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EFFECT OF LEAF EXTRACT OF *Azadirachta indica* AND PLANT ASH ON *Callosobruchus maculatus* ATTACKING *Vigna unguiculata* SEEDLINGS.

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ABSTRACT

Seedlings of Vigna unguiculata L. Walp attacked by the pulse beetle, Callosobruchus maculatus were treated with water extracts of fresh leaves of Azadirachta indica A. Juss and plant ash separately. The extract was found to exhibit an insecticidal effect. It has an antifeedant and growth regulating effects on the pulse beetle. It inhibited molting, caused sterility, loss of flying ability, disrupted sexual communication and reduced motility. The dry plant ash caused mortality of the pulse beetle. The results obtained showed that C. maculatus caused a great negative effect on the leaf size, pod length, and number of seeds per pod of affected V. unguiculata. This effect is controlled or minimized by the right dosage of extract of A. indica and plant ash on affected seedlings of V. unguiculata.

INTRODUCTION

The low yield of crops is linked to the irregularities of the rains, the non adapted cultural techniques and particularly to pest infestations causing damage to crops. Many problems have been faced due to the use of synthetic pesticides. Such problems as resistance of insect pests to synthetic pesticides and environmental hazards caused by these pesticides have buttressed the need for effective biodegradable organic fungicides and pesticides to be preferred (Elhag, 2000). This awareness has created worldwide interest in the development of alternative strategies, including the re-examination of plant-based insecticides.

Some medicinal plants have been tested for their insecticidal and fungicidal activities on insects and pathogens and some of them were reported to have actually minimized and restricted pests and pathogens infestation (Rise, 2002). Extracts of different medicinal plants have attracted the special interest of entomologists and phytochemists all over the world. This is due to the numerous ingredients contained in these plants. In recent years, plant-based products have been developed and they have proven to be very effective against insects (Rise, 2002).

Many medicinal plants have secondary metabolites that have biological activities. Liquids and oils extracted from parts of some medicinal plants such as roots, fruits, seeds, flowers, leaves etc when applied to plants have positive effect of increasing yield and minimizing disease and pest infestations. The tree *Azadirachta indica* commonly called neem tree has been used for many things. Of primary interest to research scientists is its activity as an insecticide. Parts of this tree have been used in medicine, fuel, lubrication and most of all as pesticide. It is the use of this tree as an insecticide that now draws interest from industrialized countries. It is seen as an environmental safe alternative to synthetic pesticides. Insects that have become resistant to synthetic pesticides are controllable with the extracts from this tree (Talukder and Howse, 2005).

Azadirachta indica is a shady tree and a member of the Meliaceae family. It is an evergreen tree and can reach heights of 30 meters. It can live for over two centuries. It has deep root system which is adapted to retrieving water and nutrients from the soil. This deep root system is very sensitive to water logging. This tree thrives in hot, dry climates. The tree can withstand many environmental adversities including drought and infertile, stony, shallow or acidic soils. *A.indica* produces drupes borne on axillary clusters. These fruits contain kernels that have high concentrations of secondary metabolites (National Research Council, 1992).

Plant ash is the residue of burnt plant parts like bark, wood, leaves, pulp, etc. plant ash is a good liming material to neutralize soils. Plant ash is used as a pesticide. Although synthetic pesticides are effective at controlling pests, the environmental hazards of these chemicals are of increasing concern. Ashes of plant material are an alternative to synthetic pesticides. Plant ash is generally environmental bening and low in contaminants such as heavy metals and organic compounds (Walt and Ewete 2007).

Vigna unguiculata is an annual herb, erect, sub-erect or spreading with glabrous taproot. The roots have large nodules. Stems are usually procumbent. First leaves above cotyledons are simple and opposite. Subsequent trifoliate leaves are alternate. Leaflets are ovoid-rhombic, entire or slightly lobed. Lateral leaflets are oblique (Imrie, 2004).

