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# Use of essential oils of aromatic plants for the management of pigeon pea infestation by pulse bruchids during storage

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This study investigated the insecticidal and deterrent behaviour of volatile constituents derived from leaves and twigs of four aromatic plants such as *Chenopodium ambrosioides* Linn. (Chenopodiaceae), *Clausena pentaphylla* (Roxb.) DC (Rutaceae), *Mentha arvensis* Linn. (Lamiaceae) and *Ocimum sanctum* Linn. (Lamiaceae) towards pulse bruchids *Callosobruchus chinensis* L. and *C. maculatus* F. All tested oils showed significant lethality and ovipositional deterrency of test insects as compared to control set. *Chenopodium* oil was more toxic to both adults with $LC_{50}$ value ranges from 9.3-9.9 µl followed by *Clausena* (9.9-10 µl), *Mentha* (9.9-10.2 µl) and *Ocimum* (11-12.8 µl) oils. Egg laying and adult emergence of both beetles were drastically reduced by *Chenopodium* and *Clausena* oil when applied at 5µl dose than other oils. During *in vivo* study fumigant application of *Chenopodium* and *Clausena* oil's formulation at 20 and 40 µl concentration significantly enhanced feeding deterrence in insects and reduced grains damage as well as weight loss. In view of overall pesticidal potential of aforesaid oils, they can be successfully exploited as fumigants against insect infestation of pigeon pea seeds during storage and strengthen the possibility of using it as an alternative preservative to the commercial pesticides.

**Key words:** *Callosobruchus* spp., essential oils, insecticidal, ovipositional deterrency, formulations

## Introduction

Insect damage of stored grains and pulses may amount to 10-40% in countries where modern storage technologies have not been introduced. Pulse bruchids (*Callosobruchus* spp.) are the most serious insect pests of stored pulses throughout the tropical countries. It causes substantial loss and damage to seeds of many legumes especially pigeon pea (*Cajanus cajan* L.) which is major source of

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dietary protein and other essential nutrients. The species responsible for annual losses of pigeon pea seeds all over the worlds are *C. chinensis* L. and *C. maculatus* F. (Ali *et al.,* 2004). In order to keep these stored grains free from pest attack, various synthetic pesticides have been used (Opolot *et al.,* 2006). Although they are effective, their repeated use for decade has disrupted natural biological control system and lead to outbreak of resistant pests to various types of insecticides, undesirable effects on non target organism, environment and human health concern (Owens, 1986). Therefore environment needs some other alternative of chemical pesticides. Plant essential oils are alternative of synthetic pesticides possess insecticidal, ovicidal, repellent and ovipositional activities against various stored product insects (Chiasson *et al.,* 2004; Tripathi and Kumar 2007; Tripathi *et al.,* 2009; Aboua *et al.,* 2010). Plant essential oils are potential source of alternative compounds to currently use as contact or fumigant pesticides because they include a rich source of bioactive compounds. In the laboratory described herein, we have examined the toxicity of essential oils on mortality, oviposition and adult development of *C. chinensis* and *C. maculatus*. Further we had also report the *Chenopodium* and *Clausena* oil's formulations as *in vivo* fumigants to protect pigeon pea seeds from insect pests.

## Materials and methods

### *Insects rearing*

The cultures of *Callosobruchus chinensis* (L.) and *C. maculatus* (F.) used for the present study were established from infested stored pigeon pea seeds collected from 35 places of Eastern Uttar Pradesh, India (identified by literatures, Drees and Jackman, 1999; Beck and Blumer, 2007) and authenticated from Entomology Lab., Department of Zoology, DDU Gorakhpur University, Gorakhpur. The cultures of both insects were maintained subsequently on insecticide free newly harvested pigeon pea (*Cajanus cajan* L.) seeds at laboratory (28 ± 2ºC temperature) in darkness to obtained same aged insects.

### *Extraction of volatile constituents*

Essential oils from leaves and twigs of *Chenopodium ambrosioides* Linn. *Clausena pentaphylla* (Roxb.) DC, *Mentha arvensis* Linn. and *Ocimum sanctum* Linn. (250 g each) were extracted separately using Clevenger's apparatus (Clevenger, 1928) at 90±2ºC for 4h. Each essential oil was dried over anhydrous sodium sulphate and was stored at 4ºC in clean glass vials.

