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Effect of Callosobruchus Maculatus (Coleoptera: Bruchidae) and Acanthoscelides Obtectus Say Infestation on the Qualitative and Quantitative Phytochemicals of Vigna Unguiculata, Vigna Aconitifolia, Phaseolus Vulgaris, Phaseolus Lunatus and Phaseolus Autifolius

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Abstract: *Infestation of grains has been reported in many stored grains. This research was carried out to determine the effect of infestation on the qualitative and quantitative phytochemicals of five beans species. Results of qualitative analysis revealed the presence of seven and the absence of three phytochemicals in infested and uninfested samples T-test analysis of results revealed increase in alkaloids in infested V. unguiculata, V. aconitifolia and P. autifolius with decease in infested P. vulgaris and P. lunatus. Reductions in glycosides were significant ($p<0.05$) in infested V. unguiculata and P. lunatus, with increases in P. vulgaris, P. autifolius and V. aconitifolia. Saponins were reduced in P. lunatus, V. aconitifolia and P. vulgaris with significant increases in infested V. unguiculata and P. autifolius. Flavonoids and tannins showed reductions due to infestation. Effect of infestation resulted in polyphenols decreases of 30.1%, 25.9%, 14.5% in P. vulgaris, P. lunatus, P. autifolius respectively with associated increases of 69.4%, 58.0% for V. aconitifolia and V. unguiculata. Infestation caused increases in reducing compounds in all the beans species studied with the exception of P. vulgaris. This study concluded that infestation engendered significant reductions and increases in infested seeds of the beans species investigated.*

Keywords: *Beans species, Storage pest infestation, Qualitative and quantitative phytochemicals,*

I. Introduction

Phytochemicals are a large group of non nutritive bioactive compounds hypothesized to be responsible for much of the disease protection conferred from diet high in fruits, vegetables, beans, cereals and plant-based beverages [1]. Phytochemicals present in foods of plant origin play an important role in disease prevention [2]. Plants have the potential to synthesize secondary metabolites like flavonoids, flavones, flavonols, phenols, phenolic acids, quinines, tannins and comarins [3]. Secondary metabolites mostly found in legumes seeds are polyphenol compounds: which include phenolic acids, flavonoids and tannins. These have been reported as health-promoting effects on human health. Phenolic compounds have strong antioxidant properties and content in bean seeds depend on factors such as environmental, characteristics of the cultivar in addition to seed coat colour at the time of collection [4].

Several researches have provided evidence for the pharmacological effects of phytochemicals [5]. Alkaloids have been reported by [6] to be haemolytically active, toxic to microorganisms and are widely used as therapeutic agents in cancer management. Glycosides are a group of molecules in which, a sugar molecule is bond by a non sugar molecule. Many plants store medically important chemicals in the form of inactive glycosides. They are medically important; anthracene glycosides function as purgative, and for the treatment of skin diseases, cardiac glycoside (acts on the heart), chalcone glycosides (anticancer), cyanogenic glycosides are used in many pharmaceutical preparations as flavouring [7]. Glycosides inhibit turmour growth and also protect against gastrointestinal infections [8]. Flavonoids, flavones and flavonols are important group of polyphenols widely distributed in plants. Flavonoids and saponins possess antioxidative action, promote protective enzymes in the liver, antiseptic properties, may prevent cancer cell from multiplying, anti-inflammatory effects and they have been reported to manifest their actions through effects on membrane permeability [9]. Reports abound on the varied uses of triterpene, saponins and their aglycones as antiinflammatory, analgesic, antioedema and antimicrobial, antipyretic, antiulcerogenic and fibrionolytic agents [10], [11]. Tannins are phenolic compounds of high molecular weight widely distributed in plants. Tannin-rich plants have been used in Ayurvedic medicine for the treatment of leucorrhoea, rhinnorrhoea and diarrhea, as well as astringent [12]. Anthelmintic effects of tannins have also been reported by [13], its potential for anticancer prevention [14]. Phenols or polyphenols are chemical constituents found naturally as colour pigments responsible for fruit colour of plants. They are natural

