Menthol containing formulation inhibits adzuki bean beetle, *Callosobruchus chinensis* L. (Coleoptera; Bruchidae) population in pulse grain storage

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ABSTRACT

Plants synthesize many industrially useful chemicals for the benefit of humanity. Plant biopesticides are species specific, less harmful to beneficial arthropods, biodegradable and require low cost in developing formulations than synthetic pesticides that are directly developed from petrochemical derivatives. Twenty different tablet formulations prepared from various combinations of promising plant products have been evaluated for stored pulse grains protection. The natural menthol (9.09%) based tablet formulation containing natural binder and carrier agent-*Vigna mungo* seed powder (73.63%), liquid preservative-Acetic acid (11.36%), solid powdered preservative - sodium benzoate (5.68%) and edible lemon yellow colour (0.22%) (Treatment - XVII) applied once was found to be the most suitable for the management of adzuki bean beetle, *Callosobruchus chinensis* L. adult during pulse grains storage period of three and six months. The two tablets/250g grains comprising menthol (XVII) was found to reduce significantly 100% *C. chinensis* population in jars containing grains of both *Cicer arietinum* and *Lens esculenta* up to six months storage period. The beetle population did not increase as compared to average increase range of 245 to 1515 adult beetle populations in *C. arietinum* and 1825 to 3346 in *L. esculenta* in other treatments and untreated control during three months of storage. Result indicates that menthol containing cost effective tablets formulation may be developed at commercial level to protect the pulse grains attack of adzuki bean beetles during storage in homes and market places.

Key words : Biopesticides, adzuki bean beetle, pulse grains, menthol, tablet formulation.

INTRODUCTION

The adzuki bean beetle, *Callosobruchus* spp. (Coleoptera: Bruchidae) are major pests of stored pulse grains which cause 15 to 20% damage of total pulse grains production throughout the world (Kumar *et al*., 2003). To manage this serious pest, various physical and chemical pest management methods have been employed globally. Synthetic fumigants developed and used to control this pest in storage are found to leave residues in/ on grains and beetles have been found to develop resistance against Ethylene-di-bromide (EDB) and aluminium phosphide (phosphine) during storage (Bond, 1984; Zettler and Cuperus, 1990; Zettler and Keever, 1994). Both developed and developing countries are gradually banning most of the insecticides as they have harmful effects on human health and environment. Therefore, demand of plant-based pesticides increased globally to manage serious pests of agriculture.

During the last 50 years, more than 2000 plant species have been found to contain different insecticidal properties (Balandrin *et al*., 1985, Dayan and Duke, 2003) The insecticides developed from plant molecule (s) and/ or extract are known to have a relatively short life and their structure as well as formulation can be easily altered. In traditional African farming practice, vegetable oils, ash, sand and other plant products admixed with chilies and other stored grains or other fumigants, goat and cow urine or dung etc. have been used to protect the common beans from the attack of *Callosobruchus* spp. in storage granaries (Gahurkar, 1988; Getu and Gebre-Amlak, 1998; Abate *et al*., 2000, Isman, 2006). In Uganda, banana juice, pepper, Mexican marigold and eucalyptus leaves are also used for bruchid control (Giga *et al*., 1992). The black bean treated with coconut oil has been reported to reduce the egg oviposition of *C. chinensis* and number of exit holes in grains (Ani, 2010). However, thyme oil has shown significant mortality of *C. chinensis* adults (Righi - Assia *et al.*, 2010). Several plant products, essential oils and phyto-molecules have been screened against *C. chinensis* (McLaren, 1986; Singh and Agrawal, 1988; Lydon and

Duke, 1989; Singh *et al*., 1989; Shaaya *et al*., 1997; Singh and Mehta, 1998; Ketoh *et al*., 2002, Kim *et al*., 2003, Sathyaseelan *et al*., 2008). The direct application of grain protecting plant powders/oils on food grains are harmful as they may stick to seed coat of grain, create cleanness problem, leave high pungency, bitterness, oiliness and may toxic for human consumption. Although, the tablet formulation is usually considered safer than direct mixing of active materials with food grains, formulation may influence the bioactivity of even most promising plant products (Cox, 2004). The aim of the study is to identify the most active tablet formulation using different combinations of some promising plant products against *C. chinensis* which could be useful during storage.

MATERIALS AND METHODS

Insects

Both male and female adzuki bean beetles were collected from local markets of Lucknow, India and were reared on untreated healthy grains of chickpea, *Cicer arietinum* L. (Fabaceae) and lentil, *Lens esculenta* Moench. (Fabaceae), separately in 10 liter capacity glass jars (30mm x 23.5mm) at 25 ± 1º C and 75 ± 5% RH in insect culture room of laboratory.

