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Evaluation of Methanol, Ethanol and Acetone extracts of four plant species as repellents against *Callosobruchus maculatus* (Fab.)

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Abstract

Methanol, ethanol and acetone extracts of leaves of four plants were evaluated for repellent activity against adult *Callosobruchus maculatus* using a multi-choice bio-assay apparatus. The experimental plant species studied were *Ocimum gratissimum* (L.), *Ocimum sanctum* (L.), *Mentha viridis* (L.), and *Hyptis suaveolens* (L.) in the family Lamiaceae. The extracts were applied to the food source (mung beans, 25 g) at 1.0, 5.0 and 10.0 ml concentrations and repellant activity was recorded. Observations were made after three hours for each solvent-plant extract and Percentage Repellency (PR) was determined. Among four study plant species and three solvents tested, methanol extract of *M. viridis* (10.0 ml) showed the strongest and highest repellent effect on *C. maculatus* (81%). With all three solvent extracts, the percentage repellency of the *C. maculatus* increased significantly with the increase of concentration producing the highest at 10.0 ml. When methanol and acetone based extracts were tested against the cowpea beetles, the highest percentage repellent activity was obtained with *M. viridis* followed by *O. gratissimum* and *H. suaveolens* respectively. The lowest percentage repellency was observed with *O. sanctum.* In contrast, ethanol-based extracts of *O. gratissimum* facilitated the highest percentage repellency (78%) and *M. viridis* produced the lowest percentage repellency (55%). Significantly high PR observed in all extracts strongly indicates the presence of repellent properties in all four plants. Solvents methanol and acetone appeared to be more effective in extracting bio-active compound/s from *M. viridis* whereas ethanol as a solvent seemed to be more efficient with *O. gratissimum.*

Key Words: *Callosobruchus maculatus*, Percentage repellency, Solvent-plant extracts

Introduction

To alleviate insect pest problems in storage, synthetic insecticides and fumigants are recommended. However, due to the negative environmental impacts and human health hazards of these synthetic chemicals, there is a growing interest in research concerning the development of alternative strategies including the possible use of higher plants as storage pest control agents (Isman, 2006; Talukder, 2006; Dubey *et. al.*, 2008; Tripathi *et. al*., 2009). The incorporation of materials from locally available plants for the use in storage by the farmers in developing countries appear to be quite safe and promising (Shukla *et.al*., 2007). Their main advantage is that they may be easily and inexpensively produced by local farmers and small-scale industries as crude or partially purified extracts. In the last three decades, a substantial effort has been directed at screening plants in order to develop new pest control agents as an alternative to the currently used insecticides. Various parts and products of plants have been tried with a good degree of success as protectants against a number of grain insect pests (Boeke *et. al.*, 2004; Mulungu *et. al.*, 2007; Ayvaz1a *et. al.*, 2008; Dubey *et.al.*, 2008). Tissues of higher plants are known to contain a rich source of bioactive secondary metabolites or allelochemicals which may show antifeedant, oviposition deterrent, repellent, toxic and growth and reproduction inhibitory effects in a wide range of insects (Powel, 1989; Talukder, 2006; Ukeh *et. al*., 2009).

It is a traditional practice of rural farmers in developing countries like Sri Lanka to use various plants and plant parts to repel insect pests from stored grains and cereals. Repellents are desirable substances as they drive away the insect pests from the treated materials by stimulating their olfactory or other receptors and as they offer protection with minimal impact on the ecosystem. Repellents from plant origin are considered safe in storage pest control as they minimize pesticide residues and ensure safety of the people, food and environment (Talukder, 2006). Moreover, plant extracts, powders and essential oils from different bioactive plants were reported as repellents against different economically important stored product insects (Xie *et. al*., 1995; Boeke *et. al*., 2004; Ishii *et. al.*, 2010).

The present study was thus conducted to evaluate the percentage repellency of three (ethanol, methanol and acetone) extracts of four plant species from the family Lamiaceae against the cowpea beetle and thereby exploring their potential as effective repellents in storage insect pest management.

