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The Phytochemical Screening and Insecticidal Efficacy of Powdered and Ethanolic Extracts of *Ageratum conyzoides* (Goatweed) and *Hyptis suaveolens* (Bushmint) Against Stored Cowpea Infested Weevils, *Callosobruchus maculatus* in Abuja, FCT, Nigeria

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Abstract: *A comparative investigation of the insecticidal efficacy of two botanicals; Hyptis suaveolens (Bush mint) and Ageratum conyzoides (Goatweed) against storage cowpea weevils; Callosobruchus maculatus L, was undertaken in the laboratory of Biological Sciences Department, University of Abuja. Phytochemical screening of the Ethanolic extracts of the test plants/botanicals, revealed the presence of the various active ingredients, such as reducing sugars, alkaloids, glycosides, flavonoids, saponins, tannins, anthraquinones and terpenes; thus ascertaining their insecticidal property. A follow up confirmation of the insecticidal/pesticidal efficacy or otherwise of these two test plants/botanicals, was conducted, using four (4) treatment regimens; designated D1-D4, lasting between seven (7) to fourteen (14) days in the laboratory; during which observation on the mortality rates of Callosobruchus maculatus weevils; as well as the weight loss of cowpea seeds were observed and recorded for each successive days of observation. Results obtained showed that the different treatment regimens had varying degrees of effectiveness in the killing (death) effects (mortality rates) of Callosobruchus maculatus: such that, though, a single treatment with powdered samples/extracts of H. suaveolens (D1,) had greater efficacy than that of a single treatment with Ageratum conyzoides (D2); the combined treatment regimens (D3,) comprising both extracts of H. suaveolens and A. conyzoides, had the greatest efficacy in the control of C. maculatus. Data obtained were subjected to ANOVA test; and significant differences at P< 0.05; amongst the various treatment regimens D1-D3 were also ascertained. The results of the investigation confirm the efficacy and the safe use of these two plants extracts as biopesticides in the control of stored cowpea weevils. Recommendation for their posible incorporation into the traditional pest management system is hereby emphasised.*

Keywords: *Hyptis suaveolens, Ageratum conyzoides, Insecticidal efficacy, phytochemical screening, biopesticides, traditional pest management.*

INTRODUCTION

The use of both naturally occurring and synthetic plant materials to protect agricultural crops and products against a variety of insect pests, is an old age practice in most developing countries of the world (Peter, 1985; & Oparaeke *et al.*, 2006). Similarly, Imam (1997); Lale (2001); & Yahaya & Magaji, 1997, reported that extracts and powdered samples from different plants have been shown to possess *insecticidal* properties against a wide range of insect pest population.

Cowpea plant from which the cowpea seed (Common beans) are produced, has a protein rich food component, which is used as a stable food for Human consumption and also, as a fodder for livestock feeds, in many societies/communities of the dry savanna regions of the sub-Saharan Africa; including Nigeria. Cowpea (common beans) is also rich in minerals, fats, oils, vitamins, and is also recommended as a food supplement for loss of blood (anaemia) and diabetic patients. Similarly, the young leaves and immature pods are eaten as vegetables in most of our rural settlements (IITA, 2006).

Cowpea yields are generally low, with sometimes total yield loss, and even crop failure occurring due to the devastating activities of a wide range of insect pests, which ravage the crop in the field at different stages of growth and even in the store (Olatunji *et al.*, 2009; IITA, 2006; & NCRI, 2011).

Though, several losses of cowpea seeds have been reported from insect pests damage in the field, one of the major constraints yet confronting the cultivation and harvesting of cowpea is in its *stored pests infestations*, the most common of which is the common beans weevil: *Callosobruchusmaculatus*. The larvae and adults of this insect pests constitute the destructive stages attacking cowpea seeds in storage, such that severely damage seeds are turned into powdery substances (of mass) which is unfit for human consumption, thereby constituting a major constraint on the food availability and security.

Thus, in view of the economic importance of cowpea to us and consequent upon the severity of

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damage caused by insects pests, the scientific application of synthetic insecticides (contact and fumigant poisons; methyl bromide oil and fractions), in the field and store have been adopted by crop protection experts and agricultural entomologist in the sub-Saharan Africa, including Nigeria.

