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Changes in Germination and Biochemical Composition of *Phaseolus lunatus* (Lima bean) as Affected by *Acanthoscelides obtectus* Say Infestation

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Aim: To assess changes in germination and biochemical (proximate, elemental and vitamin) composition of *Phaseolus lunatus* (*P. lunatus*) (Lima bean) caused by *Acanthoscelides obtectus* (*A. obtectus*) Say Infestation.
Experimental Design: The study was carried out in a complete randomized block design.
Place and Duration of Study: Department of Botany, University of Calabar, Calabar, Nigeria, between January and April, 2016.
Methodology: A mix of infested and non infested seeds of *P. lunatus* was bought, sorted and kept for three months after which the seeds were planted to determine the effect of *A. obtectus* infestation on germination. The other seeds were sundried, ground into powder and used for proximate, elemental and vitamin analysis.
Results: Analysis of variance revealed a progressive decrease in seed germination with severity of infestation on all soil types with sandy and loamy soils being more effective than clay soil. Changes in mean germination count and percentage germination showed similar pattern of an initial increase in germinated (day 3-5) followed by a progressive decline (day 6-9) in infested at all levels of infestation and non infested seeds. Infestation increased or decreased with severity of infestation.

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Highest decrease or increase in germination and biochemical nutrients occurred at slight level of infestation while lowest decrease or increase occurred at severe level of infestation. Infestation led to significant (*P = .05*) decrease in moisture, ash, fibre, fat and carbohydrate with highest reduction of 12.35, 48.1%, 22.4%, 24.1% and 11.9% at severe infestation (SI) and lowest reductions of 3.3%, 22.2%, 9.2%, 10.7% and 7.25 at slight infestation (SLI) respectively. Infestation of *P. lunatus* by *A. obtectus* engendered significant changes in elemental composition with decrease in K, Na, Fe, Zn, Cu, Mn and with increase in Ca, Mg and Ni. Infestation led to increase in the content of vitamin B_1, B_2, A, and C and decrease in vitamin B_3, E and biotin when compared to the non infested.

Conclusion: *Acanthoscelides obtectus* infestation adversely affected the physiological and biochemical composition of *P. lunatus* necessitating control of the pest to maximize cultivation and nutritional quality.

Keywords: Phaseolus lunatus; Acanthoscelides obtectus infestation; seed germination; biochemical changes.

1. INTRODUCTION

Phaseolus lunatus L. (Lima bean, butter bean, Madagascar bean) is a New World legume that has been domesticated in areas corresponding to present day Peru and Mexico and is presently being cultivated in many tropical regions of the world [1]. It is an important legume crop cultivated in Nigeria. *Phaseolus lunatus* is cultivated for its valued and edible seeds which are enjoyed by millions of people throughout the world. In tropical Africa, immature and dry seeds are boiled, fried in oil or baked. In Nigeria they are also cooked with maize, rice or yam and used in making special kind of soup and stew. The Yoruba tribe in Nigeria processes the seeds into porridges, puddings and cakes. Fresh and dry Lima bean in the United States, are processed on an industrial scale involving canning and freezing. Lima bean has been grown as a cover crop and for green manure. The leaves and stems may be turned into hay or silage. The leaves of *P. lunatus* are also occasionally used as vegetable and straw for fodder [2]. In Ghana and Malawi, immature green seeds, young pods and leaves are eaten as vegetable. In many Asian countries sprouts and young plants are cooked and eaten. Medicinally, in Nigeria the seeds are powdered and rubbed into small cuts on tumours and abscesses to promote suppuration. In traditional Asian medicine the seeds and leaves are valued for their astringent qualities and used as diet against fever. Juice from the leaves is used in nasal instillations against headache and as eardrops against otitis in Senegal and DR Congo. The legume is considered as an important source of dietary protein for the majority of the people who cannot afford expensive animal protein thus, helps to eliminate human malnutrition [3]. *Phaseolus lunatus* is also rich in energy, carbohydrate, dietary fibre, mineral elements, vitamins and amino acids.