V. unguiculata is one of the most widely used legumes in the tropical world. The grain is used widely for human nutrition especially in Africa. Mixing of *V. unguiculata* with some carbohydrates such as maize is a common food in Eastern Nigeria. Cowpea is the most dual-purpose legume being used as vegetables (leaves and flowers).*V. unguiculata* has a high potential as a green manure. It can be incorporated into the soil or spread on the soil surface 8 to 10 weeks after sowing, and can provide the equivalent of 80kg/haN to a subsequent crop. *V. unguiculata* may be potential as a forage plant. Some accessions have been used for its use as key pasture on alkaline clay soils in Southern Queensland (Fatokun *et al*; 2002). Roots of *V. unguiculata* are eaten in Sudan and Ethopia. Scorched seeds are occasionally used as a coffee substitute. Peduncles are rated for fibre in Northern Nigeria. It is used as a folk medicine in Hausa and Yoruba tribes to abate evil and to pacify the spirits of sickly children (Imrie, 2004).

MATERIALS AND METHODS

Collection and preparation of plant materials

Fresh leaves of *A. indica* were collected from the Botanical Garden of University of Nigeria, Nsukka. Dry leaves and dry barks of some plants were also collected from the garden for burning.

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Extraction of ash and liquid from plant materials

Both cold and hot leaf extracts of *A. indica* were prepared. With the aid of a scale balance, 20g of neem leaves was weighed out twice for both the cold and hot extractions respectively.1000ml of water was added to one portion of neem leaves (i.e. 20g) and boiled at a temperature of 100^{o}C after which the leaves were sieved out and the remaining solution served as the hot water extract. 1000ml of water was also added to the other portion of neem leaves. The leaves were squeezed by sterile hand until the water turned green. The remaining leaves were sieved out and the remaining solution yielded the cold water extract.

100g of dry leaves and barks were burnt. The ash gotten from the burning was allowed to cool and kept in a dry place out of rain water or moisture before used. This was done in order for the ash not to loose its solubility.

Planting of *Vigna unguiculata*

Healthy seeds of *Vigna unguiculata* were brought from the Department of Botany, University of Nigeria, Nsukka. The seeds were planted in perforated clay pots containing top soils. Three seeds were planted in each clay pot. After germination, two of the seedlings were thinned down leaving one seedling per pot. A total of one hundred and twenty seedlings were used for the experiment.

Treatment procedure and duration

The cold water extract of *Azadirachta indica* prepared by squeezing the leaves in 1000ml of water was diluted in order to get a concentration of 50% of the liquid extract. This was done by adding water of the same volume as the liquid extract to the already prepared extract. The solution obtained from the dilution was applied to the first three treatments (i.e treatments A, B and C). The hot water extract prepared by boiling the leaves in 1000ml of water was diluted to get a concentration of 50% of the extract by adding water of the same volume as the liquid extract to the already prepared hot water extract. The solution obtained from the dilution was applied to the next three treatments (i.e treatments D, E and F).the ash obtained by burning dry plant materials was applied to the 7th treatment (i.e treatment G).

The liquid extracts and the ash were applied to the different treatments once every two weeks for eight weeks. These applications started ten days after germination of the *Vigna unguiculata* seeds. The last treatment (i.e treatment H) served as the control; neither the liquid extract nor ash was applied to it.

Data collection

The leaves of *Vigna unguiculata* seedlings were measured every two weeks before the application of extracts and ash. This was done for eight weeks yielding four sets of data. When pods started developing, each pod was plucked at maturity; the pod length and number of seeds per pod was determined and recorded. At the end of the experiment, the number of leaves and number of pods per *V. unguiculata* seedling were also counted and recorded.

Data collected were subjected to statistical analysis using Analysis of Variance (ANOVA).

RESULT

The results of the effect of leaf extract of *Azadirachta indica* and plant ash on *Callosobruchus maculatus* attacking *Vigna unguiculata* seedlings are shown in (Tables 1 to 8). The results showed that both the plant leaf extract and plant ash used led to sterility of the pulse beetles, preventing them from multiplying thereby reducing their population and attack on the *V. unguiculata* seedlings treated. The results also showed that the extracts used inhibited molting of the pulse beetles. However, the level of inhibition differed depending on the extract used.

The hot water leaf extract of *A. indica* was able to cause mortality and reduce motility of the pulse beetles. The *V. unguiculata* seedlings treated with hot water leaf extract of *A. indica* grew well with numerous sizeable leaves and pods of reasonable length containing many seeds.