Currently, alternative control strategies requiring the use of potent botanical extracts from a variety of plants are being adopted to replace the traditional and synthetic insecticides due to insect resistance and harmful effects of fumigants to field and storage workers. These botanical extracts have been found to affect the biology of target insects in different modes, either as ovicides repellents, antifeedants, fumigants, contact toxicants or insecticide (Emosairee *et al.*, 2000).

Despite all these efforts at control, most field and storage insect pest infestations of cowpea have largely remained unchecked in the tropical Africa, including Nigeria, in the last five decades. It is in the light of these challenges that prompted the current attempt to assess the efficacy of locally available plant botanicals against insect pest infestations and the attendant economic losses suffered in the field and in the store, by our rural farmers, is undertaken.

MATERIALS AND METHODS

Harvesting and Preparation of Test Plant samples (extracts/powdered substances)

Fresh and matured leaves/stems of the test *A. conyzoides* and *H. suaveolens* were harvested from the cultivated farmlands and then air dried in a shade with diffused light, and grounded into a fine powder, using an electric blender. Each portions of the dried powder samples weighing 100gm, was dissolved in 500ml of 95% of ethanol and extracted using the extractor after shaking, stirring and filtering through a funnel plunged with white cotton and what mann filter paper No. 1. The resultant filtrate was then concentrated to a powdered form through complete evaporation, using an extractor solvent by a rotator evaporator, to give a 35% solid residue of greenish colour and then stored in a refrigerator until further use or the next experimental test (Harbone, 1998). The solvent/liquid extracts was used to assess its effects on larval/immature stages of weevils/insects, and also to determine their phytochemical constituents.

Collection and Rearing of Insect Pests (Cowpea weevils *C Maculatus)*

Adult cowpea weevils (*C. maculatus)* were isolated from already infested cowpea *Vigna ungucuilata)seeds* obtained from the markets and house hold stores, among farmers and traders in the six area councils; and placed in a plastic container covered with a muslin net material tightly fastened with rubber band and taken to the laboratory for rearing/culturing. The rearing was done to adapt the weevils to the prevailing laboratory condition, thereby ensuring the emergence of new adults, for the purpose of the experiment.

Phytochemical Screening of the Test Plant extracts.

This was carried out using procedures suggested and adopted by Harbone (2010); & Aboubakry *et al.* (2010). The chemical constituents tested were reducing sugars, glycosides, saponins, Sterol/steroids, flavonoids, tannins, anthraquinones, Terpenes and alkaloids, using their characteristic colour changes, on application of standard procedure and reagents (Harbone, 1998).

Test for reducing sugars

This is also called fehlings test. Here 2mls of water/ethanol extract was added to a mixture of equal volume of fehling re-agents A and B and then boiled on water bath. A brick red colour at the bottom of the test tubes indicated the presence of free reducing sugar.

Test for Glycosides

Ten (10) mls each of H_2SO4 and Fehlings solutions were added to one ml (1) of extracts and the mixture was heated in boiling water for 15 minutes. A brick red precipitate was confirmatory of the presence of glycosides.

Test for Alkaloids

A ml (1) of HCL was added to 3mls of the ethanolic test extracts in a testtube and about 2-3 drops of Meyer's reagent added. A creamy and turbid precipitate indicated the presence of Alkaloids.

Test for saponins. It is also called the Frothing test. About 2 mls of the extracts (ethanolic) was poured into a test tube and vigorously shaken for about 6 minutes. The presence of the frothing in the testtube indicated the presence of saponins in the extract.

Flavonoids Tests: To a ml (1) of the ethanolic extracts, was added or dropped *magnesium Ribbon* followed by the addition of HCL in a drop wise fashion/manner. A *magenta coloration*indicated the presence of flavonoids.

Sterols/Steroids and Terpenes Tests

- **Preparation:** Powdered samples of about 5gm was extracted by maceration with 50mls of 95% ethylacetate and filtered. The filtrate was then evaporated to dryness (i.e residues). The residues was dissolved in 10mls of anhydrous chloroform and then filtered again.
- **Test for Steroids/sterols:** one of the portions of the filtrate was mixed with 2mls of conc. H_2S0_4. A reddish brown – like colour, indicated the presence of a sterol, in the form of steroidal ring. This is called salk workis test for sterols.
- **Test for Terpenes,** the other portion of the filtrate was mixed with 1ml of acetic anhydride, followed by the addition of 1ml of conc. H_2S0_4, carefully down the wall of the test-tube, to form a layer

***Corresponding Author:** Ahmad-Alizaga, S. L.

underneath. The resultant formation of a redish-violet colour indicated the presence of terpenes. This is called Liebermann-Burchards test for Terpenes.

