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Assessment of Damage Due to Callosobruchus Maculatus (Coleoptera: Bruchidae) Infestation on Germination and Nutrient Quality of Vigna Unguiculata L. (Walp)

A. A. J. Mofunanya[1] and E. E. Namgbe[2]
[1 & 2] *(Department Of Botany, Faculty Of Biological Sciences, University Of Calabar, Calabar, Nigeria)*

Abstract: *This research was conducted to assess Callosobruchus maculatus infestation effect on germination and nutrient quality of Vigna unguiculata L. ANOVA depicted that infested grains had lower germination when compared with the non-infested. Results revealed an initial increase in germination followed by a progressive decrease for infested and non-infested seeds. Highest percentage germination for infested and non-infested seeds were (16.4 and 48.9%) for sandy soil, (18.4 and 50.0%) for loamy soil and (17.4and 49.2%) for clay soil. The study revealed significant ($p \leq 0.05$) reductions and increases in all the nutritional components investigated in infested and non-infested V. unguiculata. Infestation caused reductions in moisture, protein and carbohydrate with increase in ash, fiber and fat. Infested samples had higher amount of Zn, Mn, K, Ca, Na and Co and lower amount of Ni, Fe, Cu and Mg compared to non-infested samples. C. maculatus infestation led to significant reduction in vitamin B and A with increase in Vitamin E and C. Data obtained from this research has shown that infestation of stored V. unguiculata by C. maculatus reduced germination potential making them unsuitable for planting and deteriorated the quality of the infested grains, depleted nutrient value and made them unsuitable for human consumption.*

Keywords: *Callosobruchus maculatus, Germination, Nutrient quality,Vigna unguiculata*

I. Introduction

Beans in Nigeria is consumed in many forms as beans cake, beans puddy, baked beans, beans soup, fried beans amongst others [1]. The beans *V. unguiculata* or *V. Sinensis* L. (Walp) also known as ‘’black eyed pea’’ is one of the most important legume food crops in the tropics; Asia, Africa, Southern Europeans, Central and South America. In Nigeria, the beans is commonly called ‘’iron beans’’. The crop has an advantage over others by being a cheap protein source apart from being the most practical source of storage and transportable protein. Bean is a key staple food for the poor people in developing countries of the world [2]. It is a good source of vitamins, minerals and provides a significant quantity of calories, dietary protein and fiber [3]. In addition, the cowpea possesses many health-promoting compounds such as vitamins, minerals and phytochemicals [4], [5].

Vigna unguiculata is susceptible to many diseases and pest attack from growth to storage [6]. The cowpea weevil *C. maculatus* Fab. (Coleoptera: Bruchidae) is a major pest of economic importance to leguminous grains [7]. At least twenty species of the genus Callosobruchus originated mostly from Africa and Asia, occurring mainly in the tropical and subtropical regions of the world [8]. The pest can cause up to 100% damage to legume seeds during storage [9]. [10], documented an average pulse damage of 5-10% and 20-30% for temperate and tropical countries caused by bruchid insects during storage. Infestation of freshly harvested grains in the store begins in the field where eggs are lay on green or drying pods by adults [11] or contamination of the materials through which they are brought to the store from the field. The female adults, lays eggs on the surface of the grain and the hatching larvae burrow into the grains. [12], reported that the whole development takes place inside a single seed and within a single grain. Storage pest damage to grains reduced the grain weight (dry matter reduction), makes them unsuitable for human and animal consumption, food contamination with live or dead insects, dejection and fragments, and depreciation of the nutritional and commercial values of the infested product and cause poor germination ability [13]. Heavy grains infestation can lead to mouldiness thereby causing a reduction in commercial value of the grains [10]. Field infestation has no significant implications as damage in the field is low. But once infested grains are stored, great damage occurs in a short period due to rapid multiplication of insects [14], [15]. Reports exist on the effect of weevil infestation on germination [16], [17], the nutritional composition of stored grains. Inconsistent increases and decreases on the nutrient contents due to storage pest infestation have been documented [15], [1], [18]. The quality deteriorating characteristics and damaging potential of *C. maculatus* has been recognized. The objective of this study is to assess the damage caused by *C. maculatus* infestation on seed germination and nutrient quality of *V. unguiculata*.

II. Materials and Methods

2.1 Seed and soil collection

Seeds of *V. unguiculata* were procured at the Watt Market Calabar, Calabar, Nigeria. The various soil types were obtained at different locations in the University of Calabar Campus. Infested seeds were sorted from the non-infested and placed in a transparent glass jar covered with a net mesh size of 1 cm by 1 cm in order to enhance infestation continuity. The non-infested (control) seeds were sealed tightly with a metallic lid. These seeds were allowed to stay for a period of three months.

