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EFFECTS OF CRUDE EXTRACTS OF *ARTEMISIA ANNUA, ALOE VERA, AND MORINGA OLEIFERA SEED* ON EXPERIMENTAL *CALLOSOBRUCHUS SUBINNOTATUS* INFESTATION OF BAMBARA NUTS

[1]Abdullahi, J., [2]Mohammed, F. I., [2]Sadiq, I. A. and [2]Magaji, N
[1]Federal Ministry of Labour and Productivity, Abuja
[2]Samaru College of Agriculture Ahmadu Bello University, Zaria

Abstract
The effect of the plant products *Artemisia annua* crude aqueous extract, *Aloe vera* gel and oil of *Moringa oleifera* on the mortality of *C. subinnotatus* at three levels of application 2 ml/kg, 4 ml/kg and 8 ml/kg were investigated. The treatments were compared with Actellic 2% dust at 10 g/kg and *Azadirachta indica* seed oil at 2 ml/kg as standards and an untreated control. The bioactivity of the plant products was studied under prevailing laboratory conditions for a period of two years. The design of the experiment was completely randomized design with three replicates. Data was analyzed using Microsoft Excel 2003 and SPSS 13.0. The plant oil from *M. oleifera* at all levels of application achieved 100% mortality of the bruchid after 24h of application and compared favourably to the synthetic insecticidal dust and neem seed oil at 5% level of significance (single factor ANOVA and SNK). The mode of action of the plant product demonstrated contact, repellent and fumigant toxicities against the bruchid. Treatment with *A. vera* at the rate of 8 ml/kg compared favourably to the *M. oleifera* and standard treatments from 24h in the second trial. It showed positive fumigant and contact toxicities but had no repellent effect against the bruchid. All treatments demonstrated some biological activity against the bruchid and can be used as a cheap, readily available and environmentally friendly alternative biopesticide.
Keywords: Phytotoxicity, neem seed oil, Moringa seed oil, *Aloe vera* oil and gel, bruchids

Introduction
The place of agriculture in any society cannot be quantified given its importance in the life of human beings. It plays an important role in ensuring adequate food to the masses for a healthy and productive life. In terms of dietary balance, grain legumes or pulses contain more proteins than cereals and about 10x as much protein as most roots and tubers (FAO 1968; Lale, 2000). Food legumes have a major role to play in the fight against malnutrition and diet related diseases. It is therefore necessary that their level of consumption, which is already too low in many developing countries, should be increased (Borget, 1992; Ebony, 2005; Beverly, 2007). Legumes serve as a source of protein to a large proportion of the population in the poor countries of the world by being the least expensive and easily stored and transported non-processed protein source for rural and urban dwellers (Rachie and Silvester, 1997; nature.com, 2009). The high carbohydrate (65%) and relatively high protein (18%) content of bambara groundnut makes it a complete food (Doku, 1995; Tanimu, 2005). There is a concerted call for the introduction of bambara groundnut as a cash crop in Nigeria (Okonkwo and Opara, 2010). They also have important nutritional value as extenders of animal proteins that are scarce and costly. Even though they are not as widely grown as other food crops, they can enhance nutritional status, increase household food security, increase crop productivity by reducing buildup of pests and diseases, conserve soil, increase soil fertility and aid in increasing household and national income by trading in the crop. Though the crop may be neglected globally, they are staples at National or Regional levels. However, they are grossly underutilized in Africa where it originates (Bamshaiye *et al*., 2011). This is important since they are well known for their adaptations to marginal growing conditions. The neglect and underutilization

of foods like bambara groundnuts can lead to genetic erosion which depletes gene pool available for natural selection processes and at the same time increases the vulnerability of agricultural crops to sudden and abrupt changes in climate or weather, or to appearances of new pests and diseases. The unpredictable climate in Africa highlights the importance of hardy crops as bambara groundnut (Danida, 2012). Generally, the crop is relatively tolerant to insect pests and diseases and loss is mostly found in storage. The bruchid *Callosobruchus subinnotatus* is regarded as its most important storage pest in Africa**.**

Materials and Methods

Experimental Treatments on *C. subinnotatus* Pic.:Two hundred and fifty grammes each of pre-fumigated bambara groundnut was placed in plastic containers and the plant products were applied at the rates of 2.0, 4.0 and 8.0 ml/kg (i.e. 0.5, 1.0 and 2.0ml/250g bambara groundnuts). Actellic 2.0% dust [Pirimiphos-methyl] was applied at the rate of 10 g/kg. Neem oil used as a standard was applied at the recommended dose of 2 ml/kg. There is an untreated control. Five pairs of day old adult (0-24 hr old) *C. subinnotatus* were placed in each treatment. The design was Completely Randomized Design and each treatment was replicated three times. Mortality readings for the bruchids were taken at 24, 48 and 72 hours after application of treatment. Thereafter readings were taken at weekly intervals for two weeks. This was done by sieving out the insects, counting and removing dead ones. The live ones were returned to their respective Kilner jars. Data obtained was analyzed using the one-way analysis of variance and differences between means were compared at the 5% level of probability with Student's Newman's Keuls (SNK).

