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PLANT PRODUCT AS A FUMIGANT FOR THE MANAGEMENT OF STORED-PRODUCT PESTS

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ABSTRACT

Of some essential oils screened against dominant storage fungal pests *viz., Aspergillus flavus, Penicillium italicum, Altemaria alternata* as well as stored-product insects *viz., Rhyzopertha dominica, Trogoderma granarium, Sitophilus oryzae, Corcyra cephalonica* and *Ephestia cautella*, the oil of *Cymbopogon flexuosus* (Steud.) Wats. [Poaceae], was the strongest toxicant. The oil killed the tested fungi within 4-6 days but required only 6-8 h to kill insect pests at 10 μL mL^{-1}. In addition to its broad pesticidal spectrum, the oil was found to inhibit heavy doses of inocula, was thermo-stable and persisted up to 36 months. Encouraged by these results, further tests were conducted along with *in vivo* investigations. By observing the maximum phyto-tolerant concentration, formulations were developed. Further investigations have been carried out on various toxicological, organoleptic and pharmacological parameters, to determine the minimum effective dose of the formulation. A preliminary comparison of some common characteristic features of the 'formulated herbal fumigant' with some synthetic fumigants showed that the herbal formulation is superior. As such, after a successful multi-site trial, it was recommended that the fumigant formulation of *Cymbopogon flexuosus* oil could be commercialized for the management of stored-product pests.

INTRODUCTION

Effective pest control is no longer a matter of heavy application of pesticides, partly because of the rising cost of petroleum derived products but largely because excessive pesticide use promotes speedier evolution of resistance in insect pests, destroys natural enemies, turns formerly innocuous species into pests, harms other non-target species, and contaminates food (Anon. 1980). Concern about the widespread use of broad-spectrum pesticides has led to a surge of research into alternative pest control technologies. The pesticidal formulations based on herbal products have attracted particular attention because of their specificity to insect pests, their biodegradable nature and their potential for commercial application (Mishra and Dubey, 1994; Singh *et al.*, 1983; Bishop and Thronton, 1997; Shaaya *et al.,* 1997; Tiwari, 1998; Shukla *et al.,* 2000).

Knowledge of the toxic plants, their toxic principles and their biological activity is of paramount importance not only to enable them to be utilized as natural pest control agents and replace the commercial synthetic pesticides but also to enable us to understand the nature of their toxicity to non-targeted animals. Over 2,000 plant species have been reported to possess pesticidal activity (Crosby, 1971; Chakraboraty and Basu, 1997) out of about 2,500,000 angiosperms so far documented, only a fraction of which have been analysed.

Therefore, with these objectives in mind, this study was undertaken to formulate a renewable, biodegradable, non phytotoxic and harmless bio-pesticide to control losses in stored wheat (*Triticum vulgare*) and groundnuts (*Arachis hypogea*), two of the major cereal and oil seed crops.

The volatile secondary metabolite (essential oil) was isolated from the fresh leaves of the selected plant *Cymbopogon flexuosus* (Steud) Wats. (Family-Poaceae and tested against three prevalent storage fungi: *Aspergillus flavus* Link, *Penicillium italicum* Wehmer and *Alternaria alternata* (Fr.) Keissler as well as some major stored-product insect pests namely the beetles: *Rhyzopertha dominica* F., *Sitophilus oryzae* L., *Trogoderma granarium* Everts, and the moths: *Corcyra cephalonica* Staint, and *Ephestia cautella* (Walker), isolated from infested and infected samples of wheat (*Triticum vulgare*) and groundnut (*Arachis hypogea*). Besides the investigations undertaken into its various physical and chemical characteristics, and its fungicidal/insecticidal action, the oil was also subjected to comparison with synthetic fumigants. Studies into its minimum effective dose, organoleptic tests and pharmacological investigations were also undertaken.

MATERIALS AND METHODS

The essential oil was extracted from 1 kg of chopped fresh leaves of *C. flexuosus* that were subjected to hydro-distillation through Clevenger's apparatus (Clevenger, 1928). A clear light yellow coloured oily layer was obtained on the top of the aqueous distillate. This was then separated and dried over anhydrous sodium sulphate. The various physical and-chemical properties of the oil were determined and characterized, according to the methods of Langenau (1948).

