Original Research Article

- 1 Alterations Induced by Cowpea Weevil Callosobruhus maculatus F. (Coleoptera: Chrysomelidae) Infestation on Seed 2 Germination Potential and Nutrient Quality of Vigna aconitifolia (Jacq.) 3 4 Abstract 5 Aims: To evaluate alterations induced by cowpea weevil Callosobruhus maculatus (C. maculatus) infestation on seed germination 6 potential and nutrient quality of Vigna aconitifolia (V. aconitifolia). 7 **Experimental design:** The research was carried out in a complete randomized block design. 8 Place and duration of study: Department of Botany, University of Calabar, Calabar, Nigeria, between March and June, 2016. 9 Methodology: A mix of V. aconitifolia seeds was bought, infested seeds sorted from the non infested ones at different levels of 10 11 infestation and kept for three months before planting to ascertain alteration induce by C. maculatus on seed germination potential. The other seeds were sundried, milled into powder and used for proximate, minerals and vitamins analysis. 12 **Results:** Significant (*P* = .05) alterations induce by *C. maculatus* on seeds germination ability and nutrient quality of *V. aconitifolia* 13 were observed. These changes varied according to the severity of infestation. The germination potential of seeds was affected with 14 significant reductions observed at all levels of infestation with respect to soil types compared to seeds before infestation (BI). Seed 15 16 germination was highest in sandy and loamy than in clay soil. Percentage germination observed on the eleventh day for seeds 17 planted on clay, sandy and loamy soil were 61.7%, 87.1% and 66.7% (BI) compared to values of 4.2%, 6.1% and 5.3% respectively at severe infestation (SI). Results revealed that after infestation (AI) seeds of V. aconitifolia had significant decrease at SI level of 18 10.1% for moisture, 44.2% (ash), 25.5% (fat), 18.0% (fibre) and 12.4% (carbohydrate) while protein had increase of 40.2%. P, Na, 19 20 Cu and Ni showed decrease in content while K, Ca, Mg, Fe, Zn, Mn, Co depicted increase at all levels of infestation compared to seeds content BI. Lead was not detected. Significant decrease in vitamins A, B₁, B₂, B₃, B₅, B₆, B₉, C and biotin contents of the 21 cowpea with decrease in severity of infestation was found. Vitamin E revealed increase with increase in severity of infestation. 22
- Conclusion: Callosobruchus maculatus infestation damaged seeds resulting in a reduction in the germination ability and 23 marketability of V. aconitifolia seeds with severe alteration in nutritional quality. 24

25	Keywords: Seed	germination potential,	Vigna aconitifolia,	Callosobruchus	maculatus infestation,	Nutrient q	uality	
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Introduction

The cowpea weevil, C. maculatus F. (Coleoptera: Chrysomelidae) is a devastating pest of V. aconitifolia. The life cycle of stored 28 29 product beetle and moth includes an initial egg stage, several larval stages, a pupal stage, and finally an adult stage. The first instar larva burrows into the seed to start consuming it from the inside. All larval stages are spent inside the seed, where they remain 30 31 concealed and protected as they feed. The last instar lava pupates inside the seed. After emergence from the pupa, the adult beetles chews its way out of the seed, leaving a characteristics round hole in the shell. Soon after adult (active, strong fliers) emergence from 32 33 the pupa, there immediately begin their search for food. Insect pests need food, air and water to live. The best place for insect to live and grow is in stored grains because food, air and water are available in sufficient quantities. That is why some insect pest infests 34 stored grains and pulses [1]. 35 36 Callosobruchus maculatus is a cosmopolitan field-to-store pest ranked as the principal post-harvest pest of cowpea in the tropics and

Callosobruchus maculatus is a cosmopolitan field-to-store pest ranked as the principal post-harvest pest of cowpea in the tropics and subtropics of the world [2], [3]. It posed a major threat to the production and storage of cowpea in developing countries [4]. Damage caused by cowpea weevils in seeds affected germination [5]. This pest causes substantial quantitative and qualitative losses evident by seed perforation or holes and reduction in weight, market value and germination ability of seeds [6]. *Callosobruchus maculatus* infestation significantly damaged seeds of *Vigna unguiculata* resulting in reduced seed germination and altered nutrient quality of stored beans [7]. Infestation of *Phaseolus lunatus* by the cowpea weevil, *Acanthoscelides obtectus* reduced seed germination and

42 with changes in the biochemical composition of seeds [8].

