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Mortality Effects of Some Medicinal Plants on the Pulse Beetle *Callosobruchus chinensis (Coleoptera: Bruchidae)*

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Abstract

Effect of ethanol extract of the leaf of plants *Hydrocotyl asiatica, Boerhavia diffusa, Bacopa monieri* and *Trichosanthes cucumarina* was tested against the pulse beetle *Callosobruchus chinensis*. The efficacy of the extract on the insects was dose-dependent. Different doses were checked and the results showed that high doses of the extracts were significantly more toxic to *Callosobruchus chinensis* compared to lower doses. LD50 value was assessed using probit analysis. The effect of the plant *Hydrocotyl asiatica* was found to be most significant causing highest mortality compared to other plants. DNA fragmentation studies also showed similar results and points out the significance of the medicinal plants in controlling the pest.

Introduction

Insects have been causing tremendous losses not only to the crops growing in fields but also to post-harvest commodities during storage. Stored products are attacked by insects in different ways. It can be a continuation of a field attack or the eggs may be laid in the field itself and the damage may occur in storage or the infestation may continue from the material stored earlier and be carried over to fresh material stored later in go down or storage house. Different kinds of stored product pests are seen in India like Indian meal moth, Saw toothed grain beetle, Red flour beetle, Rice weevil, Cowpea weevil, Warehouse beetle, Drugstore beetle etc. Among them the pulse beetle, *Callosobruchus chinensis L.* (Coleoptera: Bruchidae) is one of the the most widespread and destructive major insect pest of stored legumes [1]. Adult beetle is 3-4 mm long. Eggs are whitish, elongated and stuck on the grains or on pods and sometimes on the surface of the container. Hatching larvae bore inside and spend their life within the seeds. Infestation commonly begins in the field, where eggs are laid on maturing pods. Control of stored-product insect populations is primarily dependent upon continued applications of insecticides. In spite of its efficacy, their repeated use for several decades has disrupted biological control system by natural enemies and led to outbreaks of insect pests, widespread development of resistance, undesirable effects on non-target organisms, and environmental and human health concerns. These problems have highlighted the need for the development of new types of selective insect-control alternatives. Plants may provide potential alternative to currently used insect-control agents because they constitute a rich source of bioactive chemicals. Many medicinal plants and spices have been used as pest control agents [2,3]. Farmers and researchers often claim the successful use of plant materials in insect pest control, including ash [4,5], vegetable oils [6,7] plant extracts [8,9], and botanical powders [10,11]. It has been reported that certain plant preparations and traditional methods are much safer than chemical insecticides [12,13] Keeping this in view, the present study was carried out to test the efficacy of the leaf extracts of four plants *Hydrocotyl asiatica, Boerhavia diffusa, Bacopa monieri* and *Trichosanthes cucumarina*.

Materials and Methods

Test insects

Newly emerged adults were used for the experiments. Adult beetle is 3-4 mm long, female being larger, brownish in color, broader at shoulders and rounded posteriorly. There are dark patches on elytra and thorax. Adults show sexual dimorphism. Males possess deeply emarginated or indented eyes and prominently serrate antennae, while in female these characters are not distinctly marked. In females tip of abdomen is exposed while in males it is covered by elytra.

Culturing of test insects

Experiments were conducted in the Entomology Research Laboratory, Department of Zoology, University College Thiruvananthapuram. The pulse beetle, *Callosobruchus chinensis L.* adults were obtained from naturally infested green gram seeds from local markets. The adult male and female beetles were reared on clean and un-infested green gram (*Vigna radiata L*). The seeds were made uninfected by washing with clean water. . Three jars each of of 300 gm were used. Each jar was filled with 200 gm chickpea grains and about 100 beetles were added to each jar. The jars were then covered.

Preparation of ethyl alcohol extract of plants

For the extraction, soxhlet apparatus was used. About 25 gms powder of each shade dried plant leaves were extracted with 250 ml ethyl alcohol. The extraction of each plant sample was done in about 12 hrs. After soxhlet extraction; the material was run on rotary evaporator. The extracts were concentrated on rotary evaporator by removing the excess solvent under vacuum. After evaporation of solvent with rotary evaporator the remaining extracted material was kept in a water bath for removing remaining solvent from the extracts. The extracts were stored seperately at 4°C prior to application.

Treatments

The extracts were applied at different doses on Whatmann No. 1 filter paper and air-dried for an hour. The controls were treated with ethyl alcohol. The treated and control filter paper discs were placed singly at the bottom of plastic jars and 200 gms of green gram seeds were placed on the papers. Hundred insects *were* released in each plastic container. There were three replicates for each treatment and control. Observations were recorded on the seventh day of treatment.

