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Bioefficacy of four essential oils against *Callosobruchus analis* (F.) (Coleoptera: Bruchidae), A seed pest of stored legumes world wide

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Abstract
Laboratory bioassay of the essential oils extracted from *Zanthoxylum armatum* DC., *Rabdosia rugosa* Wall. ex Benth, *Artemisia maritima* Linn. and *Colebrookea oppositifolia* Sm. by hydrodistillation was carried out against the major bruchid pest *Callosobruchus analis* (F.) to evaluate their oviposition deterrence, ovicidal activity and progeny deterrence. There was a significant difference in the number of eggs laid on treated and control sets and among the different treatments of essential oils. *Z. armatum* at 100 µl/ml allowed the bruchid to lay only 19.15±3.6 eggs as compared to 82.35±4.5 in control and proved to be most effective treatment with 76.74% oviposition deterrency. *R. rugosa* and *A. maritima* oil were found most effective in reducing the egg hatchability to 48.00±3.2 and 49.52±2.2% respectively at a lowest dose of 10 µl/ml. Egg hatching inhibition percentage increased with an increase in concentration of all the treatments. *R. rugosa* oil at 100 µl/ml proved to be most effective in reducing the adult emergence with 85.48% progeny deterrence followed by *A. maritima* showing 81.67% deterrency. All the tested essential oils revealed a wide range of bioactivities against the insect pest.

Keywords: Ovipositon deterrency, essential oils, bruchid pest, progeny emergence, ovicidal activity

Introduction
Several bruchid species attack cereals and pulses in the store and causes a loss of 10- 15% with a germination loss ranging from 50- 92% (Adugna 2006) [2]. The cowpea weevil *Callosobruchus analis* (F.) (Coleoptera: Bruchidae) is considered as a pest of economic importance for stored-leguminous grain (Southgate 1979, Rehaman 1989, Khandwe *et. al*, 1997; Shafique and Ahamad, 2002) [22, 8, 20]. This pest has been observed infesting seeds of 15 genera, including peanut, chickpea, bean, pea, cowpea and soyabean (Waterworth, 1986) [23]. Although effective fumigants and contact synthetic insecticides are available, there is global concern about their negative effects such as ozone depletion, environmental pollution, toxicity to non-target organisms, pest resistance and pesticide residues (Shaaya *et al*., 1997) [18]. Due to the detrimental usage of these synthetic insecticides, there is need to look into the use of botanical insecticides, which tend to have wide spectrum of activities, readily biodegradable, less toxic to mammals, and may be selective in action and the growth of development of resistance. They may be easily or cheaply produced. Compounds extracted from plants, or the derivatives of such compounds may affect insect physiology in various ways. In the search for alternatives to conventional fumigants, essential oils extracted from aromatic plants have been widely investigated. Essential oils often constitute the bioactive fraction of plant extracts (Shaaya *et al*,. 1991; 1997; Regnault- Roger 1997) [19, 13]. Their lipophilic nature facilitates their interference with basic metabolic, biochemical, physiological and behavioural functions of insects (Nishimura, 2001) [10]. They have potential as ovicides, fumigants, insect growth regulators and insecticides against various insect species (Regnault-Roger, 1997; Shaaya *et al*,. 1997) [18, 14]. Many essential oils are known to possess ovicidal, repellent, antifeeding, and biocidal activities against various arthropod pests (Isman 1999; Saxena 1989) [7, 17]. The insecticidal constituents of many plant extracts and essential oils against stored product insects are mainly monoterpenoids such as limonene, linalool, terpineol, carvacrol, and myrcene (Ahn *et al*., 1998; Coats *et al*., 1991) [3, 6]. The present study aimed to investigate the oviposition deterrence, ovicidal effects and progeny deterrence of four essential oils extracted from *Zanthoxylum armatum* DC., *Rabdosia rugosa* Wall. ex Benth, *Artemisia maritima* Linn. and *Colebrookea oppositifolia* Sm. against bruchid pest *Callosobruchus analis* (F.)