Materials and Methods

Rearing of Test Insects

A culture of cowpea beetle *C. maculatus* on mung beans was maintained in the laboratory under laboratory conditions (29 ± 2 ^{0}C, 84 ± 2 % RH and 12h: 12h light: dark regime) under low crowding conditions. For all experiments 1-3 days old adult *C. maculatu*s were used.

Preparation of Plant Extracts

Fresh leaves of *Ocimum gratissimum* (Gasthala), *Ocimum sanctum* (Maduruthala), *Mentha viridis* (Minchi), and *Hyptis suaveolens* (Alithala) were cleaned and air-dried (under shade conditions) and ground into a fine powder using a domestic electric grinder. Powdered leaves (20.0 g) of each plant were mixed with 100 ml of Ethanol, Methanol and Acetone separately and kept for 24 hours. The plant-solvent mixture was stirred occasionally with a glass rod. Each of the plant-solvent mixture thus obtained was filtered twice using Filtermann® (125mm) filter paper. Three treatments each for three solvents and four plants were prepared by mixing 1 ml extract+9 ml solvent, 5 ml extract+5 ml solvent and 10 ml extract without any solvent. Each treatment thus is made up to 10ml of extract and solvent to ensure even smearing of mung beans.

Determination of Repellent Effects

A Multiple choice bio- assay chamber was used to determine the repellant effects of the plants. This consisted of plastic container (27 X 27 X 9 cm) into which four petri dishes (9 cm diameter) each containing 25 g of mung beans were placed at equal distance. Mung beans in three petri dishes were mixed with the three treatments separately and shaken manually for 2 min. to ensure uniform coating over the grain surface. Mung beans in the fourth petri dish treated with the corresponding solvent (ethanol, methanol and acetone) were considered as control. Treated beans were kept for 30 minutes to allow the solvent to evaporate completely before placing the petri dishes inside the bio-assay chamber. Twenty adult *C. maculatus* were then introduced into the middle of the bioassay chamber and the number of insects present on control (Nc) and treated (Nt) grains are recorded after three hours of exposure. Each treatment was replicated ten times (for three solvents and four plants). In each replicate, positions of the four petri dishes in bioassay chamber were altered randomly to prevent any bias in the results. Percent repellency (PR) was calculated by using the formula used by Udo & Epidi (2009) where PR = [(Nc – Nt) / (Nc + Nt)] X 100. Data obtained for repellency were subjected to one-way analysis of variance (ANOVA) according to the procedure "Minitab 14" and where significant differences ($p < 0.05$) existed, means were separated by Tukey's test.

Results and Discussion

Repellent effects of methanol, acetone and ethanol extracts of the four plant species against *C. maculatus* using a multiple choice bioassay chamber are depicted in Tables I, II and III. Among all four plant species and three solvents tested, methanol extract (10.0ml) of *M. viridis* was found to elicit the strongest repellent effect on *C. maculatus* with a percentage repellency of 81%. This was followed by the ethanol extract (10.0 ml) of *O. gratissimum* with 78% repellency in the *C. maculatus.* Other extracts with significantly high percentage repellent activity were the acetone extracts (10.0ml) of *M. viridis* and *O. gratissimum* showing a similar repellency level (76%).

Table I. Repellent effects of *C. maculatus* to methanol extracts of different plant species

Treatment (ml)	Percentage Repellency			
	Hyptis suaveolens	***Ocimum sanctum***	***Ocimum gratissimum***	***Mentha viridis***
1.0	42.18 ± 11.34 [a*]	23.35 ± 10.37 [a]	36.08 ± 8.89 [a]	47.92 ± 11.13 [a]
5.0	63.65 ± 14.28 [b]	38.98 ± 16.56 [ab]	62.93 ± 8.07 [ab]	54.61 ± 5.47 [a]
10.0	67.59 ± 9.68 [b]	55.79 ± 18.19 [b]	75.85 ± 19.19 [b]	76.65 ± 8.41 [b]
F Value	7.85	6.65	32.42	18.13
Probability	P<0.05	P<0.05	P<0.05	P<0.05

Table II. Repellent effects of *C. maculatus* to acetone extracts of different plant species