This bean is attacked by stored product insect *A. obtectus* Say. It is a serious pest of *P. lunatus* both in the field and storage. In storage, *A. obtectus* eat the inner content of the bean and contaminate it with excrements and the larva of the bean weevil makes flavor and seed sowing properties worse, as well as decreasing their weights from 2.1% to 23.8%. Bean weevils (*A. obtectus* Say) damages in common and snap bean varieties (*Phaseolus vulgaris* L.) decreased germination when compared to healthy seeds [4]. The feeding preference of bean weevil (*A. obtectus* Say) decreased seed germination [5]. Reduction in germination success after damage in breeding lines SSD5 and SSD9 rendered them unsuitable for planting after storage [6], Damage caused by *Callosobruchus maculatus* on *Vigna unguiculatus* reduced the germination rate of the cowpea compared to the non infested [7]

Acanthoscelides obtectus is a very destructive pest and the insect larvae begins feeding from the point of embryo and finally consumes the entire or whole seed, making the grain hollow and leaving only the seed coat. Different levels of infestation abound (slight, moderate and severe); in severe infestation, infested seeds are filled with frass, cast skins and excreta, which adversely deteriorate the quality of the grain. Hence, a common trend of zero-tolerance by buyers is increasing. Infestation by insect reduces seed germination and produce unpleasant odors, dirty appearance and abhorrent taste due to contamination by insect fragments and excretion. Severe infestation also makes the seeds unpalatable. The quality of grain may decrease due to nutrient depletion. The quality deterioration potentials of *A. obtectus* have assigned them a status of noxious pest and a bridge to trade. Keeping the destructive nature and its significance in global food security and safety in view, the present research is aimed at assessing germination and biochemical changes of *Phaseolus lunatus* (Lima bean) as affected by *Acanthoscelides obtectus* Say infestation. Specific objectives includes; Changes in mean and percentage germination of *P. lunatus* seeds resulting from infestation of *A. obtectus* (Say) with respect to soil types, changes in proximate, elemental and vitamins of *P. lunatus* seeds due to *A. obtectus* (Say) infestation.

2. MATERIALS AND METHODS

2.1 Seed Collection

Seeds of *P. lunatus* were obtained from Goldie market, Calabar, Calabar, Nigeria. The seeds were sorted according to their levels of infestation into groups; non infested with no hole emergence, slight infestation with 1-3 holes, moderate infestation with 4-5 holes and severe infestation with 6 holes and above per seed representing group 1, 2, 3, 4, respectively. Each of the sorted group was place in a transparent glass jar covered with a net of mesh size 1 cm by 1 cm to enhance infestation continuity at 25±2°C and 70±5% relative humidity. The non infested (control) group was tightly sealed with a metallic lid. These seeds were kept a period of three months after which were planted and processed for biochemical analysis.

2.2 Experimental Design

Planting bags of 16 mm in diameter were filled with soil of different types obtained from different locations in the University of Calabar. The bags were group into two; Group one for the planting of non infested seeds and group two for the planting of infested seeds of three different levels of infestation; 2 (slight), 3 (moderate) and 4 (severe). Prior to planting, the bags were arranged in randomized block design.

2.3 Seed Planting and Seed Germination

The soil types used were sandy, loamy and clay. Each soil types had 18 bags (3 bags replicated three times = 9 bags for infested and non infested). Thirty seeds from the three months post-storage from infested with the different levels of infestation and non infested seeds of *P. lunatus* were superficially placed on the various soil types. The bags were watered daily. Seed emergence began on the third day for all the soil types. Germinated seeds of infested and non infested were counted daily for a period of eleven days and expressed as a percentage of the seeds planted. Germination percentage which is an estimate of seed viability population was calculated by the formula:

$$GP = \text{Seeds germinated/Total seeds} \times 100$$

2.4 Sample Preparation for Analysis

At three months post-storage, infested with different infestation levels (2, 3 and 4) and non infested (1) seeds were removed from their storage containers, the seeds were properly checked and dissected to remove all larvae, pupae and adults of *Acanthoscelides obtectus.* The seeds were then sundried for one week, milled for each group into powder and used for biochemical analysis of changes induced by *A. obtectus* infestation of *P. lunatus*. Samples were analyzed for moisture, ash, protein, fibre, fat and carbohydrate using standard methods of Association of Official Analytical Chemists [10]. Elements; Fe, Mg, Ca, Cu, Zn, P, Mn, Co, Ni were analyzed by atomic absorption spectrophotometer [8] Na and K were analyzed by flame photometry and vitamins by Official Method Analysis [9].

2.5 Statistical Analysis

Data obtained in this study were subjected to analysis of variance (ANOVA) at P = .05 using the Statistical Package for Social Science, Version 15.0 [10].

4. RESULTS

4.1 Changes in Mean Germination of *P. lunatus* as Affected by *Acanthoscelides obtectus* Say with Respect to Soil Type

A total of 270 non infested and infested seeds of *P. lunatus* at different level of infestation were planted. The number of germinated non infested seeds planted on sandy, loamy and clay soils were 267, 268 and 265 respectively. While the number of infested seeds at different levels of infestation; SLI, MI and SI were 173, 112 and 70 (for seeds germinated on sandy soil), 178, 114 and 73 (for seeds germinated on loamy soil) and 154, 95 and 54 (for seed germinated on clay soil). The number of germinated seeds of *P. lunatus* was drastically reduced due *A. obtectus* infestation. Seed germination commenced on the third day after planting with an initial increase in germination followed by a progressive decrease in germination. Mean germination count showed significant (*P = .05*) decrease with infestation at day 3 and 9 for infested seeds planted on all soil types at different levels of infestation had values of 0.44 ±0.11, 2.33 ±0.11 (2), 2.11 ±0.1, 0.44 ±0.11 (3) and 1.11 ±0.11, 0.22 ±0.33 (4) for seeds germinated in sandy soil, 2.56±0.12, 0.44±1.12 (2), 2.11±0.33 and 0.33 ±0.1 (3), 1.22 ±0.01 and 0.56±0.01 (4) for seeds germinated on loamy soil and 2.22 ±0.02 and 0.56 ±0.12 (2), 1.56±0.1 and 0.33± 0.14 (3), 0.89 ± 0.01 and 0.11 ± 1.0 (4) for seeds germinated on clay soil (Table 1). Mean germination count decreased with days and with severity of infestation in both infested and healthy seeds.

4.2 Changes in Percentage Germination of *P. lunatus* as Affected by *Acanthoscelides obtectus* Say with Respect to Soil Type

Percentage germination of seeds on different soil types depicted a range in germination of 4.1% to 65.0% for sandy soil, 4.9% to 64.8% for loamy soil and 7.1% to 44.6% for clay soil for non infested seeds. Infested seeds had a range of 2.2% to 23.9% (2), 2.2% to 12.1% (3) and 1.0% to 8.4% (4) for sandy soil, 3.5% to 26.8% (2), 1.7% to 12.8% (3) and 1.4% to 8.5% (4) for loamy soil, 3.8% to 20.3% (2), 1.6% to 10.6% (3) and 0.4% to 7.0% (4) for clay soil (Table 2). Changes in percentage germination followed the same trend with mean germination.

4.3 Changes in Proximate Composition of *Phaseolus lunatus* as Affected by *Acanthoscelides obtectus* Infestation

Results of biochemical changes in nutrient contents of *P. lunatus* at different levels of infestation are shown in Table 3. Infestation resulted in significant *(P = .05)* reductions in moisture, ash, fibre, fat and carbohydrate with increase in protein in infested samples when compared to the non infested control. Reductions increased with severity of infestation. Slightly infested seeds had the lowest percentage reductions of 3.3%, 22.2%, 9.2%, 10.2% and 7.2% for moisture, ash, fibre, fat and carbohydrate respectively. Corresponding reductions for severely infested seeds were 12.3%, 48.1%, 22.4%, 24.1% and 11.9%. Protein content was observed to increase with severity of infestation. Values for percentage increase in protein for SLI, MI and SI were 24.4%, 26.1% and 62.8% respectively.