### *Contact toxicity bioassay*

A series of dilutions of each essential oil (5, 10 and 20 µl each) was prepared using ethanol (50 µl) as solvent as described by Paranagama *et al.* (2003). Aliquot of each dilution was separately applied on inner surface of glass vials (100 ml) including cap. The solvent was allowed to evaporate for 2 min. and 12 pairs of mixed sex newly emerged each bruchids with 30 pigeon pea seeds were introduced into the each vial separately and screw cap was tightened. After incubation at 28 ± 2ºC temperature and 24h exposure, mortality was observed. The insects were considered to be dead as no leg or antennal movements were observed. A control experiment was maintained in which treatment was made with ethanol. Three replicates of each control and treatment set were made.

### *Fumigant toxicity bioassay*

Filter paper discs (1.5 cm dia.) were impregnated with aliquot of 5, 10 and 20 µl dilution of essential oils as prepared earlier. After evaporating the solvent for 2 min. the filter paper discs were attached to under surface of screw cap of glass vials (100 ml) separately and 12 pairs of each bruchids were introduced into the vials with 30 pigeon pea seeds separately (Huang *et al.,* 2000). The neck of the vials was blocked with nylon cloth to avoid contact effect of insects with paper disc. The cap of each vial was screwed tightly and kept at 28 ± 2ºC temperature. Mortality was observed after 24h exposure. Each concentration and control replicated three times.

### *Effect of essential oils on oviposition and adult development of bruchids*

Experiment was designed following the method Kumar *et al.* (2008). A stock solution of the each essential oil was prepared separately by dissolving 80 µl of oil in 1 ml of ethyl alcohol. Fifty seeds of pigeon pea (*Cajanus cajan* L.) were filled in glass vials (9.5 cm height X 2 cm diameter) and treated separately with different dose i.e. 20, 15 and 5 µl/ml of the oil. The seeds were then dressed by continuous shaking for five minutes for proper mixing of the oils on the seeds. For control sets the seeds were dressed in requisite amount of ethyl alcohol in place of the oil. After 24 hours, 12 pairs of bruchids of mixed sex were introduced in each vial separately and kept at 28 ± 2ºC temperature. Observations were made after 10 days for oviposition and after 21 days for progeny emergence. The per cent deterrency was calculated following formula of Paranagama *et al.* (2003).

### *Preparation of essential oil formulations*

Formulations were prepared following the methods of Moretti *et al.* (2002) to assess their efficacy during *in vivo* storage of pigeon pea seeds. Formulations of *Chenopodium* and *Clausena* essential oils were prepared separately by dispersing 1 and 5% (v/v) essential oil in glycerin (as emulsifier) and acetone. Acetone was used as a co solvent for addition of essential oils in glycerin. All the formulas were homogenized at 10,000 rpm for 10 minutes. The formulations prepared were stored separately in glass vials under air tight condition (4ºC) for further need.

### *Fumigation of pigeon pea seeds by developed formulations*

To see the fumigant effect of formulations on pigeon pea seeds during storage, 500 g of pigeon pea samples were kept separately in tin containers (45 cm diameter x 16 cm). Care was taken to use un-infested freshly harvested pigeon pea seeds. 20 individual of each insects i.e. *C. chinensis* and *C. maculatus* of mixed sex were introduced separately in tin containers. Variable concentration of oil based formulations (1 and 5%) of each essential oil was introduced separately in tin containers by soaking in cotton swab so as to procure concentration of 20 and 40µl. The containers were made air tight. The un-infested non fumigated pigeon pea seeds with insects were also run parallel as control set. Each experiment replicated three times. After six months the efficacy of formulations due to insects infestation was determined by calculating grains injured/punctured (%), weight loss (%) and feeding deterrence index (%) of treated and control sets. The grains damaged/injured were determined by weighing feeding injuries and emergence hole on the surface of the grains. The weight loss of seeds was calculated following formula of Perkin *et al.* (1956) while feeding deterrence index was calculated following Xie *et al.* (1996).

### *Statistical analysis*

Data were expressed as mean ± SD which obtained during each method that was statistically analyzed by two-way ANOVA, and means were compared using Duncan's Multiple Range Test (DMRT) at 0.05% level. Probit analysis was used to estimate $LC_{50}$ and $LC_{80}$ values (1999).

## Results

### *Contact toxicity bioassay*

$LC_{50}$ and $LC_{80}$ values of each essential oil are shown in Table 1. The essential oil of *C. ambrosioides* achieved $LC_{50}$ at 9.9 µl dose for *C. chinensis* and *C. maculatus*. Among other three oils, *Clausena* was the most toxic, followed by *Mentha* and *Ocimum* oil with $LC_{50}$ values 10, 10.1-10.2 and 11 µl respectively against both test insects.