Vigna aconitifolia (Jacq) is commonly called mat bean, moth bean, matki, Turkish gram or dew bean. The bean is widely consumed in Nigeria where it serves as a valuable source of dietary proteins, vitamins and minerals [9]. It is an important economic species in the diets of many societies. Due to the drought resistant quality of *V. aconitifolia*, its ability to combat erosion, it high protein content, this bean has been identified as a more significant food source in the future [10]. *Vigna aconitifolia* food products like food products of other species of *Vigna* exhibit many excellent nutritional attributes and these products provide a needed complement in diets comprised mainly of roots, tubers or cereals [11]. Seeds occupy a central place in the life of humans. Seeds serve as food, while

- 49 other seeds are important raw materials for the manufacturing of industrial chemicals and other products. When seeds are infested
- 50 by *C. maculatus* remarkable changes in germination, nutrient content, reduction in grain weight and marketability occur [12], [7]
- resulting in great economic losses. The severity of these losses depends on the level of storage pest infestation.
- 52 This study is designed to investigate changes induced by cowpea weevil on the germination ability of seeds, to estimate the nutrient
- 53 content of *V. aconitifolia* before and after infestation. To account for the qualitative loss in cowpea during storage with a view to
- 54 highlighting the deleterious alterations induced on stored cowpea after infestation by *C. maculatus*.
- 55

56 Materials and Methods

57 2.1 Seed collection and preparation

- 58 Infested and non infested seeds of *V. aconitifolia* used in this study were purchased from the Watt Market Calabar, Calabar Nigeria.
- 59 Sorting was done according to the level of seed infestation into groups; group 1 is non infested or seeds before infestation with no
- 60 emergence hole, after infestation; group 2 slight infestation (SLI) 1-3 holes, group 3 moderate infestation (MI) 4-5 holes and group 4
- 61 severe infestation (SI) 6 holes and above. Each of group of infested seeds was placed in a transparent glass jar covered with a net
- 62 mesh size of 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 then kept for a period of three months before they were used for further
- 64 processes.

65 2.2 Experimental design

- 66 Pots used for planting were filled with different types of soil; clay, sandy and loamy obtained from different locations within the
- 67 University Campus. The pots were grouped into two; group one for the planting of non infested seeds designated before infestation
- (BI) of *C. maculatus* and group 2 for the planting infested seeds from the different levels of infestation 2, 3 and 4 designated after
- 69 infestation (AI).
- 70 2.3 Seed planting and germination
- 71 Infested and non infested seeds of *V. aconitifolia* were planted in clay, sandy and loamy soil.

- 72 Three months post-storage seeds of infested from the different levels of *C. maculatus* infestation and non infested groups were
- 73 placed superficially on the surface of the different soil types. Each soil type had 18 pots (3 replicates for each soil type repeated
- trice). A total of 270 seeds were planted on each soil types before infestation and after infestation at different levels. The pots were
- vatered daily. Seed emergence started on the third day in all soil types. Germinated seeds count BI and AI was carried out daily for a
- period twelve days and expressed as a percentage of the seeds planted. Germination percentage is an estimate of seed viability
- population calculated by the formula: GP = Seeds germinated/Total seeds x 100.
- 78 2.4 Preparation of samples for analysis
- 79 At the end of three months storage period, seeds of V. aconitifolia BI and AI at different levels of infestation were removed from their
- storage containers, checked for eggs with a low power (magnification x 10) dissecting microscope VT-11. The seeds were dissected
- to remove the larvae, pupae and adults of *C. maculatus* with a pair of forceps. After which seeds BI and AI were sun-dried for five
- days, ground into powder and used for nutrient quality analysis. Proximate and some mineral nutrients were analyzed by the method
- of Association of Official Analytical Chemists (13), Na and K by flame photometry. Vitamins were analyzed by AOAC [14].
- 84 2.5 Statistical analysis
- Results obtained were subjected to analysis of variance (ANOVA) according to [15] to determine the level of significance BI and AI at
 different levels of infestation.
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Results

88 **3.1** Mean Germination of *Vigna aconitifolia* Seeds BI and AI *Callosobruchus maculatus* Infestation with Respect to Soil

Type.

Of the two hundred and seventy seeds (270) of Vigna aconitifolia planted on clay, sandy and loamy soil, the number of seeds that 90 germinated before infestation were 241, 269 and 268 respectively. Corresponding number of seeds that germinated after infestation 91 at SLI, MI and SI on clay soil had number of germinated seeds of 123, 78 and 43, sandy soil had germinated seeds of 147, 98 and 92 60 while loamy soil had 131, 95 and 57 germinated seeds at each level of infestation. Before infestation seeds planted on the 93 94 different soil types had higher germination ability when compared to seeds planted after infestation with highest seed germination potential observed with seeds planted on sandy soil followed by loamy soil while clay soil had the lowest seed germination potential 95 BI and AI. Significant (P = .05) alteration in mean germination was observed in seeds of V. aconitifolia after C. maculatus infestation 96 (AI) when compared to seeds before infestation (BI). Reduction in seeds ability to germinate varied according to severity of 97