4.4 Changes in Elemental Composition of *Phaseolus lunatus as Affected* by *Acanthoscelides obtectus* Infestation

Table 4 showed significant (*P = .05*) variations in elemental composition of infested *P. lunatus* at different levels of infestation. *Acanthoscelides obtectus* infestation caused reductions in K, Na, Fe, Zn, Cu, Mn and Co with lowest reductions of 4.6, 3.3%, 6.7%, 0.4%, 13.5, 6.7 and 6.3% respectively observed in slightly infested seeds compared to highest reductions of 38.8%, 12.9%, 12.0%, 26.4%, 23.1%, 20.0% and 31.3% observed for severely infested seeds. A pattern of decrease with severity of infestation was observed. Calcium, Mg and Ni were observed to increase with increase in level of infestation. The lowest increase was found in slightly infested seeds with percentage increase of 13.5%, 3.6% and 3.6% and highest increase of 16.2%, 6.8% and 18.3% for severely infested seeds respectively. A similar trend of increase with severity of infestation was also observed.

4.5 Changes in Vitamin Composition of *Phaseolus lunatus* as Affected by *Acanthoscelides obtectus* Infestation

Infestation caused considerable changes in vitamins content of *P. lunatus* with significant increases and decreases. Vitamin B_3 (niacin), vitamin E and biotin were found to decrease with severity of infestation. Slight infestation produced the lowest percentage decrease of 11.1%, 44.5% and 22.3% compared to severe infestation which had values of 47.9%, 56.3% and 33.8% respectively. Vitamins B_1, B_2, A and C were observed to increase with severity of infestation. Lowest percentage increase for these vitamins were 80.5%, 21.4%, 80.7% and 13.9% for seeds with slight infestation compared to reductions of 85.1%, 35.7%, 87.2% and 28.0% for seeds with severe infestation (Table 5). Thus, the level of infestation of *Acanthoscelides obtectus* produced significant changes in the biochemical content of *P. lunatus*.

Table 1. Changes in mean germination of *P. lunatus* as affected by *Acanthoscelides obtectus* Say with respect to soil type

GD	Sandy soil				Loamy soil				Clay soil			
	Non infested (Control)	Infested			Non infested (Control)	Infested			Non infested (Control)	Infested		
	1	2	3	4	1	2	3	4	1	2	3	4
1	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-
3	4.44 ±0.01	2.33 ±0.11	2.11 ±0.1	1.11 ±0.11	4.56±0.01	2.56±0.12	2.11±0.33	1.22 ±0.01	3.89 ±0.33	2.22 ±0.02	1.56±0.1	0.89 ± 0.01
4	7.44 ±0.12	4.11 ±0.17	2.44 ±0.1	1.67 ±0.15	7.67±0.01	4.22±0.11	2.56±0.33	1.56 ±0.06	6.67 ±0.03	3.67 ±0.02	2.22±0.3	1.11 ± 0.01
5	8.89 ±0.01	5.33 ±0.22	3.00 ±0.1	2.11 ±0.17	8.78±0.33	5.44± 0.1	3.11±0.12	2.22 ±0.06	7.22 ±0.03	4.44 ±0.03	2.67±0.1	1.89 ± 0.04
6	4.44±0.01	3.56 ±0.22	2.30 ±0.02	1.44 ±0.01	4.67±0.33	3.56±0.13	2.22±0.02	1.44 ±0.02	4.33 ±0.06	3.22 ±0.02	1.89± 0.88	1.11 ± 0.01
7	2.44±0.03	2.11 ±0.03	1.22 ±0.15	0.67 ±0.01	2.22±0.12	2.00±0.17	1.33±0.02	0.78±0.01	2.11 ±0.02	1.89 ±0.03	1.11± 0.11	0.67 ±1.18
8	1.11 ±0.03	1.44 ±0.03	0.89 ±0.12	0.56 ±0.33	1.11±0.11	1.56±0.11	1.00±0.02	0.56±0.01	1.89 ±0.01	1.11 ±0.11	0.78± 0.13	0.22 ± 1.22
9	0.22 ±0.06	0.33 ±0.06	0.44 ±0.11	0.22 ±0.33	0.22±0.01	0.44±1.12	0.33 ±0.1	0.33± 0.01	1.11 ±0.01	0.56 ±0.12	0.33± 0.14	0.11 ± 1.0
10	0.0 ± 0.00	0.0 ± 0.00	0.0 ±0.00	0.0 ± 0.00	0.0± 0.00	0.0 ±0.00	0.0±0.00	0.0 ±0.00	0.0 ±0.00	0.0 ±0.00	0.0±0.00	0.0 ±0.00
11	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ±0.00	0.0±0.00	0.0 ±0.00	0.0±0.00	0.0 ±0.00	0.0 ±0.00	0.0 ±0.00	0.0 ±0.00	0.0 ±0.00