- **Test for Anthraquinones:** About 5gm of the powder was taken into a test tube and 10mls of chloroform was added and shaken for 5 minutes. The extract was then filtered and another 5mls of Ammonia (NH3) was added to the mixture and shaken. A *bright pink colour* in the upper aqueous layer indicated the presence of anthraquinones.
- **Test for Tannins.** To 1ml of aqueous extract, was added about 7ml of feric chloride ($feCl_3$). The presence of *blue-black* and or *blue-green* precipitate indicated the presence of Tannins.

Determination of the mortality of cowpea weevils

C. maculatus 15gms each of the leaves powder of the two experimental plants:*H. suaveolens*and *Ageratum conyzoides* separately, and another 15gms, each of the test plants, totaling 30gms were mixed with 100gms of cowpea seeds and each introduced into three (3) containers containing the admixture. Also, another 100gms of cowpea seeds was taken into another container of similar size and type, without the plants powder as control. These preparations were designated A-D, and labelled with their contents (treatment regimens), containing the cowpea seeds; and gently shaken to ensurea thorough mixture. To each of these containers A-D, was introduced about 50 to 100 number of *Callosobruchus maculatus*weevils, and covered with a net fastened material tightly with rubber band. The four containers, A – D, were weighed with metlers weighing balance daily for 14 days noting both the weight loss of the seeds and mortality rate of the *C. maculatus* weevils, during the period of investigation.

RESULTS

DATA PRESENTATION

Results of Phytochemical Screening/analysis of plant extracts of *A. conyzoides* and *H. suaveolens*

The results/analysis of the phytochemical determination of the constituents/active ingredients of the extracts/powdered substances of the test plants:*A. conyzoides* and *H. suaveolens*, showed the presence of the following active ingredients/chemical constituents as presented in table 3:1 as follows:

Table 1. Phytochemical Constituents of *A. conyzoides* and *H. suaveolensharvested,in Abuja, FCT*

Chemical constituents (active ingredients)	**Results/Analysis (+ve-ve)** ***A. conyzoides***	***H. suaveolens***
Reducing sugars	++	++
Alkaloids	+	+
Glycosides	+	+
Flavonoids	+	+
Anthraquinones	+	+
Tannins	+	+
Terpenes	+	+
Saponins	+	++
Sterol/steroids	-	-

Results of Insecticidal activity (efficacy) of extracts/powdered samples of *A. conyzoides* and *H. suaveolens* against *C. maculatus*

The results of the assessment of the insecticidal efficacy of the leaf and stem extracts/powdered samples of A. *conyzoides* and *H. suaveoelens* botanicals against the storage insects weevils (*C. maculatus)* of cowpea (*Vigna unquiculata)* seeds/cops, using different treatments or test regimes, indicated variousrates of mortality(death rates) of these weevils as well as observable economic loss manifested in weight loss and depreciation value of cowpea seeds/cops, as presented in tables 2, 3 and 4, below:

Table 2. The mortality rates of *Callosobruchus maculatus (cowpea weevils) in relation to the various treatment regimens*

Days	**Treatment regimens**			
	D_1	**D_2**	**D_3**	**D_4**
1	04	08	12	-
2	02	06	10	-
3	-	-	09	2
4	04	02	04	-
5	-	04	05	01
6	05	-	05	-
7	-	05	05	02

Table 3. Daily weight losses of various test treatment regimens of cowpea seeds during the successive days of assessment/investigation

***Corresponding Author:** Ahmad-Alizaga, S. L.

Days	Treatment regimens/weights			
	D_1 (grams)	**D_2 (grams)**	**D_3 (grams)**	**D_4 (grams)**
1.	125.70	125.70	140.71	110.69
2.	125.58	12.51	140.47	110.64
3.	125.39	125.20	140.17	110.55
4.	125.18	124.89	139.84	110.40
5.	124.94	124.56	139.50	110.19
6.	124.69	124.23	139.16	109.94
7.	124.40	123.89	138.81	109.66

