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Bioefficacy Of Plant Derivatives On The Repellency, Damage Assessment And Progeny Production Of The Cowpea Weevil, *Callosobruchus Maculatus* (F) (Coleoptera: Bruchidae)

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Abstract

A laboratory experiment was conducted to investigate the efficacy of different plant derivatives that affect the development of the cowpea weevil, Callosobruchus maculatus(F) (Coleoptera:Bruchidae)fed on cowpea, Vigna unigulculata (W) seeds. The leaf extracts of the aromatic plant, Anisomeles malabarica (Br.ex.Sins) and Azadirachta indica (A.Juss) were evaluated for their repellency, damage assessment and progeny production of Callosobruchus maculatus. The results revealed that the extracts of the two plant species caused a considerable reduction in the number of weevils. The combination of neem seed kernel extract and leaf extracts of Anisomeles malabarica was the most effective in checking the insect infestation and allowing the least number of F_1 *adults emerging from the seeds over the other treatments. Acetone extracts of leaves of Anisomeles malabarica were more toxic to adult beetles compared to ethanolic plant extracts. It was concluded that the botanical products acted as insect antifeedant and the order of repelleny of the two plant leaf and kernel extracts on cowpea weevil were: combination of neem seed kernel extract + Anisomeles malabarica leaf extract > neem > Anisomeles malabarica*.

Keywords: Repellency; Damage assessment; Progeny production

Introduction

Grain Crops are one of the most important food staples due to their valuable nutrient content. The production of most of the crops is seasonal and farmers must store the harvested crops to ensure continuous food supply for their household until the next harvest. Furthermore, those resource-poor farmers can sell the crop for cash or exchange it for other products when they have a better market than at the time of harvest (Proctor, 1994). The storage time varies from one month to more than a year (Proctor, 1994). Grain crops are commonly stored on-farm in a small scale due toothier valuable nutrient content and relative ease in storage when they are dried after harvest (Duke, 1981). However, storage is one of the most crucial post-harvest operations.

Stored product insects can infest grain all year round under favourable conditions.All storage insect pests undergo complete metamorphosis, that is they reproduce by laying eggs, which produce immature forms (larval stages and pupae) and become adults. Most insect pest species have short developmental periods from egg to imago and thus complete several generations a year (Zakladnoi, 1987). The fast development, high fecundity and fertility of stored grain insects under optimal conditions and their high resistance to unfavourable habitat conditions, i.e. temperature/humidity variations, can lead to very high damage during storage (Zakladnoi, 1987).

The cowpea bruchid, Callosobruchus maculatus (Fabricus) (Coleoptera: Bruchidae) is a cosmopolitan field-to-store pest ranked as the principal post harvest pest. It caused substantial quantitative and qualitative losses manifested by seed perforation, reduction in weight, market value and germinability of seeds (Anonymous, 1989). About 4% of

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the total annual production or about 30,000 tonnes values at over 30 million US dollars is lost annually in Nigeria alone to cowpea bruchid(Casewell,1980).

With the limitations on the use of current pest control methods, there is scope for the discovery of safe, non-polluting, bio-rational pest management technologies for stored products. Extrapolation of the number of plant species studied and the number of compounds known suggests that millions of different compounds possess activity against pests which could be isolated from different plant species Harborne.1998).

The use of plant-based pesticides in grain protection has a long history (Chimbe and Galley, 1996) and there are a number of bibliographic databases on the use of different botanicals or parts of plants (leaves, twigs, roots,seeds) or their extracts (hot/cold water) or residues (ash, husk) by farmers in developing countries against stored product insect pests (Dales, 1996). These databases provide information on the plant materials, target organism, toxic level of the compound or extract or whole plant material, economic value and active principles when they are known.

Small-scale farmers have evolved their own grain protection techniques using botanicals available in their surroundings (Belmain & Stevenson, 2001). This could be due to the fact that plant materials, which are being used by farmers, are readily available to them, they have a broad knowledge of the traditional use of those plants (ethnopharmacology) as medicine or as spices or the knowledge about the plant's ecology (Cobbinah et al., 1999). Farmers use this information to select the plant part that can be used, to make their own preparations; either powdered plant material (fresh/dried) or easy water extracts or oil to treat their commodities. They believe that plant protectants are cost-effective alternatives to conventional insecticides and have appropriate advantages such as that they are available locally and do not require any packaging.