2.2 Experimental design

Planting bags with the different soil types were grouped into two; group one for the planting of infested seeds and group two for the planting of non-infested seeds. Prior to planting, the 54 bags were arranged in a randomized block design.

2.3 Seed planting

Planting bags were filled with soils of different types; sandy, clay and loamy soil. Each soil type had 18 bags (3 bags replicated three times = 9 bags for infested and non-infested). Thirty seeds from the three months infested and non-infested *V. unguiculata* were planted in soil type. These seeds were superficially planted on different soil type.

2.4 Seed Germination

Seed sprouting began on the third day in all the bags. Germinated infested and non-infested seeds were counted daily for a period of twelve days and expressed as a percentage of the seeds planted. Percentage germination which is an estimate of population seeds viability was calculated by the formula: GP = Seeds germinated/Total seeds x 100.

2.5 Samples preparation and analysis

At the end of three months storage period, infested and non-infested seeds were removed from storage containers, sundried for five days, ground into powder and used for proximate, mineral elements and vitamins analysis. Moisture, ash, protein, fiber, fat and carbohydrate were analyzed by the method of Association of Official Analytical Chemists [19]. Minerals; Mg, Ca, Fe, Cu, Zn, P, Mn, Co, Ni were analyzed by atomic absorption spectrophotometer [19], Na and K by flame photometry. Vitamins A, B, C and E [20].

2.6 Statistical analysis

Data obtained for proximate, minerals and vitamins were subjected to t-test analysis and also expressed as percentage difference. Results for germination were subjected to one-way ANOVA and the means were compared by Duncan's Multiple Range Tests at 99% confidence level (21).

III. Results

Table 1: Mean germination of infested (Callosobruchus maculatus) and non-infested Vigna unguiculata L. seeds planted on different soil types

GD	Sandy soil		Loamy soil		Clay soil	
	Infested	Non-infested	Infested	Non-infested	Infested	Non-infested
1	-	-	-	-	-	-
2	-	-	-	-	-	-
3	3.33 ± 0.33^{e}	8.33 ± 0.33^{f}	3.89 ± 0.33^{e}	9.00 ± 0.10^{e}	3.44 ± 0.33^{e}	8.78 ± 0.33^{e}
4	4.33 ± 0.33^{f}	9.78 ± 0.17^{g}	4.67 ± 0.46^{f}	10.00 ± 0.33^{f}	4.44 ± 0.33^{f}	9.89 ± 0.33^{f}
5	2.78 ± 0.33^{d}	5.00 ± 0.10^{e}	3.11 ± 0.33^{d}	5.11 ± 0.43^{d}	3.00 ± 0.33^{d}	4.22 ± 0.88^{d}
6	1.78 ± 0.57^{c}	2.78 ± 0.33^{d}	2.11 ± 0.33^{c}	2.78 ± 0.10^{c}	1.89 ± 0.33^{c}	2.67 ± 0.10^{c}
7	1.11 ± 0.49^{b}	1.44 ± 0.17^{c}	1.22 ± 0.17^{b}	1.67 ± 0.33^{b}	1.22 ± 0.17^{b}	$1.56\pm0.33b^{c}$
8	0.33 ± 0.57^{b}	1.11 ± 0.10^{c}	0.44 ± 0.33^{b}	0.89 ± 0.33^{b}	0.44 ± 0.33^{b}	0.89 ± 0.33^{b}
9	0.00 ± 0.00^{a}	0.89 ± 0.33^{b}	0.00 ± 0.00^{a}	0.67 ± 0.33^{b}	0.00 ± 0.00^{a}	0.78 ± 0.48^{b}
10	0.00 ± 0.00^{a}	0.44 ± 0.33^{b}	0.00 ± 0.00^{a}	0.33 ± 0.33^{b}	0.00 ± 0.00^{a}	0.33 ± 0.66^{b}
11	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
12	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

- = No germination, GD = Germination days
- Means of three replicates ± SEM
- Means followed by same superscript letters in each column are not significantly different, while means followed by different superscript letters are significantly different at ($p \leq 0.05$) by Duncan Multiple Range Test.