Bioactivity of Test Materials on *C. subinnotatus* Pic.

***Contact Mode of Action*:** 0.5µl of test materials was topically applied dorsally to the thorax of ten adult day old *C. subinnotatus* of both sexes using a micro applicator. The insects were then transferred to 50 g of the culture media and observed daily for a week [Liu and Ho, 1999]. Distilled water was used as control.

***Fumigant Mode of Action*:** Filter papers were impregnated with 25 µl of the test materials with distilled water as control and placed on the underside of a clean glass specimen bottle (capacity 55ml) containing ten day old adult bruchids [five pairs] and screwed tightly. These were incubated for 24 hours. After 24 hours, the insects were then transferred to clean specimen bottles containing some culture media [50g] and observed for a week as described by Liu and Ho (1999).

***Repellency Action*:** Half filter paper (Whatman No. 40, 9 cm diameter filter paper) discs were prepared and 750 µl of each of the treatment or distilled water as control was added to each half disc, each treated half was attached lengthwise, edge to edge to a control half disc with a thin strip adhesive tape and placed in a Petri dish. The orientation of the seam was changed in the replicates to avoid the effect of any external directional stimulus affecting the distribution of the insects (Macdonald *et al*., 1970; Telukder and Howse, 1993; 1995; Liu and Ho, 1999). Twenty adult day old *C. subinnotatus* were released in the middle of each filter paper circle and transparent netted cloth was placed on top for ventilation and visibility. Each treatment was replicated five times. The number of insects that settled on each half of the filter paper were counted after one hour and then at hourly intervals for five hours.

The average counts were converted to percentage repellency (PR) using the formula of Telukder and Howse [1993; 1995].

$$PR = 2(c-50)$$

$$\% R = \frac{Nc-Nt}{Nc+Nt} \times 100$$

Where **Nc** is number of insects on the untreated (control) half of the disc; **Nt** is the number of insects on the treated half of the disc ; **c** is the percentage of insects on the untreated half of the disc. Positive values will express repellency and negative values attractancy. The data (PR) was analyzed using ANOVA and DNMRT after transforming them into arcsine percentage values.

Results and Discussions

Mortality Studies: When analysed using single factor (One-way) ANOVA and means separated using SNK at the 5% level of probability in the first year of the trial, the effect of treatments on the mortality of *C. subinnotatus,* from 24 hours after treatment showed that the standards Actellic and neem caused significant mortality of the bruchid that was statistically not different from each other (P>0.05) in both years of the experiment (Tables 1 and 2). The oil treatment of *M. oleifera* at all the three levels under investigation caused mortality of the bruchid that compared favourably to the standard treatments with neem and Actellic (P> 0.05) in both years (Tables 1 and 2) and was statistically higher than the other treatments (P<0.05). This indicates that the environmentally friendly oil of *M. oleifera* can be used for the control of the bruchid at the lower application rate of 2 ml/kg seed. This agrees with a later study on the flour moth pupa where it caused significant mortality (De Oliveira *et al*., 2011) and on the mosquito *Aedes aegytii* in Pakistan where significant control was achieved using the oil (Ashraq and Ashraq, 2012). The treatments of *A. annua* and *A. vera gel* and the untreated control did not compare favourably to the standards (P<0.05) and were not significantly different from each other (P>0.05) in the first year. One week after treatment, all the treatments and untreated control at the application compared favourably to the standard treatments and *M. oleifera* in causing the mortality of the bruchid. The plant product *A. vera* gel may be used as a plant based pesticide for the control of the bruchid at the higher rate of 8 ml/kg but may require longer exposure period for it to cause mortality. This may not however protect the grains from oviposition by the bruchid. Seventy two hours after introducing adult *C. subinnotatus* on bambara groundnuts treated with the plant products, those treated with *M. oleifera* (2, 4 and 8 ml/kg), *G. arborea* (2 and 4 ml/kg), *A. annua* (4 and 8 ml/kg) caused mortality of the bruchid that was comparable to the standard treatments (P>0.05). By the first week of investigation into the effect of the plant products on the mortality of the bruchid, were not significantly different from each other when compared using SNK at the 5% level of probability. When analysed using single factor (One-way) ANOVA and means separated using SNK at the 5% level of probability in the second trial, the effect of treatments on the mortality of *C. subinnotatus,* from 24 hours after treatment showed that the standards, Actellic and neem caused significant mortality of the bruchid that was statistically not different from each other P>0.05) just like in the first trial (Table 2). The oil treatment of *M. oleifera* at all the three levels under investigation (2, 4, and 8 ml/kg bambara groundnut seed) caused mortality of the bruchid that compared favourably to the standard treatments with neem and Actellic (P> 0.05) (Table 1).