- *- = No Germination, GD = Germination Day*
- *Infestation levels; 1 = Non infested, 2 = Slight infestation (SLI), 3 = Moderate infestation (MI), 4 = Severe infestation (SI).*

Table 2. Changes in percentage germination of *P. lunatus* as affected by *Acanthoscelides obtectus* Say with respect to soil type

GD	Sandy soil (%)				Loamy soil (%)				Clay soil (%)			
	Non infested	Infested			Non infested	Infested			Non infested	Infested		
	1	2	3	4	1	2	3	4	1	2	3	4
1	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-
3	14.8	7.8	7.0	3.7	15.2	8.5	7.0	4.1	13.0	7.4	5.2	3.0
4	33.0	14.9	8.8	5.8	34.3	16.4	9.3	5.5	28.6	13.9	8.0	3.8
5	65.0	23.9	12.1	8.4	64.8	26.8	12.8	8.5	44.6	20.3	10.6	7.0
6	48.2	18.9	10.3	6.1	52.5	21.2	10.1	5.8	36.7	17.3	8.1	4.3
7	36.1	12.7	5.7	2.9	33.3	13.5	6.4	3.2	21.8	11.3	5.0	2.6
8	19.6	9.5	4.3	2.5	20.0	11.8	5.1	2.4	13.0	7.1	3.6	0.9
9	4.1	2.2	2.2	1.0	4.9	3.5	1.7	1.4	7.1	3.8	1.6	0.4
10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

- *- = No Germination, GD = Germination Days, Infestation levels;*
- *1 = Non infested, 2 = Slight infestation (SLI), 3 = Moderate infestation (MI), 4 = Severe infestation (SI).*

Table 3. Changes in proximate composition of *Phaseolus lunatus* as affected by *Acanthoscelides obtectus* infestation

	g/100 g dry matter						
Proximate content	**Non infested 1**	**Infested 2**	**% difference**	**Infested 3**	**% difference**	**Infested 4**	**% difference**
Moisture	66.20 ± 0.02	64.07 ± 0.02	3.3	62.51 ± 0.02	5.6	58.06 ± 0.2	12.3
Ash	2.70 ± 0.1	2.10 ± 0.1	22.2	1.98 ± 0.1	26.7	1.40 ± 0.1	48.1
Protein	24.60 ± 0.1	30.62 ± 0.02	24.4	30.99 ± 0.01	26.1	40.06 ± 0.1	62.8
Fibre	3.70 ± 0.1	3.36 ± 0.1	9.2	3.01 ± 0.01	18.6	2.87 ± 0.03	22.4
Fat	12.70 ±0.1	11.60 ± 0.01	10.7	11.00 ± 0.03	14.5	9.75 ± 0.03	24.1
Carbohydrate	56.09 ± 0.02	52.07 ± 0.02	7.2	51.61 ± 0.03	8.0	49.40 ± 0.03	11.9