Mean germination values of infested and non-infested seeds of *V. unguiculata* germinated on different soil types are shown in Table 1. Results revealed significant ($p \leq 0.05$) reduction in seed germination due to infestation. Infested seeds planted on all soil type depicted significant reduction in germination compared to non-infested ones. Mean germination values for infested seeds at day 4 for sandy, loamy and clay soil were 4.33 ± 0.33^{f}, 4.67 ± 0.46^{f}, 4.44 ± 0.33^{f}, compared with non-infested values of 9.78 ± 0.17^{g}, 10.00 ± 0.33^{f} and 9.89 ± 0.33^{f} respectively. Loamy soil enhanced the germination of *V. Unguiculata* but not significantly different when compared with clay and sandy soil. Soil types did not affect seed germination. Germination of infested seeds ended at day 8 while that of non-infested seeds ended at day 10.

Table 2: Percentage germination of infested (*Callosobruchus maculatus*) and non-infested*Vigna unguiculata* L. seeds planted on different soil types

GD	Sandy soil		Loamy soil		Clay soil	
	Infested seeds	Non-infested seeds	Infested seeds	Non-infested seeds	Infested seeds	Non-infested seeds
1	-	-	-	-	-	-
2	-	-	-	-	-	-
3	11.1	27.8	12.9	30.0	11.5	29.3
4	16.4	48.9	18.4	50.0	17.4	49.2
5	12.1	27.3	14.0	28.6	11.7	26.6
6	8.4	21.2	10.5	21.7	9.1	20.2
7	5.6	12.0	6.5	15.0	6.3	13.3
8	1.7	10.2	2.4	8.7	2.3	8.2
9	00.0	8.9	00.0	6.9	00.0	7.8
10	00.0	4.7	00.0	3.6	00.0	3.5
11	00.0	00.0	00.0	00.0	00.0	00.0
12	00.0	00.0	00.0	00.0	00.0	00.0

GD = Germination days

There were significant differences in percentage germination of infested and non-infested seeds of *V. unguiculata* planted on different soil type. There was an initial percentage increase in germination in days 3 and 4 followed by a progressive decrease in all the soil types for infested and non-infested seeds of *V. unguiculata*. Lowest and highest percentage germination values for infested and non-infested seeds planted on sandy were (1.7 and 14.4%, 4.7 and 48.9%), loamy soil (2.4 and 18.4%, 3.6 and 50.0%) and clay soil (2.3 and 17.4%, 3.5 and 49.2%) respectively. Highest percentage germination occurred at day 4 in all the soil types.

Table 3: Effect of *Callosobruchus maculatus* infestation on proximate content of *Vigna unguiculata* L.

	g/100 g				
Proximate	Infested	Non-Infested	% difference	T_{cal}	$T_{tab\ 0.05}$
Moisture	62.60 ± 0.1	67.50 ± 0.1*	7.3	50.0	2.78
Ash	4.10 ± 0.1	2.52 ± 0.02*	62.7	34.8	2.78
Protein	21.18 ± 0.2	27.10 ± 0.1*	21.8	25.0	2.78
Fat	14.50 ± 0.1	14.20 ± 0.1*	2.1	3.0	2.78
Fiber	4.30 ± 0.1	3.18 ± 0.2*	35.2	17.5	2.78
Carbohydrate	55.40 ± 0.02	55.44 ± 0.02*	0.07	3.8	2.78

Values are mean ± SD, N=3 replicates, ($p \leq 0.05$), * = significant

Effect of *C. maculatus* infestation on proximate content of *V. unguiculata* is high- lighted in Table 1. T-test analysis of results revealed that infestation caused significant ($p \leq 0.05$) reductions in protein, moisture and carbohydrate contents with significant increases in ash, fiber and fat contents. Percentage reductions and increases recorded for protein, moisture, carbohydrates and for ash, fiber, fat were 21.8%, 7.3%, 0.07% and 62.7%, 35.2%, 2.1% respectively.

Table 4: Effect of *Callosobruchus maculatus* infestation on the mineral content of *Vigna unguiculata* L.

	mg/100 g				
Elemental	Infested	Non-Infested	% difference	T_{cal}	$T_{tab\ 0.05}$
Potassium (K)	3.42 ± 0.1	3.10 ± 0.02*	10.3	7.8	2.78
Sodium (Na)	24.30 ± 0.1	23.70 ± 0.1*	2.5	8.6	2.78
Calcium (Ca)	163.43 ± 0.02	153.41 ± 0.02*	6.5	213.2	2.78
Magnesium (Mg)	178.93 ± 0.03	190.62 ± 0.02*	6.1	1402.8	2.78
Iron (Fe)	45.38 ± 0.02	47.17 ± 0.2*	7.7	4.4	2.78
Zinc (Zn)	3.22 ± 0.02	2.30 ± 0.1**	40.0	13.3	2.78
Copper (Cu)	1.76 ± 0.02	1.88 ± 0.02*	6.4	10.7	2.78
Manganese (Mn)	0.52 ± 0.02	0.45 ± 0.02*	15.6	8.4	2.78
Cobalt (Co)	0.30 ± 0.02	0.29 ± 0.01*	3.4	0.67	2.78
Nickel (Ni)	0.61 ± 0.01	0.73 ± 0.01*	16.4	20.7	2.78
Lead (Pb)	ND	ND	ND	ND	ND

