J Food Sci Technol (September 2014) 51(9):2210–2215
DOI 10.1007/s13197-012-0698-8

ORIGINAL ARTICLE

Insecticidal activity of *Ageratum conyzoides* L., *Coleus aromaticus* Benth. and *Hyptis suaveolens* (L.) Poit essential oils as fumigant against storage grain insect *Tribolium castaneum* Herbst

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Revised: 9 March 2012 / Accepted: 4 April 2012 / Published online: 24 April 2012

Abstract Essential oils (EOs) from *Ageratum conyzoides* L., *Coleus aromaticus* Benth. and *Hyptis suaveolens* (L.) Poit were extracted and tested against *Tribolium castaneum* Herbst, the storage grain insect. The EOs were found effective against *Tribolium castaneum* during *in vitro* as well as *in vivo* fumigant testing. The EOs of *H. suaveolens* and *A. conyzoides* showed 100 % mortality of test insect at 250 ppm while *C. aromaticus* at 350 ppm. During *in vivo* fumigant testing of wheat samples against *Tribolium castaneum*, the essential oils of *A. conyzoides* and *C. aromaticus* completely checked the damage of wheat grains by the insect at 1000 ppm while essential oil of *H. suaveolens* checked the grain damage completely even at 500 ppm concentration. There was no adverse effect on seed germination as well as on seedling growth of EOs treated seeds showing non-phytotoxic nature of the oils. Hence, these EOs may be recommended as botanical insecticide against insect invasion of stored food commodities, thereby enhancing their shelf life.

Keywords Essential oil · Fumigant activity · Phytotoxicity · *Tribolium castaneum*

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Introduction

Stored grain insect pests severely infest agricultural stored products and are responsible for worldwide loss of stored grains up to 10-40 % annually (Rajashekar et al. 2010).The continuous increasing pressure of expanding human population has also created a critical problem of food scarcity. In such a situation, to manage stored grains and other agricultural products from insect infestation, various synthetic insecticides have been used . However, reports on acquired resistance of insects against these synthetics and the residual toxicity of such chemicals have necessitated to search some biodegradable sources of pesticides (Zettler and Cuperus 1990; Jembere et al. 1995).

Earlier attempts to control stored grain pests relied on methods such as mixing gains with dry soil and wood ash for lethal dehydratiotion and fumigation with certain plant materials (Levinson and Levinson 1989). In one form or another, the use of admixture and fumigants for control of storage pests has continued present day also. In addition, growers are moving away from using methyl bromide as a post harvest fumigant because it is an ozone-depleting substance (Zhang and van Epenhuijsen 2004). Plant origin pesticides are much safer than conventionally used synthetic pesticides. The biological activity in some plants may be due to synergistic effects of different active principles. They may impart different mode of action during their pesticidal action (Varma and Dubey 1999). Amongst different plant products, the use of essential oils extracted from aromatic plants to control these pests has been investigated and is well documented (Isman 2006; Devi and Devi 2011). Some studies have assessed the ability of the essential oils and their constituents as fumigants and repellents against a number of insect pests (Clemente et al. 2003; Kumar et al. 2007) and some formulations of essential oils are in use for control of losses of stored food commodities due to biodeterioration caused by insect infestation (Dubey et al. 2010).

The rust-red flour beetle*, Tribolium castaneum* is a major pest of wheat grain flour (Howe 1965) and other stored cereals in tropical and subtropical regions of the world. The present study has been focused on the *in vitro* contact and fumigant activity of the chemically characterized essential oils of *Ageratum conyzoides* L., *Hyptis suaveolens* (L.) Poit. and *Coleus aromaticus* Benth. on mortality of *Tribolium castaneum* as well as on the *in vivo* fumigation of stored wheat samples by the oils for management of deterioration caused by insects in order to find out their practical efficacy as plant based insecticides. *Ageratum conyzoides* L. and *Hyptis suaveolens* (L.) Poit are native to tropical America while *Coleus aromaticus* Benth is native to East Indies and it has been distributed all over the tropical and subtropical region of India, Pakistan, Sri Lanka, tropical East Africa, Brazil, Egypt, Arabia and Ethiopia. All the three plants are well-known as ethnomedicinal and grow luxuriantly in India (Guha bakshi et al. 2001).

