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Screening and synergistic action of medicinal plant powders against cowpea weevils *Callosobruchus maculatus* as adulticidal and fecundity and fertility inhibitor

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Abstract

In India agriculture is the main stay in rural population representing 80% of the country and depends on it for their livelihood. In all over the world formers incur losses of stored products due to insect and pests damage. Chickpea is cultivated throughout the world and is placed third in the importance list of the food legumes but suffer heavy qualitative and quantitative losses from the attack of cowpea weevils, *Callosobruchus species*. In the present paper, the evaluations were aimed to generating natural product treatments suitable for post-harvest grain protection in the control of the cowpea weevils, *Callosobruchus maculatus* attacking pulses in storage condition. Seven medicinal plant powders *viz*. *Chrysanthemum cinerariiaefolium* (CC), *Cymbopogon nardus* (CN), *Cymbopogon flexuosus* (CF), *Eucalyptus hybrid* (EH), *Eucalyptus citriodora* (EC), *Pelargonium graveolens* (PG), *Rabdosia melisoides* (RM) and their combinations were evaluated for adulticidal activity as contact and fumigant toxicities and fecundity and fertility inhibition against *C. maculatus* to find out value added suitable grain protectants. CF powder was highly toxic with LC_{50} values of 18.0 g/100 g and 12.3 g/litre in contact and fumigant toxicity assays, respectively. CN, CF and PG gave complete inhibition of egg laying at maximum concentration of 5g/100 g food. Combination of EC + EH in equal ratio also provided 100% inhibition of egg laying. Powders of CN, CF, PG and combinations of EC + EH, EC + RM, EH + RM in 1:1 ratio and EC + RM + PG in 1:1:1 ratio provide complete fertility inhibition in terms of F1 progeny production at the concentration of 2.5 g/100 g food and above. The findings indicate the use of these plant materials as stored grain protectant with added advantage of their medicinal value.

Keywords: Medicinal plant powders, cowpea weevils fecundity and fertility, fumigant, adulticidal

Introduction

In tropical and subtropical regions, millions of populations depends on pulses for proteins [25, 31]. Pulses are the members of family Leguminosae, are main source of dietary proteins and minerals in humans across the world. Pulses constitute 27% of the global crop production and contribute about 33% of the dietary requirement in humans. India is the biggest pulses producer about 22.08 m tones and consumes about 25-28% of total global production. [15, 16, 36, 6, 10]. Pulses are severely damaged by bruchids in Asia and Africa under storage conditions [43]. Among the bruchids, cowpea weevils, *Callosobruchus maculatus* (Coleoptera: Bruchidae) is the most notorious insects causing damage both under field and storage conditions [34] and causing about 10-15 % loss [3].

In tropical and sub-tropical regions of the world, this weevils have involved the great attention because it is widely distributed and it is an important pest of several pulses including cowpea [*Vigna unguiculata* (L.) (Walp.)], chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* Medik.), soyabean (*Glycine max* Mer.) and haricot beans (*Phaseolus vulgaris* L.). The damaged pulses lose weight and market value and they are generally unacceptable for human consumption [19]. Stored seed legumes are being widely protected from insect pest damage by the use of two commercially available synthetic fumigants, aluminium phosphide and ethylene dibromide in India. Besides hazardous effects and cost associated with these insecticides, there are problems of development of insect resistance. Thus it is necessary to re-look the potential of locally available plant materials as stored grain protectants [14]. Synthetic pesticides are currently the method of choice to protect stored grain from insect damage. But, continuous or heavy uses of synthetic pesticides has created serious problems arising from factors such as direct toxicity to parasites, predators, pollinators, fish and man. It also develops pesticides resistance [44, 26] susceptibility of crop plant to insect pests [33] and increased environmental and social cost [32]. Therefore, environment needs some other alternatives of chemical pesticides.

Plant products may be a better solution for the damage done by cowpea weevils due to their biodegradable nature. Plant powders can have protective effect on stored grains based on several mechanisms. In addition to direct toxic effect, the plant materials may produce odours that may act as repellent, which could protect further invasion. Thus development of botanicals to replace chemical insecticide as fumigants and minimize insecticidal residual effects would be of significant social and health benefits [29]. The aromatic medicinal plants used in the present work have wide applicability in medicine and drugs, but work on their potential as stored grain protectant remains meager. The present study has been aimed to investigate powders of certain medicinal plants for their adulticidal, and fecundity and fertility inhibitors activities against the cowpea weevils, *Callosobruchus maculatus*. Further, their synergistic combinations have also been evaluated for search of a suitable formulation product acting as stored grain protectant.

