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LABORATORY EVALUATION OF FOUR MEDICINAL PLANT ESSENTIAL OILS AS PROTESTANTS AGAINST THE COWPEA WEEVIL *CALLOSOBRUCHUS MACULATUS* (COLEOPTERA : BRUCHIDAE)

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A B S T R A C T

The four medicinal plant essential oils; *Anethum sowa*, *Cinnamomum zeylanicum*, *Cuminum cyminum* and *Citrus reticulata*, were evaluated as grain protectant against the Cowpea weevil *Callosobruchus maculatus* in the laboratory at 250, 500 and 750 ppm Concentrations, parameters assessed were adult mortality, rate of adult emergence, grain damage effect and weevil perforation index (WPI). There was increase in adult mortality with days of exposure in all concentrations. *A. sowa* followed by *C.zeylanicum* were most potent both in adult mortality and adult emergence. This study reveals *A.sowa* to be a potent biopesticide, for protecting cowpea grains from *C. maculatus* infection and damage. The details of the bioassay procedures used and the results obtained in the present experimentation were discussed in the light of recent literature. These findings are first hand information about efficacy of selected oils against *C. maculatus*.

INTRODUCTION

In the field, the crop is susceptible to many pests (Singh *et al*., 1990). The dry, ripe seeds, however, before harvest or in storage are vulnerable to only few pests of which the Cowper Weevil, *Callosobruchus maculatus* (Coleoptera : Bruchidae) is the most important insect pest. Infestation by this beetle commences in the field (prevett, 1961), but most damage is done during storage. Over 90% of the insect damage to Cowper seeds are caused by *C.maculatus* (Caswell, 1981). Infection may reach 100% within 3-5 months of storage (singh, 1977). The germination of the beans is adversely affected due to the beetle's emergence holes (Baier and Webster, 1992). Damages seeds lose weight and market value and they are generally unacceptable for human consumption (Javaid and poseal, 1995). To protect the stored beans against *C.maculatus*, many methods can be mixed with ash, sand or other try fine substances that fill up the space between the beans and provide a barrier to insect movement (Golob and webley, 1980).

Fresh, dry or processed plant materials can be applies as insecticides or to repel the pest insect. These methods, in combination with the natural occurrence of parasitoids, should keep the beetle infection as low a possible. Nowadays, methods such as storage in airtight Plastic or steel containers, application of chemical insecticides, gamma irradiation, freezing the beans or heating them, are some of the additional possibilities. However, most of these methods require high inputs, offer unavailable and unaffordable for subsistence farmers. As for ash and sand, the main disadvantage is that to be effective they have to be applied in such large quantities that they are practical only for small amount of beans (Wegmann, 1983). With the Introduction of – often subsidized – Chemical pesticides much of the traditional knowledge of the use of plant materials of pesticides is diminishing (Kone, 1993). Move over, the development of synthetic pesticides has limited the move wide spread use of botanical insecticides (Delobel and Malonga, 1987). Mean while *C. maculatus* continuous to destroy Stocks of beans that could prevent famine.

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The plant products that are traditionally used and produced by the farmers in developing countries appear to be quite safe and promising. It is age-old practice of traditional farmers in the tropics to mix a local plant with the grain legumes. Using plants with insecticidal properties is therefore an alternative to the more expensive synthetic pesticides. Various plant by – products have been tried recently with the good degree of success as protectants against a number of stored grain insect pests (Gill and Lewis, 1971; Teotia and Tawari, 1971; Ketkar, 1976; Pandey *et al*., 1986; Dixit and Saxena, 1990). Mixing with plants oils is an ancient Indian and African method of protecting grains against insect attack (Pereira, 1983) and most of the reported studies with plant oils have involved we against stored a grain insect pests. An increasing number of plant oils have been screeched for preventing post-harvest losses due to insects (Golob and Webley, 1980; Raja *et al*., 2001; Papachvistos and Stamopoulos, 2002). Plant oils have repellent and insecticidal (Shaaya *et al*,. 1997; Kestyukovsky *et al*., 2002;). Essential oils produced in various external and internal glands of these plants are a very complex mixture of terpehes, sesquiterpenes, their oxygenates derivatives and other aromatic compounds. The actual chemical composition is a function of species, chemo type, climate, Soil conditions and geographical location. (OKa *et al*., 2000; shaaya and Kostyukovsky. 2006). Alternatives to synthetic chemical insecticides are highly desirable (Xie *et al*., 1995) Research on the use of natural pesticides for crop protection and storage is increasing because of their low toxicity to human beings (Ivibjaro, 1983; Zehrer, 1984; Fagoonee, 1987; Schmutter *et al*, 1987; Tanzubil, 1987; Raja *et al*., 1988). Plant essential oils have been used in the protection of stoved grains (EL – Nahal *et al*., 1989; Saxens and mathur, 1976; Risha *et al*., 1990; Gbolade and Adebaya, 1993). In the present study, plant essential oils were studied for their determent effects on Insecticidal and Grain damage actions against *C. maculatus.*

MATERIALS AND METHODS

Insects rearing

Callosobruchus maculatus was collected in the Koothur, Sirkali Taluk, Nagapattinam District, from the local varieties of cowpea field. The beetles were reared on cowpea in our laboratory for about a year (Approximately 10 generations) prior to the experiments. The insect culture was done in a climate chamber at 30 ±1ºC with 12h photo period at ambient R.H (50-80%) For the experiment, newly emerged (1-1.5h) insects were used. In the experiments, the day of death of the adult beetles was determined as the day the antennae and legs did not move upon gentle disturbance with forceps.