Naturally occurring compounds can affect the physiology of insects or they can modify the behaviour of insects (Bell et al., 1995). Either the compounds in thevapour phase (volatile) or non-volatile compounds can affect insects to change theirbehaviour. Those compounds can impede development, kill insects or cause losses in fecundity or viability of egg production and, therefore, reduce the number of offspring. They may act by ingestion, cuticle contact or fumigant action (Städler, 1983).

The present study revealed that the efficacy of Neem and Anisomeles malabarica (seed kernel and leaf extracts) afford better protection in cowpea against the infestation of the cowpea weevil, Callosobruchus maculatus.This study has shown that the tested plant materials when applied to cowpea significantly reduced progeny emergence and killed adults. It was obvious that the combined effect of neem seed kernel and leaf extracts of Anisomeles malabarica were observed to be more effective in checking repellency, damage assessment and percent weight loss of the seed than the control.

Materials and Methods

Biology of *Callosobruchus maculatus*

C.maculatus is a cosmopolitan pest of stored legumes(Fabaceae),particularly of the genus *Vigna.* Females cement eggs to the surface of the host (Messina, 1991).Larvae burrow into the seeds where their entire development is completed. Larvae cannot move among seeds and are thus restricted to the seed that their mother has chosen for them. Beetles emerge from seeds reproductively mature. Emerging adults are well adapted to storage conditions, requiring neither food nor water to reproduce. Because beetles most commonly occur in seed stores, lab conditions do not significantly vary from their their natural conditions.

Rearing of *Callosobruchus maculatus* :

A small population of beetles was reared and bred under laboratory conditions on the seeds of cowpea (*Vigna unguiculata*) inside a growth chamber at *30* ± 2^0C, 12:12 L: D and with 70% RH. Initially, 50 pairs of 1-2 day old adults were placed in a jar containing cowpea seeds. The jars were sealed and a maximum of 7 days were allowed for mating and oviposition. Then parent stocks were removed and cowpea seeds containing eggs was transferred to fresh cowpea seeds in the breeding jars that were covered with pieces of cloth fastened with rubber band to prevent the contamination and escape of beetles. The subsequent progenies of the beetles were used for all experiments.

Test Plant Materials

The leaves of *Anisomeles malabarica* (Br.ex.Sins) and *Azadirachta indica* (A.Juss) seeds were collected from plants growing in and around the Bharathiar University Campus, Coimbatore, Tamil Nadu, India. The leaves were thoroughly washed and air-dried in the shade; the dried leaves were manually ground into powder with the help of a mortar and pestle.

Bioassays

Experiment 1: Repellency bioassay

An olefactometer was constructed from a large (19 cm diameter) plastic petridish.Ten small (5 cm diameter) petridishes were attached to the central dish around its circumference and a small hole was made to allow free passage between each small petridish and the large central dish.In the lid of the large dish,a small hole was made to allow the release of insects into the chamber. This hole was closed during the experimental tests preventing insect escape.

Twenty seeds were thoroughly mixed with 2 ml of each plant extract. Residual extract was allowed to evaporate from the seeds. This experimental procedure was repeated for plant extract in each of the solvent.Each experimental test was replicated 4 times. For each replicate, fifty *C.maculatus* adults were released into the large dish and twenty grains, soaked in individual plant extract were placed in the small peripheral dishes. The direction of movement of beetles was recorded at 15 min,30 min,1 hr, 2 hr and 24 hr intervals.

Experiment 2: Damage assessment

Damage assessment was carried out on treated and untreated grains. Samples of 100 g of grains were taken from each jar and the number of damaged grains was counted and weighed.

$$\text{Percentage seed weight loss} = \frac{UNd - Dnu}{U\,(Nd + Nu)} \times 100$$

where U = weight of undamaged grain
D = weight of damaged grain
Nd = number of damaged grains
Nu = number of undamaged grains

Experiment 3: Progeny production

20 pairs of beetles were introduced into treated and control grains and after 30, 15 and 7 days oviposition period for *C.maculatus* respectively, the parent adults were removed. Insect subsequently emerging were counted to estimate the F1 progeny production. Counting was stopped after 63. 42 and 42 days *C.maculatus* respectively, to avoid overlapping of generation [Bokel *et al.*, 1997]

Statistical Analysis

All data were subjected to analysis of variance (ANOVA) and the means were separated using Duncan's multiple range test (Duncan, 1955).