Treatment (ml)	Percentage Repellency			
	Hyptis suaveolens	***Ocimum sanctum***	***Ocimum gratissimum***	***Mentha viridis***
1.0	42.18 ± 11.34 [a*]	23.35 ± 10.37 [a]	36.08 ± 8.89 [a]	47.92 ± 11.13 [a]
5.0	63.65 ± 14.28 [b]	38.98 ± 16.56 [ab]	62.93 ± 8.07 [ab]	54.61 ± 5.47 [a]
10.0	67.59 ± 9.68 [b]	55.79 ± 18.19 [b]	75.85 ± 19.19 [b]	76.65 ± 8.41 [b]
F Value	7.85	6.65	32.42	18.13
Probability	P<0.05	P<0.05	P<0.05	P<0.05

According to the results obtained for all three solvent extracts, it is evident that the percentage repellency of the *C. maculatus* increased significantly with the increase of concentration producing the highest at 10 ml of the dose. When methanol based extracts were tested against the cowpea beetles, the highest repellent activity was obtained with *M. viridis* followed by *O. gratissimum* and *H. suaveolens* respectively. The lowest level of repellency was observed with *O. sanctum* (Table 1). Furthermore, a similar pattern was seen when acetone-based plant extracts were bio-assayed (Table II). In contrast, ethanol-based extracts of *O. gratissimum* facilitated the highest percentage repellent activity in the beetles. This was followed by *H.suaveolens* and *O. sanctum.* It is of interest to note that *M. viridis* produced the lowest PR activity when ethanol-based extracts were tested (Table III).

Table III. Repellent effects of *C. maculatus* to ethanol extracts of different plant species

Treatment (ml)	Percentage Repellency			
	Hyptis suaveolens	***Ocimum sanctum***	***Ocimum gratissimum***	***Mentha viridis***
1.0	33.68 ± 12.53[a*]	29.76 ± 10.11 [a]	49.76 ± 10.00 [a]	33.34 ± 8.17 [a]
5.0	64.16 ± 7.02 [b]	50.09 ± 14.90 [b]	70.23 ± 10.76 [b]	56.62 ± 8.93 [b]
10.0	73.19 ± 7.50[b]	62.78 ± 12.54 [b]	78.29 ± 8.59 [b]	54.84 ± 10.75[b]
F Value	29.37	10.37	13.44	11.42
Probability	P<0.05	P<0.05	P<0.05	P<0.05

*Repellent effects given as % Mean repellency ± SD in all treatments.

Means followed by the same letters in each column are not significantly different according to Tukey's Test at P=0.05

The analysis of the repellency effect of each plant species, regardless of the solvent used shows that the extracts of four plant species tested elicit considerably strong repellent effects against *C. maculatus*. Furthermore, significantly high PR activity observed in 5 and 10 ml of all three extracts against *C. maculatus* is indicates the presence of repellent properties in all four plants varieties. Moreover, it is worthy in stating that the solvents methanol and acetone appeared to be more effective in extracting bio-active compound/s from *M. viridis* than from the other three study plants.

However, the comparison among solvents indicates that ethanol as a solvent is more efficient in extracting bioactive compound/s form *O. gratissimum* even though the other two solvents also produced considerably higher effects (Figure 1).

When the overall results of the experiments are scrutinized, it is evident that determination of the most suitable solvent is crucial to extract bio-active compounds or secondary metabolites effectively from different plants.

It has long been observed that certain plants repel pests from stored food products, cultivated fields and are connected to the existence of compounds bio synthesized by plants and related mostly to the secondary metabolism. Biological activity of some plant extracts is the result of these various metabolites dissolved in these extracts (Roger and Philogene, 2008). Results of the present study indicate that presence of these bio active compounds in the extracts of four plant species may have independently or jointly contributed to cause repellant action in adult *C. maculatus*.