Materials and Methods
Extraction of essential oils
Essential oils were extracted from leaves of *A. maritima, C. oppositifolia, R. rugosa* and *Z. armatum* collected from the local areas of Shimla district of Himachal Pradesh, India. The leaves were dried in shade at room temperature (30±5 ^{0}C) and grounded by domestic mixer. The dried powdered material was hydro-distilled in Clevenger apparatus. Conditions of extraction were: 50 g of air-dried sample in 1:10 plant material /water volume ratio for 4 hrs distillation. Anhydrous sodium sulphate was used to remove water after extraction. Oil yield (2.9% w/w) was calculated on a dry weight basis. Extracted oil was stored in a refrigerator at 4^{0}C for further analysis.

Insect Culture
Culture of *C. analis* was maintained in the laboratory without exposure to any insecticide on cowpeas, in glass containers at 25 ±5^{o}C and 50 - 60% rh. Initially, 50 pairs of 1-2 day old adults were placed in a jar containing black gram seeds. The

jar was sealed and a maximum of 7 days were allowed for mating and oviposition.

Bioassays

Oviposition deterrency by essential oils

Experiment was designed by following the method of Kumar *et al.* (2008) [9] to study effect of essential oils on the oviposition behaviour of pulse beetle. A stock solution of all essential oils was prepared by dissolving 100 µl of essential oil in 1 ml of methanol. Fifty seeds of chickpea were filled in glass vials (6.3 × 2 cm diameter) and treated separately with different dose i.e. 10, 30, 50 and 100 µl of the oils. The seeds were then dressed by continuous shaking for five minutes for proper mixing of the oils on the seeds. 1 ml solvent alone was used as control. The treated seeds were placed on filter paper to evaporate the solvent. The seeds were then transferred into Petridishes and after 24 hours, 12 bruchids (6 males and 6 females) were introduced in each Petridish separately. At different time intervals the mortality of insects was observed in each Petridish and dead insects were removed. The number of eggs laid on treated and control seeds were then enumerated after ten days of oviposition.

The % deterrency of oviposition was calculated according to the equation:

$$\% \text{ Deterrency} = \frac{\text{NC - NT}}{\text{NC}} \times 100$$

Where NC is the number of eggs laid on control seeds, and NT is the number of eggs laid on treated seeds.

Ovicidal activity of essential oils

The female adults (gravid beetles) were placed on chickpea seeds and the numbers of eggs laid were enumerated. The eggs laid were collected just after oviposition to test the effect of essential oils on the eggs laid by *C. analis.* A stock solution of all the essential oil was prepared by dissolving 100 µl of essential oils in 1 ml of methanol. Plastic jars of 250 ml capacity with screw lids were used as exposure chambers. Different doses of 10, 30, 50 and 100 µl of each oil prepared in solvent were applied to a circular filter paper (Whatman No. 1). The treated filter paper discs were then introduced into the plastic jars and attached to the inner surface of the screw lid of the jar by using adhesive tape. 1 ml solvent alone was used as control. In each jar a small glass Petri dish containing about 25 eggs was placed carefully. After an exposure period of 24 hours to above compounds the eggs were transferred to clean Petri dishes and observed for hatching after 8 to 10 days. Percentage egg hatching was calculated as:

$$\% \text{ Egg hatching} = \frac{\text{No. of eggs hatched}}{\text{Total no. of eggs}} \times 100$$

Numbers of unhatched eggs in each Petri dish were counted and the percent mortality of egg was calculated by Abbott's formula