Results

The repellent activity of extracts of neem seed kernel (NSKE) and AMLE at three concentrations against *Callosobruchus maculatus* at one- hour interval showed that maximum repellent activity (81%) was observed for NSKE at 2% concentration after 1 hour.(Tab.1) While increasing in time, the repellent activity was decreased for all the other extracts. When the plant powders of neem seed kernel and powdered leaves of *Anisomeles malabarica* at three different concentrations against *Callosobruchus maculatus* at one-hour interval were treated, maximum repellent activity (76%)was observed for NSKP (2% conc) after 1-hour treatment, followed by 62% repellency in AMLP.(Tab.2).

The results of damage assessment of *Callosobruchus maculatus* on cowpea after the treatment of acetone plant extracts was discussed in Tab3.Percentage of damage were decreased while increasing the concentration of the extract. Among the acetonic extracts studied, NSKE showed better activity (14%) than AMLE (19%). The damage assessment of *Callosobruchus maculatus* on cowpea after the treatment of ethanolic plant extractsshowed that increase in concentration causes a decrease in damage of the seeds(Tab.4). Among the ethanolic extracts tested, NSKE (18%) showed higher activity than AMLE (23%).However, the acetone extracts of NSKE showed maximum protection than the ethanolic extracts of NSKE.The damage assessment of *Callosobruchus maculatus* on cowpea after the treatment of plant powders of neem seed kernel and powdered leaves of *Anisomeles malabarica*.showed that a decrease in percentage of damage was observed while increasing the concentration of the powder. Among the treatments, NSKP exhibited better activity(12%) than AMLP(33%).But the NSKE treated grains caused less seed damage than NSKP treated grains(Tab.5).

The emergence of F1 progeny of *Callosobruchus maculatus* after the treatment of different plant powders proved that various plant powders caused a significant reduction of progeny of *Callosobruchus maculatus.* NSKP significantly reduces the progeny production of. *Callosobruchus maculatus* than AMLP. (Tab.6). But the combined treatment of NSKP and AMLP showed maximum progeny reduction. The progeny production of *Callosobruchus maculatus* after the treatment of acetone and ethanolic plant extracts was provided in Table 7.The acetone plant extracts of NSKE was more effective in reducing the F_1 progeny than the ethanolic extracts. Among the extracts tested, the combinations of acetone extracts of NSKE and AMLE proved to be the best plant materials in controlling the emergence of F_1 individuals

Discussion

In the present investigation, the efficacy of neem seed kernel and *Anisomeles malabarica* afford better protection to the infestation of the cowpea weevil, *Callosobruchus maculatus*. In the present study to evaluate the toxic effect of neem and *Anisomeles malabarica* ,it was observed that the seed kernel extracts were more effective in checking the mortality and oviposition than the control.

Pradhan *et al* (1963) reported that neem seed kernel possess an extra ordinary gustatory repellent properties, much higher than neem leaf powder against the desert and migratory locusts. Rouf *et al* (1996) studied that mixing of neem leaf powder with lenthil seeds resulted in reduced oviposition and adult emergence of the pulse beetle, *Callosobruchus chinensis*. Pandey *et al* (1986) reported that a petroleum ether extract of neem leaves and twigs mixed with green gram seeds inhibited the oviposition of *Callosobruchus chinensis*. Butterworth and Morgan(1971) revealed that the most active antifeedant is reported to occur in neem seed kernel powder, Further, the results are confirmed by who also reported that Azadirachtin is a major compound in the seed kernel responsible for the reduced oviposition and adult emergence in beetles. Neem has many other activities against insects disrupting or inhibiting development of eggs, larvae or pupae, preventing the molting of larvae or nymphs, disrupting mating and sexual communication, repelling larvae and adults, deterring females from laying eggs, sterilizing adults, poisoning larvae and adults, feeding deterrent, blocking the ability to swallow by reducing the motility of the gut preventing metamorphosis, thus preventing adult maturation, inhibiting the formation of chitin, the substance essential for the insect to form an exoskeleton This huge array of insecticidal properties of Neem is thought to be due to it's adversely effecting the insects hormone system. (Joshi and Sitaramaiah, 1979).