Treatments

Twenty different tablet formulations (I - XX) were prepared by nine plant parts namely *Azadirachta indica* A. Juss. (Meliaceae), *Capsicum annum* L. (Solanaceae), *Cedrus deodara* (Roxb.) Loud. (Pinaceae), *Curcuma longa* L. (Zingiberaceae), *Mentha arvensis* L. (Labiatae), *Mentha piperita* L. (Labiatae), *Piper nigrum* L. (Piperaceae), *P. retrofractum* Vahl (Piperaceae), and *Syzygium aromaticum* (Linn.) Merr. & Perry (Myrtaceae). Seeds of *Triticum aestivum* L. (Gramineae), *Oryza sativa* L. (Gramineae), *Cicer arietinum* L. (Fabaceae), and *Vigna mungo* (L.) Hepper (Fabaceae) were used as carrier agents for the preparation of tablet formulation. Acetic acid (vinegar) was used as natural liquid preservative and sodium benzoate as dry powder preservative. Lemon yellow edible colour was used for the colour of each tablet. A consistent thick paste was made by distilled water and the quantity was standardized for each kind of tablet composition separately. Blank tablet was also prepared from carrier agent, preservatives, colour and water. The untreated control was designated as XXI. The quantitative details and per cent combination used for preparation of each kind of tablet/ treatment (I-XXI) is presented in Table 1.

Preparation of tablets

The method of preparing tablet formulation was developed and standardized by us in our laboratory (data unpublished). The healthy fruits, rhizomes, and seeds of plant species were shade dried and ground to a fine powder using an electric blender. The oil of *Azadirachta indica* A. Juss. (Meliaceae) was extracted from the seed kernel by compression method. The essential oils were obtained from the leaf of *Mentha piperita* L. (Labiatae) and wood chips of *Cedrus deodara* (Roxb.) Loud. (Pinaceae) by hydro-distillation method (Guenther, 1948-1952). Each botanical material was weighed with a Mettler electronic balance (M/S Mettler Instrumente AG, CH-8606 Grefensee-Zurich, Switzerland). The raw materials were then homogenized separately by electric blender. Distilled water (Quantity standardized) was added to prepare a consistent thick paste to formulate the tablet (25mm x 6mm) with an average weight of 5.76g each manually with a wood plank. A poly vinyl roller was used for spreading the dough, punched with cork and then tablets were shade dried at room temperature.

Bioassay

Two hundred and fifty gram of healthy, fresh, clean, unbroken and untreated seeds of chickpea, *C. arietinum* L. (Fabaceae) and lentil, *L. esculenta* Moench. (Fabaceae) were placed separately in clean and dry Perl Pet plastic jars (400 ml capacity) for each treatment. Two tablets of each treatment were placed inside the central place of the grain layer once in each jar (2 tablets/250g pulse grains). Ten unmated adult (6-7 day old) pulse beetles from the insect culture of respective hosts were kept in jars and covered with lids. The quantity of grain (250g) was based on our observation of preliminary experiments which was found sufficient for 10 adults and their progeny on each host during study period beyond six months of storage. Each treatment (I-XXI) was replicated five times. After three and six month's storage, total adult beetle populations (live and dead) were recorded in chickpea and lentil. In another experiment, similar observations were also carried out on different doses of menthol (0.288, 0.144, 0.72, 0.34, 0.17, and 0.08 g) containing tablet formulation by mixing with chalk powder to the total weight of 5.76 g as tablet formulation was prepared with the above mentioned method.

Data analysis

The data obtained on total number of beetles from each treatments were analyzed in a randomized complete block design. Insect counts at three months were log transformed to (X+5) before statistical analysis. Original mean values are given in parentheses. Least significant differences (LSD) were used to detect the difference of treatment means from the control mean (Steel and Torrie, 1960).

Table 1. Different natural products and their combinations used to prepare tablet formulations.

