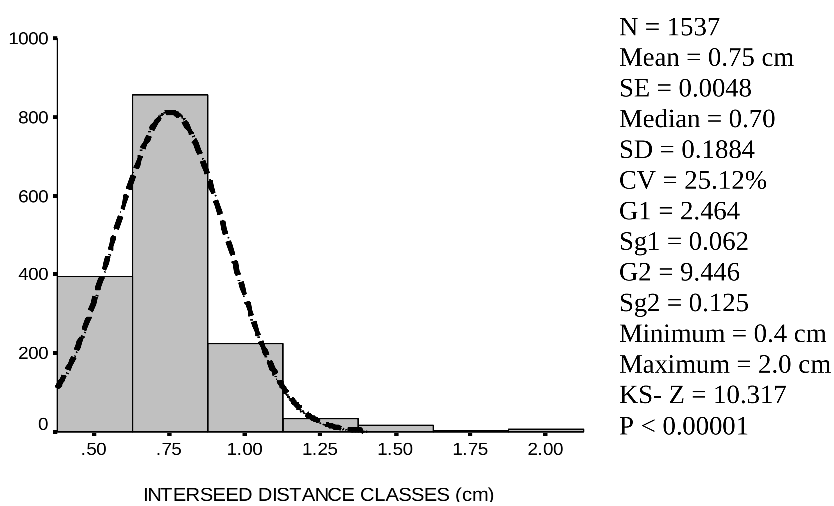



Fig. 5. *L. leucocephala* - *d*istribution of inter-seed distances in pooled sample of 90 normal pods.

**DISTRIBUTION PATTERN OF SEED SIZE WITHIN PODS (Positional Effect)**

To investigate within-pod positional effect of seed on the seed size, four approaches were employed 1) comparison of weight of the first proximal seed with the mean weight of the rest of the seeds in the pod, 2) comparison of the weight of the last (distal seed) with the mean weight of the seeds lying above the distal seeds, 3) comparison of the weight of middle seed (s) of the pod with the mean weights of seeds lying proximally or distally and 4) the mean seed weight of each seed in healthy pods was determined in sequential order from proximal to distal seed.

In the composite sample of healthy pods of three mother plants and individually in plant B and C the frequency of proximal seed to be lesser in weight than the mean of the rest seeds was significantly higher as compared to the frequency of proximal seed weight to be higher than the mean of the seeds towards distal end (Table 5a).

The comparison of weight of distal seed with the mean weight of seeds above the distal seeds is presented in Table 5b. In the composite sample of *Leucaena* as well as in all the three mother plants there was significantly higher frequency of occurrence of heavier distal seed compared to the mean weight of seeds above (Table 5b).

The results of the third approach to determine positional effect of seed on seed size are presented in Table 5c. No clear cut pattern of seed size distribution could emerge, however, in composite sample, the weight of the middle seeds (s) was found to be significantly higher than the mean weight of the upper seeds ($t = 3.74$, $df = 89$; $p < 0.0001$).

Table5a. The comparison of proximal seed mass with the average mass of the rest (lower) seeds in the pods.

| Parameter | Percent Occurrence | | |
|---|---|---|---|
| | Plant A N = 30 | Plant B N = 30 | Plant C N = 30 |
| NE | 6.66 | 10.0 | 3.33 |
| H | 50.0 | 3.33 | 0.0 |
| L | 43.33 | 86.70 | 97.7 |
| Composite sample (N = 90) | | | |
| NE | 5.55 ± 1.93 a | | |
| H | 17.77 ± 1.616 b | | |
| L | 76.66 ± 1.637 c | | |

Key to the acronyms: NE, the first (proximal) seed is near equal in mass to the mean mass of the rest seeds in the pod. H, the first proximal seed's mass is higher in magnitude than the mean mass of the rest seeds. L, The first proximal seed mass is lesser in magnitude than the mean mass of the rest of the seeds. Dissimilar letters following figure indicates the significant difference at $p < 0.05$ as given by t-test.