Mortality studies

Data on mortality was recorded on seventh day of treatment. LD 50 for each plant was assessed using probit analysis [14].

DNA fragmentation studies

DNA was isolated from cells of both control and treated insects (Qiagen DNA isolation kit using spin column method). Transfer 100 cells of control and treated sample to 1.5 ml sterile micro-centrifuge tubes. Centrifuged at 2000 rpm in an Eppendorf table top centrifuge for 5 minutes at 4°C and removed supernatant. Added 20 µl of TES lysis buffer. Mix cell pellet with TES lysis buffer bystirring with a wide-bore pipette tip. Add 10 µl of RNase Cocktail and mixed well by flipping the tip of the tube. Do not vortex. Incubate for 30-120 minutes at 37°C. Add 10 µl of proteinase K, mix by flipping the tip of the tube, and incubate at 50°C for at least 90 minutes or, if preferred, overnight. Added 5 µl of 6X DNA loading buffer and loaded DNA samples into dry wells of a 1-1.5% agarose gel in TAE containing 0.5 µg/ml ethidium bromide. For size reference, also loaded a 100-bp size ladder. Instead of adding ethidium bromide directly into the agarose gel, DNA can also be visualized by staining the gel after electrophoresis in 1µg/ml ethidium bromide-containing TAE buffer for 1 hour and de-staining in water. Ran the gel at a low voltage, which improves resolution of DNA fragments DNA ladders are finally visualized by a UV light source and documented by photography.

Observation and Result

Effect of plant extract on mortality of insects

The total number of adult insects surviving after the treatment was recorded for seven days consecutively. The percent mortality was then calculated. No mortality was seen in the case of control. LD 50 was calculated using probit analysis (Tables 1 and 2).

Effect of plant extract on DNA fragmentation

DNA fragmentation studies showed the effect of plant extract. The four plants contain different chemicals which caused DNA fragmentation in the treated insects. Fragmentation in DNA was seen in insects treated with ethanol extract of the plants. In control there was no fragmentation of DNA (Figure 1).

Discussion

Higher plants are rich sources of novel natural substances that can be used to develop safe methods for insect control [15]. In India the ethanolic extracts of plant leaves like Bakain (*Meliaazedarach*), Mint (*Mentha longifolia*), Habulas (*Myrtus communis*), Lemongrass (*Cymbopogon citratus*) and Datura (*Datura stramonium*) were tested against stored grain pests [16] Research reveals that extracts prepared from plants have a variety of properties including insecticidal activity, repellency to pests, anti feedant effects, insect growth regulation, toxicity to nematodes, mites and other agricultural pests, also antifungal, antiviral and antibacterial properties against pathogens [17]. Plants like *Hydrocotyl asiatica*, *Boerhavia diffusa*, *Bacopa monieri* and *Trichosanthes cucumarina* contains various compounds which are known to be insect repellent and toxic to insects. DNA fragmentation indicates the effect of plant extracts in impairing the cells and genomic content of the insect. The various compounds like triterpenoids, rotenoids, alkaloids and glycosides present in the plants probably caused DNA fragmentation. Anti-mitotic, anti-proliferative, anticancer activity and DNA fragmentation was obtained by using ethanol and chloroform extracts of *Revia hypocrateriformis* on human leukemic cells [18]. Willow leaf extracts also caused DNA fragmentation in human tumor cells [19].

Plants	dose	LD 50	Probit value
Hydrocotyl asiatica	0.5	1.2	1.34
Boerhavia diffusa	1.5	1.5	1.53
Bacopa monieri	2.5	1.8	1.85
Trichosanthes cucumarina	3.5	2	2.05

Table 1: LD 50 of ethanol extract of *Hydrocotyl asiatica, Boerhavia diffusa, Bacopa monieri* and *Trichosanthes cucumarina* on *Callosobruchus chinensis.*

Plants	dose	Mortality %
Hydrocotyl asiatica	0.5	48
	1.5	54
	2.5	58
	3.5	68
Boerhavia diffusa	0.5	46
	1.5	50
	2.5	58
	3.5	66
Bacopa monieri	0.5	44
	1.5	46
	2.5	56
	3.5	64
Trichosanthes cucumarina	0.5	44
	1.5	48
	2.5	54
	3.5	60