Materials and methods

Rearing of test insects The storage insect pest *Tribolium castaneum* Herbst was collected from stored wheat grain flour and its identity was confirmed from Deparment of Entomology, Institute of Agriculture Sciences, Banaras Hindu University, Varanasi, India. The culture of the insects *Tribolium castaneum* Herbst (family: Tenebrionidae) was maintained on wheat (*Triticum aestivum* L.) grains following Perez-Mendoza et al. (2004). 200 adult insects were released separately in 250 g of wheat grain flour in plastic containers covered by muslin cloth. After 48 hours, the adult insects were removed from the wheat grain flour and the samples were stored in the laboratory at 28±2 °C to get insects of same age.

Isolation of essential oils of the selected angiospermic plants Essential oils were isolated from fresh leaves of *Ageratum conyzoides* L., *Coleus aromaticus* Benth. and *Hyptis suaveolens* (L.) separately through hydrodistillation by Clevanger's apparatus for 3 h (Prakash et al. 2011). The isolated oils (yield 0.5%V/W, 1.1%V/W and 0.8%V/W respectively) were dried over anhydrous sodium sulphate and the essential oil was stored in clean glass vials.

In vitro insecticidal activity of the essential oils against T. castaneum the stored grain pest by fumigant method The essential oils were tested for their fumigant activity on mortality of *T. castaneum* following the method of Kim and Ahn (2001) with slight modification. Plastic containers of 500 mL capacity with screw lids were used as exposure chambers. Requisite amount of EOs were dissolved separately in 0.25 mL acetone and applied to a circular filter paper (WhatmanNo. 1, 3 cm diameter) were introduced into the plastic jars to achieve final concentrations (50–500) ppm with respect to volume of the jars.

Controls received only 0.25 mL acetone. Thereafter allowing the acetone to evaporate by keeping the jars in a fume chamber for 10 min at room temperature, the filter paper was attached to the inner surface of the screw lid of the jar using adhesive tape. 25 adult insects of same age were placed in plastic containers. Treated sets were held at 28±2 °C (80±5 % RH). Insect mortality was determined at 1,2,3,4 and 5 days after treatment. Test insects were considered dead if appendages did not move when podded with brush. The LC_{50} and LC_{90} values of essential oils were calculated by Probit analysis (Finney, 1971).

All the experiments were repeated three times and % corrected mortality was recorded by Abott's formula.

$$P = \frac{T - C}{100 - C} \times 100$$

Where

- P % corrected mortality
- T % kill in treatment
- C % kill in control

In vivo fumigation of stored wheat samples by the oils for management of deterioration caused by insects The wheat samples were separately fumigated by the oils by the usual methods of Dubey et al. (1983a), Dikshit et al. (1983) and Shaaya et al. (1997). Prior to experimental, the grain sample were cleaned and sterilized at 45 °C for 6 h in order to kill the eggs and developing larvae of pre infested insect pest (Jayakumar 2010). Different lots, each containing 500 g of wheat samples (moisture content 12 %) were taken separately in closed plastic containers (volume 1 litre). The insect *Tribolium castaneum* (25 adults of insect) were introduced in each lot. Requisite amount of the essential oils were introduced separately in the plastic containers by soaking in cotton pieces so as to procure concentrations of 250, 500 and 1000 ppm (v/v) for each oils. Control sets contained wheat samples infested by insects (without any treatment with the essential oils). After twelve months of storage at laboratory conditions temperature 28±2 °C and RH 80±5 % the analysis of insects associated with wheat samples of treated and control sets were made. The efficacy of *Ageratum conyzoides* L., *Coleus aromaticus* Benth. and *Hyptis suaveolens* (L.) EOs on insect infestation were determined

Table 1 LC_{50} and LC_{90} value of essential oils of *Ageratum conyzoides, Hyptis suaveolens and Coleus aromaticus* oils against *T. castaneum* after 24 h exposure time

Essential oil	LC 50(ppm) (95 % fiducial limits)	LC90 (ppm (95 % fiducial limits)	Slope ± S.E.	Chi Square (χ2)	df
Ageratum conyzoides	213.02 (192.81 - 232.95)	351.12 (312.43 - 416.76)	5.91±0.37	15.27	6
Hyptis suaveolens	229.33 (201.03 – 259.47)	414.39 (349.14 – 557.16)	4.98±0.34	22.43	6
Coleus aromaticus	256.71 (238.99-275.32)	414.20 (372.97-482.30)	6.17±0.43	10.19	6

by calculating grain damage (%), weight loss (%) and feeding deterrence (%) of treated and control sets. The damage was observed as appearance deformation and flour on grains in treatment and control sets.