The seedlings of *V. unguiculata* subjected to plant ash treatment also grew well but the leaves and pods were not as big as those treated with hot leaf extracts of *A. indica.*

The cold water leaf extract of *A. indica* inhibited the lowest molting and also caused sterility of the pulse beetles just like the other treatments.

Some *V. unguiculata* seedlings were left untreated and these served as the control experiment. The seedlings werew severely damaged by the pulse beetle (*Callosobruchus maculatus* leading to great loss of yield).

Table 1: Mean length of leaves of *Vigna unguiculata* seedlings (in cm) ten days after germination.

Determinations Treatment	**1**	**2**	**3**	**4**	**5**
A	6.19±0.58	6.41± 0.52	4.33± 0.31	5.46± 0.56	5.19± 0.55
B	6.09±0.56	6.23± 0.59	6.45± 0.49	5.35± 0.52	5.24± 0.65
C	5.20± 0.49	5.45± 0.51	5.60± 0.58	6.33± 0.71	6.21± 0.73
D	6.20± 0.42	6.43± 0.63	5.32± 0.46	6.07± 0.49	6.41± 0.71
E	6.38± 0.51	6.00± 0.61	6.15± 0.57	6.31± 0.65	6.14± 0.68
F	6.17± 0.58	6.07± 0.59	6.68± 0.31	5.97± 0.49	5.26± 0.72
G	5.78± 0.61	6.58± 0.69	6.03± 0.37	5.50± 0.78	5.64± 0.61
H	5.62± 0.53	5.56± 0.51	6.22± 0.79	5.90± 0.73	5.84 ± 0.55

Mean of determinations with three replications ± Standard Error.

Table 2: Mean length of leaves of *Vigna unguiculata* seedlings (in cm) two weeks after the first application of extracts.

Determination Treatment	**1**	**2**	**3**	**4**	**5**
A	6.40± 0.82	6.79± 0.89	4.78± 0.41	5.77± 0.51	5.55± 0.57
B	6.31± 0.71	6.52 ±0.92	6.63± 0.89	5.70± 0.58	5.69 ±0.56
C	5.66± 0.52	5.84± 0.42	6.05 ±0.91	6.62± 0.58	6.53± 0.86
D	6.82± 0.72	6.96± 0.96	5.82± 0.61	6.70 ±1.23	7.14± 1.37
E	6.82 ±0.83	6.66± 0.71	6.76 ±0.82	7.06± 1.18	6.62± 0.66
F	6.70± 0.71	6.68± 0.97	6.97 ±0.87	6.57± 1.01	6.02± 0.73
G	6.24 ±0.77	6.97± 0.91	6.44 ±0.82	6.04± 0.92	6.15 ±0.79
H	5.83± 0.61	5.75± 0.81	6.26± 1.13	6.00± 0.88	6.09± 0.85

Mean of determinations with three replications ± Standard Error.

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Table 3: Mean length of leaves of *Vigna unguiculata* seedlings (in cm) four weeks after the first application of extracts.

Determination Treatment	**1**	**2**	**3**	**4**	**5**
A	6.74± 0.83	7.02± 0.73	5.28± 0.51	6.13± 0.65	5.93± 0.63
B	6.67± 0.98	6.82± 0.97	6.94± 0.71	6.25± 0.71	6.03 ±0.69
C	6.02± 0.51	6.32± 0.91	6.51± 0.82	7.01± 0.62	6.90 ±0.91
D	7.54± 1.19	7.68 ±1.17	6.63± 0.69	7.43± 0.75	7.73± 1.12
E	7.68± 1.19	7.33 ±1.02	7.45± 1.23	7.63 ±1.17	7.33± 1.18
F	7.24± 1.27	7.53 ±1.29	7.53± 1.29	7.38 ±1.21	6.81± 1.11
G	6.74± 1.29	6.92± 0.98	6.92± 0.98	6.50± 0.89	6.63± 0.83
H	5.92± 0.55	6.20± 0.91	6.20± 0.91	6.00± 0.83	6.02± 0.64

Mean of determinations with three replications ± Standard Error.

Table 4: Mean length of leaves of *Vigna unguiculata* seedlings (in cm) six weeks after the first application of extracts.