Table 2: Effect of plant extract on mortality of *Callosobruchus chinensis.*

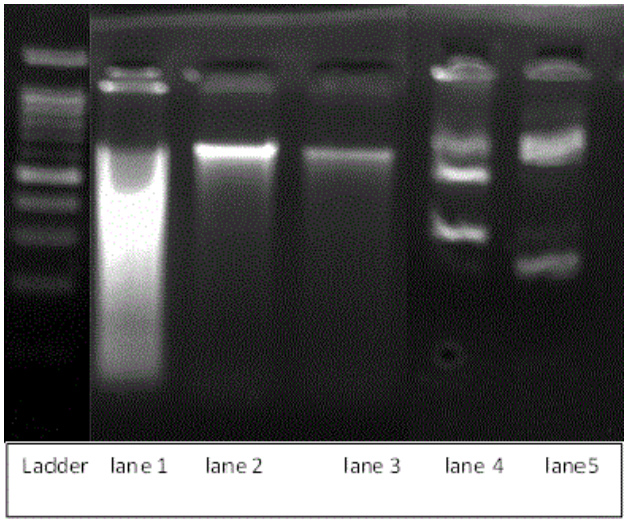

Figure 1: Fragmentation of DNA isolated from the cells of Callosobruchus chinensis treated with ethanol extract of different plants. Lane 1: Hydrocotyl, Lane 2: Boerhavia; LANE 3: control; LANE 4: Bacopa; LANE 5: Trichosanthes; LADDER (100bp)

References

1. Park I, Lee S, Choi D, Park J, Ahm Y (2003) Insecti-cidal activities of constituents identified inthe essential oil from the leaves of Chamaecyparis obtuse against C. chinensis L. and S. oryzae (L). Journal of Stored Product Research 39: 375-384.
2. Lale NES (1992) A laboratory study of the comparative toxicity of products from three species to the maize weevil. Postharv Biol Technol 2: 612-664.
3. Isman MB (1995) Leads and prospects for the development of new botanical insecticides. Rev Pestic Toxicol. 3: 1-20.
4. Ofuya TT (1986) Use of wood ash, dry chilli, pepper fruits and onion scale leaves for reducing C. Maculates damage in cowpea seeds during storage. Journal of Agricultural Science 107: 467-468.
5. Ajayi O, Arokoyo JT, Nesan OO, Olaniyan M, Ndire-Mbula M, et al. (1987) Laboratory assessment of the efficacy of some local materials for the control of storage insect pests. Samaru J Agric Res 5: 81-85.
6. Schoohoven AV (1978) Use of vegetable oils to protect stored beans from Bruchid attack. J Econ Entomol 1: 254-256.
7. Kazi SA, Takao I, Toshihide I (1999) Effects of plant oils on oviposition preference and larval survivorship Callosobruchus chinensis (Coleoptera: Bruchidae) on azuki bean. J App Entomol and Zoology 34: 547-550.
8. Chiasson H, Vincent C, Bostanian NJ (2004) Insecticidal properties of a Chenopodium-based botanical. J Econ Entomo 97: 1378-1383.
9. Devanand P, Usha Rani P (2008) Biological potency of certain plant extracts in management of two lepidopteran pests of Ricinus communis L. Journal of Biopesticides 1: 170-176.
10. Abdullahi YM, Muhammad S (2004) Assessment of the toxic potentials of some plants powders on survival and development of Callosobruchus maculatus. African Journal of Biotechnology 3: 60-62.
11. Gupta L, Srivastava M (2008) Effect of Withania somnifera extracts on the mortality of Callosobruchus chinensis L. Journal of Biopesticides 1: 190-192.
12. Verma J and Dubey NK (1999) Perspective of botanical and microbial products as pesticides of tomorrow. Current Science 76: 172-179.
13. Subramanyam B, Weaver DW (2000) Integrated management of insects in stored products. Marcel Dekker Inc., New York.
14. Muhammad Akram Randhawa, (1944) Calculation of LD50 values from the method of Miller and Tainter. J Ayub Med Coll Abbottabad 21: 184-185.
15. Arnason JT, Philogene BJR and Morand P (1989) Insecticides of plants origin. American Chemical Society Symposium Series 387.
16. Manzoor F, Nasim G, Saif S, Malik SA (2011) Effect of ethanolic plant extracts on three storage grain pests of economic importance. Pak J Bot 43: 2941-2946.
17. Prakash AJ, Rao (1997) Evaluation of botanical pesticides as grain protectants against rice weevil S. oryzae. Proc.Symp. Botanical pesticides in IPM Rajamundry, 360-365.
18. Saboo SS, Khadabadi S, Tapadiya GG (2012) In vitro evaluation of anti mitotic, anti proliferative, DNA fragmentation and anticancer activity of chloroform and ethanol extracts of Revia hypocrateriformis. Asian Pacific Journal of Tropical Disease 2:S503–S508.
19. El-Shemy HA, Aboul-Enein AM, Aboul-Enein KM, Fujita K (2007) Willow leaves' extracts contain anti-tumor agents effective against three cell types. Plos One 2: 178.