antioxidants used as nutraceuticals, prevent blood clotting, anticancer, anti-inflammatory agent and function in the prevention of heart ailment [15]. Literatures abound on evaluation of phytochemicals in plants. [16], reported the presence of phytochemicals in seeds extracts of *Lablab purpureus*, [17] reported the presence of flavonoids, alkaloids, steroids, triterpenoids, steroids, cyanogenic glycosides in *Psidium guajava* L. leaf oil, [18] documented the presence of tannins, flavonoids, saponins in two local cowpea species, [19] recorded the presence of alkaloids, glycosides, saponins, tannins, reducing sugar, terpenoids, flavonoids and resins in *Azadiracta indica,Ocimum gratissimum, Afromomum melegueta* and a host of other plants. [20], documented in *Telfairia occidentalis, Amaranthus hybridus, Phaseolus vulgaris* and *Sphenostylis stenocarpa* the presence of alkaloids, glycosides, saponins, tannins, steroids, flavonoids, reducing compounds and polyphenols. [21], also reported on the presence of alkaloids, glycosides, saponins, tannins, flavonoides, terpenoids, polyphenols and reducing sugars in *Cucurbita moschata*.

[22], reported lower values of saponins in control samples compared to stored infested samples of legumes by *Callosobruchus chinensis.* [23], reported significant reductions in total phenols, total flavonoids and reducing power with an increase in alkaloids in *Callosobruchus maculatus* infested *Vigna unguiculata.* [4], documented higher flavonoids and phenolic acid contents in *Acanthoscelides obtectus* infested seeds of *Phaseolus sp.* Nearly all dried food products are susceptible to insect infestation. Nigeria is a country characterized by a large population of the poor people who cannot afford drugs for their various ailments. Legume species are of immense importance owing to the high amounts of phytochemicals present in them. The consumption of plant-based food rich in phytochemicals would go a long way in meeting the health needs of the people. The presence research investigates the effect of *Acanthoscelides obtectus* and *Callosobruchus maculatus* infestation on the qualitative and quantitative phytochemical of five legume species in Nigeria.

II. Materials And Methods

2.1 Seed collection and processing

Seeds of *V. unguiculata, V. aconitifolia, P. vulgaris, P. lunatus, P. autifolius* were bought from the Watt Market in Calabar, Calabar, Nigeria. Infested seeds of the beans species were sorted from the uninfested. Both seeds were allowed to stay for a period of three months to allow for more weevil infestation and damage to the grains. At the end of the period the weevils were carefully sorted, the infested and uninfested seeds were then sun-dried for five days, milled into powdered form and used for qualitative and quantitative analysis of phytochemicals in the beans species investigated.

2.2 Phytochemical analysis

2.2.1 Qualitative determination of phytochemicals

Qualitative analysis of plant chemicals in infested and uninfested samples was carried out using standard methods described by the following researchers; Alkaloids and glycosides were identified by the method of [24], tannins, flavonoids, reducing compounds, polyphenol, phlobatanins, anthraquinones and hydroxymethyl anthraquinones employed the method of [25].

2.2.2 Quantitative determination of phytochemicals

Percentage crude phytochemicals of flavonoids, alkaloids and saponins in infested and uninfested species of beans were determined by the method of [26], reducing compounds and polyphenols by methods described by [27].

2.3 Data Analysis

Results of this study were analyzed using the student t-test. Results were also expressed as percentage difference and difference between mean values were determined at 5% probability.

III. Results

Table 1: Effect of *Callosobruchus maculatus* and *Acanthoscelides obtectus* infestation on qualitative phytochemicals of *Vigna unguiculata, Vigna aconitifolia, Phaseolus vulgaris, Phaseolus lunatus and Phaseolus autifolius*

	V. unguiculata *Callosobruchus maculatus*		*V. aconitifolia* *Callosobruchus maculatus*		*P. vulgaris* *Acanthoscelides obtectus*		*P. lunatus* *Acanthoscelides obtectus*		*P. autifolius* *Acanthoscelides obtectus*	
Chemical Constituents	Infested	uninfested	Infested	Uninfested	Infested	Uninfested	Infested	Uninfested	Infested	Uninfested
	E A	E A	E A	E A	E A	E A	E A	E A	E A	E A
Alkaloids	+ ++	+ ++	+ ++	+ ++	+ ++	+ ++	+ ++	+ ++	+ ++	+ ++
Glycosides	+ ++	+ ++	+ ++	+ ++	+ ++	+ ++	+ ++	+ ++	++ +	++ +
Saponins	+ ++	+ ++	++ +++	++ +++	+ +	+ +	+ ++	+ ++	+ +	+ +++
Tannins	+ ++	+ +	+ +	+ +++	+ +	+ +	+ +	+ +	+ +	+ +
Flavonoids	+ ++ +++	++	++ +++	+ +++	++ +++	++ +	+++ ++	++ +	+ +	+++ ++
Reducing Compounds	++ +++	+ ++	+ +++	+ ++	++ +++	+ ++	+ +	+ +	++ +++	++ +++

Polyphenols	++	+++	+	++	+	++	++	+++	+	++	++	+++	+	++	++	+++	+	+++	++	++
Phlobatanins	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydroxymethyl anthraquinones	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

E = Ethanol extract, A = Aqueous extract, + = Present in low amount (LA), ++ = Present in moderate amount (MA), +++ = High amount (HA), - = Absent.