- *Values are means ± SD of three replicates*
- *Infestation levels; 1 = Non infested, 2 = Slight infestation (SLI), 3 = Moderate infestation (MI), 4 = Severe infestation (SI).*

Table 4. Changes in elemental composition of *Phaseolus lunatus as affected* by *Acanthoscelides obtectus* infestation

	m g/100 g dry matter						
Elements	**Non infested 1**	**Infested 2**	**% difference**	**Infested 3**	**% difference**	**Infested 4**	**% difference**
Potassium (K)	3.25 ± 0.01	3.10 ± 0.1	4.6	2.86 ± 0.01	12.0	1.99 ± 0.01	38.8
Sodium (Na)	20.27 ± 0.2	19.60 ± 0.1	3.3	19.00 ± 0.01	6.3	17.66 ± 0.02	12.9
Calcium (Ca)	68.94 ± 0.01	78.27 ± 0.2	13.5	78.95 ± 0.1	14.5	80.10 ± 0.02	16.2
Magnesium (Mg)	89.70 ± 0.1	92.92 ± 0.02	3.6	93.58 ± 0.03	4.3	95.79 ± 0.02	6.79
Iron (Fe)	44.32 ± 0.02	41.42 ± 0.02	6.7	41.00 ± 0.03	7.5	39.01 ± 0.01	12.0
Zinc (Zn)	2.31 ± 0.01	2.30 ± 0.1	0.4	2.00 ± 0.1	13.4	1.70 ± 0.01	26.4
Copper (Cu)	22.15 ± 0.01	19.17 ± 0.2	13.5	18.80 ± 0.01	15.1	17.03 ± 0.2	23.1
Manganese (Mn)	0.15 ± 0.01	0.14 ±0.02	6.7	0.13 ± 0.02	13.3	0.12 ± 0.1	20.0
Cobalt (Co)	0.16 ± 0.01	0.15 ±0.01	6.3	0.14 ± 0.2	12.5	0.11 ± 0.03	31.3
Nickel (Ni)	0.82 ± 0.02	0.85 ± 0.01	3.6	0.90 ± 0.01	9.8	0.97 ± 0.01	18.3
Lead (Pb)	ND	ND	-	-	-	-	-

- *Values are means ± SD of three replicates.*
- *Infestation levels; 1 = Non infested, 2 = Slight infestation (SLI), 3 = Moderate infestation (MI), 4 = Severe infestation (SI).*
- *ND = Not detected.*

Table 5. Changes in vitamin composition of *Phaseolus lunatus* as affected by *Acanthoscelides obtectus* infestation

Vitamins	Non infested	Infested	% difference	Infested	% difference	Infested	% difference
	1	2		3		4	
Thiamin (Vit. B1) (µg/dl)	104.30 ± 0.02	188.59 ± 0.2	80.8	190.02±0.02	82.2	193.01 ± 0.1	85.1
Riboflavin (Vit. B_2) (µg/dl)	0.14 ± 0.01	0.17 ± 0.03	21.4	0.18 ± 0.02	28.6	0.19 ± 0.01	35.7
Niacin (Vit. B_3) (µg/dl)	2.61 ± 0.01	1.92 ± 0.03	11.1	1.63 ± 0.02	24.5	1.36 ± 0.02	47.9
Vitamin A (µg/dl))	69.45 ± 0.01	125.50 ± 0.1	80.7	126.10 ± 0.1	81.6	130.00 ± 0.4	87.2
Vitamin E (µg/100 g)	2.56 ± 0.02	1.42 ± 0.02	44.5	1.30 ± 0.1	49.2	1.12 ± 0.03	56.3
Ascorbic acid (Vit. C)	7.41 ± 0.03	8.44 ± 0.1	13.9	8.88 ± 0.2	19.8	9.41 ± 0.02	28.0
Biotin (mg/100 g)	1.48 ± 0.03	1.15 ± 0.1	22.3	1.07 ± 0.2	27.7	0.98 ± 0.1	33.8

