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Biopesticidal potential of green chemicals against *Callosobruchus analis* (f.) (Coleoptera: Bruchidae)

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Abstract

Pulses have 20-27% proteins which is 2- 3 times higher than traditional cereals. These constitute the main source of proteins for developing countries, like India where per capita consumption of the animal protein is low, thus they are rightly considered the **poor man's meat.** India is largest pulse consumer, importer and producer country of the world occupying an area of 228.47 lakh hectares with the production of 17380 million tones every year. With the United Nations declaration of 2016 as International Year of Pulses to replace the social evil of malnutrition by legume, the research pertaining to the biology and bio intensive management of bruchids pests has become increasingly important. Therefore, laboratory bioassay of essential oils which are regarded as "Green Chemicals" extracted from *Zanthoxylum armatum* DC., *Rabdosia rugosa* Wall. ex Benth, *Artemisia maritima* Linn. and *Colebrookea oppositifolia* Sm. by hydro distillation was carried out against *Callosobruchus analis* (F.) to evaluate biopesticidal potential in terms of oviposition and progeny deterrence and ovicidal activities. 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 deterrence. *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% deterrence. All the tested essential oils revealed a wide range of bioactivities against the bruchid pest.

Keywords; Ovipositon deterrence, essential oils, bruchid pest, progeny emergence, ovicidal activity.

Introduction

Bruchids attack cereals and pulses both in fields and store and responsible for 10- 15% loss along with a germination inability varying from 50- 92% (Adugna 2006). The cow pea weevil *Callosobruchus analis* (F.) (Coleoptera: Bruchidae) a pest of economic importance for stored-leguminous grain worldwide (Southgate 1979, Rehaman 1989, Khandwe *et. al,* 1997 and Shafique and Ahamad, 2002). Due to the persistence usage of the synthetic insecticides, there is need to unveil the bio-pesticides and insecticides, which tend to be specific on the target species and biodegradable and less toxic to mammalian species. In the search for alternatives to conventional fumigants, essential oils, now designated as "green chemicals" extracted from aromatic plants have been widely investigated. Essential oils have the bioactive fraction of plant extracts (Shaaya *et al,.* 1991; 1997; Roger and Hamraoui 1997). They have potential as fumigants, ovicides, insect growth regulators and lethal against wide range of insect pests (Roger, 1997 and Shaaya *et al.,* 1997). 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* L. and *Colebrookea oppositifolia* Sm. against bruchid pest *Callosobruchus analis* (F.) a pest of stored legumes worldwide.

Material and methods

Leaves of *R. rugosa, C. oppositifolia, A. maritima* and *Z. armatum* were dried in shade and grounded followed by 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. Extracted oil was stored in a refrigerator at 4^0C for further analysis. Cultures of *C. analis* were maintained in the laboratory on cowpeas, in glass containers with their open mouth covered with muslin cloth. Initially, forty pairs of 24 hours old adults were placed in a jar containing host seeds. Experiment was designed by following the method of Kumar *et al.* (2008). Fifty seeds of chickpea filled in glass conical flask were treated separately with different doses of the oils. After 24 hours, 6 males and 6 female bruchids were introduced in each Petridish separately. Mortality of insect was recorded and the number of eggs laid on treated and control seeds were 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.

The number of eggs laid by gravid females were enumerated and exposed to different doses of essential oils. After an exposure period of 24 hours the eggs were 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

Five gm of food media for each insect species was treated separately with different doses of oils. The seeds were then transferred into Petridishes and 6 pairs of *C. analis* (male and female of almost equal age) were introduced into them. The mortality of insects was observed and % 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, NT is the number of adults emerged from treated food media.

Results

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* proved to be the most effective treatment with 76.74% oviposition deterrence. *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% deterrence. *C. oppositifolia* resulted in least oviposition deterrence. (Table 1).

Table 1. Oviposition deterrence of *C. analis* under different doses of four essential oils.

	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.

The results revealed that *A. maritima* and *R. rugosa* oil were most potent in reducing the egg hatchability to 49.52±2.2 and 48.00±3.2 % respectively at a l dose of 10 µl/ml whereas, egg mortality was calcu 40.94±1.2 and 39.07±2.4% respectively. *Z. armatum* at a dose of 100 µl/ml obtained 55.70±4.8% egg mortality. *C. oppositifolia* was least toxic than any other resulted in a low mortality of 44.63±1.5% against eggs of *C. analis* even at a highest dose of 100 µl/ml. (Table 2).

Table 2. Ovicidal action of essential oils against *C. analis eggs.*

	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.