infestation. Seeds with slight infestation (SLI) had the highest germination potential, followed by seed with moderate infestation (MI) 98 while seeds with severe infestation (SI) had the lowest germination potential with respect to all soil types. Seeds of V. aconitifolia 99 sown on sandy soil BI and AI showed highest mean germination potential, followed by seeds sown on loamy soil while seed planted 100 on clay soil had the least germination potential. Seed germination began on the third day and ended on the eleventh day. Highest 101 and lowest seed germination potential BI had values of $5.56 \pm 0.12^d \pm 0.12^d$, 0.44 ± 0.11^a (clay soil), 6.67 ± 0.15^e , 6.67 ± 0.15^e (sandy 102 soil) and 6.00 ± 0.03^e, 5.56 ± 0.10^d (loamy soil). Corresponding values AI at SLI, MI and SI were 1.89 ± 0.12^b, 2.56 ± 0.01^c (SLI), 2.11 103 $\pm 0.03^{\circ}$, 0.22 ± 0.03^{a} (MI), 1.11 ± 0.33^{b} , 0.00 ± 0.00^{a} (SI) on clay soil, $2.89 \pm 0.10^{\circ}$, 0.33 ± 0.16^{a} (SLI), $2.22 \pm 0.03^{\circ}$, 0.22 ± 0.02^{a} (MI) 104 and 1.44 ± 0.03^{b} , 0.11 ± 0.11^{a} (SI) on sandy soil while values for loamy soil were 2.22 ± 0.03^{c} , 0.33 ± 0.02^{a} (SLI), 1.78 ± 0.15^{b} , 0.22 ± 0.02^{c} 105 0.01^{a} (MI) and 0.67 ± 0.23^{a} , 0.11 ± 0.01^{a} (SI). 106

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108 Table 1: Alterations Induced by Cowpea Weevil *Callosobruhus maculatus* Infestation on Seed Germination Potential of

- 109 *Vigna aconitifolia* with Respect to Soil Type.
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						Manage and the state of a second state of the				NA 1 11 11 11			
	Mean germi	nation on clay	y soll		Mean ge	rmination on	sandy soil		Mean ge	ermination or	loamy soil		
	BI		ΔΙ		BI		Δ1		BI		ΔΙ		
					Ы								
GD	1	2	3	4	1	2	3	4	1	2	3	4	
1	-	-	-	-	-	-	-	-	-	-	-	-	
2	-	-	-	-	-	-	-	-	-	-	-	-	
3	0.44 ± 0.11 ^a	0.33 ± 0.02^{a}	0.22 ± 0.03^{a}	0.00 ± 0.00^{a}	0.56 ± 0.11 ^a	0.33 ± 0.16 ^a	0.22 ± 0.02^{a}	0.11 ± 0.11 ^a	0.55 ± 0.01 ^a	0.33 ± 0.02^{a}	0.22 ± 0.01 ^a	0.11 ± 0.01 ^a	
4	0.56 ±0.12 ^a	0.44 ± 0.02^{a}	0.33 ± 0.03^{a}	0.22 ± 0.01^{a}	0.89 ± 0.10 ^a	0.56 ± 0.01 ^a	0.67 ± 0.02 ^a	0.44 ± 0.16 ^a	0.78 ± 0.03 ^a	0.67 ± 0.02^{a}	0.56 ± 0.02 ^a	0.33 ± 0.12 ^a	
5	2.00 ± 0.11 ^b	1.11 ± 0.33 ^b	0.56 ± 0.02 ^a	0.33 ± 0.01 ^a	2.22 ± 0.12 ^b	1.33 ± 0.01 ^b	0.89 ± 0.01 ^a	0.56 ± 0.16 ^a	2.44 ± 0.01 ^b	1.11 ± 0.02 ^b	0.33 ± 0.13 ^a	0.56 ± 0.10 ^a	
6	2.56 ± 0.10 ^b	1.56 ± 0.22 ^b	0.67± 0.01 ^a	0.33 ± 0.11 ^a	3.44 ± 0.10 ^c	1.78 ± 0.01 ^b	1.11 ± 0.03 ^b	0.67 ± 0.15 ^a	$3.22 \pm 0.06^{\circ}$	1.67± 001 ^b	1.00 ± 0.01 ^b	0.67 ± 0.10 ^a	
7	2.89 ± 0.12 ^b	1.67 ± 0.12 ^b	0.78 ± 0.33 ^a	0.44 ± 0.11 ^a	3.78 ± 0.10 ^c	2.00 ± 0.11 ^c	1.00 ± 0.12 ^b	0.67 ± 0.03^{a}	$3.44 \pm 0.06^{\circ}$	1.89 ± 0.01 ^b	1.11 ± 0.01 ^b	0.67 ± 0.22 ^a	
8	3.00 ± 0.01 ^c	1.89 ± 0.12 ^b	1.00 ± 0.33 ^b	0.67 ± 0.11 ^a	$3.33 \pm 0.03^{\circ}$	2.11 ± 0.12 ^c	1.11 ± 0.13 ^b	0.67 ± 0.03^{a}	3.67 ± 0.01 ^c	2.00 ± 0.12 ^c	1.11 ± 0,21 ^b	0.67 ± 0.23 ^a	
9	3.33 ± 0.22 ^c	2.00 ± 0.01 ^c	1.56 ± 0.03 ^b	0.89 ± 0.03^{a}	$3.67 \pm 0.03^{\circ}$	2.22 ± 0.13 ^c	1.78 ± 0.11 ^b	1.00 ± 0.06 ^b	3.44 ± 0.01 ^c	2.11 ± 0.03 ^c	1.78 ± 0.33 ^b	1.00 ± 0.01 ^b	
10	5.33 ± 0.22 ^d	2.11 ± 0.33 ^c	1.44 ± 0.02 ^b	0.78 ± 0.01 ^a	5.56 ± 0.15 ^d	2.56 ± 0.13 ^c	1.89 ± 0.03 ^b	1.11 ± 0.06 ^b	5.56 ± 0.10 ^d	2.22 ± 0.03 ^c	1.78 ± 0.15 ^b	1.00 ± 0.22 ^b	
11	5.56 ± 0.12 ^d	2.56 ± 0.01 ^c	2.11 ± 0.03 ^c	1.11 ± 0.33 ^b	6.67 ± 0.15 ^e	2.89 ± 0.10 ^c	$2.22 \pm 0.03^{\circ}$	1.44 ± 0.03 ^b	6.00 ± 0.03 ^e	2.78 ± 0.03 ^c	2.11 ± 0.15 ^c	1.33 ± 0.13 ^b	
	111		-	•	•	-	-	-					

