

EFFECT OF PLANT OILS ON CALLOSOBRUCHUS CHINENSIS L.

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Abstract: Oils obtained from neem (*Azadirachta indica* A. Juss.) seed kernel, the Himalayan cedar wood (*Cedrus deodara* Roxb) and their combination (1:1) each at 3, 2 and 1% concentrations were tested against adults of pulse beetle (*Callosobruchus chinensis* L.). Cedar wood oil exhibited highest fumigant potential at 3, 2 and 1% concentrations showing corrected inhibition (knock-down) of 100, 100 and 96%, respectively. While neem oil + cedar wood oil (1: 1) at 3, 2 and 1% concentrations inhibited 96, 84 and 51.66%. The least corrected % knock-down of 62, 45 and 32 were observed by application of neem oil at 3, 2 and 1%, respectively, by Potters spray tower in 24 h of treatment. However, corrected percent mortality of the same study reveals highest mortality in the cedar wood oil rather than neem and their combinations. Topical application by Arnold hand micro applicator at the rate of 0.5 III per insect at 3, 2 and 1% concentrations of both the oils and their combinations showed negligible mortality after 24 and 48 h of treatment. In general observations on the behavioural effects due to treatment under Potter's spray tower method showed that cedar wood oil and its combination with neem oil treated insects quickly recovered from the anesthetic effect when compared to insects in neem oil treatment and control. In ovipositional behavioural experiment, cedar wood oil treated chickpea, *Cicer arietinum*, grains had 134.3, 114.0 and 112.3 mean number of eggs/5females while neem oil had 98.3, 118.7 and 57 at 3, 2 and 1% concentrations, respectively. Methanol + acetone (1:1) treated control had only 89.67 eggs/5 females. However, negligible difference was noticed among 3, 2 and 1% concentrations of cedar wood oil + neem oil and control. Joint action potential of Himalayan cedar wood oil with neem oil and its possible use in the formulation of botanical insecticides are discussed.

Keywords: *Azadirachta indica*, biopesticide, *Callosobruchus chinensis*, *Cedrus deodara*, fumigant, oviposition behaviour, toxicity.

Introduction

Synthetic pesticides lack quick biodegradability and lead to accumulation of residues, development of resistance in insect species, destruction to beneficial arthropods and aquatic animals etc. This has necessitated the search for naturally occurring pesticidal sources which are considered to be species specific with many fold biopotentials: biodegradable and altogether ecofriendly (Raguraman, 1987). During the last two decades, the neem tree, *Azadirachta indica*, has been under close scrutiny for both medicinal and pesticidal potentials leading to commercialization. So far, more than 400 insect species have been found to be

sensitive to neem products (Schmutterer and Singh, 1995). Neem compounds/derivatives are also reported to affect behaviour and physiology of major stored grain insect pests: *Sitophilus oryzae*, *Trogoderma granarium*, *Tribolium castaneum*, and *Corcyra cephalonica*; *Callosobruchus* spp. Another tree of Indian origin, the Himalayan cedar wood (*Cedrus deodara* Roxb) has also been identified as toxic to the pulse beetle (Singh and Rao, 1985), and grain protectant against *Sitophilus oryzae* (Singh et al., 1989) Considerable bioactivities of neem oil and cedar wood oil on various insect species reported led us to investigate the comparative study of both oils and their joint action potential through knock-down effect, mortality and ovipositional behaviour against an important pest of stored grains, the pulse beetle, *Callosobruchus chinensis* L.

Materials and methods

Insect Culture

The experiment was conducted in the Entomology Laboratory, Division of Plant Quarantine, ICAR-National Bureau of Plant Genetic Resources, New Delhi during June 2020 to August 2020. The pulse beetle, *Callosobruchus chinensis* was mass reared in the Entomology Laboratory, Division of Plant Quarantine, ICAR-National Bureau of Plant Genetic Resources, New Delhi during June 2020 to August 2020 on chickpea grains in jars at an average temperature of $25 \pm 1^\circ\text{C}$ and $69 \pm 5\%$ RH with alternating light and dark periods of 12 h.