The results of qualitative phytochemicals of legumes as shown in Table 1 revealed the presence of alkaloids, glycosides, saponins, tannins, flavonoids, reducing compounds and polyphenols and the absence of phlobatanins, anthraquinones and hydroxymethyl anthraquinones in infested and uninfested seed samples investigated. Chemical compounds were more in aqueous extracts of uninfested and infested extracts than in ethanol extracts. Saponins and tannins were more in *V. aconitifolia* while flavonoids, reducing compounds and polyphenols were more in all the bean varieties examined.

Table 2: Effect of *Callosobruchus maculatus* infestation on percentage crude alkaloids, glycosides, flavonoids, saponins, tannins, polyphenols and reducing compounds of *Vigna unguiculata*

	(mg/100 g)				
Phytochemicals	Infested	Uninfested	% difference	Tcal	Ttab (p< 0.05)
Alkaloids	1.90 ± 0.1	1.60 ± 0.1*	18.8	3.06	2.78
Glycosides	1.13 ± 0.02	1.40 ± 0.1*	19.3	4.74	2.78
Saponins	4.00 ± 0.1	3.20 ± 0.1*	25.0	3.00	2.78
Tannins	0.36 ± 0.01	0.42 ± 0.01*	14.3	3.01	2.78
Flavonoids	5.22 ± 0.02	6.50 ± 0.1*	19.7	22.98	2.78
Polyphenols	6.61 ± 0.01	4.17 ± 0.01**	58.0	2.44	2.78
Reducing compounds	5.78 ± 0.02	4.59 ± 0.01*	25.9	79.30	2.78

Values are mean ± SD, N= 3 Replicates, * Significant, p< 0.05.

Results in Table 2 revealed that C. *maculatus* infestation of *V. unguiculata* resulted in significant (p< 0.05) decreases in glycosides (19.3%), flavonoids (19.7%) and tannins (14.3%) in infested seeds with corresponding increases in polyphenols (58.0%), reducing compounds (25.9%), saponins (25.0%) and alkaloids (18.8%) infested seeds. T-test analysis of results also revealed significant decreases and increases.

Table 3: Effect of *Callosobruchus maculatus* infestation on percentage crude alkaloids, glycosides, saponins, tannins, flavonoids, polyphenols and reducing compounds of *Vigna aconitifolia*

	(mg/100 g)				
Phytochemical	Infested	Uninfested	% difference	Tcal	Ttab (p< 0.05)
Alkaloids	3.00 ± 0.1	2.30 ± 0.1	30.4	12.12	2.78
Glycosides	1.58 ± 0.02	1.51 ± 0.02	4.6	5.16	2.78
Saponins	2.20 ± 0.1	2.30 ± 0.1	22.0	21.69	2.78
Tannins	0.40 ± 0.1	0.48 ± 0.02	16.7	1.70	2.78
Flavonoids	7.30 ± 0.1	9.70 ± 0.1	24.7	41.78	2.78
Polyphenols	5.35 ± 0.01	3.16 ± 0.02	69.3	21.69	2.78
Reducing compounds	8.22 ± 0.02	7.19 ± 0.01	14.3	52.47	2.78

Values are mean ± SD, N= 3 Replicates, * Significant, p< 0.05.

In Table 3, infestation of *V. aconitifolia* by *C. maculatus* led to significant (p< 0.05) reductions in flavonoids (24.7%), saponins (22.0%) and tannins (16.7%) in *V. aconitifolia* while increases were recorded for polyphenols (69.3%), alkaloids (30.4%), reducing compounds (14.3%) and glycosides (4.6%) in infested seeds.