Determination Treatment	**1**	**2**	**3**	**4**	**5**
A	7.14 ±1.35	7.31± 1.33	5.71± 0.83	6.62± 0.85	6.44± 0.97
B	6.92± 0.71	7.12± 1.61	7.07 ±1.53	6.73± 0.92	6.41± 0.83
C	6.41± 0.88	6.83± 0.72	7.22 ±1.43	7.45± 1.49	7.39 ±1.48
D	8.21± 1.62	8.14± 1.56	7.29± 1.21	8.05±1.51	8.35± 1.77
E	8.32± 1.79	8.04± 1.57	8.19± 1.69	8.28± 1.69	8.11± 1.55
F	7.92± 1.42	8.13± 1.58	8.27± 1.65	8.03± 1.52	7.63± 1.42
G	7.26± 1.58	7.55± 1.21	7.41 ±1.21	7.01± 1.31	7.13 ±1.33
H	6.01± 0.59	5.81± 0.68	6.14 ±0.92	6.02± 0.95	6.14 ±0.85

Mean of determinations with three replications ± Standard Error.

Table 5: Mean number of leaves of *Vigna unguiculata* seedlings (in cm) at the end of the experiment (77 days after planting).

Determination Treatment	**1**	**2**	**3**	**4**	**5**
A	12.67± 1.06	12.67± 1.13	14.00 ±1.34	12.67± 1.01	13.33± 1.11
B	13.00± 1.11	13.33 ±1.18	13.33 ±1.18	13.33± 1.12	14.00± 1.34
C	12.67± 1.03	14.00± 1.28	14.00± 1.25	13.33 ±1.21	13.33± 1.23
D	16.67 ± 1.49	17.00 ±1.60	17.00 ±1.66	16.67± 1.48	17.33 ±1.81
E	16.33 ±1.43	18.00 ±1.77	16.67± 1.42	17.00± 1.62	17.33 ±1.61
F	17.00± 1.72	18.00± 1.77	17.67± 1.65	17.33± 1.75	17.33± 1.79
G	16.00± 1.39	15.33± 1.52	15.67 ±1.48	15.00 ±1.57	15.33± 1.51
H	9.00± 1.07	10.00 ±1.02	11.00± 1.01	11.00 ±1.09	9.67 ±1.04

Mean of determinations with three replications ± Standard Error.

Table 6: Mean length of pods of *Vigna unguiculata* (in cm) after plucking (77 days after planting).

Determination Treatment	**1**	**2**	**3**	**4**	**5**
A	6.85± 0.71	7.95± 0.75	8.04± 0.92	7.45± 0.71	6.03± 0.61
B	7.20± 0.68	7.70 ±0.86	9.20 ±0.83	6.75 ±0.77	7.78± 0.83
C	8.85± 0.92	6.25 ±0.59	7.03 ±0.81	8.07± 0.81	8.80± 0.88
D	9.85± 1.01	11.20± 1.13	10.47± 1.09	11.17± 1.53	9.91 ±0.93
E	11.87± 1.31	9.89 ±0.92	10.61± 1.02	11.50± 1.68	12.23± 1.77
F	11.12 ±1.87	11.89± 1.27	11.11± 1.24	11.07± 1.66	11.51± 1.21
G	9.62 ±1.01	10.01± 1.29	9.05± 1.07	9.71 ±0.91	10.47± 1.34
H	4.23± 0.46	4.52 ±0.53	4.93± 0.58	5.21± 0.71	5.14 ±0.69

Mean of determinations with three replications ± Standard Error.

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Table 7: Mean number of pods of *Vigna unguiculata* at the end of the experiment (77 days after planting).

Determination Treatment	1	2	3	4	5
A	2.67± 0.27	2.69± 0.26	3.33± 0.21	3.00 ±0.19	4.00± 0.31
B	3.33± 0.31	3.67± 0.13	2.67± 0.28	2.33± 0.18	3.67± 0.24
C	3.00± 0.29	3.67 ±0.21	3.67± 0.21	3.00± 0.31	3.33± 0.33
D	4.33± 0.55	4.33 ±0.52	5.00 ±0.61	4.00± 0.42	3.67± 0.28
E	4.00 ±0.62	4.67± 0.55	4.00± 0.45	5.00 ±0.43	4.67 ±0.38
F	4.67 ±0.71	4.67 ±0.52	5.00± 0.51	5.00 ±0.41	4.00 ±0.39
G	3.67± 0.29	4.00 ±0.31	4.00± 0.42	4.00± 0.52	3.67 ±0.49
H	1.00± 0.13	1.00± 0.31	1.00 ±0.17	1.33± 0.23	1.00± 0.14

Mean of determinations with three replications ± Standard Error.