Plant species / materials	*Part* sused	Amount of different materials (%) in tablet formulation of various treatments																			
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX	XX
P. nigrum	fruit	nil	4	nil	4	4	4	4	4	4	4	4	4	4	4	4	4	nil	18.51	17.85	18.51
P. retrofractum	fruit	nil	2	2	nil	2	2	2	2	2	2	2	2	2	2	2	2	nil	nil	nil	nil
S. aromaticum	fruit	nil	2	2	2	nil	2	2	2	2	2	2	2	2	2	2	2	nil	nil	nil	nil
C. annum	fruit	nil	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	nil	0.1	0.1	0.1	0.1	nil	x	x	0.46
C. longa	rhizo-me	nil	4	4	4	4	nil	4	4	4	4	4	4	4	4	4	4	nil	nil	nil	nil
A. indica	oil	nil	1	1	1	1	1	nil	1	1	1	1	1	1	1	1	1	nil	4.62	4.46	4.62
M. piperita	oil	nil	6	6	6	6	6	6	nil	6	6	6	6	6	6	6	6	nil	x	x	x
C. deodara	oil	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	4.46	nil
T. aestivum	flour	25.94	25.94	25.94	25.94	25.94	25.94	25.94	25.94	25.94	25.94	25.94	25.94	nil	25.94	25.94	25.94	nil	nil	nil	nil
O. sativa	flour	25.94	25.94	25.94	25.94	25.94	25.94	25.94	25.94	25.94	25.94	25.94	25.94	25.94	nil	25.94	25.94	nil	nil	nil	nil
C. arietinum	flour	12.98	12.98	12.98	12.98	12.98	12.98	12.98	12.98	12.98	12.98	12.98	12.98	12.98	12.98	nil	12.98	nil	nil	nil	nil
V. mungo	flour	12.98	12.98	12.98	12.98	12.98	12.98	12.98	12.98	12.98	12.98	12.98	12.98	12.98	12.98	12.98	nil	73.63	60	57.85	60
M. arvensis	men-thol	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	9.09	nil	nil	nil
Acetic acid (Vinegar)	vinegar natural	2	2	2	2	2	2	2	2	nil	2	2	2	2	2	2	2	11.36	9.25	8.92	9.25
Sodium benzoate	synt-hetic	1	1	1	1	1	1	1	1	1	1	nil	1	1	1	1	1	5.68	4.62	4.46	4.62
Colour (Lemon Yellow edible)	edible colour	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	nil	0.04	0.04	0.04	0.04	0.04	0.04	0.22	0.18	0.17	0.18
Water	distilled water	QS	QS	QS	QS	QS	QS	QS	QS	QS	QS	QS	QS	QS	QS	QS	QS	QS	QS	QS	QS

Nil = Not added in formulation, QS = Quantity Standardized.

RESULTS

During three and six months of storage, the number of adzuki bean beetles was recorded significantly lowest in the menthol (XVII) treatment of *C. arietinum* (df = 20.00, F = 54.99, P $\leq$ 0.05) and *L. esculenta* (df = 20.00, F = 146.94, P 0.05), respectively. The average number of beetle population in treatments ranged from 245 to 1515 (Standard Deviation ranged from ± 0.00 to ± 420.13) adult in *C. arietinum* and 1825 to 3107 (Standard Deviation ranged from ±0.00 to 777.2) in *L. esculenta* and control during three months of storage. It was visually observed that the menthol containing tablet formulation did not kill the beetle but prevented the laying of eggs on the seed grain surfaces of both *C. arietinum* and *L. esculenta*. It also completely checked the outbreak of secondary pest (microbial) development. The seeds treated with menthol containing tablet formulations were found fresh, healthy, free from microbial infection, no eggs on grain coat surfaces and suitable for human consumption even after six months of storage period over other treatments and control. In contrast, the grains stored in jars containing other treatments and controls were highly damaged, high growth and development of beetles led to serious damage, infected with microbes, and seeds were not found fresh and healthy (Table 2).

In another experiment, tablet formulation containing menthol mixed with chalk powder was found reduce population of *C. chinensis* adults in seeds of *C. arietinum*. A minimum dose of 0.144g of menthol per 250g of chickpea grain in jar capacity of 400g found significantly (P 0.05) superior in effectiveness over other doses during 3 months of storage of *C. arietinum* with no increase in adzuki bean beetle population. However, in jars comprising untreated control and natural infestation, the pulse beetle population was recorded on an average 1861 and 1336 times, respectively (Table 3).