The oil thus obtained was evaluated at different concentrations in order to determine minimum killing times (MKT) against the above mentioned storage fungi and stored-product insects. In the case of fungi, the mycelial disc killing technique of Shahi *et al., (*1997) was followed with a slight modification by Shukla *et al.* (1997).

For the fungi, the nature of toxicity of the oil, whether fungistatic or fungicidal, was determined by the method of Garber and Houston (1959). The relationship between inoculum density of the fungi and the fungicidal toxicity of the oil at 10 μL/mL was determined following the procedure outlined by Dikshit and Dixit (1982) with a slight modification of Shukla *et al.* (1997). For the stored-product insects, the dosage of 10 μL/mL was prepared in distilled water, which was placed separately in a petri dish (7.5 cm diam) inside a larger petri dish (15 cm diam), containing the five different insect species separately. The petri dishes were then sealed with vacuum grease. Un-treated controls were prepared in a similar manner. After a fixed interval of 1 hour the insects were gently transferred to the grease exposed petri dish. The data recorded was mean of the 5 replicates, repeated twice. Corrected insect mortality (CM) was by applying Abbott's formula (1925).

The fungicidal toxicity of the oil at 10 μL/mL against the storage fungi: *A. parasiticus* Speare, *Cladosporium cladosporioides* (Fresenius) de Vries, *Curvularia lunata* (Wakker) Boedijin, *Colletotrichum capsici* (Syd.) Butler & Bisby, *C. falcatum* Went, *Fusarium oxysporum* Schlecht, *F. udum* de Vries, *Helminthosporium maydis* Nisikado & Miyakel, *H. oryzae* Breda de Haan, *Penicillium implicatum* Biourge and *P. minio-luteum* Dierckx was also studied, as was the susceptibility of *Tribolium castaneum* (Herbst).

The shelf-life of the oil was also determined by storing it at room temperature and testing its toxic activity at the minimum lethal dose at a regular interval of 6 months for a period of 36 months. The effect of temperature on the toxicity of the oil was determined by incubating it at 40 and 80°C in an incubator for one hour and then testing for toxicity at the minimum lethal dose rate.

The minimum phytotoxic concentration of the oil as a fumigant was determined following the method of Krishnaswami *et al.,* (1974) with slight modification by Shukla *et al.,* (1997). The requisite quantity of the oil samples loaded on some 'tiller compounds' were used to prepare the formulations, ranging from 5 μL/mL to 50 μL/mL.

To fumigate the seeds, petri dishes containing 10-20 gm wheat and groundnut seeds were placed separately in 5-L desiccators and then, formulations of different concentrations were introduced into the different desiccators that were sealed with vacuum grease. In the control set, wheat and groundnut seeds were given a similar treatment with fumigant-free formulations using only tiller compounds. After 10 d exposure, the seeds were stored for six to twelve months and then subjected to germination tests as well as evaluation of seedling growth and general health of the plants.

The minimum effective doses (MED) of the formulated essential oil defined as the ratios between the concentrations (50, 40, 20 and 10 μL/mL) and the 5-L volume of the desiccator, was prepared according to the technique of Krishnaswami *et al.,* (1974) with slight modification (Shukla *et al.,* 1997). Seeds of wheat and groundnut, 300 gm each, were fumigated following the method as used in case of the maximum phyto-tolerant concentration with formulations of 50, 40, 20 and 10 μL/mL) of concentration in 5-L desiccator, separately.

To determine which treatment had the most effective residual effect, the treated samples were fumigated for 10 days and then stored for 12 months in 100 g lots in polythene, cotton and gunny (jute) bags.

On the basis of the above investigations the MED was determined. The joint fungicidal and insecticidal analyses of these samples was carried out following the methods of Chakraverty (1995) and Samson *et al.* (1995).

Phytotoxicity analysis and organoleptic evaluations were made using the technique of Tiwari and Dixit (1995) with slight modification. The organoleptic tests were based on appearance, tenderness, flavor, taste and general acceptability. The wheat and groundnut samples were prepared to a consumable form. These samples were then served to a panel of 30 persons comprising equal numbers of males and females of different age groups, namely: teenagers (10), middle age group (10) and old age group (10). The experiment was repeated twice. The persons were asked to score the aforementioned organoleptic parameters on a scale of 1 to a maximum of 10. Mean scores were recorded.