### *Fumigant toxicity bioassay*

The *Chenopodium* oil again demonstrated highest fumigant toxicity to both species of beetles than other oils (Table 1). In contrast to contact toxicity, $LC_{50}$ values of *Chenopodium* oil were inferior for *C. chinensis* (9.3 µl) and *C. maculatus* (9.5 µl) followed by *Clausena* (9.9 µl) and *Mentha* (10-10.1 µl) oil. However it enhanced with *Ocimum* oil where 12 µl value was obtained for *C. chinensis* and 12.8 µl for *C. maculatus*.

**Table 1.** $LC_{50}$ and $LC_{80}$ values of tested essential oils at 24h exposure against test insects

| Methods | Essential oils | *Callosobruchus chinensis* | | | *C. maculatus* | | |
|---|---|---|---|---|---|---|---|
| | | Slope function | $LC_{50}$(µl) | $LC_{80}$(µl) | Slope function | $LC_{50}$(µl) | $LC_{80}$(µl) |
| Contact test | *C. ambrosioides* | 1.76 | 9.9 | 15.5 | 1.74 | 9.9 | 15.5 |
| | *C. pentaphylla* | 1.70 | 10 | 15.5 | 1.70 | 10 | 15.5 |
| | *M. arvensis* | 1.66 | 10.2 | 15.9 | 1.67 | 10.1 | 15.9 |
| | *O. sanctum* | 1.78 | 11 | 17.9 | 1.85 | 11 | 18.6 |
| Fumigant test | *C. ambrosioides* | 1.75 | 9.3 | 15.2 | 1.78 | 9.5 | 15.4 |
| | *C. pentaphylla* | 1.76 | 9.9 | 15.5 | 1.74 | 9.9 | 15.5 |
| | *M. arvensis* | 1.68 | 10.1 | 15.8 | 1.70 | 10 | 15.5 |
| | *O. sanctum* | 1.75 | 12 | 19.1 | 1.72 | 12.8 | 20.1 |

### *Effect on oviposition and adult development*

The effect of essential oils on oviposition and adult development of *C. chinensis and C. maculatus* in treated pigeon pea samples is depicted in Fig. 1(a), 1(b), 2(a) and 2(b). The presence of essential oils vapour significantly deters the majority of females of both bruchids from laying their eggs on the seeds than control sets. *Chenopodium* and *Clausena* oil exhibited superior oviposition deterrent activity for *C. chinensis* and *C. maculatus* than other oils. The oviposition due to both insects on seeds was reduced to 100% by them at 20µl oil dose (Fig. 1(a) and 2(a)). Hence progeny emergence was failed. The reduction of eggs hatching was also directly proportional to oil dose. *Chenopodium* and *Clausena* oils checked more than 84% of adult emergence of both bruchids at different doses and considered to be more potent than *Mentha* and *Ocimum* oil as shown in Fig. 1(b) and 2(b).