This study also demonstrated the potential of using *Anisomeles malabarica* (Br.ex.Sins) to control *Callosobruchus maculatus* in stored cowpea. *Anisomeles malabarica*, commonly called as Malabar catmint is a highly aromatic plant belonging to the family Lamiaceae. (Joshi,2000). The plant powder obtained from this plant in this study can be used as the most conventional insecticides, which are less toxic to humans(Duke,1985). This may be due to the presence of volatile compounds such as anisomelic acid, betulinic acid, citral , gerainic acid and ovatodiolide in the plant extract(Guha Bakshi *et al*,1999). Of the above phytochemicals, it is supposed that Citral plays a key role as insecticide in controlling the bruchid population and disrupt the physiological body mechanism of *Callosobruchus maculatus*. (Prajapati and Kumar, 2003). Treatment of various plant extracts and powder treatment caused repellency effect to *Callosobruchus maculatus,* after the treatment of NSKE, AMLE, NSKP and AMLP. Neem seed kernel extract and AMLE extract interacted together and caused higher repellency index. The phytochemicals like azadirachtin present in neem extract and citral present in Anisomeles plant extract may suppress the phago stimulation and arrest the physiological events of the beetle. The antifeedant effects of azadirachtin are well known (for reviews see Jacobson, 1989; Schmutterer, 1990; Ascher, 1993; Mordue and Blackwell, 1993). Both primary and secondary antifeedant effects have been observed in the case of azadirachtin Ascher, 1993). Primary effects include the process of chemoreception by the organism (e.g. sensory organs on mouthparts which stimulate the organism to begin feeding) whereas azadirachtin results from blockage of input receptors for phagostimulants or by the stimulation of deterrent receptor cells .

The damage assessment of *Callosobruchus maculatus* on cowpea after the treatment of NSKE, AMLE, NSKP and AMLP was studied at three different concentrations as individual and combined treatment. Higher concentrations of extracts and powders studied reduced the seed damage effectively than individual treatment. Among the treatments, NSKE and AMLE showed higher activity than other combinations. This clearly suggests that the plant extracts contain powerful phytochemicals, which suppress the chemoreceptors in the mouthparts of the beetle and reduced the feeding in *C.maculatus.*

Neem's efficacy to non-target and beneficial organisms has been documented in previous and recent literature (Schmutterer, 1995; Ascher, 1993; Murugan *et al*., 1999). Many biologically active compounds can be extracted from neem, including triterpenoids, phenolic compounds, carotenoids, steroids, and ketones. The tetranortriterpenoid azadirachtin has received the most attention as a pesticide because it is relatively abundant in neem kernels and has shown biologicalactivity on a wide range of insects. Azadirachtin is actually a mixture of seven isomeric compounds labeled as azadirachtin-A to azadirachtin-G with azadirachtin-A being present in the highestquantity and azadirachtin-E regarded as the most effective insectgrowth regulator (Verkerk *et al.*, 1993). Many other compoundshave been isolated that shows antifeedant activity as well asgrowth regulating activity on insects. This cocktail of compounds significantly reduces the chances of tolerance or resistance developing in any of the affected organisms. However, only four of the compounds in neem have been shown to be highly effective in their activityas pesticides: azadirachtin, salannin, meliantriol, and nimbin(Jacobson, 1990; National Research Council, 1992).

The emergence of F_1 progeny of *Callosobruchus maculatus* on cowpea after the treatment of NSKE, AMLE, NSKP and AMLP suggests that the acetone plant extracts of NSKE was more effective in reducing the F_1 progeny than the ethanolic extracts. The insect groyh regulatory effect of azadiracthin and citral causes various developmental, post-embryonic, reproductive and growth inhibitory affects in insects so that the emergence of F_1 generation is prevented.

Thus it is concluded that the botanical materials tested here could be useful and further studies are recommended to determine the possibility of the use of these plant species in the control of other storage pests also and to elucidate the effectiveness of the adult emergence deterrency of the plant species against storage pests. The results of the investigation would indicate a significant potential for these plants as a possible source of natural products that could be used as an alternative to synthetic insecticides(Zebitz, 1987).