Grain damage (GD)(%),

$$GD(\%) = \frac{C - T}{C} x\ 100$$

Where

C number of infested grain (flour appearance) in control set.
T number of infested grain (flour appearance) in essential oil treated set

The weight loss (%) of wheat samples in the treated and control sets was calculated by fresh weight basis using the formula suggested by Parkin (1956).

$$\%\, Weight\, loss = \{(WI - W)/WI\} \times 100$$

Where

W_I Weight of grains before the experiment.
W Weight of grains after experiment.

Feeding deterrence was calculated using the feeding deterrent index following Isman et al. (1990)

$$FDI\ (\%) = \frac{C - T}{C} x\ 100$$

Where

C Weight loss in control set.
T Weight loss in fumigated set.

Phytotoxicity of the essential oils The phytotoxicity of the EOs in terms of seed germination and seedling growth of wheat was assayed with respect to control sets following Mishra and Dubey (1994). Requisite quantity of the oils were dissolved in one ml of 0.01 % of Tween-80 and it was then pipetted in a flask containing 19 ml water so as to procure the final concentration of 500 ppm for *H. suaveolense* and 1000 ppm for *A. conyzoides and C. aromaticus* essential oils. 50 g seeds of wheat were soaked separately in each of the solution for 30 and 60 minutes. After soaking for requisite period 20 seeds were placed on two layered Whatman No.1 moistened filter paper in sterilized Petri plates. For controls, requisite amount of sterilized water was used in place of the essential oils. The experiment was repeated for five times. Percent germination of wheat seeds as well as length of radicle and plumule was recorded at 24, 48, 72 and 96 h interval.

Data analysis

Experiments were performed in triplicate and data analysis was done on mean ± SE subjected to one way ANOVA. Means are separated by the Tukey's multiple range test when

Table 2 Effect of essential oil s on grain damage, Weight loss and Feeding deterent index in treated wheat during storage (Insect: *Tribolium castaneum*) (n=3)

Insect *Tribolium castaneum*	Concentrations of oil (ppm)	*Hyptis suaveolens* (L.) Poit.	*Ageratum conyzoides* L.	*Coleus aromaticus* Benth.
% grain damage	250	$8.0±0.11^b$	$8.3±0.13^c$	$12.5±0.08^c$
	500	$0.0±0.00^a$	$3.8±0.05^b$	$8.8±0.11^b$
	1000	$0.0±0.00^a$	$0.0±0.00^a$	$0.0±0.00^a$
	Control	$96±0.00^c$	$96±0.00^d$	$96±0.00^d$
% weight loss	250	$5.2±0.05^b$	$7.1±0.21^c$	$10.0±0.17^c$
	500	$0.0±0.00^a$	$3.2±0.11^b$	$6.3±0.10^b$
	1000	$0.0±0.00^a$	$0.0±0.00^a$	$0.0±0.00^a$
	Control	$23.2±0.15^c$	$23.2±0.15^d$	$23.2±0.15^d$
% Feeding Deterent Index (FDI)	250	$77.42±0.11^a$	$68.86±0.35^a$	$56.39±0.25^a$
	500	$100.0±0.0^b$	$86.11±0.06^b$	$72.31±0.26^b$
	1000	$100.0±0.0^b$	$100.0±0.0^c$	$100.0±0.00^c$

Means followed by the same letter in the same column for each parameter do not significantly ($p<0.05$) different according to ANOVA and Tukey's multiple comparison tests

Table 3 Effect of *Hyptis* oil, *Ageratum* oil and *Coleus* oil on germination (%) of seeds of treated wheat (n=3)

Essential oil	Germination period (h)	Control	½h treatment (500 ppm)	1 h treatment (500 ppm)
Hyptis oil	24	90±0.28^{c}	73±0.28^{c}	68±0.60^{c}
	48	97.5±0.40^{b}	87±0.34^{b}	86±0.15^{b}
Ageratum oil	24	90±0.17^{b}	80±0.40^{c}	75±0.00^{c}
	48	100±0.00^{a}	97.5±0.17^{b}	89±0.20^{b}
Coleus oil	24	93.2±0.11^{c}	90±0.850^{c}	73±0.32^{c}
	48	97.5±0.17^{b}	96±0.11^{b}	90±0.00^{b}
*	72	100±0.00^{a}	100±0.00^{a}	100±0.00^{a}
	96	100±0.00^{a}	100±0.00^{a}	100±0.00^{a}

* absolute germination for all the treatment. Means followed by the same letter in the same column for each parameter do not significantly $p<0.05$) different according to ANOVA and Tukey's multiple comparison tests

ANOVA was significant ($p<0.05$) (SPSS 10.0; Chicago, IL, USA).