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• GD = Germination Days, - = No Germination, BI = Before Infestation (Non Infested = 1)

• AI = After Infestation (2= Slight Infestation SLI, 3 = Moderate Infestation MI, 4 = Severe Infestation SI).

- Means followed by the same superscript letters in each column are not significantly different, while means followed by different superscript letters are significantly different at (*P* = .05) according to Duncan Multiple Range Test.
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3.2 Alterations in Seed Percentage Germination Before and After *Callosobruhus maculatus* Infestation with Respect to

- 118Soil Type.
- Results revealed that BI seeds of V. aconitifolia planted on all soil types had significant (P = .05) percentage germination compared 119 to seeds AI at all levels (2, 3 and 4). Sandy soil had the highest percentage germination BI and AI followed by loamy soil and lastly 120 clay soil. Alteration in seeds percentage germination AI varied according to levels of infestation with SLI having the highest 121 germination, followed by MI while SI had the least germination. A trend of lowest percentage germination at initial period (day 3) and 122 123 highest at later period (day 11) was observed in seeds BI and AI. A range in percentage germination values of seeds BI planted on 124 clay, sandy and loamy soils were 0.44 to 60.7%, 1.9 to 87.1% and 1.9 to 66.7% respectively. A range in values of seeds AI at 2, 3 and 4 levels of infestation were 1.1 to 23%, 0.7-9.0% and 0.7 to 4.2% respectively for seeds planted on clay soil, 1.1 to 16.9%, 0.7 to 125 9.9% and 0.4 to 6.1% for seeds planted on sandy soil while loamy soil had a range in values of 1.1 to 15.4% at SLI, 0.7 to 9.8% at MI 126 127 and 0.4 to 5.3% at SI (Table 2).
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Table 2: Alterations Induced by Callosobruhus maculatus Infestation on Seed Germination

Potential of Vigna aconitifolia with Respect to Soil Type.

	% seed ge	on clay s	soil % se	eed germ	ination or	n sandy soil	% se	% seed germination on loamy soil				
	BI		AI		BI		AI		BI		AI	
GD	1	2	3	4	1	2	3	4	1	2	3	4
1	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-
3	1.5	1.1	0.7	0.00	1.9	1.11	0.7	0.4	1.9	1.11	0.7	0.4
4	1.9	1.5	1.12	0.7	3.0	1.9	2.2	1.5	2.6	2.2	1.9	1.11
5	6.9	3.8	1.9	1.12	7.8	4.6	3.1	1.9	8.5	3.8	3.0	1.9
6	9.5	5.5	2.3	1.13	12.7	6.4	3.9	2.4	12.3	6.0	3.5	2.4

7	11.8	6.3	2.8	1.5	16.4	7.7	3.7	2.5	15.0	7.2	4.1	2.4
8	13.9	7.6	3.6	2.3	17.3	8.8	4.3	2.5	18.8	8.2	4.2	2.4
9	18.0	8.7	5.9	3.2	19.1	10.2	7.1	3.9	21.7	9.5	7.1	3.7
10	35.0	10.1	5.8	2.9	44.4	13.0	7.8	4.5	35.7	11.0	7.6	3.8
11	61.7	13.5	9.0	4.2	87.1	16.9	9.9	6.1	66.7	15.4	9.8	5.3

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132 3.3 Proximate Composition of Vigna aconitifolia Before and After Callosobruchus maculatus Infestation

133 Results of proximate nutrients before infestation revealed significant (P=.05) increase in moisture, ash, fat, fibre and carbohydrate.