Beans

Cowpea (*V.unguiculata)* of the variety susceptible to *C. maculatus* (Baker *et al*., 1989). Were stores in a freezer at -18ºC for a week and subsequently dried in a store at 60ºC for about a week to guarantee the absence of viable insects without having to use chemicals. The beans were stored in airtight plastic containers at room temperature before use. Only visually uninfected beans were uses for the experiments.

Plant Materials

The seed of *Anethum sowa*., (Apiaceae). The bark of *Cinnamomum zeylanicum.*, (Lauraceae) The seed of *Cuminum cyminum*., (Apiaceac) and the seed of *Citrus reticulata*., (Rutaceae). All plant was collected from various parts of Tamilnadu and the harvesting period in year 2010. The voucher specimen has been deposited in the laboratory of Zoology, Annamalai University, Annamalai nagar, Tamilnadu.

Essential oil distillation

The essential oil was obtained by hydro distillation in a Clevenger type apparatus for eight hours. The oils thus obtained were dried over anhydrous magnesium suplhate o extract the oil.

Effect of essential oil on weevil mortality

The toxic effect of essential oil on adult *C. maculates* was accomplished in Petri-dishes (9cm diameter) Containing 25g of cowpea with concentrations of 250, 500 and 750 ppm essential oils. The essential oils were thoroughly mixed with the aid of a glass rod and agitated for 5-10min to ensure uniform coating. The dishes were left open for approximately 30 min so as to allow traces of solvent to dry off; after which 20 newly emerged adult *C. maculatus* were introduces into the dishes and mortality was observed daily for four days. Grains that were solvent treated served as the control experiment. Adults were considered dead where no response was observed after probing them with forceps.

Effect of essential oils on adult emergence and grain damage

Another experiment was performed with the infected and treated grain left for 49 days (7 weeks). At the end of the 49th day observation period, the extent of weevil damage was assessed using the exit-hole counted as a measure of damage to the grains. Grains that were riddle with exit-holes were counted; the percentage damage (PD) and Weevil Perforation Index (WPI) were calculated using the methods is Fatope *et al*. (1995) respectively.

$$PD = \frac{\text{Total number of treated grains perforated}}{\text{Total number of grains}} \times 100$$

$$WPI = \frac{\text{\% of treated grains perforated}}{\text{\% of control grains perforated + \% of Treated grain perforated}} \times 100$$

Data analysis

Statistical analysis of the data was done using SPSS 10.0 software package. The result were showed significant difference at $P<0.05$ level.

RESULT AND DISCUSSION

The use of plant extracts in the control of stores products insects is an ancient practice. Oils are commonly used in insect control because the oils are relatively efficacious against virtually all life stages of insects (Don-pedro, 1989, 1990). The toxicity bioassay of the essential oils on adult *C.maculartus* is presented in Table-1. Adult mortality significantly increases with increasing concentration and days of exposure. The highest value of 100% Mortality was observed in the treatment with *Anetnum sowa* and *Cinnamomum zeylanicum* by the second day and followed by *Cuminum cyminum* (90.0%) and *Citrus reticulata* (80.0%) recorded in the fourth day (All at 750 ppm concentration). There was no mortality with the control.

Table 3 present the effects of essential oils on grain damage. A similar trend of plant activities was observed among the plants used. *Anethum sowa* gave the lowest value of 0.833% grain damaged, followed by *Cinnamomum zeylanicum* (0.858%), *Cuminum cyminum* (3.41%), and *Citrus reticulata* (4.166%) grain damage. Another observation from this research is that plant materials that acted as stomach and contact poison were found to be active in suppressing growth or development of insects. This is what may be responsible for the result obtained in Table3. The percent damage values show the activities of one plant material at different concentrations

Table 1 Effect of plant essential oils on adult weevils of *C. marculaltus.*

Plants name	Conc. ppm	*Mean Mortality (±S.E) (%) at 1-4 days past treatment DAY1	DAY2	DAY3	DAY4
Anethum sowa	250	23.3±3.3[e]	40.0±5.8[e]	83.3±3.3[h]	100.0±0.0[h]
	500	26.7±3.3[f]	53.3±3.3[i]	86.7±3.3[i]	100.0±0.0[h]
	750	60.0±0.0[k]	100.0±0.0[k]	100.0±0.0[j]	100.0±0.0[h]
Cinnamomum zeylanicum	250	16.7±3.3[d]	36.7±3.3[d]	70.0±5.8[f]	100.0±0.0[h]
	500	30.0±0.0[g]	43.3±3.3[f]	83.3±3.3[h]	100.0±0.0[h]
	750	53.3±3.3[j]	100.0±0.0[k]	100.0±0.0[j]	100.0±0.0[h]
Cuminum cyminum	250	6.7±3.3[b]	16.7±3.3[b]	46.7±3.3[c]	60.0±0.0[b]
	500	10.0±5.8[c]	46.7±3.3[g]	60.0±0.0[e]	86.7±3.3[f]
	750	43.3±3.3[i]	60.0±0.0[j]	86.7±3.3[i]	90.0±5.8[g]
Citrus reticulata	250	16.7±3.3[d]	26.7±3.3[c]	43.3±3.3[b]	60.0±5.8[c]
	500	23.3±3.3[e]	36.7±3.3[d]	53.3±3.3[d]	70.0±5.8[d]
	750	36.7±3.3[h]	50.0±0.0[h]	73.3±3.3[g]	80.0±0.0[e]
Control(Solvent-treated)	0.00	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]