- *Values are means ± SD of three replicates*
- *Infestation levels; 1 = Non infested, 2 = Slight infestation (SLI), 3 = Moderate infestation (MI), 4 = Severe infestation (SI) by A. obtectus infestation.*

4. DISCUSSION

Results of the present study revealed a decrease in germination in infested seeds with respect to soil type compared to non infested seeds at different levels of infestation with more decrease in germination obtained at the level of severe seeds infestation than at the level of slight and moderate infestation. The soil type on which seeds are sown has some effect on germination. Germination is either enhanced or delayed on the basis of soil type. Finding on seed germination with respect to soil type revealed that loamy soil enhanced germination of *P. lunatus* seeds better than sandy and clay soil. This finding agrees with previous work by [7] who also reported that loamy soil enhanced germination better than clay and sandy soil in seeds of *Vigna unguiculata* infested by *Callosobruhus maculatus*.

The study revealed decrease in seed germination due to the laying of eggs on the seed surface by adult female *A. obtectus* and the burrowing into the grain by the hatching larvae. This correspond with previous results by [4] who also reported a decreased in seed germination resulting from *A. obtectus* compared to the healthy seeds [5,7]. Internal infestation resulted in a progressive reduction of germination directly proportional to seed infestation levels. Internal damage of seeds induced by insect infestation affects the physiological quality of seeds because most insect species feed on the embryo. Insects that spend a considerable part of their life in the seed decrease the amount of endosperm. Grain quality is related to external and internal infestations of seeds, depicting that the higher the level of insect infestation the greater the changes in the commercial standard; meaning that insect infestation lowers the grain quality. Insect infestation damaged seeds lowering their germination potential. Thus, infestation by insect significantly reduces the physiological quality of *P. lunatus* seeds for planting as well as its marketability.

The large germination percentage reduction of viable seeds induced by *A. lunatus* after storage in this study is disturbing. Seeds serve as the only viable means of sustaining life on this planet Earth. The life of the plant is in the seed. Plants are the foundation of human diets across the world. The seeds of some plant species are important to humans as sources of food, while other seeds are important as raw materials for the manufacture of industrial chemicals and other products. Seeds are the primary dispersal units of higher plants containing the complete genetic make-up of species. The seed consist of nutrient reserve storage, an embryo and encapsulating structures which aim at protection and also regulate germination [11]. With the seed, the independence of the next generation of plants begins. Seeds are the most important contributors to the establishment of villages, towns and countries. The development of agriculture was necessary at the beginning of civilization, which allowed people to stay at the same place for long periods; it led mankind to produce their own food at different times of the seed production. Propagation of agricultural plants is mostly based on seeds [12]. Also, the success of any plant propagation program, among other things, hinges on a continuous supply of high quality seeds for the production of the desired quantity of seedlings in nurseries or for successful stand establishment by direct sowing out in the field. Seed quality is defined as ‘’a measure of characters or attributes that will determine the performance of seeds when sown or stored’’. It is a multiple concept encompassing the physical, physiological, genetic, pathological and entomological attributes that affect seed lot performance [13,14].

Several factors affect the production of high quality seeds, such as insect infestation, pollination failure and post-zygotic degeneration, infection by seed borne pathogens, environmental conditions during seed development, cone collection time as well as the genetic constitution [15,16]. Stresses that impact up on seeds can affect plant reproduction and productivity and hence, agriculture and biodiversity [17]. But, one of the most important factors is seed damage. When seeds are damaged, the food security, safety and success of plant propagation of any nation are hampered. So seeds should be protected from damage by maintaining hygienic conditions during storage and a routine check of grain in store houses to guide against pest infestation [18].