Materials and Methods

Powders of Medicinal Plant Parts

Seven medicinal plant parts listed in Table 1, were dried in shade and ground to fine powder mechanically just before the start of each set of experiments. The powders were packed in cheese cloth bags. These bags were used in all the experiments.

Table 1: Leaf powder of medicinal plants tested for activity against *Callosobruchus maculatus*

Common name	Scientific name	Code	Family	Part used	Major constituents
Pyrethrum	*Chrysanthemum cinerariaefloium*	CC	Asteraceae	Flower	Pyrethrin I, Pyrethrin II, Cinerin, Jasmolin and sesquiterpene lactones (Sashida *et al*., 1983, Glynne-Jones, 1981)
Lemongrass	*Cymbopogon flexuosus*	CF	Poaceae	Leaf	Citral, Geraniol, citronellol, (Chisowa *et al*., 1998)
Citronella	*Cymbopogon winterianus*	CN	Poaceae	Leaf	Citral, citronellol, geraniol, nerol, citral, citronellal, camphene (Silva *et al* 2010) Wijesekara, 1973
Eucalyptus	*Eucalyptus hybrid*	EH	Myrtaceae	Leaf	1,8-Cineole (Singh & Naqvi, 2003)
Eucalyptus	*Eucalyptus citriodora*	EC	Myrtaceae	Leaf	1,8- Cineole Citral (Singh and Naqvi, 2003)
Geranium	*Pelargonium graveolens*	PG	Geraniaceae	Leaf	Citronellol, linalool, geraniol, citronellyl formate (Rao, 2000)
Patti	*Rabdosia melisoides*	RM	Labiatae	Leaf	Thymol,P- Cymene menthol (Singh *et al*., 1983; Singh and Naqvi, 2005)

Rearing of Cowpea weevils

C. maculatus was reared on chickpea seeds (*Cicer arietinum* var. 'Pant G 114') in a growth cabinet at a constant temperature of 30^{0}C and 70% relative humidity, with a photoperiod of 12h light:12h darkness. Adults of *C. maculatus* (0-1- day old) from the F1 progenies were e used in all the experiments.

Adulticidal as contact bioassay

The medicinal plant powders contained in cheese cloth bags were kept at the bottom of screw cap jar (300 ml capacity) along with food substrates as chick pea seeds. 5, 10, 15, 20, 25 and 30 g of ground powders/100g of sterilized (steam sterilization in autoclave) food materials. Untreated food materials served as control. Batches of 10 adult insects were introduced in each bottle and closed air-tight with lid. Five replications were used for each dose. Mortality was recorded daily for 7 days but the analysis was based on mortality percentage after 7d of exposure periods.

Adulticidal as fumigant bioassay

The medicinal plant powders were tested for fumigant toxicity in space trial test. Plant powders (5, 10, 15, 20, 25 and 30 g of each plant) were taken in a muslin cloth bag and placed at the bottom of 1 liter glass bottle. Batches of ten adult insects of *C. maculatus* were introduced into each bottle and their caps were screwed tightly. In control, no plant material was kept. Ten replicates were set up for each treatment and for control. Mortality was observed at 48h intervals until end-point mortality, which was reached after 7d. The LC_{50} values were determined by the adult mortality data.

Fecundity inhibition bioassay

For fecundity inhibition bioassay, plant powders were thoroughly mixed with healthy and beetle damage free chickpea seeds at the concentrations of 1.0, 2.0, 3.0, 4.0 and 5.0 g/100 g seeds. Five pairs of freshly emerged 0-1 d old adults of *C. maculatus* were released into each vial containing treated seeds and considered as one replication.

Five replications were used for each concentration of plant powders. In control only untreated seeds were used. After 7 days adults were removed. The total number of eggs laid on chickpea seeds were counted and average percent egg laying per pair of beetles were calculated. The data obtained were analyzed for percent fecundity inhibition.

Fertility inhibition bioassay

Medicinal plant powders found toxic towards cowpea weevils were tested at lower doses to verify their effects on adults emergence, Powders at the rate of 0.5, 1.5, 2.5 and 4.5 g /100g seeds were placed in cylindrical jars (25 x 10 cm). Five pairs of *C. maculatus* were introduced separately in each jar and then covered. Each treatment was replicated five times. The parent adults were removed after 7 d. Observations on F1 adults emerged were made after 30 d of introduction of parent adults.

