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INSECTICIDAL ACTION OF CERTAIN ESSENTIAL OILS AGAINST *CALLOSOBRUCHUS MACULATUS* (COLEOPTPERA: BRUCHIDAE).

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ABSTRACT

Certain important medicinal plant essential oils such as *Curcuma longa, Zingiber officinale, Murraya koenigii* and *Ocimum caasiym* were investigates for their insecticidal, antiovipositional and antifeedant activity against *Callosobruchus maculatus* among four essential oils highest percentage of mortality were showed in (58-81%) *Ocimum canum* at 500 ppm concentrations. All essential oils showed complete feeding deterrent action at 500ppm concentrations. Loss of fecundity was also noticed in all essential oils at higher concentrations. The maximum antiovipositional activity was recorded in 500 ppm of *O.canum* followed by *M. koentigii*, *Z.officinale* and *C.longa.*

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INTRODUCTION

The bruchid *Callosobruchus maculatus* develops in the seeds of 14 leguminosae species, but the cowpea *Vigna unguiculata* (Walp) is its main host-plant (Decelle, 1981;Delotel and Tran, 1993), It is mainly cultivated in dry lands. Cowpea is a very good source of vegetable protein for millions of people. The estimated global posot-harvest losses caused by insect damage, Microbial deterioration and other factors are of the order of 10-25% (matthews, 1993). In india 12.5 million tones of edible legumes are produced every year and nearly 18.6% of cowpea alone is damaged by bruchids during storage (Agarual *et al*., 1988). The brichid beetle *C.maculatus* has been associated with dried legumes for thousands of year (Mitchell,1983; Messina,1991). Synthetic chemical pesticides have been uses to protect grain but their wide spread use has led to the development of pest resistance (Champ and Dyte, 1976; settler and Cuperus, 1990; white, 1995). Alternative to synthetic chemical insecticides are highly desirable (xie *et al*., 1995).

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toxicity to human beings (lvibjaro, 1983; zehrer, 1984; fagoonee, 1987; Schmutterer,1987; Tanzubil, 1987) plant volatile oils have been uses in the protection of stored grains (EL-Nahal *et al*., 1989; Saxena and mathur, 1976; Risha *et al*., 1990; Gbolade and Adebaya, 1993).

Various plant byproducts have been tried recently with a good degree of success as protectants against a number of stored grains. Pests (Teotia and Tiwari, 1971; Ketkar, 1976; Pandey *et al*., 1976, 1986; Atri and Prasad, 1980; Yadav and Bhatnagar, 1987; Ayyangar and Rao, 1989; Dixit and Saxena, 1990). Plant powder can have a protective effect on the beans on several mechanisms. In addition to direct toxic effects, the plant materials may produce invasion or cause emigration from the treated stock. Many plants have been tested in laboratories for their toxic effects on storage beetles but few of them were tested for their repellent effect. Comparison of results on trained under laboratory conditions with the situation under actual storage conditions is problematic, but a hierarchy for the potential efficacy of plants can be established. Unfortunately, the outcomes of such test are often contradictory to others and few authors have been able to recommend a certain plant or an application method (reviewed by Boeke *et al*., 2001).

A laboratory experiment was therefore conducted to study the insecticidal, antifeedant and antiovipositional action of certain essential oils against *C.maculatus*, a stored grain pest.

MATERIALS AND METHODS

Plant collection and Essential oils distillation

The rhizome of *Curcuma longa*., (Cyperaceae) The rhizome of *Zingiber officinale*., (Zingiberaceae) The leaf of *Murraya koenigii*.,(Rutaccae) and the leaf of *Ocimum canum*., (Lamiaccae). All medicinal plant essential oils were collected from various parts of Tamil nadu and harvesting period in year 2010. The voucher specimen has been deposits in the laboratory of Zoology Annamalai University, Annamalai Nagar, Tamilnadu.

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.

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.

Biological assay methodology

Insecticidal activity

Insecticidal activity of certain essential oils 0.4ml of each essential oils at three different concentrations (100,250 and 500 ppm) was poured into clean, grease-free glass tubes of 100ml capacity and a uniform film of the essential oils was made by rolling the vials. Freshly emerged adults 20 were then released in each of the treated vials which were then covered with a piece of muslin cloth held by a rubber band to prevent escape of the adults. Un treated healthy cowpea seeds were provides to the adults during the bioassay. Observations on insecticidal activity were made at 1-7 days after treatment.

