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Effect of Plant Powders on Survival, Oviposition and Progeny Development of *Callosobruchus Maculatus* for Protection of Leguminous Seeds during Post Harvest

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Abstract: ***Insect infestation on leguminous plants particularly cowpea causes high destruction that lead to nutritional and economic losses. Hence powders from two medicinal plants namely Bersama abyssinica and Aristolochia elegans were tested for their efficacy as survival, oviposition and progeny development inhibitors of Callosobruchus maculatus to ensure food security. The experiments were conducted under laboratory temperature and relative humidity conditions and the powders were applied at rates 0.5g per 30g of cowpea seeds as contact toxicity to insects placed in Petri dishes. Results of contact toxicity assay showed that powders of Bersama abyssinica root bark and Aristolochia elegans seeds were effective against the adult C. maculatus by inducing 100% mortality within 5 days of application at the rate of 0.5g/30g of cowpea seeds. Few number of eggs approximately 1.50 ± 0.65 - 4.25 ± 0.65 per 50 seeds of cowpea seeds were oviposited and there was no progeny development of the cowpea weevil in seeds treated with plant powders. In untreated experiment the adult Callosobruchus maculatus survived and were able to lay eggs. The eggs were hatched and developed to F_1 generation where 3.75 ± 0.25 approximately 45.8% adult C. maculatus emerged after 4 weeks .In the positive control, 100% mortality of C. maculatus occurred within 24 h and there was negligible seed loss in treated seed where as in untreated cowpea seed loss incurred by 37.00 ± 0.00 %. This study showed that all the tested plant powders were toxic to cowpea weevil thus could be applied by farmers by mixing with cowpea seeds to avert hatching of the eggs and development of larva, a destructive stage for management of insects and maintain nutritional and seed value in cowpeas.***

Keywords: Cowpea weevil, legumes, *Bersama abyssinica*, *Aristolochia elegans*, Nutrition

1. Introduction

Callosobruchus maculatus is an insect belonging to a species of beetle commonly known as the cowpea weevil, cowpea seed beetle or bean beetle as it commonly feed and infest different parts of the regumes [1]. *Callosobruchus maculatus* is a member of the leaf beetle family, Chrysomelidae, and not a true weevil [2]. It is a field to store pest since its infestation on cowpea begins in the field as the mature pods dry and when such seeds are harvested and stored [3]. This insect is major constraint of cow pea production as it begins from field by feeding on its inner protein thus causing nutrients loss [4]. The pest population increases rapidly under favorable conditions and results in total destruction within a short duration of time [5]. The larvae of this species feed and develop exclusively on the endosperm seed of legumes (Fabaceae),whereas adults do not require food and spend their lifetime approximately 1–2 weeks for mating and laying eggs on leguminous seeds hence extra efforts are required to control larva, a destructive stage so as to protect seeds from damage [6] [7]. *Callosobruchus maculatus* has been reported also to be a world threat of leguminous seed [8] and it costly affect agricultural sector of Africa and Asia that produce large amount of this kind of regume [9] [10]. So far various efforts has been employed to protect seed damage so as to ensure food security although currently pest control in stored food products relies greatly on the application of gaseous fumigants and residual contact insecticides that are hazardous [11]. For example it has been claimed that synthetic pesticides kills untargeted organisms, are carcinogenic to human and environmentally unfriendly [12] [13]. Having seen impacts caused by synthetic pesticides, there is an urgent need to searching for effective and affordable pesticide from plant sources. However there is no study conducted to determine insecticidal property of *Bersama abyssinica* and *Aristolochia elegans* against cowpea seeds destructive insects. Hence in this study *Bersama abyssinica* and *Aristolochia elegans* powders were evaluated for insecticidal and protectant effect against bruchid beetle, *Callosobruchus maculatus*.