Table 4: Effect of *Acanthoscelides obtectus* infestation on percentage crude alkaloids, glycosides, saponins, tannins, polyphenols, reducing compounds of *Phaseolus vulgaris*

	(mg/100 g)				
Phytochemicals	Infested	Uninfested	% difference	Tcal	Ttab (p<0.05)
Alkaloids	2.10 ± 0.1	2.50 ± 0.1	16.0	10.89	2.78
Glycosides	1.73 ± 0.02	1.56 ± 0.02	10.9	426	2.78
Saponins	1.20 ± 0.1	1.40 ± 0.1	14.3	17.17	2.78
Tannins	0.34 ± 0.02	0.40 ± 0.01	15.0	5.22	2.78
Flavonoids	12.17 ± 0.02	10.70 ± 0.1	13.7	10.89	2.78
Polyphenols	5.23 ± 0.02	7.47 ± 0.01	30.1	312.4	2.78
Reducing compounds	7.19 ± 0.01	8.80 ± 0.02	18.3	3.01	2.78

Values are mean ± SD, N= 3 Replicates, * Significant, p< 0.05.

T- test analysis of results depicted in Table 4 revealed that *Acanthoscelides obtectus* infestation of *P. vulgaris* caused significant ($p<0.05$) reductions in polyphenols, reducing compounds, alkaloids, tannins and saponins when compared to the uninfested. This implies higher amount in uninfested seeds and lower amount in infested ones. On the contrary, flavonoids and glycosides were significantly higher in infested seeds and lower in uninfested seeds of *P. vulgaris.*

Table 5: Effect of *Acanthoscelides obtectus* infestation on percentage crude alkaloids, glycosides, saponins, tannins, flavonoids, polyphenols and reducing compounds of *Phaseolus lunatus* L.

	(mg/100 g)				
Phytochemicals	Infested	Uninfested	% difference	Tcal	Ttab ($p<0.05$)
Alkaloids	2.70 ± 0.1	2.97 ± 0.2	9.1	10.20	2.78
Glycosides	1.31 ± 0.01	1.72 ± 0.02	23.8	56.71	2.78
Saponins	1.41 ± 0.02	1.83 ± 0.02	23.0	37.02	2.78
Tannins	0.31 ± 0.01	0.29 ± 0.01	2.0	1.08	2.78
Flavonoids	8.40 ± 0.1	11.30 ± 0.1	25.7	89.1	2.78
Polyphenols	5.79 ± 0.1	7.81 ± 0.1	25.9	30.4	2.78
Reducing compounds	3.60 ± 0.02	3.31 ± 0.01	8.8	16.7	2.78

Values are mean ± SD, N= 3 Replicates, * Significant, $p< 0.05$.

Acanthoscelides obtectus infestation of *P. lunatus* led to significant reductions in polyphenols, flavonoids, glycosides, saponins and alkaloids with percentage reductions of 25.9%, 25.7%, 23.8%, 23.8%, 23.0% and 9.1%. Reducing compounds had an increase of (8.8%) in infested seeds with marginal increase of 2.0% recorded for tannins (Table 5). However, T-test analysis of results revealed insignificant increase of tannin in infested seeds of *P. lunatus.*

Table 6: Effect of *Acanthoscelides obtectus* infestation on percentage crude alkaloids, glycosides, saponins, tannins, flavonoids, polyphenols and reducing compounds of *Phaseolus autifolius L.*

	(mg/100 g)				
Phytochemicals	Infested	Uninfested	% difference	Tcal	Ttab ($p< 0.05$)
Alkaloids	2.17 ± 0.2	1.80 ± 0.1	20.6	3.67	2.78
Glycosides	1.38 ± 0.02	1.30 ± 0.02	6.15	5.62	2.78
Saponins	2.45 ± 0.2	1.80 ± 0.1	36.1	6.25	2.78
Tannins	0.46 ± 0.01	0.41 ± 0.01	12.2	7.14	2.78
Flavonoids	0.93 ± 0.1	10.30 ± 0.1	9.7	1.00	2.78
Polyphenols	3.72 ± 0.02	4.35 ± 0.02	14.5	64.2	2.78
Reducing compounds	5.30 ± 0.1	3.54 ± 0.01	49.7	50.18	2.78

Values are mean ± SD, N= 3 Replicates, * Significant, $p< 0.05$.

Results of infestation as presented in Table 6 showed that polyphenols (14.5%) and flavonoids (9.7%) were higher in uninfested and lower in infested seeds (implying a reduction in polyphenols and flavonoids) while reducing compounds (49.7%), saponins (36.1%), alkaloids (20.6%), tannins (12.2%) and glycosides (6.15%) were significantly ($p< 0.05$) higher in infested seeds of *P. autifolius* and lower in uninfested seeds.