Results from 48 hours post application of plant materials indicated that treatments of *A. vera* at application rate of 8 mg/kg bambara groundnut seed showed comparable performance to the standards and *M. oleifera* at all three levels of application by not being significantly different from each other (P>0.05).

Table 1: First Year Study on the Effect of Test Materials on the Mortality of *C. subinnotatus*

Treatment	24 Hours	48 Hours	72 Hours	One week	Two weeks
A. vera coc1	.00[a]	.33[a]	2.67[ab]	6.33[ab]	7.00[ab]
Control	.00[a]	.67[ba]	2.67[ab]	5.67[ab]	6.67[ab]
A. annua coc1	.33[a]	2.00[bac]	4.00[ab]	8.00[ab]	9.33[b]
A. vera coc3	.67[a]	1.67[bc]	3.00[ab]	6.00[ab]	9.33[b]
A. annua coc3	1.67[a]	4.00[b]	6.00[abc]	8.00[ab]	10.00[b]
A. vera coc2	2.00[a]	3.00[bac]	3.67[ab]	5.33[ab]	6.00[ab]
A. *annua* coc2	2.00[a]	3.00[bac]	5.00[abc]	8.33[ab]	10.00[b]
Neem	8.67[b]	10.00[d]	10.00[d]	10.00[b]	10.00[b]
Actellic	9.67[b]	10.00[d]	10.00[d]	10.00[b]	10.00[b]
M. oleifera coc1	10.00[b]	10.00[d]	10.00[d]	10.00[b]	10.00[b]
M. oleifera coc2	10.00[b]	10.00[d]	10.00[d]	10.00[b]	10.00[b]
M. oleifera coc3	10.00[b]	10.00[d]	10.00[d]	10.00[b]	10.00[b]

Means separated by the same letter within the same column are not statistically different from each other SNK (P=0.05). Legend: coc1= 0.5m (g)l/kg, coc2= 1.0ml (g)/kg, coc3 =2ml (g)/kg

Table 2: Second Year Study on the Effect of Test Materials on the Mortality of *C. subinnotatus*

Treatment	24hrs	48hrs	72hrs	1week	2weeks
AloeCoc1	0.00[a]	1.67[ab]	1.67[a]	1.67[a]	1.67[a]
Blank	0.33[a]	1.33[a]	2.67[a]	5.00[b]	7.33[b]
ArtmCoc1	2.00[ab]	4.67[ac]	7.00[bc]	9.33[c]	10.00[c]
ArtmCoc3	1.33ab	3.0ab	5.0b	8.ab	10.0b
AloeCoc3	4.00[bc]	10.00[e]	10.00[c]	10.00[c]	10.00[c]
AloeCoc2	2.00[ab]	3.00[ab]	6.00[b]	8.67[c]	9.00[c]
ArtmCoc2	3.00[ab]	5.00[c]	7.00[bc]	8.67[c]	10.00[c]
Neem	9.00[e]	10.00[d]	10.00[d]	10.00[b]	10.00[c]
Actellic	10.00[e]	10.00[d]	10.00[d]	10.00[b]	10.00[c]
MoriCoc1	10.00[e]	10.00[d]	10.00[d]	10.00[b]	10.00[c]
MoriCoc2	10.00[e]	10.00[d]	10.00[d]	10.00[b]	10.00[c]
MoriCoc3	10.00[e]	10.00[d]	10.00[d]	10.00[b]	10.00[c]

Means separated by the same letter within the same column are not statistically different from each other LSD (P=0.05)

Mortality caused by the different treatments from 72 hours showed that *A. annua* at the application rate of 8 ml/kg seed compared favourably to the standards. However, the untreated control and treatment with *A. vera* at the application rate of 2 ml/kg did not compare to the standards and were statistically different from the standards (P<0.05). When tested at the 5% level of probability and

means compared using SNK, results for the first week post treatment showed all the treatments at the different levels performing comparatively similar (P>0.05) except for the untreated control and *A. vera* at application rate of 2 mg/kg, which were not similar to the other treatments (P< 0.05) and were significantly different from each other (P<0.05). Results for the second and third weeks showed all the treatments including the untreated control not being statistically different from each other (P>0.05) and all being statistically higher than treatments of *A. vera* at an application rate of 2 mg/kg.