Statistical analysis

Data from all experiments were subjected to analysis of variance (ANOVA) and means were separated by Least Square Difference (LSD test) [38]. LC_{50} was determined by Probit analysis [12]. Mortality data was corrected using Abbott's formula [1, 42].

Results

Contact bioassay

Effects of medicinal plant powders as contact toxicant towards cowpea weevils is given in Table 2. CF showed highest toxicity with LD_{50} values of 18.0g/100g food. In combination EC + EH gave highest level of toxicity followed by EC + RM with LD50 values of 16.3 and 17.5 g/100 g food, respectively. Both EC and RM were least effective among all the plant powders tested but in combination they provide better toxicities towards *C. maculatus* adults indicating synergistic effects (Table 2). Similar activities were observed in case of EC, EH and their combination. However plant powders in triplet combination could not provide better results than binary mixtures.

Table 2: Effect of plant powders and their combinations (1:1/1:1:1) on adults emergence of *Callosobruchus maculatus* (F)

Rate Powder of admixing plant material (g/100 g food)					
Rate of powder	0.0	0.5	1.5	2.5	4.5
Mean number* of F_1 adults emergence					
CC	52^a	38^b	15^b	12^a	0^a
CN	40^b	25^b	10^e	0^b	0^b
CF	32^c	25^b	5^a	0^a	0^a
EH	46^d	28^b	19^e	11^b	5^b
EC	28^e	36^b	24^e	17^b	11^b
PG	52^a	38^b	15^b	0^a	0^a
RM	44^b	23^b	21^e	17^b	4^b
EC + EH	28^c	22^b	6^a	0^a	0^a
EC + RM	52^d	23^b	15^e	0^b	0^b
EH + RM	26^e	28^b	19^e	0^b	0^b
EC + RM + PG	28^e	30^b	16^e	0^b	0^b
EH + RM + PG	23^e	34^b	24^e	17^b	5^b

* Each value is an average of observations on five replicates of 10 adult beetles each. Means in the same column followed by the same letters are not significantly different at P = 0.05

Fumigant bioassay

Plant powder of CF showed promising fumigant toxicities towards *C. maculatus* adults followed by PG and CC with LC50 values of 12.3, 16.3 and 17.2g/100g food respectively (Table 2). Binary mixture of EC + EH and EC + RM showed LC50 values of 13.2 and 14.2g/100g food, respectively. The most effective combination of plant powders was EC + EH + PG with LC50 value of 11.4g/100g food which was lower than LC_{50} values of individual components showing true synergistic effect (Table 2).

Fecundity inhibition bioassay

Data on fecundity inhibition effect of plant powders towards *C. maculates* showed that chickpea seeds treated with plant powders of CN, CF, PG and RM completely prevented egg laying at the highest dose of 5g/100g food (Fig. 1). Plant powders when treated in combinations, only binary mixture of EC + EH showed 100% fecundity inhibition followed by EC + RM. However, triplet mixtures of plant powders were not found to be effective in preventing egg laying (Fig. 1).

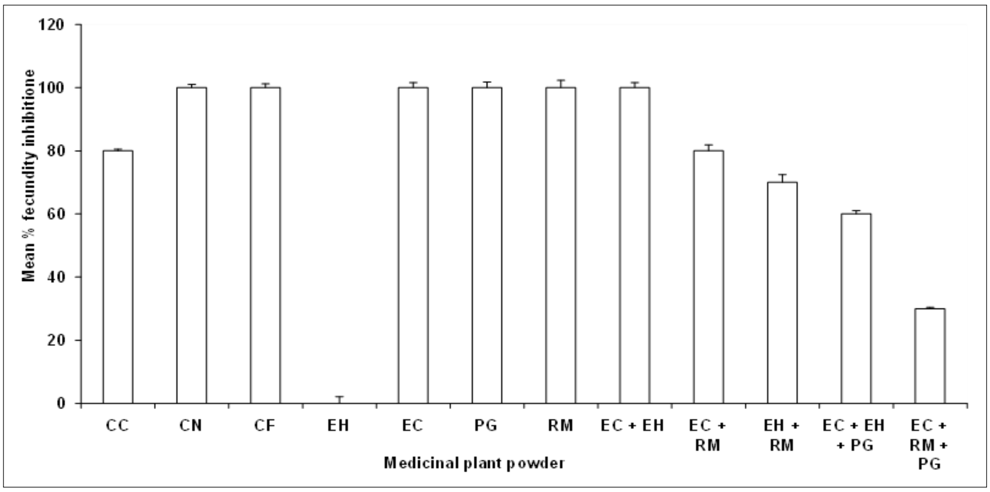

Fig 1: Effect of medicinal plant powder as fecundity inhibition against *C. maculatus at the dose of 5g/100g food*