IV. Discussion

This research was conducted to determine the effect of *Callosobruchus maculatus* and *Acanthoscelides obtectus* on the phytochemicals of five beans species. Results of this study have shown that pest infestation of stored grains produced significant ($p<0.05$) differences in phytohemicals of investigated species. *Callosobruchus maculatus* engendered general reductions in flavonoids, tannins, saponins and glycosides in *V. unguiculata and V.aconitifolia* while *A. obtectus* caused reductions in polyphenols, flavonoids, glycosides, saponins, alkaloids, tannins, reducing compounds in *P. vulgaris, P. lunatus* and *P. autifolius* respectively. Phytochemicals that were reduced in one species showed increase in other species. The reductions and increases in these medically important phytochemicals are in agreement with reports by [23], [24], [4] due to storage pest infestation. The reductions in phytochemicals due to infestation may be due to insect feeding activities and their stages of development [28] during which a high level of minerals will be utilized. Increase in infested seeds may be attributed to presence of eggs, egg cases and excretory products of the pest. Earlier report by [4] stated that secondary metabolites present in legumes were mostly polyphenols. Results of this research have however, added the presence of alkaloids, glycosides, flavonoids, saponins and reducing compounds as secondary metabolites also found in legumes.

According to Davis of the University of California, phytochemicals are thought to be responsible for much of the disease protection granted by high fruits diet, vegetables, beans, cereals, and plant-based beverages

(tea and wine). Many experts have suggested that people can reduce their risk of cancer meaningfully by eating foods that contain phytonutrients, according to American Cancer Society. Phytonutrients may work by helping in the prevention of potential carcinogens formation, blocking the action of carcinogens on their targeted organs and tissues, or suppression of cancer development on cells [29]. It has been reported by researchers that flavonoids may be a key phytochemical contributing to mortality reduction rates observed in people consuming high amount of plant-based foods, Davis report. Myocardial infarction was found to decrease as flavonoids consumption increased in Zutphen study of the elderly. In another seven countries study which compared men diets in various Western countries including the U. S, it was reported that the intake of flavonoids was responsible for 25 percent difference in mortality rates observed in different countries [29].

In recent times, health-promoting and disease preventing properties of plants have been attributed to phytochemicals with antinutrient effects [30] thus arousing the interest of researchers and food manufacturers. Phytochemicals function through various mechanisms to help the body ward off disease: some of which include, their function as antioxidants, example, caroenoids can delay or prevent some of the oxidative damage associated with free radicals, improving cancer and heart disease prognosis ease. They also influence the function of hormones; isoflavonoids and lignans can mimic body estrogen by blocking estrogen receptor sites and decreasing its effects on certain tissues. Certain liver enzymes are capable of making estrogen less effective. These enzymes however, can be up-regulated by indoles phytochemical in cruciferous vegetables.

According to Davis report, the Standing Committee on Scientific Evaluation of Dietary Reference Intakes and its Panel on Dietary Antioxidants and Related Compounds chose not to create a Dietary Intake Reference (DRI) due to the absence of food data and a poor understanding of the absorption and metabolism of plant nutrients. Owing to the absence of information on DRI, many health authorities such as American Cancer Society and the American Heart Association have recommended the consumption of diets high in fruits and vegetables to ensure that citizens get an adequate amount of phytochemicals.

V. Conclusion

The results have shown the presence of phytocomponuds in the different beans species to which the biological activity may be attributable. Beans are a miracle food, according to The Daily Times [30]. Beans regulate blood sugar and insulin production, lower cholesterol level, promote digestion and protect against cancer. A single package of beans offers fiber, protein and antioxidants. The reductions in phytochemicals in infested seeds caused by *Callosobruchus maculatus* and *Acanthoscelides obtectus* infestation and their increases in uninfested legumes investigated is disturbing and should not be treated lightly because a recommended amount of phytochemicals intake does not exist. And also because excessive intake is risky and low amounts will not yield the desired health effects. The knowledge provided by the differences in quantities of some of these phytochemicals as revealed by this research should serve as a guide to their intake. Some phytochemicals example, cyanogenic glycosides are poisonous and excessive intake of cyanogenic glycosides can be fatal. Some foodstuff containing cyanogenic glycosides can cause poisoning (severe gastric and damage) if not properly taken [7]. Beans weevil is a high risk factor in storage of dry bean seeds [4] as it reduces seed quality and nutritional benefits. Beans seeds intended to be used for consumption or other needs must be prevented from storage pest infestation. Further studies should be conducted to authenticate phytochemical dosage for ultimate benefit.

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