2. Materials and Methods

This study was conducted in the Life science and bio-engineering laboratory at Nelson Mandela African Institution of Science and Technology, Arusha-Tanzania

2.1 Preparation of plant materials tested

Leaves, stem bark and root bark of *Bersama abyssinica* were collected from Ilolo village of Rungwe district in Mbeya whereas Aristolochia elegans leaves and seeds were collected from Nambala village in Arusha, Tanzania. The plant materials were authenticated by Mr. Daniel Sitoni of Tropical Pesticides Research Institute (TPRI) and then air dried under shade for two weeks before pulverization. Powders of *Bersama abyssinica* leaves, stem bark and root bark and *Aristolochia elegans* leaves and seeds were prepared by separately grinding approximately 200g each plant part in an electric grinding machine. Then the resulting powders were sieved through a 25-mesh sieve to obtain fine powders.

2.2 Collection and Preparation of Cowpea Seeds

Cowpea seeds used for this study were obtained from a newly stocked seeds free of insecticides at Arusha market .The cowpea seeds were first cleaned and disinfested by washing in sterilized clean water then dried in air and kept in fridge for at -5 ^{0}C for 7 days to kill all hidden insects. The disinfected cowpeas were warmed in an oven at 40 °C for 4 h and then air dried in the laboratory to prevent moldiness before they were stored in plastic containers with perforated tight lids [14].

2.3 Insect culture

Callosobruchus maculatus used in this study were obtained from Sokoine University of Agriculture, Morogoro-Tanzania. The culture was maintained in plastic containers with perforated lids under ambient temperature of 25 ± 5°C and 70 – 75 % relative humidity as described by [15].The insects were raised on uninfested local cultivar of cowpea and subsequent insects were drawn from this culture.

2.4 Survival, Oviposition and Progeny Development Inhibition assay

The toxicity assay was done according to method employed by [16] with some minor modifications whereas the oviposition and progeny inhibitory assay was adopted from [17] with some improvement. Thirty gram (30 g) of seeds were placed in Petri dishes arranged in seven treatments each replicated four times .The treatments were powders of *Bersama abyssinica* leaves, stem bark, root bark *Aristolochia elegans* leaves and seeds including positive control, the Actellic dust and untreated cowpea seeds which saved as negative control.. The plant part powders and Actellic dust were administered on cowpea seeds at the rate of 0.5 g per 30 g of seeds. Then 3 pairs of copulating adult *C. maculatus* less than ten hours old were introduced and confined in each Petri dish for five days. The experiments were arranged in Completely Randomized Design in the laboratory for seven days before data collection commenced. The cowpea weevils were left to oviposit on the seeds for seven days and were carefully shifted out after they had died. Each group of the experiment was suspended in a bowl of water so as to prevent predation of eggs laid by red ants.

Data were collected on the total number of egg oviposited, number of eggs hatched (eclosion) and number of adults that emerged from each treatment and analyzed using analysis of variance from Statistical Package for Social Science version 21.0 (SPSS).

2.5 Seed loss assay

The assay was performed according to the method of [18] with some modification but at maintained room temperature in the laboratory. Then 3 copulating pairs of adult cowpea beetle were introduced into each of the boxes and left for a week to allow egg deposition. After egg deposition, adult insects were removed from the boxes and the development of the eggs was monitored for another 4 weeks. In the positive control trial Actellic dust was used to treat cowpea seed at the doses of 0.5 of plant powder per 30g of seeds before 5 copulating pairs of the cowpea beetles were introduced into the boxes. In negative control only 30g of cowpea was left with 3 pairs of cowpea beetle. The controls were also left for a week to allow egg deposition before adult weevils were relocated. The developments of the eggs were monitored for another 4 weeks and each of the experiments was performed in four replicates. At the end of the fourth week, the components of each box were poured in a dish and cowpea seeds were sorted out of the plant powders. The seeds were further sorted into those with developing eggs or insects or holes and those without. Those having developing insects/holes were counted as lost seeds. The percentage seed loss was calculated by:

$$\% \text{ seed loss} = \frac{\text{Weight of seeds + insect/holes x 100}}{\text{Initial weight of seeds/No of seeds}}$$

3. Results

The effect of plants powders on survival, oviposition and development of *Callosobruchus maculatus* as contact insecticides are depicted in Table 1, 2 and 3.The result presented in this report shows that the plant powders were able cause mortality within five (5) days in which *Bersama abyssinica* root bark and *Aristolochia elegans* seeds powders caused 100% .The Bersama abyssinica stem bark was able to cause an average of 93.33% mortality whereas *Bersama abyssinica* and *Aristolochia elegans* leaves caused 80.00 and 79.16% respectively. Comparing with controls, in untreated cowpea seeds no any insect mortality was observed while in positive control, the Actellic dust induced 100% within 48 hr thus is claimed for causing side effects. The mortality effects caused by powders had consistence effects on oviposition, eclosion and adult development as observed in this study.