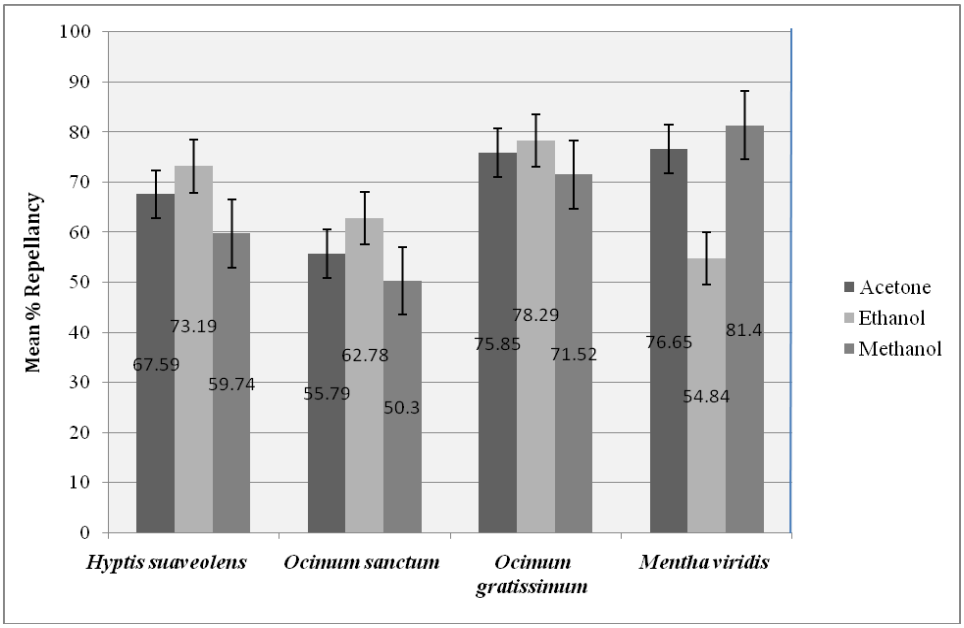

Figure 1. Comparative efficacy of three solvent extracts of four plant species when tested at the highest dose (10.0 ml)

In general, the extracts of *M. viridis, O. gratissimum, and O. sanctum* and *H. suaveolens* produced considerably high repellent effects on *C. maculatus*. The performance of *O. gratissimum* in the present study coincides to a certain extent with previous findings of some researchers. Ogendo *et. al.* (2008) in their studies stated that 37% -100% repellency was observed in five storage insect pests *Sitophilus oryzae*, *Tribolium castaneum*, *Oryzaephilus surinamensis*, *Rhyzopertha dominica* and *Callosobruchus chinensis* with the oil of *O. gratissimum.* However, Al-Jabr in 2006 stated that oil of *M. viridis* produced only a weak repellent activity in *T. castaneum* and *O. surinamensis.* It was also reported that when the essential oil of *O. gratissimum* was applied to adult *C. maculatus* as an insecticidal fumigant and powder a70% mortality was recorded. Similarly, this oil has exhibited a significant effect both on the egg hatch rate and on the emergence of adults (Kéita *et. al.*, 2001). Tripathi and Upadhay (2009) have evaluated the efficacy of *Hyptis suaveolens* essential oil on four stored grain coleopteran pests and reported a considerably high range of repellency against the tested insects. According to Iloba and Ekrakene (2006) powdered leaves of *H. suaveolens* and *O. gratissimum* were very effective enhancing adult mortality in *Sitophilus zeamais* and *C. maculatus* and performed well in reducing adult emergence.

The findings of the present study indicates it is observantly clear that the four plants tested, particularly *M. virids* and *O. gratissimum* are highly promising candidates in the treatment of grain in storage. These plant species are also available in Sri Lanka and can be grown in a vast scale for commercial use. Furthermore, the effective use of these natural plant materials/compounds may minimize the use of synthetic pesticides and reduce the development of insect resistance. Moreover, the outcome of the present study indicates that the use of natural plant products such as these would help controlling the storage pests in an environmental-friendly way. Since adult *C. maculatus* do not feed on pulses but invade them to deposit their eggs, the use of bio-active compounds that repel adult insects effectively away from the food source would be a highly strategic method of protecting grains from *C. maculatus.* However, the biological activities of the four tested plants merit further investigation to determine the active ingredients responsible for their repellent properties.

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