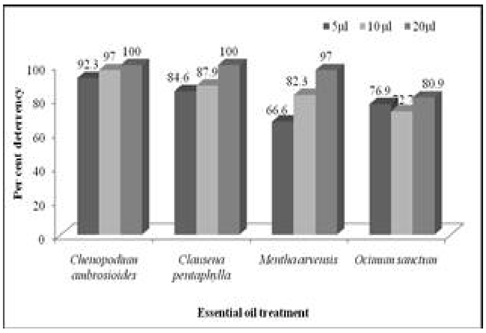


**Fig 1a.** Per cent deterrency in oviposition of *Callosobruchus chinensis* caused by oils

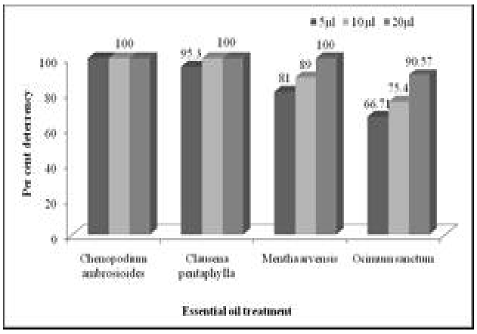


**Fig 1b.** Per cent deterrency in progeny emergence of *C. chinensis* caused by oils

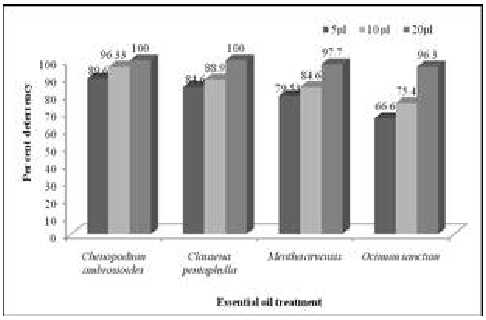


**Fig 2a.** Per cent deterrency in oviposition of *C. maculatus* caused by oils

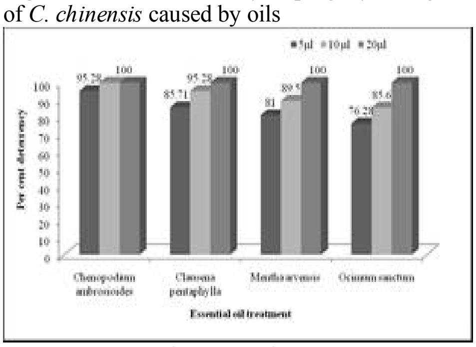


**Fig 2b.** Per cent deterrency in progeny emergence of *C. maculatus* caused by oils

### *Fumigant effect of formulations*

It revealed that both the formulations significantly protected stored grains from *C. chinensis* and *C. maculatus* (Table 2). The feeding deterrency index of *C. chinensis* was the utmost against 5% *Chenopodium* oil formulation (100%) at 40 µl concentration while lowest against 1% *Clausena* oil formulation (71%) at 20 µl concentration. For *C. maculatus*, both formulations of 5% exhibited 100% feeding deterrency at 40 µl dose. There were 72.87% grains damaged due to *C. chinensis* and 76.67% due to *C. maculatus*. However a significant reduction in weight loss was found in all fumigated seeds. The reduction in weight loss is directly proportional to formulation concentration. In *C. chinensis* the weight loss and grains damage due to 1% formulations of *Chenopodium* (2.67 and 5.86%) and *Clausena* (5.06 and 7.86%) at 40 µl concentration were significantly different from *Clausena* oil formulation (8.40 and 14.13%) respectively when treated at 20 µl concentration.

**Table 2.** Fumigant efficacy of botanical formulations on stored grains and infest with insect pests

| Treatment with formulations in µl | Dose (%) | *Callosobruchus chinensis* | | | *C. maculatus* | | |
|---|---|---|---|---|---|---|---|
| | | Weight loss (%)±SD | Grain damaged (%)±SD | FDI Index (%) | Weight loss (%)±SD | Grain damaged (%)±SD | FDI Index (%) |
| ***Chenopodium*** | | | | | | | |
| 20 | 1 | 5.60±1.80c | 8.27±1.15ab | 81 | 6.26±1.52c | 10.93±2.08ab | 76 |
| | 5 | 3.47±0.56b | 5.73±0.28b | 87 | 3.46±0.28b | 5.6±1.70b | 86 |
| 40 | 1 | 2.67±0.58b | 5.86±1.52b | 90 | 2.26±1.25b | 4.53±0.15b | 91 |
| | 5 | 0.00±0.00a | 0.00±0.00a | 100 | 0.00±0.00a | 0.00±0.00a | 100 |
| ***Clausena*** | | | | | | | |
| 20 | 1 | 8.40±0.76b | 14.13±1.52c | 71 | 9.68±0.87c | 13.47±0.66c | 66 |
| | 5 | 2.67±0.58b | 7.33±0.35ab | 90 | 4.66±0.75b | 9.87±0.41ab | 82 |
| 40 | 1 | 5.06±1.80c | 7.86±1.50ab | 82 | 3.33±1.52b | 6.93±0.57a | 87 |
| | 5 | 2.53±0.52b | 4.93±0.57a | 90 | 0.00±0.00a | 0.00±0.00a | 100 |
| **Control** | | 49.73±3.60d | 72.87±1.52d | | 46.8±3.60d | 76.67±1.25d | |

Each data represents the mean of three replicates. Per cent weight loss/ grains damaged (±SD) followed by same later within a column are not significantly different at 0.05 level (DMRT).
FDI- Feeding Deterrency Index.

## Discussion

Of the four oils assayed, all had distinct insecticidal and deterrent properties against *C. chinensis* and *C. maculatus*. Plant essential oils contain many volatile compounds jointly or independently, they might contribute to insecticidal activity; however the use of crude plant essential oil instead of purified or synthetic compounds may result in beneficial effect beyond mere pest control and therefore, convey additional economic benefits (Kim and Park, 2008). In present study, the minimum of 9.3 and 9.5 µl dose and 24h exposure of *Chenopodium* oil in fumigant test was effective to instill 50% mortality of *C. chinensis* and *C. maculatus* respectively by the end of exposure. This value prolonged in contact test (9.9 µl). Although *in vitro* and *in vivo* studies with *Chenopodium*, *Mentha* and *Ocimum* oils against *Callosobruchus* spp. had been conducted by earlier workers (Obeng-Ofori *et al.,* 1998; Tapondjou *et al.,* 2002; Tripathi *et al.,* 2009) but no report on pesticidal properties of *C. pentaphylla* oil against *C. chinensis* and *C. maculatus* was made till date. The toxicity of *Clausena* oil was first time reported in present study.