With regard to oviposition effect, the result showed that in cowpeas treated with *Bersama abyssinica* leaves, stem bark and root bark only 3.25 ± 0.48, 4.50 ± 0.65 and 1.50 ± 0.96 respectively eggs were oviposited whereas the cowpea seeds treated with *Aristolochia elegans* leaves and seed only 4.25 ± 0.65 and 1.50 ± 0.65 eggs were oviposted as compared to untreated experiment where 18.50 ± 0.65 eggs were recorded within 5 days only. This shows that plant powders tested have ability to protect cowpea seeds from infestation.

On the other hand the result on eclosion showed that even the oviposited eggs were not able to hatch due to contact toxicity caused by plant powders. In experiment treated with *Bersama abyssinica* leaves, stem bark and root bark an average of 1.25 ± 0.48, 0.50 ± 0.50 and 0.25 ± 0.25 new *C.maculatus* emerged respectively and an average of 1.50 ± 0.50 and 0.25± 0.25 emerged in cowpea treated with *A.elegans* leaves and seeds respectively. In untreated experiment an average of 4.00 ± 0.28 new insect emerged showing that the plant powders significantly possessed insecticidal activity. This activity impacted heavily on the development of C.maculatus into adult as an average of 0.25 ± 0.25 -0.75 ± 0.25 adults survived after 4 weeks whereas in untreated cowpea seeds an average of 3.75 ± 0.25 adults developed and survived within after weeks.

In the seed loss assay result showed that cowpeas treated with Bersama abyssinica leaves, stem bark, root bark prevented seed loss by 93.75, 96.00 and 98.5% respectively whereas Aristolochia elegans leaves and seeds prevented cowpea seed loss by 98.10 and 100% showing that plant powders are effective seed protectant. However in untreated cowpeas 37.00% of cowpea seeds lost within 7 weeks whereas in Actellic dust treated cowpea no seed loss was observed.

Table 1:. Mortality of *C. maculatus* on cowpea seeds treated with powders of different plant parts of *Bersama abyssinica* and *Aristolochia elegans* at the rate of 0.5g/30g of cowpea seeds

Plant powder	*% Mortality within 5 days*				
	Day 1	*Day 2*	*Day 3*	*Day 4*	*Day 5*
B.abyssinica leaves	17.50	20.00	22.50	55.00	80.00
B.abyssinica stem bark	\22.50	47.50	47.50	62.50	93.33
B. abyssinica root bark	33.33	50.00	75.00	72.50	100
A.elegans leaf	20.00	37.50	50.00	70.83	79.16
A.elegans seeds	50.00	62.50	83.33	87.50	100
Actellic dust	95.80	100	-	-	-
Untreated	0.00	0.00	0.00	0.00	0.00

Table2. Insecticidal effect of *Bersama abyssinica* and *Aristolochia elegans* powders from different plant parts on Oviposition, hatching and development of *Callosobruchus maculatus* on cowpea seeds

Plant/Control Powder	**Mean No. of eggs laid ± SE in 5 days**	**Mean eggs hatched ± SE in 4wks**	**Mean of adult ± SE after 4 wks**
A. elegans leaves	4.25 ± 0.65	1.50 ± 0.50	0.75 ± 0.25
B.abyssinica leaves	3.25± 0.48	1.25 ± 0.48	0.75 ± 0.25
B. abyssinica stem bark	4.50 ± 0.65	0.50 ± 0.50	0.50 ± 0.50
B.abyssinica root bark	1.50 ± 0.96	0.25 ± 0.25	0.25 ± 0.25
A.*elegans* seed	1.50 ± 0.65	0.25± 0.25	0.25 ± 0.25
Actellic dust(+control)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Untreated(-control)	18.50 ± 0.65	4.00 ± 0.28	3.75 ± 0.25