Table 8: Mean number of seeds per pod of *Vigna unguiculata* after plucking.

Determination Treatment	1	2	3	4	5
A	4.67± 0.56	5.00± 0.56	4.33± 0.45	5.00± 0.71	3.67± 0.18
B	4.67± 0.51	5.00±0.53	4.67± 0.49	4.33± 0.43	4.00± 0.27
C	5.00± 0.80	4.00± 0.40	3.33± 0.31	6.33± 0.61	5.00± 0.56
D	7.33± 0.64	9.67± 1.36	9.00± 1.21	8.67± 0.92	7.67± 0.81
E	9.67± 1.39	8.33± 0.89	8.67± 0.97	9.33± 1.17	9.67± 1.27
F	8.33± 1.05	9.33± 1.20	9.00± 1.13	8.67 ±1.38	9.33± 1.22
G	7.33± 0.79	7.67± 0.73	8.67± 1.02	6.33± 0.61	7.33± 0.71
H	2.33± 0.22	2.67± 0.26	3.00± 0.25	3.33± 0.17	2.00± 0.22

Mean of determinations with three replications ± Standard Error.

From the (Tables 1-8), the results obtained indicates that the length of leaves, number of leaves, length of pods, number of pods and number of seeds per pod of *Vigna unguiculata* seedlings treated with the hot water exract of *Azadirachta indica* are bigger than those subjected to the other treatments.

DISCUSSION

The findings of this research indicate that both extracts of *Azadiradhta indica* and plant ash are useful as insect control agents. Both of the two extracts tested were effective in reducing the ovipositional preferences, increasing the inhibition rates and causing mortality of the pulse beetle, *Callosobruchus maculatus* thereby reducing their attack on *Vigna unguiculata*. The hot water leaf extract of *A. indica* and the plant ash treatments exhibited significant results whereas the cold water leaf extract of *A. indica* showed moderate pesticidal effect on the mean number of pods per seedling of *Vigna unguiculata*.

The use of indigenous plant products and other locally available materials to protect cereals and legumes have been reported by many workers (Kim *et al.*, 2003; Katembwe, 2003; Ewete, 2007). The present study showed that the leaves of *A. indica* in extracted form effected the mortality of *C. maculatus* thereby reducing its attack on *Vigna unguiculata.* Plant ash also gives a good control of pulse beetles (*Callosobruchus maculatus*) when applied (Walt *et al.*, 2007).

The ability of *A. indica* to control *Callosobruchus maculatus* infestation on *Vigna unguiculata* is attributed to the protectant effect of the presence of azadirachtin (Ashamo *et al.*, 2001; Omotoso, 2004). The insect-control substances are primary azadirachtin A,B and G (National Research Council, 1992). Azadirachtin has deterent, anti-feedant, growth disrupting, anti-ovipositional and fecundity reducing properties on a range of insects (Elhag, 2000). The reduction in the attack of *Vigna unguiculata* by *Callosobruchus maculatus* brought about by the results is probably due to the above inherent properties.

Serious problems of genetic resistance by insect pests, resurgence, photo toxicity, vertebrate toxicity, widespread environmental hazards and increasing costs of application of the presently used synthetic insecticides have directed the need for the effective biodegradable pesticides (Rise, 2002; Schwin *et al.*, 2004). The main advantage of using biodegradable insecticides is that they may be easily and cheaply produced by farmers and small-scale industries as crude or partially purified extracts. It has been reported that the leaf, bark, seed powder or oil extract of plants reduce oviposition rate and suppress adult emergence of bruchids (Elhag, 2000; Talukuder *et al*., 2005).

In conclusion, a study to improve the effectiveness of botanical derivatives as insecticides will benefit agricultural sectors of developing countries, as these substances are not only of low cost, but also have less environmental impact in terms of insecticidal hazard.

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