Table 5b .The comparison of distal seed mass with the average mass of the rest (upper) seeds in the pods.

| Parameter | Percent Occurrence | | |
|---|---|---|---|
| | Plant A N = 30 | Plant B N = 30 | Plant C N = 30 |
| NE | 143.33 | 6.66 | 6.66 |
| H | 60.0 | 56.66 | 56.66 |
| L | 26.66 | 36.66 | 36.66 |
| Composite sample (N = 90) | | | |
| NE | 8.88 ± 2.20 a | | |
| H | 57.78 ± 1.33 b | | |
| L | 33.33 ± 3.13 c | | |

Key to the acronyms: NE, the last (distal) seed is near equal in mass to the mean mass of the rest (upper) seeds in the pod. H, the last (distal) seed's mass is higher in magnitude than the mean mass of the rest (upper) seeds. L, The mass of the last seed is lesser in magnitude than the mean mass of the rest of the seeds. Dissimilar letter following figure indicates the significant difference at $p < 0.05$ as given by t-test.

Table 5c. Mean weight (mg) of mid seed (s) in a pod and seeds falling above and below the mid seeds in healthy pods of three *L. leucocephala* mother plants (30 pods each)

| Seeds location | Plant A | Plant B | Plant C | Pooled |
|---|---|---|---|---|
| Seeds above mid seeds | 40.30 ± 0.730 | 38.24 ± 0.692 | 37.87 ± 0.629 | 38.80 ± 0.4255 |
| Mid seed (s) | 41.01 ± 1.02 | 39.82 ± 1.335 | 41.49 ± 0.932 | 40.79 ± 0.6472 |
| Seeds below mid seeds | 40.83 ± 0.580 | 39.53 ± 0.645 | 43.72 ± 2.285 | 41.36 ± 0.4302 |

The results of the fourth approach to determine distribution pattern of seed size within pods are presented in Fig. 6. The first seed of *L. leucocephala* pods averaged to 33.30 ± 0.00090 mg and was lighter than the subsequent seeds (number II to 25) by a quantum of c 4 to 10.8 mg. The seeds of all positions varied in weight by 11.21 to 18.2%.

**PLANT - INDUCED VARIATIONS IN POD AND SEED CHARACTERISTICS**

Pod length and breadth although varied predominantly within trees (the within tree component variations for these parameters explained for the total variation by the quantum of 72.49 and 65.65%, respectively). However, there was substantial magnitude of amongst-tree variation also (the amongst-trees component variations for these parameters explained for 27.5 and 30.4% of the total variation, respectively). Seed yield per pod and brood size varied little amongst trees as the amongst–tree variation explained for only 7% of the total variance. Within-trees variances of these parameters explained 92-93 % of the total variation (Table 6).

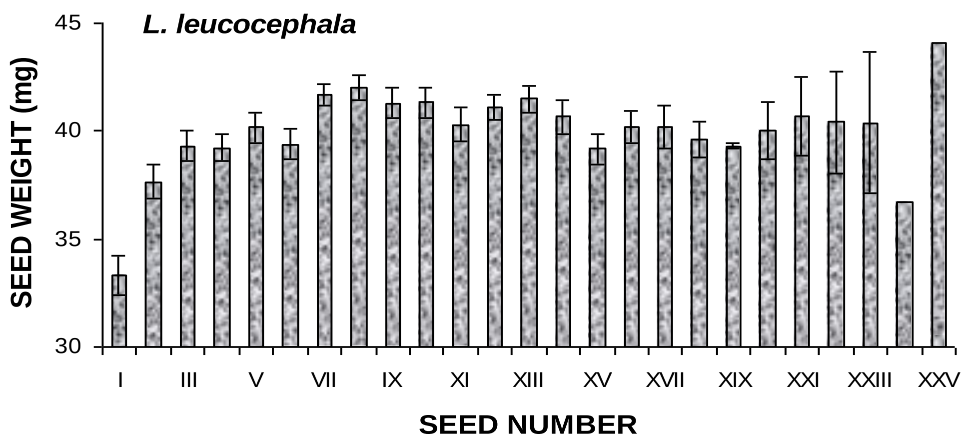



Fig. 6. Mean weight of seeds for specific position in pods (N = 30) sequentially from proximal to distal end in healthy pods..