Fertility inhibition bioassay

Plant powders of CN, CF and PG completely inhibited adult emergence of *C. maculates* at the dose of 2.5g/100g food and above (Table 3). Among the five combinations of plant powder tested except EH + RM + PG, all effectively prevented complete adult emergence at the dose of 2.5g/100g food and above.

Table 3. Response of plant powders and their combinations (1:1) towards mortality of *C. maculatus* F. in contact and fumigant bioassay

Plant powder		Contact bioassay			Fumigant toxicity			
	LD_{50} (g/100 g food) (Fiducial limits)	Slope ± SE	χ^2	P-value	LC_{50} (g/liter) (Fiducial limits)	Slope ± SE	χ^2	P-value
CC	21.4 (19.4–21.6)	2.95 ± 0.28	7.8	0.167	17.2 (16.4-18.5)	3.14 ± 0.14	5.9	0.310
CN	24.2 (23.2-25.6)	2.71 ± 0.28	3.5	0.617	20.5 (19.1-21.6)	2.78 ± 0.20	6.2	0.285
`CF	18.0 (15.6-21.2)	3.28 ± 0.27	20.9	0.001	12.3 (10.1-14.7)	3.53 ± 0.26	15.3	0.009
EH	20.1 (18.1-22.3)	2.50 ± 0.33	9.8	0.081	18.9 (17.1-21.2)	3.21 ± 0.17	9.8	0.079
EC	30.7 (24.6-36.8)	1.96 ± 0.26	12.3	0.031	19.2 (17.6-20.9)	2.92 ± 0.33	11.0	0.051
PG	25.6 (22.7-29.1)	2.42 ± 0.21	16.1*	0.007	16.3 (13.8-18.7)	3.32 ± 0.13	16.5	0.005
RM	29.8 (24.6-33.8)	1.92 ± 0.19	11.5	0.042	22.8 (21.5-23.8)	2.78 ± 0.20	7.9	0.158
EC + EH	16.3 (14.6-18.2)	3.57 ± 0.28	10.9*	0.053	13.2 (11.5-15.4)	3.42 ± 0.16	11.9	0.035
EC + RM	17.5 (16.0-19.5)	3.50 ± 0.27	10.5*	0.062	15.7 (12.9-18.1)	3.35 ± 0.11	18.6	0.020
EH + RM	26.6 (25.3-27.3)	2.25 ± 0.54	1.5	0.904	14.2 (12.5-16.4)	3.53 ± 0.22	12.6	0.026
EC + EH + PG	27.8 (24.6-31.5)	1.89 ± 0.15	8.4	0.137	11.4 (9.6-13.3)	3.50 ± 0.41	11.6	0.040
EC + RM + PG	28.4 (27.0-31.0)	1.75 ± 0.26	6.6	0. 244	23.5 (21.2-26.0)	2.82 ± 0.27	9.5	0.088

CC (*Chrysanthemum cineraiaefolium*); CF (*Cymbopogon flexuosus*); CN (*Cymbopogon camphora*); EH (*Eucalyptus hybrid*); EC (*Eucalyptus citriodora*); PG (*Pelargonium graveolens*); RM (*Rabdosia melissoides*)

* Chi square values are significant Not active.

Discussion

Our findings on bioactivities of plant powders against *C. maculatus* indicates that powders of lemongrass (*Cymbopogon flexuosus*) showed prominent contact and fumigant effect, and fecundity and fertility inhibition effect. Apart from this plant powders of *Cymbopogon winterianus* (*Citronella*) and *Pelargonium graveolens* (*Geranium*) also showed bioactivities either alone or in combination. But in case of *Eucalyptus hybrid*, *Eucalyptus citriodora* and *Rabdosia melisoides* exhibited contact and fumigant toxicity, fecundity and fertility inhibition in combination only. Pyrethrin (I & II) is a potent natural insecticide nds pyrethrin derived from the seeds or flower of *Chrysanthemum cinerariiaefloium* (CC). Kumrungsee *et al*.,[24] reported the synergistic action of Thymol and Pyrethrin against *Spodoptera exiqua*. Chandel *et al*.,[7] also reported that plant powder of *Chrysanthemum cinerariiaefolium* as a repellent against *Callosobruchus maculatus*. Not many known natural insecticides encompass such superior qualities as pyrethrin very high efficacy against a broad spectrum of pests. It has broad range of activity against flies fleas, beetles, and spider mites. Essential oil and powder of