Table 2. Effect of different treatments on population of *C. chinensis* on grains of *C. arietinum* and *L. esculenta* during storage

Treatment numbers	*Cicer arietinum.*		*Lens esculenta*	
	Three months	Six months	Three months	Six months
I	6.76 (0865.2 ± 143.6)	-	7.95 (2873.0 ± 525.9)	-
II	7.17 (1294.0 ± 096.4)	-	7.79 (2431.8 ± 250.1)	-
III	6.99 (1119.0 ±390.2)	-	7.49 (1825.0 ± 455.5)	-
IV	6.93 (1032.6 ±180.9)	-	7.74 (2311.0 ± 401.8)	-
V	6.68 (840.4 ± 308.8)	-	7.75 (2362.6 ± 436.9)	-
VI	7.10 (1214.8 ± 093.4)	-	7.80 (2488.8 ± 511.2)	-
VII	7.15 (1267.2, ±100.3)	-	8.04 (3106.8 ± 389.4)	-
VIII	7.12 (1271.8 ± 356.2)	-	7.86 (2657.0 ± 704.6)	-
IX	6.94 (1039.2 ± 135.5)	-	7.83 (2567.6 ±599.5)	-
X	7.06 (1161.2 ±141.5)	-	7.99 (2984.6 ± 551.5)	-
XI	7.15 (1293.8 ±303.1)	-	7.86 (2614.6 ± 330.3)	-
XII	7.32 (1515.0 ±226.2)	-	7.76 (2417.2 ± 693.2)	-
XIII	6.38 (626.4 ± 267.6)	-	7.70 (2317.6 ± 777.2)	-
XIV	7.07 (1201.6 ± 275.4)	-	7.84 (2569.4 ± 455.7)	-
XV	6.94 (1063.2 ± 310.1)	-	7.80 (2467.6 ± 407.5)	-
XVI	7.23 (1422.2 ± 396.9)	-	7.94 (2873.6 ± 765.6)	-
XVII	2.71 (10.0 ± 0.00)^*	09.6*	2.71 (10.0 ± 0.00)^*	10.0*
XVIII	6.59 (800.8 ± 371.3)	-	8.12 (3346.0 ± 205.9)	-
XIX	5.41 (245.0 ± 116.7)	-	7.94 (2856.4 ± 560.9)	-
XX	6.26 (540.4 ± 175.6)	-	7.76 (2422.4 ± 799.9)	-
XXI	6.70 (884.6 ± 420.1)	-	7.93 (2790.0 ± 183.6)	-
F value calculated	54.99	NC	146.94	
SEM	0.135	NC	0.931	NC
LSD at 5%	0.379	NC	0.262	NC

*Statistically significant, NC = Not calculated due to high damage and grains decay infested with fungus ; Insect counts at three months were log transformed to (+ 5) before statistical analysis; Original mean values of population and, Standard Deviation (± SE) are given in parentheses.

Table 3. Effect of single application of different doses of menthol (natural) in the form of tablet formulation on population of *C. chinensis* in stored *C. arietinum*

Weight of natural menthol (g)	Number of total adult beetles inoculated initially/ replication	Mean up beetle population based on Log value
0.288	10	1.00* (10.0 ± 0.00) ^
0.144	10	1.00* (10.0 ± 0.00) ^
0.72	10	1.96 (472.5 ± 611.5)
0.34	10	2.70 (1415.0 ± 964.0)
0.17	10	2.06 (697.5 ± 822.8)
0.08	10	3.18 (1626.2 ± 539.8)
Control $	10	3.26 (1861.0 ± 353.1)
Natural infestation(Control) #	-	3.07 (1336.0 ± 646.7)
SEM	-	0.31
LSD at 5%	-	1.18

*Statistically significant, $ inoculated ten insect as in treatment, # natural infestation status in grains, no insect was inoculated, @Mean of four replications, ^ only inoculated insect, neither eggs on seed coat nor larva found inside the grains, healthy and clean grains for human consumption. Insect counts at three months were log transformed to (+5) before statistical analysis. Original mean values of population and, Standard Deviation (± SE) are given in parentheses.