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Tables

Table 1. Repellent activity of different plant extracts on *Callosobruchus maculatus*

Treatment	**Conc. (%)**	**Repellency (%)**			
		1 HAT	**2 HAT**	**3 HAT**	**4 HAT**
NSKE	0.5	61 [c]	55 [d]	50 [d]	47 [c]
	1	72 [ab]	64 [ab]	60 [ab]	55 [b]
	2	81 [a]	72 [a]	67 [a]	64 [a]
AMLE	0.5	52 [bc]	50 [cd]	46 [cd]	43 [cd]
	1	61 [c]	56 [c]	51 [c]	46 [d]
	2	73 [b]	65 [b]	62 [b]	54 [ab]
Control	0	0	0	0	0

Within a column means followed by a same letter is not significantly different 5% level of DMRT

NSKE = Neem Seed Kernel Extract, AMLE = *Anisomeles malabarica* Leaf Extract

Table2: Repellent activity of different plant powders on *Callosobruchus maculatus*

Treatment	Conc. (%)	Repellency (%)			
		1 HAT	2 HAT	3 HAT	4 HAT
NSKE	0.5	52 ^{d}	41 ^{d}	34 ^{cd}	27 ^{cd}
	1	68 ^{b}	53 ^{c}	40 ^{c}	35 ^{c}
	2	76 ^{a}	64 ^{a}	59 ^{a}	42 ^{ab}
AMLE	0.5	46 ^{cd}	40 ^{cd}	37 ^{d}	32 ^{d}
	1	54 ^{c}	53 ^{c}	50 ^{ab}	45 ^{b}
	2	62 ^{ab}	58 ^{b}	54 ^{b}	49 ^{a}
Control	0	0	0	0	0

Within a column means followed by a same letter is not significantly different 5% level of DMRT

NSKE = Neem Seed Kernel Extract, AMLE = *Anisomeles malabarica* Leaf Extract

Table 3. Damage assessment of *Callosobruchus maculatus* on cowpea after the treatment of acetone plant extracts.

Treatment	Conc. (%)	% of damage
NSKE	2	35 ^{b}
	4	27 ^{c}
	6	20 ^{d}
	8	14 ^{e}
AMLE	2	64 ^{b}
	4	48 ^{c}
	6	33 ^{d}
	8	19 ^{e}
Control	0	97 ^{a}

Within a column means followed by a same letter is not significantly different 5% level of DMRT

NSKE = Neem Seed Kernel Extract, AMLE = *Anisomeles malabarica* Leaf Extract

Table 4. Damage assessment of *Callosobruchus maculatus* on cowpea after the treatment of ethanol plant extracts.

Treatment	Conc. (%)	% of damage
	2	42 [c]
	4	36 [d]
NSKE	6	27 [cd]
	8	18 [f]
	2	72 [b]
AMLE	4	51[ab]
	6	42 [c]
	8	23 [e]
Control	0	97 [a]

Within a column means followed by a same letter is not significantly different 5% level of DMRT

NSKE = Neem Seed Kernel Extract, AMLE = *Anisomeles malabarica* Leaf Extract

Table 5.Damage assessment of *Callosobruchus maculatus* on cowpea after the treatment of certain plant powders.

Treatment	Conc. (g)	% of damage
	2	45 [b]
NSKP	4	31[c]
	6	20 [d]
	8	12 [e]
	2	75 [b]
AMLP	4	56 [c]
	6	42 [d]
	8	33 [e]
	2+2	36 [b]
	4+4	24 [c]
NSKP+AMLP	6+6	15 [d]
	8+8	9 [e]
Control	0	95 [a]

Within a column means followed by a same letter is not significantly different 5% level of DMRT

NSKP= Neem Seed Kernel Powder, AMLP = *Anisomeles malabarica* Leaf Powder

Table 6. Emergence of F_1 progeny of *Callosobruchus maculatus* on cowpea after the treatment of certain plant powders.

Treatment	No: of F_1 adults
NSKP	7 ^{c}
AMLP	9 ^{b}
NSKP+AMLP	6 ^{ab}
Control	18^{a}

Within a column means followed by a same letter is not significantly different 5% level of DMRT

NSKP= Neem Seed Kernel Powder, AMLP = *Anisomeles malabarica* Leaf Powder

Table 7. Emergence of F_1 progeny of *Callosobruchus maculatus* on cowpea after the treatment of certain plant extracts.

Treatment	No: of F_1 adults	
	Acetone extract	**Ethanol extract**
NSKE	5 ^{ab}	6 ^{ab}
AMLE	7 ^{b}	8 ^{b}
NSKE+AMLE	4 ^{c}	5 ^{c}
Control	15 ^{a}	15 ^{a}

Within a column means followed by a same letter is not significantly different 5% level of DMRT

NSKE = Neem Seed Kernel Extract, AMLE = *Anisomeles malabarica* Leaf Extract