Mode of Action Study

***Contact toxicity*:** Twenty four hours after topical application of treatments, the synthetic pesticide actellic showed the most significant effect on the bruchid by causing mortality. This compared favourably to treatments with neem seed oil which is statistically comparable to treatments with *M. oleifera* (SNK P= 0.05). Forty eight hours after application, treatments of *A.* vera and *M. oleifera* compared favourably to the standard treatments actellic powder and neem seed oil by causing the mortality of the bruchids that were statistically superior to the treatments with *A. annua* and the untreated control which are not statistically different from each other (Table 3). By the seventh day (166 hrs) of the treatment, all the treatments showed significant control of the bruchids that compared favourably to each other (SNK) at the 5% level of significance and were statistically higher than the untreated control.

Table 3: Contact Toxicity of Treatments on *C. subinnotatus*

	Mean number of dead insects/ hours post application						
Treatment	24	48	72	96	120	144	168
A.vera	2.33^{c}	7.33^{a}	9.0^{a}	9.33^{ab}	9.33^{a}	9.67^{a}	10.0^{a}
A.annua	0.33^{c}	0.67^{b}	0.67^{b}	1.67^{cd}	3.0^{c}	6.0^{b}	8.33^{a}
M. oleifera	4.67^{b}	8.33^{a}	9.33^{a}	10.0^{a}	10.0^{a}	10.0^{a}	10.0^{a}
Neem	7.67^{ab}	10.0^{a}	10.0^{a}	10.0^{a}	10.0^{a}	10.0^{a}	10.0^{a}
Actellic	10.0^{a}	10.0^{a}	10.0^{a}	10.0^{a}	10.0^{a}	10.0^{a}	10.0^{a}
Untreated Control	0.0^{c}	0.0^{b}	0.0^{c}	0.33^{d}	0.33^{d}	0.67^{c}	0.67^{b}
SE ±	0.66 ±	0.66 ±	0.64 ±	0.66±	0.73±	0.81±	0.88±

Means followed by the same letter within each column are not significantly different (P>0.05) SNK.

Table 4: Mean Fumigant Toxicity Effect of Treatments on *C. subinnotatus*

Treatment	**First trial**		**Second trial**	
	Hours after treatment		Hours after treatment	
	72	120	72	120
A. vera	8.0^{a}	10.0^{a}	10.0^{a}	10.0^{a}
A. annua	7.0^{ab}	7.0^{a}	4.0^{cb}	10.0^{a}
M. oleifera	10.0^{a}	10.0^{a}	5.0^{b}	10.0^{a}
Neem	10.0^{a}	10.0^{a}	9.0^{a}	10.0^{a}
Actellic	10.0^{a}	10.0^{a}	10^{a}	10.0^{a}
Untreated control	5.0^{b}	9.0^{a}	1.0^{d}	3.0^{b}
SE ±	0.88 ±	0.97 ±	0.96 ±	0.94 ±

Means separated by the same letter within the same column are not statistically different from each other SNK (P=0.05)

Table 5: Result of Duncan New Multiple Range Test (Using SAS 9.0 Statistical Software)

Treatment	Mean % Repellency	Mode of action
A. vera	-13.8	Non repellent
A. annua	-10	Non repellent
M. oleifera	47.2	Repellent
Neem	43.6	Repellent
Actellic	55	Repellent

***Fumigant Toxicity*:** Fumigant toxicity of the plant materials in the first trial when compared using SNK at the 5% level of significance indicated that all the plant materials under investigation showed significant fumigant toxicity by causing mortality of the bruchids from 24 hrs post treatment which compared favourably to each other and to the standard treatments actellic powder and neem seed oil but are significantly different from the untreated control (table 4). In the second trial, the two standards compared favourably to treatments of *A. vera* and the three treatments showed significantly higher fumigant toxicity to the other treatments and untreated control. Treatments of *M. indica* and *A. annua* compared to each other and are statiscally higher than the untreated control (Table 4). By the 120^{th} hour, all the treatments compared favourably to each other and showed fumigant toxicities that are statistically higher than the untreated control (SNK) at the 5% level of significance. This is indicating that the plant materials potency may be enhanced when used in an airtight environment

Repellent toxicity: Treatments of *M. oleifera* as well as the standard treatments of actellic 2% dust and neem seed oil showed positive repellency whereas treatments of *A. annua* and *A. vera* were non-repellent in their mode of activity. When compared using the single factor analysis of variance and the transformed means compared using the Duncan New Multiple range Test (Using SAS 9.0 Statistical Software), the treatments were not significantly different from each other ($P>0.05$) at the 5% level of significance (Table 5).

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