Antifeedant activity

0.5 ml of each essential oil at three different concentrations (100,250 and 500ppm) was poured into glass vials of equal volume containing 20 healthy cowpea seeds. Before being places in the vials, the seeds were kept for 24hr in an oven at 70ºC. The solvents were allowed to evaporate completely before release of five pairs of freshly emerged larvae in each vial. The solvent treated used as control. The numbers of damaged seeds were recorded daily for seven days.

Antiovipositional and ovicidal activity

Dry and healthy seeds (20g) were kept in the Petri dishes in two lots and treated with each essential oil at 1 ml $100g^{-1}$ seed. For this experiment 10 pairs of 1-3 day old laboratory-cultured pulse beetles were released in each dish. Oviposition observed after seven days and adult emergence recorded after 1 month. The ovicidal effect was observed after soaking the seeds on watered blotting paper for 3 hr followed by observation under 20 x magnifications for egg mortality. Eggs were removed to observe the pin holes in the seed coat. Five replications of each treatment were use and all experiments were conducted at 24±2ºC and 75±5º% R.H.

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.

Table 1 Mortality of adults *C.maculatus* at different time intervals after exposure to certain essential oils

		*Mortality (%)						
Plants name	**Conc.ppm**	**Day1**	**Day2**	**Day3**	**Day4**	**Day5**	**Day6**	**Day7**
Curcuma longa	100	6^{a}	12^{b}	20^{b}	28^{b}	38^{c}	42^{a}	50^{a}
	250	12^{c}	18^{e}	24^{e}	30^{d}	40^{e}	48^{d}	54^{c}
	500	18^{g}	23^{i}	35^{h}	41^{g}	52^{h}	60^{g}	70^{g}
Zingiber officinale	100	10^{b}	16^{d}	22^{d}	28^{b}	36^{a}	44^{b}	52^{b}
	250	14^{d}	20^{f}	25^{f}	32^{e}	43^{f}	49^{e}	56^{d}
	500	20^{h}	24^{j}	37^{i}	43^{h}	55^{i}	62^{h}	80^{i}
Murraya koenigii	100	6^{a}	11^{a}	18^{a}	26^{a}	37^{b}	44^{b}	52^{b}
	250	10^{b}	18^{e}	24^{e}	29^{c}	38^{c}	46^{c}	56^{d}
	500	17^{f}	22^{h}	35^{h}	48^{j}	59^{k}	68^{j}	78^{h}
Ocimum canum	100	10^{b}	15^{c}	21^{c}	26^{a}	39^{d}	48^{d}	58^{e}
	250	15^{e}	21^{g}	26^{g}	38^{f}	49^{g}	54^{f}	68^{f}
	500	$\mathbf{21^{i}}$	$\mathbf{25^{k}}$	$\mathbf{39^{j}}$	$\mathbf{47^{i}}$	$\mathbf{58^{j}}$	$\mathbf{67^{i}}$	$\mathbf{81^{j}}$

*20 freshly emerged adults were taken in each test in replicates of five. There was no Mortality of the insects in control. Values

RESULTS AND DISCUSSION

The results in Table1 showed that all the concentrations of the plant essential oils caused 50-70% of mortality recorded in *C. longa* 52-80 % of mortality recorded in *Z.officinale*, 52-78% of mortality recorded in *M.koenigii*

Table 2 Extent of feeding by adults of *C. maculatus* of treated essential oils grams seeds.

Plants name	Conc. ppm	*Damaged (%) Day1	Day2	Day3	Day4	Day5	Day6	Day7
Curcuma longa	100	-	3^{a}	5^{b}	9^{c}	12^{e}	16^{e}	18^{e}
	250	-	-	-	1^{a}	3^{c}	6^{c}	8^{c}
	500	-	-	-	-	-	-	-
Zingiber officinale	100	-	-	-	-	2^{a}	5^{b}	7^{b}
	250	-	-	-	-	-	-	-
	500	-	-	-	-	-	-	-
Murraya koenigii	100	2^{a}	5^{b}	7^{c}	13^{d}	18^{f}	21^{f}	26^{f}
	250	-	-	3^{a}	6^{b}	8^{d}	11^{d}	16^{d}
	500	-	-	-	-	-	-	-
Ocimum canum	100	-	-	-	-	1^{a}	3^{a}	5^{a}
	250	-	-	-	-	-	-	-
	500	-	-	-	-	-	-	81^{h}
Control	0	19^{b}	24^{c}	31^{d}	39^{e}	46^{g}	53^{g}	64^{g}