Table 6. Results of one way ANOVA for pods and seeds characteristics in healthy pods of *L. leucocephala* (30 pods each for three mother plants).

| Characteristics | MS | F | p | Variance Explained (%) | |
|---|---|---|---|---|---|
| | | | | Between | Within |
| PL | 61.86844 | 11.50 | 0.00001 | 27.50 | 72.49 |
| PB | 0.533348 | 19.07 | 0.00001 | 30.44 | 65.56 |
| PW | 0.093243 | 1.56 | 0.2154 | 3.47 | 96.53 |
| Pericarp | 0.008578 | 0.35 | 0.7066 | 0.80 | 99.20 |
| Seed Yield ( per pod) | 0.114350 | 3.42 | 0.0373 | 7.28 | 92.72 |
| Brood (SN) | 42.03333 | 3.28 | 0.0423 | 7.01 | 92.98 |

Acronyms: PL, Pod Length,; PB, Pod breadth; PW, pod weight; pericarp, Pericarp weight per pod; SW, Seed weight per pod; SN, Number of seeds per pod.

**SEED PACKAGING COSTS**

SPC was quite low averaging to 0.7497 ± 0.0458, 0.9498 ± 0.0020 and 0.7799 ± 0.0357g pericarp mass.$g^{-1}$seed in three mother plants of *L. leucocephala*, respectively. SPC varied 33.5, 55.0, and 24.04%, respectively in these plants. SPC on the basis of g pericarp mass.$seed^{-1}$ was highly comparable in the three plants – 0.0305 ± 0.00195, 0.0350 ± 0.0024, and 0.0306 ± 0.0012g pericarp mass.seed$^{-1}$. In all plants, it tended to be normally distributed (Table 7). In the composite sample of 90 healthy pods, SPC1 and SPC2 averaged to 0.8265 ± 0.0366 and 0.0321 ± 0.0011g, respectively. SPC1 didn't appear to follow normal distribution (positively skewed and highly leptokurtic (KS-z: 1.834, p < 0.002) where as SPC2 tended to be marginally deviated from the normalcy (KS-z: 1.258, p< 0.084).

**PLANT-BASED VARIATION IN SEED PACKAGING COSTS**

One-way ANOVA of The SPC data (Table 8) indicated that SPC expressed as g.pericarp.g$^{-1}$ seed or g pericarp.seed$^{-1}$ didn't vary significantly among the mother trees. Within tree variance in both cases (SPC1 and SPC2) was predominantly accounted for the total variance by a quantum of 93-96%. The amongst-tree variance was low (6.52 and 3.89% for SPC1 and SPC2, respectively).

Table 7. Seed packaging cost (SPC1 and SPC2) in three mother plants of *Leucaena*.

| Descriptive Statistics | SPC (1) – g per g seed N = 30 | | | SPC (2) – g per seed N = 30 | | | Pooled sample N = 90 | |
|---|---|---|---|---|---|---|---|---|
| | Plant A | Plant B | Plant C | Plant A | Plant B | Plant C | SPC (1) | SPC (2) |
| Mean | 0.7497 | 0.9498 | 0.7799 | 0.0305 | 0.0350 | 0.0306 | 0.8265 | 0.0321 |
| SE | 0.04582 | 0.00195 | 0. 035667 | 0.00195 | 0.00244 | 0. 0012 | 0.03656 | 0.00111 |
| Q2 | 0.72603 | 0.90129 | 0.78137 | 0.02959 | 0.03405 | 0.03149 | 0.8048 | 0.0317 |
| SD | 0.25095 | 0.49393 | 0.19534 | 0.01066 | 0.01335 | 0.00640 | 0.34684 | 0.01062 |
| CV (%) | 33.47 | 52.00 | 25.04 | 34.94 | 38.14 | 20.96 | 41.96 | 33.08 |
| g1 | 1.534 | 2.480 | -0.050 | 1.677 | 1.711 | -0.103 | 2.980 | 1.824 |
| Sg1 | 0.427 | 0.427 | 0.427 | 0.427 | 0.427 | 0.427 | 0.254 | 0.254 |
| G2 | 5.728 | 8.746 | 1.838 | 5.569 | 8.495 | 1.428 | 15.588 | 9.211 |
| Sg2 | 0.833 | 0.833 | 0.833 | 0.833 | 0.833 | 0.833 | 0.503 | 0.503 |
| Minimum | 0.2930 | 0.1390 | 0.2634 | 0.0109 | 0.0059 | 0.0166 | 0.139 | 0.0059 |
| Maximum | 1.6795 | 2.8609 | 1.2859 | 0.0693 | 0.0878 | 0.0479 | 2.8609 | 0.0878 |
| KS-z | 0.824 | 1.614 | 0.611 | 1.136 | 1.087 | 0.698 | 1.834 | 1.258 |
| P | 0.506 | 0.011 | 0.850 | 0.156 | 0.188 | 0.714 | 0.002 | 0.084 |