Table 4. Daily weight losses of cowpea seeds as per successive days of treatment regimens

Days	Treatment regimens/weights			
	D_1 (grams)	**D_2 (grams)**	**D_3 (grams)**	**D_4 (grams)**
1.	0.00	0.00	0.00	0.00
2.	0.12	0.11	0.24	0.06
3.	0.19	0.31	0.30	0.09
4.	0.21	0.31	0.33	0.15
5.	0.24	0.33	0.34	0.21
6.	0.25	0.33	0.34	0.25
7.	0.29	0.34	0.35	0.26
Total	**1.30**	**1.13**	**1.90**	**1.02**

Key: D_1 – *C. maculatus* + cowpea seeds + *A. conyzoides* powder
D_2 – *C. maculatus* + cowpea seeds + *H. suaveolens*powder
D_3 – *C. maculatus* + cowpea seeds + *Aconyzoides and H.suaveolens powdered mixed*
D_4 – Cowpea seeds + *C maculatus – test plants (control)*

DISCUSSION

It is reported that over 90% of the insect damage to cowpea seeds/cops in storage is caused by the cowpea weevils, *Callosobruchusmaculatus F (coleotera: Bruchiidae)*, also reported to bea pest of various leguminous plants (pulses) such as soya beans *(Glyceine max)*, common beans *(Phaseolus vulgaris)*. Thus, over the years, interest in botanical insecticides (herbs) has increased partly, as a result of environmental concerns arising from unfavourable traditional practices, as well as a result of insect population becoming resistant to chemical insecticides, both in the field and in the store; thereby necessitating the evaluation of essential oils (ingredients) in natural plant extracts/powdered samples, against more importantly, stored product insect pests (Kim *et al.,* 2013; Ayvaz *et al.,* 2009; & Idu *et al.,* 2012).

Traditionally, it was even reported that most of our rural farmers have adopted locally available plants extracts, parts or even powdered grinded substances of the leaves or stem or both to control various insect infestations against stored crop produce as cowpea (common House beans) and Grains (Lale & Ofuya, 2011; & Asawalam *et al.,* 2007).

In the present study, the scientific evaluation of the various extracts and powdered preparations of the leaves and stems of these two locally available botanicals to ascertain their insecticidal efficacy, against the cowpea weevils (*C. maculatus*) in the store was undertaken and the results obtained confirmed the insecticidal efficacy/property of these plants extracts/powdered scores, with respect to the mortality of the cowpea weevils; though the rate of mortality was generally low during the 1st few days of assessment until at the end of the 7th day observation. However, with combined treatment regimens (D111), the potency and hence efficacy was higher, meaning that the death of the test insect pests;*C. maculatus*, was observed to be higher and faster as compared to the single DI & DII treatment regimens (table 3:3). Also, the economic value of the stored seeds was observed to be lower at the end of the seven day treatment regimes than at the early stage (1st – 3rd day). This is reflected in the quality and quantity (depreciated value and weight loss) of the seeds before, during and after the successive days of the seventh day test treatment. This is confirmed in the 1.3% decrease in the test treatments regimens DI – DIII and a 0.93% decrease in the control treatment (DIV) respectively (Tables 3 and 4).

CONCLUSION

Callosobruchus maculatus (cowpea weevils) has been reported to parasitise a wide range of legumes instore, preferably infesting members of the *Vigna unguiculata*commonly referred to as the common house beans. A single larva of *C. maculatus* is reported to diminish the weight of a cowpea bean by 3% and multiple larvae will infest one cowpea in most storage infestations (Egija, 1979). Additionally, Mahfuv & Khalaquzzaman (2007), reported that a slight damage by the larvae of *C. maculatus*can be destructive towards the viability of a cowpea seed for the next planting season.

Following the destructive prevalence of agricultural products in the field and in the store by these

***Corresponding Author:** Ahmad-Alizaga, S. L.

insect pests, several Agricultural entomologist and Crop protectionists, discovered; by ascertaining the insecticidal efficacy of both the synthetic and natural plants products' control of the pest population in the field and in the store. This research is one of such control attempts; which has proved beyond doubt that a great breakthrough in the population reduction of *Callosobruchus maculatus*, is established in the store, considering their generation time and continuity of life.

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