Results and discussion

In recent years, many essential oils have been shown to be effective against stored product insects (Don Pedro 1996; Ranaweera 1996; Jayasingha et al. 1999; Elhag 2000; Adhikari et al. 2002; Bandara et al. 2005) but the practical efficacy of only few oils have been observed. The literature is mostly silent on the *in vivo* pest controlling potency of the oils.

Fumigation has been considered to be the most effective, practical and quick method of eradicating storage pests. The tiny gas molecules easily penetrate large stacks right into the individual grains, reaching and killing all stages of development of the pest (Bond 1984). Hence, for practical application in management of biodeterioration of stored wheat samples by the insects, the efficacy of essential oils of *Ageratum conyzoides, Coleus aromaticus* and *Hyptis suaveolens* were evaluated as fumigant in the present investigation.

In the present investigation, the essential oils of *A. conyzoides, H. suaveolens* and *C. aromaticus* were found absolute insecticidal in nature to the test insect during *in vitro* testing by filter paper method. All the three oils showed 100 % mortality of the test insect *Tribolium castaneum.* During *in vitro* fumigation, the insecticidal activity of the oils was found to vary against *Tribolium castaneum*, with their concentration and exposure period. The essential oil of *H. suaveolens* and *A. conyzoides* could show 100 % mortality of *Tribolium castaneum* on 2nd day of exposure at 250 ppm while essential oil of *C. aromaticus* showed 100 % mortality on 2nd day of exposure at 350 ppm. The LC_{50} value of EO of *H. suaveolens A. conyzoides* and *C. aromaticus* were found to be 229.33, 213.02, 256.71 ppm respectively while the corresponding LC_{90} values were 414.39, 351.12 and 414.2 ppm (Table 1).The essential oils showed absolute insecticidal potency on prolonged exposure after fumigation and at enhanced concentrations. Their efficacy increased with increased concentration of doses.

During *in vivo* fumigant testing, the essential oils were highly effective in checking infestation of wheat samples by *Tribolium castaneum*. At 1000 ppm essential oils of *Ageratum conyzoides* and *Coleus aromaticus* completely checked the damage of wheat grains while essential oil of *H. suaveolens* checked the grain damage completely even at 500 ppm showing its more efficacy than other two oils. The essential oils showed zero % weight loss and 100 %

Table 4 Effect of *Hyptis* oil, *Ageratum* oil and *Coleus* oil on seedling growth of wheat (n=3)

Age of Seedlings (h)	Radical (cm)	1/2 h	1 h	Plumule (cm)	1/2 h	1 h
Hyptis oil	Control	500 ppm	500 ppm	Control	500 ppm	500 ppm
48	1.17±0.01^{c}	1.02±0.12^{c}	0.85±0.01^{c}	0.36±0.12^{c}	0.28±0.04^{c}	0.26±0.08^{c}
72	2.10±0.2^{b}	2.1±0.15^{b}	1.86±0.08^{b}	0.53±0.08^{b}	0.34±0.17^{b}	0.32±0.13^{b}
96	2.53±0.14^{a}	2.4±0.12^{a}	2.13±0.20^{a}	1.12±0.14^{a}	0.93±0.17^{a}	0.78±0.13^{a}
Ageratum oil	Radicle (Control)	1000 ppm	1000 ppm	Plumule cm	1000 ppm	1000 ppm
48	2.13±0.13^{c}	1.80±0.05^{c}	1.75±0.10^{c}	1.27±0.05^{c}	1.17±0.14^{c}	1.03±0.03^{c}
72	2.60±0.11^{b}	2.00±0.00^{b}	1.94±0.15^{b}	1.45±0.02^{b}	1.39±0.17^{b}	1.28±0.03^{b}
96	3.13±0.13^{a}	2.50±0.05^{a}	2.23±0.14^{a}	2.23±0.08^{a}	1.96±0.08^{a}	1.85±0.02^{a}
Coleus oil	Radicle (Control)	1000 ppm	1000 ppm	Plumule cm	1000 ppm	1000 ppm
48	2.10±0.10^{c}	2.01±0.11^{c}	1.93±0.11^{c}	0.99±0.05^{c}	0.90±0.15^{c}	0.84 ±0.20^{c}
72	2.50±0.10^{b}	2.32±0.00^{b}	2.18±0.05^{b}	1.32±0.10^{b}	1.10±0.05^{b}	1.07±0.20^{b}
96	2.80±0.00^{a}	2.50±0.17^{a}	2.30±0.11^{a}	1.60±0.11^{a}	1.40±0.15^{a}	1.23±0.88^{a}