Table 8. Results of one way ANOVA for seed packaging costs in healthy Pods of *L. leucocephala* (30 pods each for three mother plants)

| Seed packaging cost | MS | F | p | Variance Explained (%) | |
|---|---|---|---|---|---|
| | | | | Between | Within |
| SPC (1) | 0.34925666 | 3.0364 | 0.0531 (NS) | 6.52 | 93.48 |
| SPC (2) | 0.00019574 | 1.7608 | 0.1780 (NS) | 3.89 | 96.11 |

Acronyms: SPC (1) - seed packaging cost (g per g seeds); SPC (2) - Seed packaging cost (g per seed).

**DISCUSSION**

Our studies indicated that although pod length, pod breadth, seed yield per pod and brood size varied highly significantly within the mother trees, there was substantial variation particularly in pod length and breadth amongst the trees also. The results highlighted the significant role of genetics and the ecological history of the mother trees as well as the some variation in developmental and the environmental history of the pods. The analysis of isoenzyme systems in *L. leucocephala* has indicated high level of intraspecific variability (Harris *et al.,* 1994). It should be interesting to explore ultra specific diversity in local *L. leucocephala* populations. Its three recognized subspecies are reported to be widely distributed pantropically (Orwa *et al.,* 2009).

In a composite sample of 5583 seeds of *Leucaena*, there were 6% waste seeds, 3.46% small seeds, 39.13% medium-sized seeds and 51.08% large-sized seeds. It follows from the data that in spite of the fact that substantial number of seeds was wasted due to various random reasons like deformation, insect infestation, etc., around 80% of the seeds were the good quality seeds reflecting the reproductive success of the species and future propagation of the species.

The weight of the individual seed inclusive all seed types averaged to 39.86 ± 0.188 mg in mother plant A with variation around 21.66 %, 38.53 ± 0.197mg in mother plant B with 19.11% variation and 39.17 ± 0.194 in plant C with variation of 20.97%. The pooled sample distributed asymmetrically with mean weight of individual seed 39.21 ± 0.112mg and varying 20.60%. Earlier, the seed mass was considered to be the least plastic character (Harper, 1970). There are, however, reports of seed weight variation in several tropical species (Janzen, 1977a; Foster and Janson, 1985; Khan *et al.*, 1984; Khan *et al.* 1999, 2002; Khan and Umashanjkar, 2001; Murali, 1997; Marshall, 1986; Upadhaya *et al.*, 2007, Khan *et al.*, 2011). Seed weight variations within a species and an individual (Halpern, 2005) and even within a fruit of an individual plant as recorded in this study are common. Seed weight variation in plants may be many-fold in magnitude (Zhang and Maun, 1990). The seeds of *Prosopis juliflora* varied in weight by 16.83% (Khan *et al.* (1984) and that of *Opuntia ficus-indica* c. 18.2% (Khan, 2006). Michaels *et al.* (1988) have examined 39 species (46 populations) of plants in eastern-central Illinois and reported variability (in terms of coefficient of variation) of seed mass commonly exceeding 20% - significant variation being among the conspecific plants in most species sampled. Seed weight variation in *Thespesia populnea* is reported to be around 27% (Gohar *et al.*, 2012). Sixteen-fold variation in seed mass has been reported in *Lamatium salmoniflorum* (Thompson and Pellmyr, 1989).