After infestation, these nutrients were found to decrease significantly. Percentage decrease observed at different levels of infestation

135 (2, 3 and 4) were 1.8%, 6.8% and 10.1% for moisture, 22.6%, 29.0% and 44.2% for ash, 5.6%, 14.8% and 25.5% for fat, 2.0%, 8.6%

and 18.0% for fibre and 3.5%, 6.5% and 12.4% carbohydrate. Protein increased significantly (P=.05) after infestation with percentage

increase of 15.1%, 19.6% and 40.2% for infestation level 2, 3 and 4 respectively (Table 1). Decrease or increase in proximate

138 nutrients varied according to levels of infestation with minimum (decrease or increase) observed at slight infestation (2) and

139 maximum (decrease or increase) observed at severe (4) infestation level.

140 Table 3: Alteration in Proximate Composition of *Vigna aconitifolia* Before and After

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Callosobruchus maculatus Infestation

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	g/100 g							
	Before infestatio	n	Afte	r infestation				
Proximate								
nutrients	1	2	%	3	%	4	%	
Moisture	65.60 ± 0.1 ^c	64.40 ± 0.1 ^c	1.8	61.12 ± 0.01 ^c	6.8	58.99 ± 0.1 ^c	10.1	
Ash	3.10 ± 0.1 ^a	2.40 ± 0.1 ^a	22.6	2.20 ± 02^{a}	29.0	1.73 ± 0.01 ^a	44.2	
Protein	23.61± 0.01 ^b	27.18 ± 0.02 ^b	15.1	28.23 ± 0.1 ^b	19.6	33.10 ± 0.02^{b}	40.2	
Fat	14.10 ± 0.1 ^b	13.30 ± 0.1 ^b	5.6	12.01±0.02 ^b	14.8	10.50 ± 0.01 ^a	25.5	
Fibre	3.50 ± 0.1 ^a	3.34 ± 0.02^{a}	2.0	3.20 ± 0.01^{a}	8.6	2.87 ± 0.01^{a}	18.0	
Carbohydrate	55.36± 0.02 ^c	53.44 ± 0.01 [°]	3.5	51.77 ± 0.1°	6.5	$48.51 \pm 0.2^{\circ}$	12.4	

• 1 = Non infested, 2 = Slight infestation (SLI), 3 = Moderate infestation (MI), 4 = Severe infestation (SI)

• Means followed by the same superscript letters in each column are not significantly different, while means followed by

145 different superscript letters are significantly different at (P = .05) according to Duncan Multiple Range Test.

146 3.4 Mineral Content of Vigna aconitifolia Before and After Callosobruchus maculatus Infestation

- 147 *Callosobruchus maculatus* infestation of *Vigna aconitifolia* led to significant (*P* = .05) increases and decreases in mineral nutrients.
- Before infestation *V. aconitifolia* had higher amount of P, Na, Cu and Ni but after infestation these mineral nutrients were decreased
- significantly. Mean value for Na before infestation was 17.42 ± 0.02^{d} g/100 g and values after infestation of 15.80 ± 0.1^{b} for slight
- 150 infestation, 14.02 ± 0.01^b for moderate infestation and 12.91±0.01^b for severe infestation. A similar trend was observed with P, Cu
- and Ni. Results revealed that K, Ca, Mg, Fe, Zn, Mn and Co were higher in amount after infestation from one level of infestation to
- another. Percentage increases in infested seeds for Zn, K and Mn at different levels (SLI, MI and SI) of infestation depicted values of
- 153 34.8%, 65.7% and 78.7% (Zn), 6.5%, 11.3% and 13.5% (K) and 7.7%, 15.4% and 30.8% (Mn) (Table 2). Lead was not detected in
- 154 seeds of *V. aconitifolia.*