Values are mean ± SD, N=3 replicates, ($p \leq 0.05$), * = significant, ND = Not detected

It was observed from results in Table 3 that Zn, Mn, K, Ca, Na and Co increased significantly in infested samples with percentage increases of 40.0%, 15.6%, 10.3%, 6.5%, 3.4% and 2.5% respectively. Infestation resulted in significant decreases in Ni, Fe, Cu and Mg with percentage decreases of 16.4%, 7.7%, 6.4%, 6.1% respectively.

Table 5: Effect of *Callosobruchus maculatus* infestation on the vitamin content of *Vigna unguiculata* L.

	mg/100 g				
Vitamin	Infested	Non-Infested	% difference	T_{cal}	$T_{Tab0.05}$
Vitamin C (mg/100 g)	9.87 ± 0.01	9.46 ± 0.02*	4.3	12.7	2.78
Vitamin E (mg/100g)	2.12 ± 0.02	1.93 0.01*	9.8	59.4	2.78
Vitamin A (µg/dl)	127.40 ± 0.01	158.43 0.2*	19.6	536.7	2.78
Vitamin B (µg/dl)	191.44 0.01	247.15 0.001**	22.5	55.7	2.78

Values are mean SD, N=3 replicates, ($p \leq 0.05$), * = significant

Results of effect weevil infestation on vitamins showed that the pest caused both reductions and increases in infested samples. *Callosobruchus maculatus* engendered significant ($p \leq 0.05$) reductions in vit. B (22.5%), vit. A (19.6%) and increases in vit. E (9.8%), vit. C (4.3%) in infested *Vigna unguiculata* (Table 5.)

IV. Discussion

This study was carried out to determine the effect of *C. maculatus* infestation on germination and nutrient (proximate, minerals and vitamins) quality of *V. unguiculata*. Finding of this research have revealed that the effect of *C. maculatus* on the germination of *V. unguiculata* seeds resulted in an initial increase followed by a progressive decrease in germination of infested and non-infested seeds superficially planted on the different soil types with the infested having lower percentage germination compared to the non-infested. Loamy soil was observed to enhance seed germination of infested and non-infested seeds though germination was not significantly different when compared with clay and sandy soil. The feeding damage induced by *C. maculatus* affected the germination of infested *V. unguiculata* because the non-infested seeds had higher percentage germination. Results of germination are in line with earlier report [22] who evaluated X-ray damage caused by weevils in cowpea seeds and concluded that seed damage was associated with any resulting adverse effects on germination. [11], documented reduction in germination of bambara groundnut landraces and breeding lines due to *C. maculatus* infestation. Infestation of paddy rice seeds by *Prostephanus truncates* showed low percentage germination rate compared to uninfested seeds [17]. Internal infestation of *Sitophilus zeamais* resulted in a progressive reduction in germination of *Zea mays* directly proportional to the level of infestation. Internal seed infestation by insect pest affects the seed physiological quality reducing germination [15]. Reports abound of inconsistent increase and decrease in nutritional contents of stored grains and cereals induced by storage pest. This research has revealed that infestation of *V. unguiculata* by *C. maculatus* caused reduction in protein, moisture and carbohydrate with increase in ash, fiber and fat. Results of higher ash, fiber and fat in infested seeds in this research correspond with those of [23] who reported increase in crude fiber and lipid in infested maize and increase in ash and fat in infested wheat [24]. [1], reported that slightly infested cowpea seed had the highest moisture while infested had lowest moisture. Lowest carbohydrate was reported in uninfested and highest in infested seed. An increase in protein content with severity of infestation was reported for various cowpea varieties. Significant increase in lipid and ash content were also reported with infestation severity.