The Two-way ANOVA for seed weight data indicated that effects of both the mother trees and the pods on individual seed weight variation were significant. Seed size variation may be the result of many factors (Fenner, 1985; Wulff, 1986; Mendez, 1997). Winn (1991) has suggested that plants may not have the capability of producing seeds with a completely uniform seed weight simply as a result of variation in resource availability (e.g., soil moisture during seed development). Seed size is significantly reduced under moisture stress in mature trees of walnut (Martin *et. al.*, 1980). Seed weight has also been reported to be the function of plant height in a population of *Ranunculus acris* (Totland and Birks, 1996). Different shrubs of *Purshia tridentata* (Rosaceae) are reported to produce seeds of different mean weights as did different sites (Krannitz, 1997). Most of the variation in seed weight was attributable to variation within individual shrubs (63.2%) where different shrubs accounted for variation by 29% (Krannitz (1997). It has been suggested that within-plant variation in seed size represents an adaptation, with the variance evolving in response to variable environments (Janzen, 1977a) or because smaller seeds may generally be dispersed farther (Stergios, 1976). Hence, variably sized seeds will result in a more homogenous seed shadow (Janzen, 1978). Howe and Richter (1982), however, demonstrated variation in seed size among plants to be more than the variation within plants in case of *Virola surinamensis*. Variation of seeds in a tropical plant, *Pithecellobium pedicellare,* was almost similar to that in *Virola* (Kang *et. al.*, 1992). In contrast *to P. pedicellare* and *V. surinamensis*, the studies conducted in temperate zone had shown variation in seed size within plants to be greater than among plants (Schaal, 1980; Thompson, 1984; Mazer *et al.*, 1986; Mc Ginley *et. al.*, 1990). O'Malle and Bawa (1987) found variation in seed size within and among plants to be more or less in equal magnitude. It is therefore likely that variation in seed weight is affected by maternal genetics and environmental effects both. Of course, it is difficult to weigh the relative importance of the two groups of factors. The plasticity in seed weight may be regulated by internal and external environment of mother plants (Krannitz, 1997). Obviously the seeds collected from the plants might be a mixture of half sibs and full sibs instead of strict half sibs. Seed weight variation in plants thus appears universal which may be due to trade-off of resource allocation between seed size and number (Venable, 1992) or environmental heterogeneity (Janzen, 1977a) or the genetic reasons. Alonso-Blanco *et al.* (1999) have indeed identified several gene loci responsible for natural genetic variation in seed size in *Arabidopsis thaliana*. Doganlar *et al.* (2000) have presented seed weight variation model in tomato. It may be asserted that within a species, seed mass variation should have both genetic and environmental components. Each tree of *L. leucocephala* appears to have registered the environmental variation within their pod crops. The aspects like seed shadow and dispersal needs to be investigated in *L. leucocephala* to further elucidate the seed ecology of this species.

Seed weight distribution in *L. leucocephala* was found to be asymmetrical (negatively skewed). Several types of seed weight distributions (negatively skewed, positively skewed and normal distribution) have been reported in literature. Seed weight distribution was reported to be normal in six sunflower cultivars viz. S-278, local, Hysun 39, Hysun 33, Aussie gold 61 and Aussie gold 04 and Non-normal in NK Armoni, Hybrid 1, Aussie gold 61 and the pooled sample of all cultivars (Khan *et al.*, 2011). Seed mass was also reported to be normally distributed in *Blutapason portulacoides* and *Panicum recemosum* but not in case of *Spartina ciliata* (Cardazzo, 2002). Halpern (2005) reported normal distribution of seed mass in *Lupinus perennis*. Zhang (1998) has reported seed mass variation in *Aeschynomene americana* by weighing 150 seeds from each of its 72 populations to be normally distributed in 9, positively skewed significantly ($p < 0.05$) in 14 and negatively skewed in 49 populations. The mass of mature seeds had a normal distribution in two natural populations of *Arum italicum* (Mendez (1997). Recently

Afsar uddin and Khan (2015) have reported negatively skewed seed weight distribution in *Vachellia nilotica*. Seed weight is reported to vary within a species with site quality and year of study – varying from symmetry to skewness, from leptokurtic to platykurtic (Busso and Perryman, 2005). Seed weight distribution was reported to be skewed in *Phlox drummondii* (Leverich and Levin, 1979). It is certain that high degree of variation in seed mass may be thought to have important ecological implications forming basis of qualitative and quantitative female reproductive fitness so crucial in life history diversification (Braza *et al.*, 2010).