155 Table 4: Alteration in Mineral Nutrients Content of Vigna aconitifolia Before and After

156 Callosobruchus maculatus Infestation

157										
	g/100 g									
	Before infestation		After infestation							
Mineral										
nutrients	1	2	%	3	%	4	%			
Potassium (K)	15.10± 0.1 ^b	16.08 ± 0.03 ^b	6.5	16.92 ± 0.1 ^b	11.3	17.14± 0.2 ^b	13.5			
Phosphorus (P)	296.01±0.1 ^d	257.89 ± 0.1 ^e	12.9	231.53 ± 0.02 ^e	21.8	211.86 ± 0.1 ^d	28.4			
Sodium (Na)	17.42± 0.02 ^b	15.80 ± 0.1 ^b	9.3	14.02 ± 0.01 ^b	19.5	12.91±0.01 ^b	25.9			
Calcium (Ca)	87.70 ± 0.02 ^c	92.40 ± 0.02 ^d	5.4	93.01± 0.02 ^d	6.1	95.12± 0.02 ^d	8.5			
Magnesium (Mg)	83.42 ± 0.02 ^c	86.42± 0.02 ^c	3.6	87.00 ± 0.1 ^c	4.3	88.10±0.1c	5.6			
Iron (Fe)	60.80± 0.01°	72.30 ± 0.1 ^c	18.9	$73.03 \pm 0.02^{\circ}$	20.1	74.76 ± 0.1c	23.0			
Zinc (Zn)	2.30 ± 0.1 ^a	3.10 ± 0.1 ^a	34.8	3.81±0.1 ^ª	65.7	4.11 ± 0.1 ^a	78.7			
Copper (Cu)	10.80 ± 0.1 ^b	9.80 ± 0.1 ^a	9.3	8.44 ± 0.2 ^a	21.9	7.39± 0.1 ^ª	31.6			
Manganese (Mn)	0.13 ± 0.01 ^a	0.14 ± 0.01a	7.7	0.15 ± 0.02^{a}	15.4	0.17 ± 0.1 ^a	30.8			
Cobalt (Co)	0.12 ± 0.02^{a}	0.14 ± 0.01a	16.7	0.15 ± 0.1 ^a	25.0	0.16 ± 0.1^{a}	33.3			
Nickel (Ni)	1.20 ± 0.02^{a}	1.12 ± 0.03a	6.7	1.04 ± 0.3^{a}	13.3	0.97 ± 0.01^{a}	19.2			
Lead (Pb)	ND	ND	ND	ND	ND	ND	ND			

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- 1 = Non infested, 2 = Slight infestation (SLI), 3 = Moderate infestation (MI), 4 = Severe infestation (SI)
- Means followed by the same superscript letters in each column are not significantly different, while means followed by
- different superscript letters are significantly different at (P = .05) according to Duncan Multiple Range Test.
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163 **3.5** Vitamin Composition of Vigna aconitifolia Before and After Callosobruchus maculatus Infestation

All the vitamins investigated BI and AI were significantly (P = .05) depleted by C. maculatus infestation with the exception of vitamin

165 E which was higher in seeds AI. Vitamins content of *V. aconitifolia* seeds were significantly (*P* = .05) altered by *C. maculatus*

166 infestation compared to vitamin content of seeds BI. This alteration resulted in a decrease in vitamins content of *V. aconitifolia*. The

highest percentage reduction was found in severely infested seeds and the lowest in slightly infested seeds. Vitamins C, A, B₁, B₂,

168 B₃, B₅, B₆, B₉ and biotin were observed to decrease with severity of infestation. Reductions observed in some vitamins after

169 infestation at SLI (2), MI (3) and SI (4) were 24.5%, 27.7% and 31.3% for vitamin A, 29.4%, 43.6% and 58.7% for vitamin C and

170 15.0%, 30.0% and 75.0% for vitamin B₂. A similar pattern of decrease with severity of infestation was also found in vitamins, B₁, B₃,

171 B₅, B₆, B₉ and biotin. Vitamin E content of seeds revealed increase with severity of infestation when compared to that of seeds before

infestation. Increase observed AI were 3.7% for slightly infested seeds, 4.9% for moderately infested seeds and 22.0% for severely

- infested seeds (Table 5).
- 174

Table 5: Alterations in vitamins content of *Vigna aconitifolia* before and after *Callosobruchus maculatus* infestation

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	Before infestation (BI)		After i	nfestation (AI)			
Vitamins	Ì	2	%	` 3	%	4	%
Ascorbic acid Vit. C (mg/100 g)	9.84± 0.01 ^c	6.95 ± 0.01 ^c	29.4	5.55± 0.2 ^b	43.6	$4.06 \pm 0.2^{\circ}$	58.7
Vitamin E (mg/100 g)	1.64 ± 0.01 ^b	1.70 ± 0.1 ^b	3.7	1.72± 0.1 ^b	4.9	2.00± 0.1 ^b	22.0
Biotin (mg/100 g)	1.79 ± 0.02 ^b	1.52 ± 0.02 ^b	15.1	1.28 ± 0.01 ^b	28.5	1.01 ±0.01 ^b	43.6
Vitamin A (µg/dl)	102.75± 0.01 ^d	77.54± 0.01 ^d	24.5	74.33± 0.1°	27.7	70.61± 0.1 ^d	31.3
Thiamin (Vit.B ₁) (μg/dl)	154.29± 0.01 ^d	116.44 ±0.01 ^e	24.5	100.08±0.1 ^d	35.1	93.01± 0.1 ^e	39.5
Riboflavin (Vit. B ₂) (µg/dl)	0.2 ± 0.1^{a}	0.17 ± 0.01 ^a	15.0	0.14 ± 0.01^{a}	30.0	0.05 ± 0.02^{a}	75.0