DISCUSSION

Results of our experiment showed that among different tablet formulations and untreated control evaluated for its bio-efficacy against adzuki bean beetle, *C. chinensis*, the treatment (XVII) containing menthol (9.09%) showed significantly (P 0.01) lower counts of beetle population over other treatments in jars containing seeds of both *C. arietinum* and *L. esculenta* during three and six months of storage. The significant efficacy of menthol containing tablet formulation comprising natural menthol (9.09%) prepared with natural binder and carrier agent-*Vigna mungo* seed powder (73.63%), liquid preservative-Acetic acid (11.36%), solid powdered preservative - sodium benzoate (5.68%) and edible lemon yellow color (0.22%) (XVII) applied once was found to be the most suitable for the management of adzuki bean beetle, *Callosobruchus chinensis* L. adult in various pulse grains during six months of storage. In another experiment found authenticated that tablet formulation containing 0.144g menthol and chalk powder / 250g grains in jar capacity of 400g could check 100 per cent beetle population (Table 3). Aggarwal *et al.* (2001) has recorded similar results by application of L - menthol (one of the isomers of menthol) in controlling storage pests. However, we have evaluated the tablet formulation based on natural menthol obtained from essential oil of *Mentha arvensis* L. and earlier workers have not yet reported a suitable formulation for its application and not determined the optimum dose of menthol required to check the beetle population. Botanical insecticides are found to act as direct toxicants, deterrent and repellents to major pests and few of these are commercialized for application in agriculture (Isman, 2006). Plant powders, vegetables and neem oils have also showed effectiveness in managing *C. chinensis* in laboratories but due to stickiness of oil to seed coat, adding extra fat to the human diet, dis-tastefulness of grains for human consumption and their uneconomical status not yet found suitable for commercial feasibility (Schmutterer, 1990; Ba - Angood and Al-Sunaidy, 2003). Several workers have also reported the efficacies of different plant products against adzuki bean beetle but none has reported the suitable formulation useful to manage in storage and did not optimize the suitable dose for the management of beetle namely crude extracts of *A. indica* seeds resulted 76.8% significant reduction in ovipositional preference of *C. maculatus* reducing the further progeny (Elhag, 2000), *Syzygium aromaticum* reduced the adult longevity (Kellouche and Soltani, 2004), *Cedrus deodara* oil proved to be effective in managing *C. chinensis* over neem oil (Raguraman and Singh, 1997) and *Capsicum annum* showed 67 per cent antifeedant index against *Attagenus unicolor japonicus* larvae (Han *et al*., 2006) etc. Moreover, some fatty acids found in plant materials have also found to influence oviposition in *C. maculatus* (Parr *et al.*, 1998). Moreover, some fatty acids and other acids of natural origin have been also found to deter insects from oviposition against *C. maculatus* and other pests of economic importance (Li and Ishiwaka, 2005). Sodium benzoate is used as a preservative in various food products to control the outbreak of microorganisms (Kerbs *et al*., 1983). However, mixing plant materials with other active ingredients may

reduce the efficacies of deterrent odors/ toxicity/ sub-lethal effects of the materials in preparing the tablet formulations.

Essential oil of *Mentha arvensis* is one of the major natural sources of menthol found abundantly throughout the world. India is the world's largest producer of mint oil and menthol meeting its increasing demand. Menthol is extensively used in liquors, confectionery, perfumery, cough drops and nasal inhalers and used therapeutically as antipruritic (topical) and internally as carminative and gastric sedative in veterinary (Anonymous, 1983). Earlier studies showed that menthol LD_{50} in rats and mice are recorded as 4000 mg / Kg body weight, non-carcinogenic and has no organ specific toxicity in mice or either sex at the doses tested. Moreover, no genotoxicity has been reported by other workers [http://www.inchenm.org/documents/jeefa/jeemono/v042jeoje04.htm, International programme on chemical safety; World Health Organization, safety evaluation of certain food additives: WHO Food Additive Series 42]. The suppression of micro flora in jars treated with menthol containing tablet formulations may be due to its reported antimicrobial activity against plant pathogenic microorganisms (Iscan *et al*., 2002). However, in spite of the availability of large number of products known for their efficacies against adzuki bean beetle, so far none of the natural material (s) has been commercialized for the management of storage pests. Evidently, menthol is also known for improving human digestion. In case tablet is broken accidentally in stored pulse grains containers during application and storage or menthol molecule is attached to seed coat of grain in / on side it may not be harmful because of its medicinal value and may enhance the surface area for beetles' management during storage. . Besides, the aerosol formulation based on menthol active ingredient possibly could be another new economically viable formulation for use in grain silo and granaries.

Menthol is a volatile material at room temperature. Probably, the vapours released from it may choke the spiracles of insects, create hindrance in normal supply of oxygen to the trachea, disrupting the mating and/ or contracting the female reproductive parts not allowing the oviposition of eggs even by mated females. It is also an intriguing factor whether peripheral olfactory receptor neurons (ORNS) have been found inactive in receiving the needed signals in beetles in presence of menthol (Bruce *et al*., 2005).

Tablet formulations containing menthol as active ingredient was found as a preventive measure to protect the pulse grains from the attack of adzuki bean beetle (*C. chinesis*) during storage and simultaneously inhibit the growth of micro flora; fungus and bacteria. Being safe and eco-friendly, menthol containing tablet formulation protect the stored pulse grains by a single application for more than six months. The active ingredient- menthol is obtained from a edible herb, non-sticky with seed coat of the stored grains, non-mercury and non sulphur base products, no fear of human poisoning by fumigation, easy in handling of the final product and may be safe for human health. However, more research work is needed at the field-level for the evaluation of menthol - based formulations against adzuki bean beetle and other storage pests.

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