Results of reduction in moisture, protein and carbohydrate are in conformity with those of [25] who documented a decrease in moisture, fat, protein, ash and free fatty acid after *C. maculatus* infestation of cowpea with increase in crude fiber and carbohydrate after infestation of seed when compared with seed before infestation. [18] reported that infestation of *V. unguiculata* by *C. maculatus* caused a progressive decrease in ash, protein, fat and moisture. Effect of the weevil on the proximate parameters decreased with prolonged infestation. While fiber and carbohydrate showed an initial decrease with subsequent increase in the third and fourth month of infestation. Reduction in protein conforms to finding by [23] who reported on reduction in protein in infested maize. [26], observed significant decrease in carbohydrate with increases in moisture, ash, fat, fiber and protein contents caused by *C. maculatus* infestation of *V. unguiculata*, *V. Subterranean* and *Cajanus cajan*. The report of [3] showed that infestation brought about marked increase in antinutients (phytate and oxalate) and reductions in protein, carbohydrate with marked increase in fiber content. [27] documented increase in protein in infested cowpea flour with decrease in non infested sample. Carbohydrate, phosphorus, iron and magnesium in infested cowpea compared to the non- infested. It was found by [28] that infested *V. unguiculata* grains showed higher levels of protein and iron, which related to higher consumption of carbohydrate portion of the grains. The decrease in moisture due to *C. maculatus* infestation may be attributed to the metabolic and or respiratory activities of the pest, this is probably because the pest utilizes the moisture in infested seeds for growth and other activities. With respect to ash, increase due to infestation may be attributed to the feeding activities of *C. maculatus* generating a lot of residue which amounts to ash.

This study also revealed that infestation of *V. unguiculata* by *C. maculatus* caused significant increase in Zn, Mn, K, Ca, Na and Co and decrease in Ni, Fe, Cu and Mg. These are consistent with observation by [29] that infestation induced changes in the chemical properties of some cowpea varieties. The reduction in minerals may be due to insect feeding activities and developmental stages of insect during which large amounts of minerals will be utilized. He reported that Na, Mg, K, P, Zn, Fe, Ca were significantly higher in infested seeds of cowpea varieties. Infestation of *V. unguiculata* was observed to reduce vitamin A and B in infested seeds. These results are similar to results of [27] who reported a reduction in vitamin A, B, C, The reduction could be attributed to the use of vitamins as food by the pest to maintain life and the completion of its larval development and metamorphosis [30].*Vigna unguiculata* is one of the most important tropical dual-purpose legumes, being used for vegetables (leaves and flowers) and grain and a multipurpose legume providing leaf, grain and forage. In Africa, the grain is used widely for human nutrition. It has very high nutritive value and high palatability [31]. The reductions in the nutritional value of beans posed by *C. maculatus* infestation require urgent attention since plant nutrients remains the sure way of obtaining natural and cheapest sources of minerals. Phytonutrients are the plant- based nutrients that provide humans with protection. Evidence favouring the health benefits of phytonutrients is growing every day, so much so that the biotech industry is already researching transgenic and non-transgenic ways of largely increasing the phytonutrient levels in plants that already contain high levels of the nutrients [32]. Thus, phytonutrients reduction by *C. maculatus* and any other means is not of benefit to man. Research has shown their effectiveness in the prevention and treatment of health conditions ranging from cancer to heart diseases as well as diabetes and high blood pressure. Many experts suggest that people can reduce their risk of cancer by significantly eating the food that contains phytonutrients according to American Cancer Society [32].Despite the importance of cowpea, storage still remains a major challenge facing its long term uses. The production and consumption of cowpea often falls below demand as a result of post harvest losses due to pests. Storage pest can cause losses directly through grain consumption and indirectly by creating a favourable environment for the establishment of other pests. These direct and indirect effects of storage pests make the beans unfit for planting and feeding [17]. Seed germination is extremely important and anything that affects the germination of seeds of crop plants threatens the food security of any nation.

V. Conclusion

Grain quality is related to its appearance, storage condition, nutritional status among others [15]. Findings of the present study have shown highly significant changes in the nutritional quality of *V. unguiculata* due to *C. maculatus*. *Callosobruchus maculatus* infestation significantly damaged seeds resulting in reduced seed germination and altered nutrient quality of stored *V. unguiculata*. Protein, moisture, carbohydrate, Ni, Fe, Cu, Mg, vitamin A and B contents all declined with infestation while ash, fiber, fat, Zn, Mn, K, Ca, Na, Co, vitamin E and C increased. Thus, insect infestation significantly reduces the physiological quality of *V.*

unguiculata seeds and affects its nutrient quality. Adequate care must be provided to seeds in storage to maximize damage and improve germination and nutrient value.

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