Progeny deterrency by essential oils

A stock solution of all the essential oils was prepared by dissolving 100 µl of essential oils in 1 ml of methanol and test solutions of 10, 30 50 and 100 µl of each plant oil were used for bioassay. 5 g of food media of insect pest was filled in glass vials and treated separately with different doses of each plant oil. For control sets the seeds were dressed in requisite amount of methanol. The treated seeds were placed on filter paper to evaporate the solvent for 15-20 minutes. The seeds were then transferred into Petridishes and 6 pairs of *C. analis* of mixed sex were introduced in each vial separately. The mortality of insects was observed at different time intervals. The % progeny deterrence was calculated according to the equation:

$$\% \text{ Deterrency} = \frac{\text{NC - NT}}{\text{NC}} \times 100$$

Where NC is the number of adults emerged from control seeds and NT is the number of adults emerged from treated food media.

Statistical Analysis

All the data concerning mortality were corrected by using Abbott's formula (Abbott, 1925) [1]. Tests for different bioassays were performed in triplicate and data presented are mean ± SE. The mean values were compared by one-way ANOVA and Tukey's multiple comparison tests using software SPSS, version 11.5.

Results

Oviposition deterrency of essential oils against *C. analis*

The percentage deterrency of oviposition increased with increasing concentrations of all the treatments. There was a significant difference in the number of eggs laid on treated and control and among the different treatments of essential oils. Different doses of essential oils reduced the fecundity of female *C. analis* as compared to control where maximum egg laying was recorded 82.35±4.5. At 100 µl/ml the essential oil of *Z. armatum* allowed the bruchid to lay only 19.15±3.6 eggs as compared to control and proved to be most effective treatment with 76.74% oviposition deterrency. *R. rugosa* and *A. maritima* oil also showed a remarkable acitivity and significantly deterring the majority of females from egg laying on seeds than control sets. *R. rugosa* oil at a concentration of 50 µl/ml exhibited a high deterrent activity of 70.57% followed by *A. maritima* showing 67.15% deterrency which further increased to 75.68 and 73.18% at an increased dose of 100 µl/ml for the same essential oils. The female bruchid laid 38.12±1.2 eggs on the seeds treated with 30 µl/ml of *C. oppositifolia* with 53.70% oviposition deterrency and was found least effective among all the treatments (Table 1, Fig. 1a).

Table 1: Percent deterrency in oviposition by four essential oils (at variable doses) against *C. analis.*

	Doses µl/ml	Oviposition deterrence (%)
Z. armatum	10	56.18(36.08 ±1.6)[a]
	30	65.81(28.15±2.2)[b]
	50	73.09(22.16±4.1)[c]
	100	76.74(19.15±3.6)[c]
R. rugosa	10	54.87(37.16±4.1)[a]
	30	59.65(33.22±2.5)[a]
	50	70.57(24.23±1.2)[c]
	100	75.68(20.02±2.5)[c]
A. maritima	10	53.75(38.08±1.9)[a]
	30	57.00(35.41±3.8)[a]
	50	67.15(27.05±2.4)[b]
	100	73.18(22.08±1.1)[c]
C. oppositifolia	10	51.11(40.26±4.1)[a]
	30	53.70(38.12±1.2)[a]
	50	62.10(31.21±3.6)[b]
	100	68.16(26.22±2.1)[b]
Control		(82.35±4.5)[ab]

% values are mean (n = 3) ± SE. The means within a column followed by same letter are not significantly different from each other according to ANOVA and Tukey's comparison tests.