R. rugosa at 100 µl/ml proved to be most effective with 85.48% progeny deterrence followed by *A. maritima* oil resulted in 81.67% deterrence as compared to control (55.65±5.8). A dose of 50 µl/ml of *Z. armatum* oil resulted in 67.33% deterrent activity while *C. oppositifolia* was found to be least effective resulted in 63.89% deterrent activity even at a highest dose of 100 µl/ml (Table 3).

Table 3. F_1 progeny deterrence of *C. analis* under variable doses of 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 *Acanthosceildes obtectus* has been already reported (Roger and Hamraoui, 1995). At 100 µl/ml *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 seeds than control. *Berberis lycium* in acetone the oviposition in *C. chinensis*was decreased from 38.40±0.81 in control to 11.06±0.65 in 20 per cent concentration and from 38.40±0.65 in methanol control to 9.46 in same concentration of methanol extract. The oviposition was reduced from 37.85±1.10 in control to 6.93±0.49 in 20 per cent roots extract of *B. lycium* in acetone and from 35.50±0.40 in methanol control to 8.60±0.41in same concentration in methanol (Thakur and Devi, 2016). In a similar study Shukla *et al.* (2011) recorded that 0.1µl/ml essential oil of *Callistemon lanceolatus* showed 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% deterrence. *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 81.28% egg hatching was recorded in control. *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 ionic surfactant at concentrations of 5 and 10 µL showed 68 to 88% mortality in *C. analis* and 63 to 76% in *Sitophilus oryzae* L. respectively after 24 hours of treatment (Brari and Thakur, 2016). 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* while in controls the adult emergence was 55.65±5.8. *Z. armatum* also resulted in 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).

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Effectiveness of Essential Oils from Ngaoundere, against Post-Harvest Insect and Fungal Pests of Maize

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Abstract

Successful storage of harvest is a matter of utmost importance in the Sudano-Guinean agro-ecological zone where intense cultivation takes place only once a year. Poor and rudimentary drying/storage methods, high relative humidity as well as inaccessibility to the chemical pesticides leave stored maize at the mercy of insect and fungal attack. Insect attack favours secondary attack by fungi; both leading to a fall in the nutritional, sanitary and organoleptic qualities of the stored maize. Thus, poor peasant farmers are left with the choice of locally available botanicals as alternatives to chemical pesticides. It is against this backdrop that this study seeks to determine the insecticidal efficacy of essential oils from the leaves of *Chenopodium ambrosioides* and *Cupressus sempervirens* together with their 50/50 binary combination against the maize weevil, *Sitophilus zeamais,* and the fungi: *Rhizopus stolonifer* and *Aspergillus flavus* on stored maize. Insect mortality and progeny inhibition and the inhibition of fungal invasion were evaluated. Pesticidal activities of both essential oils increased with ascending dose of application. 200 µL/kg of the binary combination caused 100% mortality within 14 days and it completely inhibited progeny production in the weevil. The mixture of the two oils showed additive effects against the weevils and fungi. The two essential oils in isolation significantly inhibited fungal spore invasion in 21 days of storage although *A. flavus* was less susceptible than *R. stolonifer*. Therefore both plants could provide active botanical pesticides against *S. zeamais* and fungal pests in stored maize.

Key words: botanical, essential oil, fungal spore, stored maize pests, food security

1. Introduction

Sub-Saharan Africa is the most vulnerable region in the world with the average amount of food available per person per day being 1,300 calories compared to the world wide average of 2,700 calories (FAO, 2013). In 2012, maize had a yield of 70,076,591 tons in Africa (FAOSTAT, 2015). It is grown in diverse agro-ecological zones and farming systems, and consumed by people with varying food preferences and socio-economic background (Langsi *et al.,* 2017a). Cameroon with agriculture as its backbone has about 70% of its active population involved in agriculture, which contributes to about 25% of the GDP (FAO, 2008). Stored maize, especially in regions of high humidity is highly prone to attack by insect and fungal pests. High humidity and water content favour fungal growth (Pitt and Hocking, 2009). The most prolific insect is *Sitophilus zeamais* which bores holes and creates hotspots suitable for fungal growth. The fungi now produce mycotoxins thereby lowering the quality and also rendering it hazardous for consumption (Rashad *et al.*, 2013).

Plants which make excellent leads for new pesticide development (Napoleao *et al.,* 2013) could be used. Essential oils from plants generally contain chemicals which have both curative and protective potentials on stored products (Hamdani *et al.,* 2015). *Chenopodium ambrosioides* L. (Amaranthaceae) and *Cupressus sempervirens* L. (Cupressaceae) locally used as botanicals were chosen for this work. *Ch. ambrosioides* L. (Amaranthaceae) is a plant whose powders have been studied against *Sitophilus*