Seed size variation has been shown to have several important ecological implications. Seed mass is associated with seed germination (Baskin and Baskin, 1998; Navarro and Guitan, 2003), seedling vigour and survival, with both across species and within species (Manga and Sen, 1996; Shaukat *et al.*, 1999; Walters and Reich, 2000; Vaughan and Ramsey, 2001; Halpern, 2005) presumably reflecting the amount of reserves available for early seedling growth (Castro *et al.*, 2006). In short, seed size variation produces variation in seedling fitness and thus the survival (Shaukat *et al.*, 1999) in variable environment.

For all pods studied (N = 300), the brood size in *L. leucocephala* averaged to 17.33 ± 0.2029 seeds per pod varying around 20.28%. The brood size distribution was found to be negatively skewed. Within a crop of ninety healthy pods of *Leucaena*, the brood size (Mean: 18.37 ± 0.3867) was larger than that in all types pods (pods containing normal as well as shriveled seeds or deformed, fungus-infected or insect-eaten seeds). Brood size of *L. leucocephala* healthy pods varied 11.53%. Our results are in agreement with Uma Shaanker *et al.* (1988) who have reported brood size to be negatively-skewed in this species averaging to 18.04 ± 4.53 from India. The negatively – skewed distribution of brood size is a common feature of many multi-ovulated species (Lee and Bazzaz, 1982). *Tamarindus indica* L., is, however, reported to exhibit positively –skewed distribution of seeds per pod (Thimaraju *et al.*, 1989) which has been demonstrated to be mainly due to pollen grain differences onto the stigma. Our results follow the pattern of brood as suggested by Uma Shaanker *et al.* (1988) i.e. negatively skewed brood distribution in fruits. *Leucaena* has many-seeded fruits and majority of ovules (c 93%) within the ovary mature into seeds in most fruits as is suggested by very high seed number – seed chambers ratio in this species. There are examples that some species accomplish the negatively skewed brood size through a maternally regulated pre-fertilization inhibition of pollen grains germination by the stigma (Ganashaiah *et al.*, 1986, 1988). In *Leucaena*, for example the germination of pollen grains is inhibited by the stigma unless a minimum threshold number of pollen is deposited. This leads to a negatively skewed distribution of fertilized ovules. A similar mechanism has also been reported in *Tammarind* (Usha, 1986), *Moringa* (Uma Shaanker and Ganashaiah, 1987), *Epilobium* (Snow, 1986), and *Nicotiana* (Cruzan, 1986). This probably ensures the development to maturity of those flowers that receive a single load of pollen grains from a particular parent. Detailed discussion on brood size distribution is given in Uma Shaanker *et al.* (1988), which may be normal, positively or negatively skewed depending upon their genetic and ecological peculiarities.