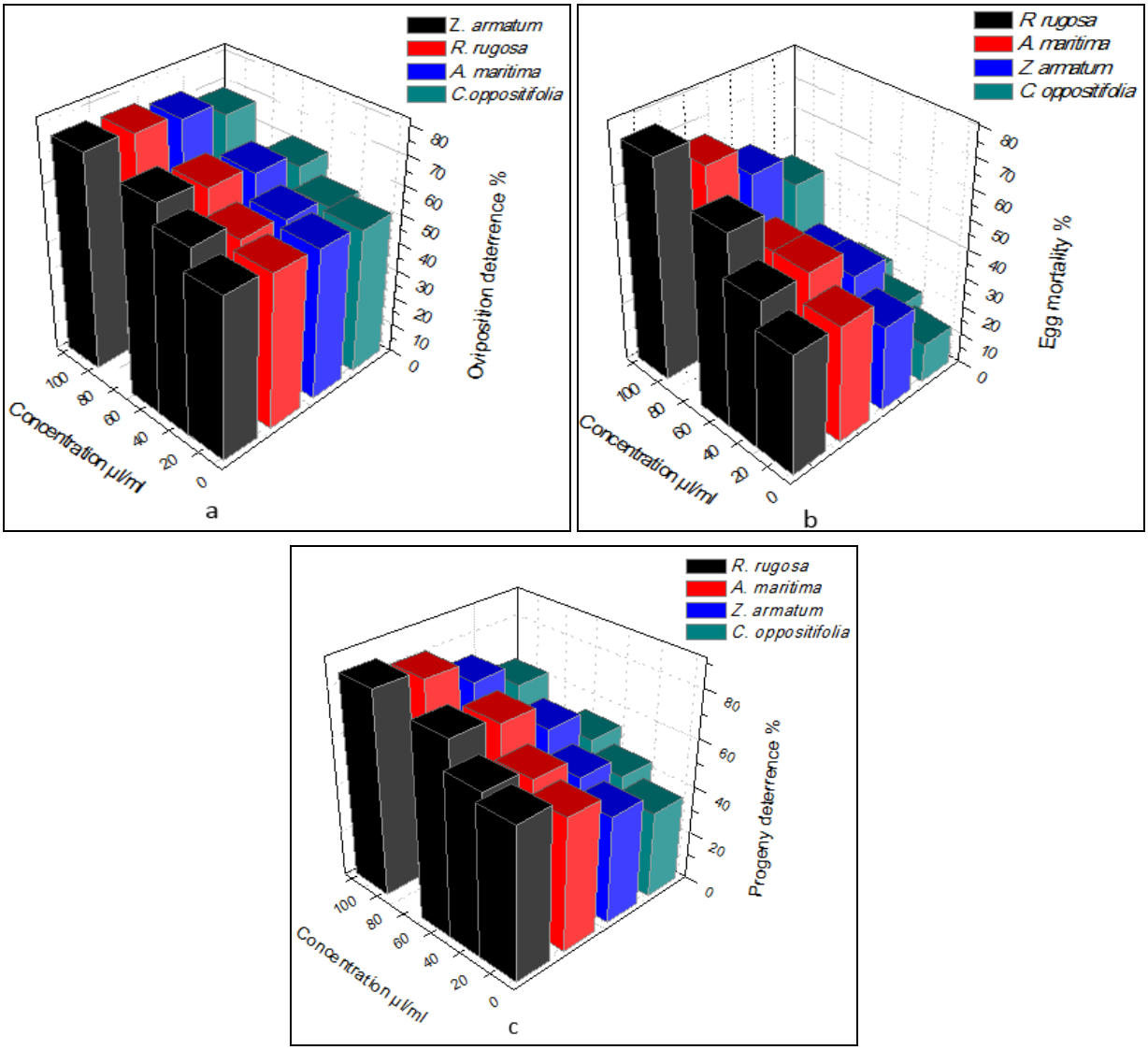

Fig 1: 3D graph representing; a) Oviposition deterrence, b) Egg mortality and c) Progeny deterrence by four essential oils against *C. analis.*