The octopaminergic nervous system has been suggested as novel target site of essential oils. The lack of octopamine receptor in vertebrates likely accounts for the profound mammalian toxicity, selectivity of essential oils as insecticides (Kostjukovsky *et al.,* 2002). In previous study *Mentha* oil was found to be effective against *C. maculatus* via fumigation which indicates that mode of delivery of oil by vapour action, likely via respiratory system (Raja *et al.,* 2001). In present study all the oils exhibited absolute toxicity via contact and fumigant test.

The mortality and deterrence records of treatment category showed positive relation with doses. The latter was more potent than former once. El-Nahal *et al.* (1994) stated that the period of exposure appears to be the most important factor affecting the efficiency of vapours of *Acorus calamus* oil to adult of five stored product insect species than the doses. On contrary in our study the insecticidal as well as ovipositional activity of all the volatiles varied according to dose as observed by Kim and Ahn (2001). In terms of mortality the efficacy of oils was recorded as *Chenopodium* > *Clausena* >*Mentha* > *Ocimum* against both test insects, by both test methods. Furthermore *Chenopodium* oil had more ovipositional deterrence of *C. chinensis* and *C. maculatus* than other oils. The marked decline in egg laying was perhaps a consequence of the mild suppressing effect exerted by these volatiles on the pulse beetles' mating, a decisive factor influencing the subsequent number of eggs laid by the beetles (Engelmann, 1970). The present findings corroborate the observation record for oil vapours on *C. maculatus* (Paranagama *et al.,* 2003). Further a drastic reduction in adult emergence that was recorded could also be due to low eggs hatchability. The oil vapours diffused into eggs and affected the physiological and biochemical process associated with embryonic development. The current results are in agreement with Ketoh *et al.* (2006) who have reported that *Cymbopogon* oil vapour treatment for 24h could be satisfactory for controlling eggs hatchability of *C. maculatus*. The reduction in adult emergence could either be due to egg-mortality or larval mortality or even reduction in hatching of the eggs. Oviposition inhibitors have the advantage of attacking a pest at the start of its life cycle. The insect is deterred from laying its eggs on the cereals/grains, thus preventing the pest population from increasing.

In the present study, the essential oil based formulations exhibited as botanical fumigants in protection of stored pigeon pea seeds up to six months by enhancing feeding deterrence and reducing grain damage as well as weight loss caused by *C. chinensis* and *C. maculatus*. Kumar *et al.* (2008) investigated that essential oil of *Aegle marmelos* protected stored grains from *C. chinensis* L. (Bruchidae) and wheat from *Rhizopertha dominica* F. (Bostrychidae), *Sitophilus oryzae* L. (Curculionidae) and *T. castaneum* Herbst. (Tenebrionidae) for first 24 months of storage thus more than the oils reported in present study. This may be due to differences in chemical composition and stability of monoterpenes of essential oils. The activity of essential oils decreased with time because of their high volatility. The rhythm of the reduction of their activity was not the same for both essential oils formulation tested. Oils with high content of hydrocarbon monoterpenes compounds lose their activity quicker than those containing mainly oxygenated monoterpene compounds (Huang and Ho, 1998). This oxidation leads to the reduction of pesticidal efficiency of the oil.

Essential oils act on insects through their aroma compound which are highly volatiles, renewable and biodegradable. Current study indicates that insecticidal mode of action of the volatiles may be largely attributable to contact and fumigant action. They may be toxic by penetrating the insect body via the respiratory system or by thorax (Aboua *et al.,* 2010). The prepared formulations enhanced feeding deterrence of *C. chinensis*, and *C. maculatus.* Therefore, insects were incapable to infest grain and cause gain damage.

In conclusion the aforesaid formulations might be useful products for managing population of pulse bruchids and can be a substitute of synthetic insecticides in preservation of stored pigeon pea seeds and other grains after successful field trials at farmer level. Application of essential oils and their formulations to grain seeds for storage is an inexpensive and effective technique, and its easy adaptability will give additional advantages leading to acceptances of this technology by farmers. A study to improve the effectiveness of botanical derivatives as insecticides will benefit agricultural sectors of developing countries, as these substance are not only of low cost, but also have less environmental impact in term of insecticidal hazards involved.

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