Naicin (Vit. B ₃) (µg/dl)	3.0 ± 0.1 ^b	2.8 ± 0.2^{b}	12.5	2.22 ± 0.02^{b}	30.6	2.00 ± 0.03^{b}	37.5
Patothenic acid (B_5) (μ g/dl)	0.6 ± 0.02^{a}	0.3 ± 0.3^{a}	16.0	0.45± 0.01 ^ª	25.0	0.3 ± 0.03^{a}	50.0
Vitamin B ₆ (µg/dl)	0.5 ± 0.02^{a}	0.39 ± 0.3^{a}	22.0	0.30 ± 0.01^{a}	40.0	0.24 ± 0.01 ^a	52.0
Folate (B ₉) (μg/dl)	6451 ± 0.01 ^f	6112 ± 0.1^{f}	7.0	5734 ± 0.02 ^e	11.1	5401 ± 0.2 ^f	19.3

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179 1 = Non infested, 2 = Slight infestation (SLI), 3 = Moderate infestation (MI), 4 = Severe infestation (SI)

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Discussion

The effect of pest on storage products (maize, wheat, cowpea etc) has been widely studied. In this research, alterations in seed germination and nutrient quality of *V. aconitifolia* caused by *C. maculatus* infestation were investigated. Results of this research

revealed that before infestation seeds had high mean and percentage germination than after infestation. The damage caused by *C*.

maculatus feeding affected germination of *V. aconitifolia* seeds because before infestation seeds showed higher mean and

186 percentage germination. This result is similar to report that control seeds of all legume hosts showed a high frequency of germination

187 while infestation by a single *C. maculatus* larva significantly reduced the frequency of germination of each host [16]. In reference to

soil type; seeds germination count was highest in sandy soil followed by loamy and lowest in clay soil. Dry sandy soil is most suitable

189 for the production of moth bean, and the bean can tolerate a variety of soil types [17]. The alteration resulting seed reduction

190 germination potential due to *C. maculatus* infestation may be attributed to embryo damage induced by the pest.

191 The germination percentage is an indicator of the ability of the seed to emerge from the soil to produce a plant in the field under

normal conditions. The loss of a seed's ability to germinate is an indication of loss of viability which involves decrease in seed vigour

and other physiological changes. Seeds can only fulfill its biological role if viable. It is important to note that physically uniform seed

of an adapted variety will be useless if it is low in germination and vigour or if it fails to germinate when planted. Seed physiologically

are built to germinate so germination is a must for seeds. This is why germination, particularly high percentage of it is an essential technical specification for seeds (18). The destruction of *V. aconitifolia* seeds in storage by *C. maculatus* reduces seeds germination

potential causing undue stress of repeated planting, time wasting and money spent in the process by farmers. Reduction in

198 germination of moth bean seeds affected yield as only little quantities of seeds germinated. It takes a germinated seed to grow,

199 mature and reproduce food.

200 Seeds are the primary basis for human sustenance. Plants require seeds to reproduction so that they do not die off and become 201 extinct. Plants are living things that operate in the natural cycle of seed, plant and fruit. Life on earth depends on plants. Without seeds there would be no vegetation. Man requires seeds for food production. The quality of seed is important in agriculture.

Agricultural production depends on a lot of factors. The seed is the basic catalyst of efficiency of all other factors. Modern crop

204 production and agricultural science also confirms that without seed quality we won't have a successful agricultural production. Seed

205 quality is that one which has a genetic purity, physical purity, is healthy and has good physiological condition in accordance to

standards prescribed for seed certification [19]. Food security therefore is dependent on seed security of farming communities or

207 growers.

208 Results revealed a decrease in moisture, ash, fat, fibre and carbohydrate content with severity of infestation. The moisture content of

the seed is an important factor in storage. The lower the temperature and relative humidity, the longer the seeds can be safely

stored. The decrease in these proximate nutrients may be attributed to metabolic activities of the pest as it utilizes these nutrients for

growth and other activities. The feeding damage of *C. maculatus* affected these proximate nutrients because before infestation

seeds had higher quantity of these nutrients. These findings agree with previous reports of decrease in moisture, and carbohydrate

213 contents of *V. unguiculata* infestation of *C. maculatus* [7]. Similar results of decrease in moisture, ash, fibre, fat and carbohydrate

with an increase in protein content of *Phaseolus lunatus* seeds infested by *Acanthoscelides obtectus* [8]. Protein content of seeds

increased with severity of infestation. This increase may be due the eggs, egg cases, excretory products left after the removal of

216 larval, pupal and adult stages of *C. maculatus* before analysis.