Ovicidal activity of four plant essential oils on *C. analis*

The impact of essential oils at doses of 10, 30, 50 and 100 µl/ml was evaluated on inhibition of egg hatching of *C. analis.* All the concentrations of essential oils were found effective in reducing the egg hatchability over control where egg hatching was 81.28%. The results in data showed that *R. rugosa* and *A. maritima* oil were most potent reducing the egg hatchability to 48.00±3.2 and 49.52±2.2% respectively at a lowest dose of 10 µl/ml whereas egg mortality was calculated as 40.94±1.2 and 39.07±2.4% respectively. The essential oil vapours of *Z. armatum* at a dose of 100 µl/ml obtained 55.70±4.8% egg mortality and hatching rate of eggs was found to be 36.00±1.9%. *C. oppositifolia* essential oil was least toxic than the others producing a low mortality of 44.63±1.5% against eggs of *C. analis* even at a highest dose of 100 µl/ml, while egg hatchability was recorded as 45.00±3.6% at similar concentration level. It was indicated from the data that the percentage of egg hatching inhibition in all the treatments increased with an increase in concentration (Table 2, Fig. 1b).

Table 2: Ovicidal action of four plant essential oils on the eggs of *C. analis*.

	Doses µl/ml	% Hatching	% Corrected mortality
R. rugosa	10	48.00±3.2^{a}	40.94±1.2^{b}
	30	40.16±1.2^{c}	50.59±2.8^{a}
	50	28.00±4.6^{b}	65.55±1.4^{c}
	100	20.20±1.2^{d}	75.14±2.2^{d}
A. maritima	10	49.52±2.2^{a}	39.07±2.4^{b}
	30	40.60±1.1^{c}	50.04±1.9^{a}
	50	40.00±2.8^{c}	50.78±3.6^{a}
	100	28.32±4.1^{b}	65.15±1.2^{c}
Z. armatum	10	57.68±3.2^{bc}	29.03±4.4^{ab}
	30	48.72±2.2^{a}	40.05±2.2^{bc}
	50	48.28±1.4^{a}	40.60±1.2^{bc}
	100	36.00±1.9^{b}	55.70±4.8^{a}
C. oppositifolia	10	70.08±1.1^{de}	13.77±2.2^{cd}
	30	65.28±2.3^{de}	19.68±3.1^{cd}
	50	60.40±2.8^{cd}	25.68±4.8^{ab}
	100	45.00±3.6^{a}	44.63±1.5^{bc}
Control		81.28^{ab}	-

% values are mean (n = 3) ± SE. The means within a column followed by same letter are not significantly different from each other according to ANOVA and Tukey's comparison tests.

Progeny deterrency of essential oils against *C. analis*

R. rugosa oil at 100 µl/ml resulted in 8.08±3.5 number of progeny production for *C. analis* and proved to be most effective in reducing the adult emergence with 85.48% progeny deterrence followed by *A. maritima* oil producing only 10.20±1.9 adults showing 81.67% deterrency as compared to control (55.65±5.8). At a dose of 50 µl/ml of *Z. armatum* oil 18±4.5 number of *C. analis* adults emerge successfully producing 67.33% deterrent activity in progeny production while *C. oppositifolia* oil was found to be least effective producing 20.09±2.8 adults showing 63.89% deterrent activity even at a highest dose of 100 µl/ml (Table 3, Fig.1c).

Table 3: F_1 progeny deterrence of *C. analis* under variable doses of four essential oils.

Essential oils	Doses µl/ml	Progeny deterrence (%)
R. rugosa	10	63.48(20.32±2.2)^{a}
	30	68.73(17.40±4.8)^{a}
	50	81.76(10.15±1.1)^{b}
	100	85.48(8.08±3.5)^{b}
A. maritima	10	55.97(24.50±1.2)^{a}
	30	63.46(20.33±3.4)^{a}
	50	78.22(12.12±2.2)^{b}
	100	81.67(10.20±1.9)^{b}
Z. armatum	10	45.94(30.08±2.8)^{c}
	30	54.30(25.43±3.6)^{c}
	50	67.33(18.18±4.5)^{b}
	100	72.47(15.32±2.8)^{b}
C. oppositifolia	10	36.17(35.52±2.1)^{d}
	30	45.39(30.39±1.8)^{c}
	50	54.19(25.49±3.2)^{c}
	100	63.89(20.09±2.8)^{a}
Control		(55.65±5.8)^{ab}

% values are mean (n = 3) ± SE. The means within a column followed by same letter are not significantly different from each other according to ANOVA and Tukey's comparison tests.