Within fruit reproductive allocation among various fruit components has scarcely been examined across the range of fruit types and taxa although it is critical in the evolutionary perspective (Chen *et al*., 2010). In the present studies, seed packaging cost (SPC) was determined on the basis of the quantum of pericarp biomass of pod per g seed (SPC1) or pericarp biomass per seed (SPC2). SPC1, in composite sample of 90 pods, averaged to 0.8265 ± 0.0366g (CV: 41.96%) and SPC2 averaged to 0.0321 ± 0.0011g (CV: 33.08%). One-way ANOVA of The SPC data, however, indicated that SPC1 and SPC2 didn't vary significantly among the mother trees of *Leucaena* (within component variance explained for was very low (6.52 and 3.89%, respectively). Conversely, *w*ithin-tree component variance accounted for 93 and 96 % of the total variances of SPC1 and SPC2, respectively. In short packaging cost of each seed of *Leucaena* was 32 mg of pericarp and packaging cost for one g seeds was 826.5 mg of pericarp. Willson *et al.* (1990) have studied seed packaging cost in twenty eight species and they noted a marked variation in average seed packaging investment in almost all 28 species surveyed. *Cassia fasciculata* included in their study showed SPC per seed to be 76.47 ± 1.89 mg. It has also been demonstrated by Mehlman (1993) to vary significantly in pods of *Baptisia lanceolata.* Seed packaging investment across 62 species of 35 families from China (No legume included) is shown to vary among species (Chen *et al.*, 2010). The lowest cost was 0.065 mg per seed in *Dicroa febrifuga* (Family Saxifragaceae) and highest 1124.897 mg / seed in *Vernicia fordi* (Family Euphorbiaceae). Highest packaging investment is, however, presented by Willson *et al.* (1990) in case of *Asimina triloba* to be 13,101.0 mg per seed. Khan and Zaki (2012) have reported that packaging cost in *C. fistula* varied from pod to pod even in case of the healthier indehiscent pods - 767.2 ± 51.4 mg per seed and 6961.3 ± 461.0 mg per g seeds. Much higher magnitude of SPC1 and SPC2 are reported for *Delonix regia* – 11912 ± 527.2 mg and 4593 ± 488.2 mg, respectively (Khan and Sahito, 2013). The economic aspects of the packaging costs demand larger number of seeds to be packed so that SPC is reduced (Bookman, 1984; Corner, 1957; Ganashaiah *et al.*, 1986, Janzen, 1982). Seed packaging cost per seed was found to relate negatively with brood size (Khan and Sahito, 2013b). If other things are equal, the maternal parent should be selected to favour a negatively skewed distribution of brood size (Ganashaiah *et al.*, 1986, Lee, 1984).

In the composite samples of healthy pods of *Leucaena* the frequency of proximal seed to be lesser in weight than the mean weight of the rest seeds was significantly higher than the frequency of proximal seed weight to be higher than the mean weight of the seeds towards distal end. In the composite sample of *Leucaena* as well as in all the three mother plants there was significantly higher frequency of occurrence of heavier distal seed compared to the mean weight of seeds lying above. As a result of the third approach to determine positional effect of seed on seed size no clear cut pattern of seed size distribution could emerge, however, in composite sample, the weight of the middle seed (s) was found to be significantly higher than the mean weight of the upper seeds. The fourth approach to investigate the pattern of within-pod seed size distribution indicated that the weight of the first seed (proximal seed) was quite lesser in magnitude than that of all the subsequent seeds. In pods studied, the proximal seed weighed 33.33 ± 0.0009 mg which was substantially lower than that of any subsequent seeds (number II to XXV) by a quantum of c 4 to 10.8mg. Similar pattern of within-pod seed size distribution has been reported by Afsar uddin and Khan (2015) in *Vachellia nilotica*.

The pattern of seed size variation in pods of *L. leucocephala* as seen here may be thought to be the result of disparity in nutrition availability to the seeds in multi-seeded fruits (Mendez, 1997). Within fruit seed mass variation i.e. unequal seed mass partitioning among seeds in a fruit is considered to be due to differential parental supply related to the genetic quality of seeds (Temme, 1986), the position effect within a fruit (Lee, 1988; Waller, 1982), parent-offspring conflict (Lloyd, 1992) or sibling rivalry (Uma Shaanker *et al.*, 1988). It may, at least in part, be due to gravity effects on photosynthates transport in *Leucaena* having an elongated and hanging pod with single vascular supply along the ventral suture. Considering a pipe-line analogy, the photosynthates moving through phloem under gravity tends presumably to be lesser availabe to the proximally developing seeds and more available to the subsequent seeds. Phloem unloading and transport, however, from source (leaves) to sink (developing seeds) may change with assimilate production at the source which may directly effect assimilate availability to the developing seeds by changing the pressure differences that governs photo-assimilate movement (Patrick, 1997). Ganashaiah and Uma Shaanker (1994), using the concept of fluids flow, assumed that probability of any given sink getting the resource molecule should be the function of 1) sink drawing ability and 2) amount of resource molecule already moved to that sink. That is to say that any molecules moving to a sink auto-catalytically increases the probability of the later molecule to move to the sink. In an elongated pod, thus differentially favourable sinks may develop. Ganashaiah and Uma Shaanker (1994) have demonstrated that even under the conditions of resource abundance seed abortion may also take. It may, however, also be mentioned that substantial amount of nutrition to seeds and pods of *Leucaena* should have been provided during their development by the green pods themselves.

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