* Value represents mean ± S.D. of five replications., each set-up with 20 adults. Values in the column with a different superscript alphabet are significantly different at $P < 0.05$ level DMRT Test.

The number of adults the emerged after 49day (7 weeks) of storage. The data is presented in Table 2.The number of emerged adults decreased with increasing concnentrations of essential oils. *Anethum sows* had the least number of emerged adult (0.0) at 750 ppm concentration. *Cinnamomum zeylanicum* (3.3) was next to *cuminum cyminum* (10.0), followed by *Citrus reticulata* (16.7). The oils extract on application covered the outer layer (testa) of the grains (there by serving as food poison to the adults insects); while some of them penetrated into the endosperm and germ layers (Thereby suppressing oviposition and larval development).

Table 2 Effect of plant essential oils on C.marculatus adult emergence (7weeks post-treatment).

Plants name	Conc. ppm	Mean number of emerges adults(±S.E)
Anethum sowa	250	6.7±3.3[c]
	500	3.3±3.3[b]
	750	0.0±0.0[a]
Cinnamomum zeylanicum	250	10.0±0.0[d]
	500	6.7±3.3[c]
	750	3.3±0.0[b]
Cuminum cyminum	250	16.7±3.3[f]
	500	13.3±3.3[e]
	750	10.0±5.8[d]
Citrus reticulata	250	23.3±3.3[i]
	500	20.0±0.0[h]
	750	16.7±3.3[g]
Control(Solvent-treated)	0.00	86.7±3.3[j]

* Means ±S.E of five replicates, each set-up with 20 adults. Values in the column with a different superscript alphabet are significantly different at $P < 0.05$ level DMRT Test.

while the weevil perforation index (WPI) compares the activities of different species of essential oils used.

Corn, groundnut, sunflower and seasome oil reduced oviposition by all three bruchid species by over 70% at a concentration of 10ml/kg cowpea seeds. At 5ml/kg results were more variable with *C. maculatus* most affects. A number of Authors have previously shown that oil coating is effective in controlling *C. maculatus* (Singh *et al*., 1978; Messina and Renwick, 1983; Pandey *et al*., 1983; Pereira, 1983). The strong fumigant toxicity of *O. gratissimum* oil could possibly be due to individual and synergistic effects of its different constituents such as engenol and β-(z)-Ocimene. However, the differential inter-Specific insect responses to essential oil and its constituents could be attributed to compound structure – activity relationships and physiological-structural induces cellular changes resulting in poisoning of injects by blocking octopimine receptors (Kostyukovsky *et al*., 2002; Enan, 2004; Priestley *et al*., 2006). In recent studies, crude powder (2.0-4.4% w/w) of *O.gratissimum* caused 87-90% and 25-50% Mortality of adult *C. maculatus* and *S. zeamais*, respectively (lloba and Ekrakene, 2006).

From this study, it is becoming evident that *Anethum sowa* and *Cinnamomum zeylanicum* displayed some potential as antifeedants, food poisons, contact poisons and repellents. The results therefore strongly suggest the possibility of using the essential oils of these plants as toxicants, repellents and food poisoning agents against *C. maculates.* Since there is very little information on the activities, and active metabolites of *Anethum sowa* an investigation is presently going on, to identify its metabolites and also to understand the metabolite(s) responsible for its high potency in insect control. The mode of action of the metabolites will also be studies.

Table 3 Effect of plant essential oils on grain damage

Plant name	Conc. ppm	Total no of grains	No of perforated grains	Unperforated grains	% Grains damage	*WPI
Anethum sowa	250	248	7	241	2.822	7.03
	500	235	5	230	2.127	5.40
	750	240	2	238	0.833	2.19
Cinnamomum zeylanicum	250	241	9	232	3.734	9.12
	500	247	6	241	2.429	6.13
	750	233	2	231	0.858	2.25
Cuminum cyminum	250	239	18	221	7.531	16.83
	500	243	13	230	5.349	12.57
	750	234	8	226	3.41	8.39
Citrus reticulata	250	239	24	215	10.041	21.25
	500	237	17	220	7.172	16.16
	750	240	10	230	4.166	10.07
Control(Solvent-treated)	0.00	242	90	152	37.190	50

* Weevil perforation Index (WPI). A value above 50 is an indication of negative protectant ability.

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