Table 3: Seed loss percentage in cowpea seeds treated with plant powders after 7 weeks

Plant powder tested	% cowpea seed loss
Bersama abyssinica leaves	6.25 ± 0.25
Bersamaabyssinica stem bark	4.00 ± 0.00
Bersama abyssinica root bark	1.5 ± 0.50
Aristolochia elegans leaf	1.90 ± 0.25
Aristolochia elegans seeds	0.00 ± 0.00
Actellic dust	0.00 ± 0.00
Untreated	37.00 ± 0.00

4. Discussion

Plant powders applied by local communities are capable of causing up to 100% mortality of *Callosobruchus maculatus* [19]. Likewise in the present study the *Bersama abyssinica* root bark and *Aristolochia elegans* seeds powders were able to cause total mortality within 5 days. Even though no plant powder caused 100% mortality within 24h as in of a positive control, Actellic dust treatment. However, the synthetic pesticides have been reported to pose side effects to human and environment thus this results are considerably effective in managing pests in stored foods [20] [21].

Plant powders also have been reported to reduce Oviposition rate, progeny development and survival of *Callosobruchus maculatus* [22]. In the present study most of insect died or unable to move when probed with sharp object showing that the plant powders affected their mobility and mating thus little number of eggs was oviposited on seeds and this could be due to intoxication caused by powders [23]. The reduction in oviposion in late days was caused by increase in days dosage of powders as compared to first days [24]. For instance seeds treated with *Bersama abyssinica* stem bark powder an average number of 4.5 egg per 50 seeds were laid but an average of only 0.5 egg was hatched, this could be due to failure of male beetle to fertilize eggs because were weakened by powder [25].

Results reported in this study showed that *Bersama abyssinica* and *Aristolochia elegans* powders have insecticidal effects on cowpea weevil, *C. maculatus* at all levels of treatment although varied with the treatment and plant powder applied. Generally the two plant species powders applied as contact insecticides were very effective against *C. maculatus* by causing 100% mortality of adult *C. maculatus* at rate 0.5g/30g of cowpea seeds within 5 days of application. They also reduced oviposition and completely inhibited progeny development by 50-100%. This shows that *Bersama abyssinica and Aristolochia elegans* species have oviposition deterrent, ovicidal and lavicidal properties. This activity could be attributed by the presence of secondary metabolites which have been reported in these plants [26]-[28]. Again, the lethal effect of tested plant powders on cowpea bruchid could be as a result of contact toxicity because insects breathe by means of trachea which usually opens at the surface of the body through spiracles [14]. These spiracles might have been blocked by the powders hence leading to suffocation.

The result reported in this study showed that the seed loss in cowpea treated with plant powders was highly prevented by 100-98% as compared to control where 37% seed loss was observed within 7 weeks. This result is comparable to the report of [29] that highlighted that insects are capable to cause loss up to 100% seed damage and 60% weight loss in untreated cowpeas within only four months of storage. The powders of *Bersama abyssinica* stem bark and *Aristolochia elegans* seeds afforded to prevent seed loss 100% of loss which was comparable with the study done by [30] that reported the efficacy of plant powders for protection of seeds. These results are also in consistence with the study done by Getu (2009) which reported that cowpeas treated with plant powders did not lose their weight and seeds as compared to untreated cowpeas. Another study reported that high weight loss in African cereals and legumes is caused by uncontrolled *Callosobruchus maculatus* [31].

5. Conclusion

The findings of present study showed that *Bersama abyssinica* and *Aristolochia elegans* powders could be potential bioorganic pesticides for control against cereal and grains pests. This is because all plant parts powders tested were able to inhibit or reduce Oviposition, emergence and development of adult *Callosobruchus maculatus*. Therefore this study acknowledges the application of botanicals for protection of cowpea seeds in storage as it is an inexpensive, effective technique and easily adapted technology by rural poor farmers. However further studies to improve the efficiency of plant natural products as insecticides are required to advance agricultural sectors in developing countries including Tanzania as these compounds are cost effective and environmentally friendly as compared to synthetic insecticides.

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