Fumigant/Contact Action Test

Healthy, active and uniform sized adults of *C. chinensis* were treated with neem oil, cedar wood oil and their combination (1:1) at 3, 2 and 1% concentration in methanol + acetone (1:1) solvent. Test insects were anesthetized by using diethyl ether. Twenty anesthetized adult beetles were kept in a clean and dry Petri dish (size: 10-cm dia X 1-cm high) and 1 ml of solution was sprayed as described by Singh and Singh (1991). Each treatment was replicated three times. Control insects were treated with 1 ml spray of methanol + acetone (1:1) solvent.

Direct Toxicity Test

Fresh, healthy and active adult pulse beetles were treated with the neem oil, cedar wood oil and their combination (1:1) at 3, 2 and 1% at a dose of 0.5 μl per insect by Arnold hand microapplicator (Mis Burkard, UK) as described by Singh and Jain (1987). All treatments were replicated three times. Observations of both fumigant and direct toxicity tests were recorded after 24 and 48 h of treatment. Data on fumigant and direct toxicity tests are presented in Table 1.

Ovipositional Behavioural Tests

The gender of freshly emerged adults of *C. chinensis* was determined. Males and females, 5 each, were released in a Petri dish containing 20 chickpea grains treated with neem oil, cedar wood oil and their combinations (1:1) 3, 2 and 1% concentrations. In each concentration 1 ml of spray fluid was used. Each treatment was replicated three times. The number of eggs laid on chickpea grains after 5 days of treatment was observed and expressed as average number of eggs laid/5 females (Table 2). All the three experiments were carried out at an average temperature of $25 \pm 10/0$ C and $69 \pm 5\%$ RH.

Results

Fumigant/Contact Action

Table 1 shows that cedar wood oil treated adults of *C. chinensis* had very high fumigant effect of 100, 100 and 96% knockdown at 3, 2 and 1% concentrations, respectively, over both neem oil alone and

Table 1. Toxicity of neem (*Azadirachta indica*) and cedar wood (*Cedrus deodara*) oils against the pulse beetle (*Callosobruchus chinensis*)

Treatment	Fumigant/Contact Action		Direct Toxicity	
	Mortality percentage after		Mortality percentage after	
	24h	48h	24h	48h
Neem oil 3%	62.66	61.30	3.33	0.00
Neem oil 2%	45.33	56.54	3.33	1.66
Neem oil 1%	32.00	47.02	3.33	0.00
Cedar wood oil 3%	100.00	89.28	1.66	3.66
Cedar wood oil 2%	100.00	68.75	5.00	0.00
Cedar wood oil 1%	96.00	40.77	0.00	0.00
Neem oil + cedar wood oil (1: 1) 3%	96.00	72.32	1.66	1.66
Neem oil + cedar wood oil (1: 1) 2%	84.00	28.57	0.00	0.00
Neem oil + cedar wood oil (1: 1) 1%	51.66	21.72	0.00	0.00

neem oil + cedar wood oil combination after 24 h of treatment. The same concentrations of cedar wood oil, after 48 h of treatment, caused mortality of 89.28, 68.75 and 40.77%, respectively. Cedar wood oil, neem oil + cedar wood oil combinations (1:1) also caused appreciable knockdown at 1, 2 and 3% concentrations, which ranged between 51.66 and

96.00%. For neem oil it ranged between only 32 and 62.66%. The 3% treatment of neem oil + cedar wood oil caused 72.32% mortality after 48 h of treatment; the other two concentrations (2 and 1%) exhibited low mortality. In the same experiment observations on the behavioural responses of *C. chinensis* adults after 2 h of treatment of oils at different concentrations showed that the cedar wood oil and its combinations had knockdown effect besides being recovered from the effect of anesthesia. The insects were very lethargic and they were unable to get up from their upside-down position due to knock-down effect. The insects in neem controls, after only 2 h, were active and fast moving, similar to controls. The same situation prevailed 20 h after treatment.

Table 2. Effect of neem (*Azadirachta indica*) oil and cedar wood (*Cedrus deodara*) oil on the oviposition of the pulse beetle (*Callosobruchus chinensis*).