Cymbopogon species had been previously reported as having reduction or inhibition of eggs laid and adults emergence against *Callosobruchus maculatus* [13, 21] and insecticidal against *Callosobruchus chinensis*. The essential oil of *Cymbopogon* species were very effective against mosquito [5] and the oil of lemongrass oil showed ovicidal and larvicidal activity against *Spodoptera exigua* [39]. Moataz *et al*., [28] reported insecticidal activity of lemongrass essential oil against *Agrotis ipsilon* and on other insects like *Spodoptera frugiperda Aedes aegypti* mosquito and *C. maculatus*. 1,8-cineol is one of the main bioactive component extracted from the leaf of Eucalyptus plant species, the essential oil of *Alpiniae zerumbet*, which possess a great medicinal value. Hussaine [18] found that essential oil or powder of *Eucalyptus gilii* provided the highest (EE) of 1,8- cineol. Ebadollahi *et al*., [11], already reported that insecticidal activity of essential oil of *Eucalyptus species* in which 1,8- cineole dominated against *Rhyzopertha dominica*. Donald *et al*., [10] have reported repellent effects of essential oils of *Zingiber officinale* containing (R) – linalool, 1,8 – Cineole, citral against *Tribolium casteaneum* and *Rhyzopertha dominica*.

Geraniol, compound extract from the certain part of leaf of the *Cymbopogon* species. The powder of Geraniol, exhibited work as a antimicrobial agent and also act as a insect repellent, anti-inflammatory and anthelminthic properties are found. Ahmed *et al*.,[4] reported as repellent, contact and fumigant activities against *Tribolium castaneum* and *Rhyzopertha dominica*. Geranium oil had the effect on the reduction of the number of emerged adults of the first generation (progeny) and higher mortality rate of *S. oryze* adults [2]. The synergistic effect of the combination of powder of CN, CF, PG, gave complete inhibition of egg laying at maximum concentration. The combination of EH and EC in a equal ratio also provided 100% fecundity inhibition. Powder of CN, CF, PG and combination of EC and EH and EC and RM and EH and RM in 1:1 ratio and apart from the 1:1 ratio of EC and RM and PG provide complete inhibition of adult emergence in F1 progeny production.

Linalool was extracted from *Pelargonium graveolens* (Geranium) is as unsaturated monoterpene component of many essential oil and powder known to exhibit several biological activities such as antibacterial & plasmodial activities. Nyamador *et al*., [30] recorded that the essential oil of *Cymbopogon nardus* showed the ovicidal activity against *C. maculatus*. Souza *et al*., [41] testing leaf extract of *Eucalyptus citriodora* observed a reduction in *C. maculatus* oviposition and adult emergence confirming the deleterious effect of secondary compounds from the genus *Eucalyptus* upon this pest. Harshani and Karunaratne, [17] studied the efficacy of citrus fruit peel powder against *C. maculatus* and also found effective. From ancient time various plant parts powder like leaf, seeds, bark and root used as fumigant in many countries as well as to inhibit seed /grain damage and have negligible effect on human health [20, 27]. To prevent grains from pests the powder form of plant parts can be directly incorporated in to grain bag and also as fumigant. It can cause death of developing stages of pests [37, 35, 23, 40, 8].

Conclusion

In this study we found that some medicinal plant powders can protect stored products against insect pest damage. Most of the medicinal plant powders evaluated in our experiments are biodegradable, edible, [22] and are classified as Generally Recognized as Safe (GRAS) by the food and drug Administration, USA. Our findings on the contact and fumigant toxicity of binary mixtures of medicinal plants especially *Chrysanthemum cinerariiefolium* (CC), *Cymbopogon nardus* (CN) are encouraging. However, the way in which these mixtures interact synergistically to result in enhanced insecticidal activity needs further investigation. Further experiments on dose optimization and formulation may lead to the development of botanical commercial stored grain protectants.

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