Means followed by the same letter in the same column for each parameter do not significantly ($p<0.05$) different according to ANOVA and Tukey's multiple comparison tests

FDI index, at 1000 ppm against test insect. But the efficacy of the oils declined at lower concentrations. However, the essential oil of *Hyptis suaveolens* was strongly efficacious even at 500 ppm showed zero % weight loss and 100 % FDI index (Table 2). The mycoflora analysis of the wheat grains has not been observed in the present investigation, although, the samples of control and treatment sets were procured from same source.

Estimation of weight loss of the fumigated commodities in comparison with untreated samples of control sets gives a clear picture of the efficacy of a product used as fumigant. Hence, this parameter was observed in the control and treatment sets before observing the efficacy of oils as feeding deterrent. Results of present study showed the efficacy of essential oils against the infestation of wheat samples by *T. castaneum*. The essential oils were found to be equally and absolutely effective at 1000 ppm showing remarkable control of the infestation of the wheat samples with respect to grain damage, weight loss as well as FDI during *in vivo* investigation. Such findings of the present investigation are in accordance with earlier reports made with essential oil of *Cymbopogon martini* (Kumar et al. 2007).

A product may be recommended in the management of post harvest biodeterioration of food commodities only when it is non phytotoxic in nature. The germination and seedling growth of the treated commodities should not be adversely affected by treatment with the pesticidal product. In the present investigation the three essential oils neither showed any adverse effect on germination of wheat seeds nor exhibited any deleterious effect on seedling growth of wheat indicating their nonphytotoxic nature (Tables 3 and 4). At 72 h period of exposure absolute germination of wheat seeds for all the treatments. It was also found that there was no visual abnormality in morphology of the plants of all the treatment sets and the plants appeared as healthy as those of control sets, thereby, further indicating the nonphytotoxic nature of the essential oils showing their prospectives as plant based insecticide. During practical application the farmer and growers may store the treated wheat samples for different period. Hence, direct phytotoxic testing of the oils was performed to know their effect on wheat germination immediately after treatment. Moreover, essential oils are tested as fumigant .The oils being volatile in nature would easily emit the vapours during sun drying. In addition, the ethnomedicinal uses of these plants may strengthen the possibility of their exploitation as safe insecticides. However, before large scale trial their safety profile on mammals should be investigated.

The essential oils as such contain different types of major and minor components in their chemical profile. Their activity may be mostly because of synergism between the components present in them (Desmarchelier 1994; Isman 2000). The intimate knowledge of essential oil composition leads to its proper exploitation in pest management. The chemical profiles of essential oils have been reported by our research group (Jaya et al. 2011). Currently some essential oils formulations have been prepared through microencapsulation technologies and are used as fumigants against storage grain pests (Dubey et al. 2010). A few EO based preservatives are already commercially available. 'DMC Base Natural' is a food preservative comprising 50 % EO from rosemary, sage and citrus and 50 % glycerol (Mendoza-Yepes et al. 1997). Recently some herbal products showing pesticidal activity are recognizes as 'generally recognized as safe' (GRAS) as food additives in some of the developed countries and modern society is attracting towards 'green consumarism' (Tuley de Silva 1996; Smid and Gorris 1999) desiring fewer synthetic ingredients in foods. Hence, EOs are currently regarded as potentially useful additives for food preservation. Moreover, recommendation of herbal products as 'generally recognized as safe' (GRAS) as food additives in the developed countries may lead scientific interest in the exploitation of essential oils as plant based antimicrobials.

Based on the findings of the present investigation the large scale trials are required with these oils so as to recommend them as gain protectant and the treated samples should be subjected to pharmacological investigations in order to find out the safety profile of the oils. Being abundantly growing in tropical and subtropical countries, sufficient raw materials would be available for formulation of insecticides from the oils of these plants.

Conclusion

The essential oils of *Ageratum conyzoides, Coleus aromaticus* and *Hyptis suaveolens* based on their *in vivo* and *in vitro* insecticidal efficacy may be recommended as nonphytotoxic herbal insecticides against *Tribolium castaneum* contamination.

Acknowledgement This work was financially supported by University Grant Commission (UGC), New Delhi, India.

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