Discussion

There was a significant difference in the number of eggs laid on treated and control and among the different treatments of essential oils. The ability of essential oils and monoterpenoids to reduce fecundity in *Acanthoscelides obtectus* has been already reported by Regnault-Roger and Hamraoui (1994, 1995) [15]. At 100 µl/ml the essential oil of *Z. armatum* allowed the bruchid to lay only 19.15±3.6 eggs as compared to maximum egg laying of 82.35±4.5 in control and proved to be most effective treatment with 76.74% oviposition deterrency. *R. rugosa* and *A. maritima* oil also showed a remarkable acitivity significantly deterring the majority of females from egg laying on treated seeds than control sets. In a similar study Shukla *et al.* (2011) [21] recorded that 0.1µl/ml essential oil of *Callistemon lanceolatus* allowed the bruchids to lay only 12 eggs as compared to the control (302 eggs) and proved to be the most effective treatments, with 96% deterrency followed by *Lippia alba* oil (66.8%) and 1,8-cineole (65.8%). Similarly *R. rugosa* oil at a concentration of 50 µl /ml exhibited a high deterrent activity of 70.57% followed by *A. maritima* showing 67.15% deterrency. The female bruchid laid 38.12±1.2 eggs on the seeds treated with 30 µl /ml of *C. oppositifolia* and producing 53.70 % oviposition deterrency. Boekea *et al.* (2006) [5] observed that *C. maculatus* laid fewer eggs on beans treated with *Capsicum frutescens* after 24 hrs.

R. rugosa and *A. maritima* oil were most potent in reducing the egg hatchability to 48.00±3.2 and 49.52±2.2% at a lowest dose of 10 µl/ml whereas egg mortality was calculated as 40.94±1.2 and 39.07±2.4% respectively. 81.28% egg hatching was recorded in control. The essential oil vapours of *Z. armatum* at a dose of 100 µl/ml obtained 55.70±4.8% egg mortality and hatching rate of eggs was found to be 36.00±1.9%. The present findings are in parallel with the

earlier findings of several workers. Papachristos and Stamopoulos (2004) [12] tested three essential oils and found that lavender and rosemary showed the highest toxicity against the eggs of *A. obtectus* while eucalyptus essential oil proved to be less toxic. Baskaran *et al.* (2010) [4] found that *Allium sativum* obtained 91.66 and 61.22% hatching rate followed by *Ocimum basilicum* with 90.80 and 64.11% of hatching at 100 and 200 ppm concentrations respectively against eggs of *C. maculatus. C. oppositifolia* essential oil was least toxic than the others producing a low mortality of 44.63±1.5% against eggs of *C. analis* even at a highest dose of 100 µl/ml. The oil vapours diffused into eggs and affected the physiological and biochemical process associated with embryonic development. *R. rugosa* oil at 100 µl/ml resulted in 8.08±3.5 progeny production for *C. analis* and proved to be most effective in reducing the adult emergence while in controls the adult emergence was 55.65±5.8. *Z. armatum* also caused a significant progeny reduction even at a dose of 50 µl/ml with a progeny deterrence of 67.33% for *C. analis.* In related studies *Chenopodium* and *Clausena* oils checked more than 84% of adult emergence of both bruchids *C. analis* and *C. maculatus* at different doses (Pandey *et al.*, 2011) [11]. *Aegle* oil checked more than 70% of adult emergence of *C. chinensis* at different doses (Kumar *et al.*, 2008) [9]. The reduction in adult emergence could either be due to reduced fecundity, egg-mortality or larval mortality or even reduction in hatching of the eggs.

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