Experimental values are significantly different from control at the 5% level according to analysis of variance. Values followed by different letters in same column different significant at P<0.05. (-) No damage

Table 3 Anti Ovipositional effects of certain essential oils against *C.maculatus*

Plants name	Conc.(ppm)	Oviposition	*Number of adults emerged from seeds.
Curcuma longa	100	+	53^{i}
	250	+	32^{h}
	500	+	18^{g}
Zingiber officinale	100	+	16^{f}
	250	+	10^{e}
	500	-	0^{a}
Murraya koenigii	100	+	9^{d}
	250	+	3^{b}
	500	-	0^{a}
Ocimum canum	100	+	7^{c}
	250	-	0^{a}
	500	**-**	**0^{a}**

* Values followed by different letters in same column different significant at P<0.05.
+ = Ovipoistion , - = No Oviposition.

and among them highest percentage of Mortality were recorded in the *O.canum* (58-81%) essential oil. During this period, there was no mortality of the insects in control checks.

Four essential oils at 500 ppm concentration provide strong antifeedant to adult *C.maculatus.* At 100 and 250 ppm Concentrations, however 8-18% recorded in *C.longa*, 7% recorded in *Z. officinale*, 16-26% recorded in *M.koenigii* and 5% recorded in *O.canum.* Feeding activities were noticed within 7 days after treatment (Table 2) in solvent treated control 64% food damage were recorded respectively during this period.

Antiovipositional effect is evidenced by the reduced number of egg and the total loss of egg deposition (Table 3). It was observed that there was some egg laying by the females in low dose treated seeds but ovipósistion was inhibites at higher concentrations. The maximum ovipositional effect were recorded in *O.canum* and lest ovipositional effect were recorded in *C.longa* essential oil. Table 3 was 250pp/100 records in *O.canum* essential oil. Table 3 shows that at low dose treatment, there was oviposition, and egg that was laid developed through to adult emergence.

The marked decline in egg laying was perhaps a consequence of the mils suppressing effect exerted by these volatile oils on the beetles mating – a decisive factor influencing the subsequent number of eggs laid by the beetle (Engelmann, 1970). The drastic reduction in adult emergence that was recorded could also in part be due to low hatchability of eggs. It is probable that oil vapors diffused into eggs and affected the physiological and biochemical process associated with embryonic development. These findings corroborate the observations recorded for peppermint and citronella oil vapors on *Earias vittella* eggs (Marimuthu *et al*, 1997) 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; Zehrer, 1983; Jadhav and Jadhav, 1984; Boughdad *et al.*, 1987; Don-pedro,1989) Reductions in adult longevity of bruchids, as reported here particularly for *C. maculatus* and *C. chinensis* with ass four oils tested, have also been shown with other vegetable oils. Uvah and ishaya (1992) reported reduction longevity of *C.maculatus* by a single applications of groundnut oil, palm oil or olive oil. Many of the plants which formers use as protectants have a strong smell which, it is believed, repels or kills insects. Other workers have previously reports that plant powders reduce oviposition by bruchids under laboratory conditions. The plants involved inchide; Neem kernel powder (Sowunmi and Akinnusi, 1983) and *Kurunegala dasia*, *Tridax proocumbers* (L) with *C. maculetus* (Bhaduri et al., 1985), and custard apple *Annona squamosa* L. Seed powder with *C. chinensis* (Ali et al., 1981) Yadav (1985) have made similar observations with residual effects lasting 90 days in which period no egg laying and new progeny were obtained. Jaipal *et al*.,(1984) and Panday *et al* (1986) have also observed insecticidal activity in petroleum ether extracts of leaves and twigs of *A.indiaca, L.camara A. conyzoides* and other plant products against *R.dominica* and *C.chinensis*.

The results of the present investigation indicate that the essential oils were positively toxic on contact and strongly antifeedant to adults of. *C maculaltus*, But their ovicidal activity was not strong enough to be used for the control at these concentrations. Never the less, this plant could be exploited for the development of bioregional agrochemicals for the control of stored grain pests.

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