Alterations induced by *C. maculatus* on mineral nutrients were significantly different in seeds of *V. aconitifolia* after infestation

218 compared to those of seeds before infestation. Infestation led to significant increase in K, Ca, Mg, Fe, Zn, Co and Mn and decrease

in P, Na, Cu and Ni contents of *V. aconitifolia*. These alterations were observed to vary according to severity of infestation. A

decrease and increase in some of these nutrients has been reported in V. unguiculata infestation by C. maculatus [7], in Lima bean

221 (*Phaseolus lunatus*) infested by *Acanthoscelide obtectus* Say [8].

222 Nutrients are components in food that an organism uses for survival and growth. Some nutrients can be stored in the body like fat-

soluble, while others are required more or less continuously. Poor health can be caused either by a lack of required nutrients or, in

extreme cases, too much of a required nutrient. This has been observed with water and salt (both absolutely required) but will cause

illness or even death in excessive amounts [20]. Changes induced by *C. maculatus* infestation resulted in decrease in content of P,

Na, Cu and Ni essential nutrients required for proper functioning of the body. Phosphorus is required component of bones; essential

for energy processing [21], Na is a common electrolyte in food not found in dietary supplements, despite being needed in large

amounts, Cu is a required component of many redox enzymes including cytochrome c oxidase, Ni is present in urease. The

reduction in these nutrients present insufficient amount for adequate body functions. Increase observed in K, Ca, Mg, Fe, Zn, Co and

230 Mn after *C. maculatus* infestation of *V. aconitifolia* should be attended to as excessive consumption of these nutrients is also

problematic to the body. Some of these nutrients are structural elements but many play a role as electrolytes [22] while others play a

catalytic role in enzymes. Potassium is a common electrolyte required for heart and nerve health, Ca is another common electrolyte,

but also needed structurally for muscle and digestive health, bone strengthening, some forms acidity, may help clear toxins, provides

signaling ions for nerve and membrane functions, Mg is needed for processing ATP and related reactions (bone building, causes

strong peristalsis, increases flexibility and alkalinity), Zn is needed for carboxypeptidase, liver alcohol dehydrogenase, and carbonic anhydrase enzymes, Co is required for vitamin B_{12} coenzymes biosynthesis and Mn is required for the processing of oxygen. The

number of nutrients known to be required by man according to the words of Marion Nestle, is almost incomplete [23].

Vitamins were also severely altered by *C. maculatus* infestation of *V. aconitifolia.* Vitamins depletion varied with severity of

infestation. Results revealed reductions in all the vitamins investigated with the exception of vitamin E that showed increase after C.

240 *maculatus* infestation in seeds. Deficient amount emanating from reductions and excesses from increase present serious health

consequences. Deficiencies in vitamins result in disease conditions such as goiter, scurvy, osteoporosis, impaired immune system,

- cell metabolism disorders, certain forms of cancer, premature aging symptoms and poor psychological health among many others
- 243 [24].

Apart from serving as a food crop moth bean also provide some form of environmental protection; its widespread tap roots and low-

lying leaf-covering on the soil surface helps the soil to retain moisture. The strong roots enable the soil tom stay and thus, help in

preventing soil erosion. Not only this, but the dense cover also help in weed control. This will not be possible if the seeds failed to

- germinate and grow. It takes a growing plant to protect the environment. Plants are environmental purifiers.
- 248

Conclusion

Alterations induced by *C. maculatus* affected seed germination with a resultant decrease in food production. Global population

growth is estimated to 9 billion persons inhabiting the Earth by 2050, a 60% increase food production must be attained. Plants

account for over 80% of the human diet and nutrition. In view of the critical role played by these nutrients; increases in these nutrients

252 (protein, vitamin E, K, Ca, Mg, Fe, Zn, Co and Mn) induced by *C. maculatus* infestation of *V. aconitifolia* should be checked in order

not to exceed acceptable levels, while attention should also be paid on decrease (moisture, ash, fat, fibre, carbohydrate fat, P, Na,

254 Cu, Ni, vitamin A, C, B₁, B₂, B₃, B₅, B₆ and B₉) to avoid the consumption of low amount so as to prevent nutritional diseases resulting

from deficiencies of these nutrient elements. Man obtained these elements by feeding on plant-based food since the body cannot

synthesize all the nutrients needed for normal body function. There is therefore the need for routine check of *V. aconitifolia* and

257 improved hygienic conditions in storage houses.

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