Results of changes in biochemical composition of *P. lunatus* as affected by *Acanthoscelides obtectus* infestation are inconsistent with increased in some of the nutrients in infested seeds and a decrease in others. Moisture, ash, fibre, carbohydrate and fat content of *P. lunatus* seeds in this study were observed to decrease with severity of *A. obtectus* infestation. This result agrees with the results of [19,7] who reported decrease in these nutrients caused by

Callosobruhus maculatus. Decrease in these proximate nutrients with severity of infestation may be attributed to the metabolic or respiratory activities of the pest, probably due to their utilization in the infested seed for growth and other activities. However, there was increase in protein content of infested seeds with severity of infestation. This is similar to the result of [20] who reported increase in protein in cowpea infested with *Callosobruhus maculatus*. Increase may be attributed to the presence of eggs, egg cases and excretory product of the pest. The decrease in moisture content obtained in this study may be linked to metabolic activities of seeds. Reduction in seed germination in this research may be partly due to reduction in moisture content of infested seeds.

Results also revealed reductions in most mineral elements; K, Na, Fe, Zn, Cu, Mn, and Co with increase in Ca, Mg and Ni infested seed compared with the non infested. These results are in line with results of [7]. The reductions induce by *A. obtectus* infestation on *P. lunatus* is of great concern because our bodies cannot produce all the nutrients that we need to function properly, so we eat them. Plant nutrients eaten in food are important for body functioning. They are necessary for building strong bones and teeth, blood, skin, hair, nerve function, muscle and for metabolic processes important for growth and development and for maintaining normal health. The reduction in these biochemical nutrients is an indication of a poor diet to millions of people who feed on this legume. A poor diet may have an injurious impact on health, causing deficiency disease such as blindness, anemia, scurvy, cretinism, preterm birth, stillbirth [21], health-threatening conditions like obesity [22], metabolic syndrome and some common chronic systemic diseases as heart disease, diabetes and osteoporosis [23]. The diet of individuals is what that individual eats and this is largely determined by the diet availability, the processing and palatability of food. When *P. lunatus* L. is infested, it is not longer said to be healthy in the diet of the people as some nutrients are low and not sufficient to meet the body's requirements while others are too high to support body functions. A diet is said to be healthy when its preparation and storage methods are such that preserve nutrients from oxidation, heat or leaching, and thus reduce the risked of food-born illnesses. It is important to note that excessively high intakes of minerals can be harmful or toxic. In the physical world everything is made up of minerals. The importance and awareness of nutrition in public health issues has resulted in the increased demand of knowledge of the nutrients of food.

This research also showed significant changes in vitamin composition as affected by *A. obtectus* infestation. The pest caused reduction in niacin (vit. B_3), vitamin E and biotin with increase in vitamin B_1, B_2, A and C when compared to the non infested. [7] also reported on vitamin A, B and C reduction in *Vigna unguiculata* infested by *Callosobruhus maculatus*. This reduction could be due to the use of these vitamins as food to maintain the life of the pest, and the completion of its larval development and metamorphosis [24]. The importance of these vitamins have been extensively discussed [25,26]. These reductions implied that *A. obtectus* infestation had a large effect on depleting the grain nutrients during storage.

5. CONCLUSION

From the physiological point of view, *A. obtectus* infestation caused significant reductions in mean and percentage germination of *P. lunatus.* The colossal damage on *P. lunatus* seeds render them unfit for cultivation thereby reducing the food supply of Nigeria a nation of over 160,000,000 people. Significant biochemical reductions in moisture, ash, fibre, fat, carbohydrate, K, Na, Fe, Zn, Cu, Mn, Co, vitamin B_3, vitamin E and biotin engendered by *A. obtectus* on *P. lunatus* reduces the nutritional quality of the legume to the poor Nigerians who cannot afford supplemented minerals. Infestation led to increase in protein, Ca, Mn, Ni, vitamin B_1, B_2, A, and C. Increase in these nutrients is not supported as excessive intake is harmful to the body. Reductions in mineral nutrient varied with levels of infestation. Severe infestation (SI = 4) resulted in more damage to seeds than slight and moderate infestation. There is therefore, the need for nutrient balance for optimal benefits. The findings of this research reveal the need for routine check of stored *P. lunatus* in order to avoid damage to seeds to enhance cultivation and improve the nutritional quality of the legume.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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