Treatment	Average number of eggs laid per 5 females
Neem oil 3%	98.33
Neem oil 2%	118.67
Neem oil 1%	57.00
Cedar wood oil 3%	134.33
Cedar wood oil 2%	114.00
Cedar wood oil 1%	112.33
Neem oil + cedar wood oil (1: 1) 3%	95.67
Neem oil + cedar wood oil (1: 1) 2%	84.67
Neem oil + cedar wood oil (1: 1) 1%	87.00
Control	89.67

Direct Toxic Effect Application of neem oil, cedar wood oil, and their combination in three different concentrations at the dose of 0.5 J.11 per insect through Arnold hand microapplicator did not show any remarkable toxic effect to the adults of *C. chinensis* after 24 and even 48 h of treatment (Table 1). Effect on Oviposition Behaviour It is evident from Table 2 that number of eggs per five females of 134.3, 114 and 112.3 were recorded at 3, 2 and 1% concentration of cedar wood oil, respectively, when compared to control (89.67 eggs/5 females) on chickpea grains after 5 days of treatment. But the neem oil treated chick pea grains at 3, 2 and 1% concentration had 98.3, 118.6 and 57 eggs/5 females and normal ovipositional behaviour was observed among cedar wood oil + neem oil (1:1) combination treatments (3, 2 and 1% concentrations) which ranged between 84.67 and 95.67 eggs/5 females, at par with control.

Discussion

Neem oil, cedar wood oil and their combinations (1:1) tested each at 3, 2 and 1% concentrations showed a dose-dependent mortality of *C. chinensis* adults (Table 1). A knockdown effect to the tune of 100% was noticed in cedar wood oil treatments. Further, cedar wood oil 3% exhibited a high mortality of 89.28% and was followed by neem oil + cedar wood oil (1:1) 3% (72.32%) and cedar wood oil 2% (68.75%) after 48 h of treatment. This might possibly be due to the fumigant effect of cedar wood oil as is evident from the present study, where the cedar wood oil and its combination treatments showed quick knock-down effect even within 2 h of treatment. Similar observations have been made by Singh and Rao (1985) where Himalayan cedar wood oil treated *C. chinensis* adults suffered 50% mortality even at 1% concentration; however, they have not worked out the combination effect of cedar wood oil with neem oil. In the present study, neem oil 3% caused 61.30% mortality of *C. chinensis* adults following next to the cedar wood oil 3 and 2% and neem oil + cedar wood oil 3% concentrations, after 48 h of treatment. It is evident from the earlier work and from the present study that the mortality under neem oil was due to both contact and fumigant effects. But the neem oil tested at 0.5 µl dose in the present study did not show any contact effect. This might be due to low dosage or low contact toxic principle applied on each individual insect in this experiment. No specific reference is available on the combined potential of neem oil with cedar wood oil. It is interesting, in the present study, that only 3% concentration of neem oil + cedar wood (1:1) showed mortality on par with cedar wood oil 3% concentration. It proves the combined potentials of both the oils, though tested at 50% dose (from individual oils) at 3% concentration. The lower concentrations (2 and 1%) of neem oil + cedar wood oil were least effective. This might possibly be due to low doses of both the oils. Ovipositional behaviour of the pulse beetle due to treatments of chickpea grains showed that Himalayan cedar wood oil treated seeds were infested with the highest number of eggs/5 females (134.33, 114 and 112.33); neem oil had 98.33, 118.67 and 57 eggs/5 females, at 3, 2, and 1% concentrations, respectively (Table 2). Normal ovipositional as observed in control was recorded in neem oil + cedar wood oil (1:1) at all concentrations. More number of eggs laid in cedar wood oil as well as neem oil treatments may be nonviables since forced egg laying. This ovipositional behaviour of laying eggs by the pulse beetle may also be attributed to the vapour effect of cedar wood oil and neem oil on females moving on the seeds to lay eggs under no choice condition. This area of investigation on physiological and biochemical approach may be studied in future. It has been pointed out by Saxena (1995) that the increased use of neem materials may lead to tolerant pest species, as

it is evidenced from a cereal grain pest, *Oryzaephilus surinamensis* and *O. acuminatus* which survive on old neem kernels itself. In this context, combination of cedar wood oil with neem oil will certainly enhance the biopotentials of neem and vice versa. Evidently, development of tolerance/resistance may be managed by using two kinds of potent materials together because chances of developing tolerance/resistance is considered to be more with one type of compound as compared to one-time application of two types of formulated materials. Further, addition of certain chemicals to slow down the evaporational loss of the essential oil which is to be mixed with neem oil or any other botanical insecticide(s) may also be attempted to use the essential oils effectively in insect pest management.

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