A preliminary cost benefit analysis was undertaken by comparing the 'formulated fumigant bio-pesticides' with that of synthetic fumigants.

RESULTS

The oil acting as a fumigant, at a concentration of 10 μL/mL killed the storage fungi *A. flavus* and *P. italicum* in 6 days and *A. alternata* in 4 days (Table 1). Against the insect pests, total mortality was obtained after 7 h for *R. dominica,* and *S. oryzae,* after 6 h for *T. granarium* and after 8 h for *C. cephalonica* and *E. cautella* (Table 2).

The toxicity of the oil persisted for up to 36 months of storage and was thermo-stable at up to 80ºC. Furthermore, phyto-tolerant concentrations of the oil were found to be at least 50 μL/mL (the maximum concentration tested) showing no adverse effects on wheat and groundnut seed germination and seedling growth (Table 2).

TABLE 1

Fumigant properties of the oil of *C. flexuosus* against three storage fungi at a concentration of 10 μL/mL

Properties studied	Control of *Alternaria alternata* (%)	Control of *Aspergillus flavus* (%)	Control of *Penicillium italicum* (%)
MKT*			
6 days	100	100	100
5 days	100	60	80
4 days	100	60	60
Inoculum			
25 fungal	100	100	100
Thermostabili			
80ºC	100	100	100
Effect of			
36 months	100	100	100

*MKT = Minimum Killing Time

Fungicidal and insecticidal analyses

Wheat: Examination of the untreated controls revealed a total of 11 storage fungi on wheat stored in gunny (jute) and cotton bags, whereas *A. alternata, A. versicolor, F. oxysporum and P. gresiofulvum* were not isolated from seeds stored in polythene bags. In the case of the storage insects, *R. dominica, S. oryzae, T. granarium, T. castaneum, S. cerealella* and *Ephestia cautella* developed in the gunny bags, though no species was isolated from the polythene bags. For the sets treated at 40 μL/mL all the fungi except *Rhizopus* sp. as well as all the insect species were controlled in the seeds stored in all the bags, in contrast to synthetic fumigants, where only about 50% of the pests could be controlled.

TABLE 2
Fumigant properties of the oil of *C. flexuosus* against insect pests Insect Pest Lethality (%) at a concentration of 10 μL/mL

Properties studied	*C. cephalonica*	*E. cautell*	*S. oryzae* %	*T. granari*	*R. domini*
MKT*					
06 h	--	--	80	100	60
07 h	60	80	100	100	100
08 h	100	100	100	100	100
Inoculum					
100 insects	100	100	100	100	100
Thermostabil					
80°C	100	100	100	100	100
Effect of					
36 months	100	100	100	100	100

*MKT = Minimum Killing Time
-- indicates not considered.

Groundnuts: In the control set of groundnuts, of the fungi isolated from seeds stored in cotton and gunny bags, *A. tereus and A. versicolor* failed to appear on seeds stored in polythene bags. For the stored-product insects, only *S. cerealella* and *C. cephalonica* were found in the control seeds stored in gunny bags, while *C. cephalonica* was also recorded in cotton bags. No insects were found in the seeds stored in polythene bags. In the treated sets at 40 μL/mL no fungi or insects were recorded from the seeds stored in all the bags, as compared with synthetic fumigants, where only about 50% of the pests were controlled.

Phytotoxic effect

The fumigation of wheat and groundnut seed showed no adverse effect on seed germination, seedling growth and general health of the plants. Moreover, in some cases the growth of preliminary radical and plume length of the fumigated seeds were healthier than the control sets.

Organoleptic investigations

These indicated that the seeds treated with the formulations of *C. flexuosus* oil were more acceptable and were preferred by the consumers of both sexes and in all age groups.

The minimum effective dose was observed as 40 μL/mL concentration and that was measured in terms of the volume of per cubic meter. Polythene bags were found to be the best container as the seeds stored in them showed least pest association in comparison with cotton and gunny bags. On comparing the fumigant activity of the formulated bio-pesticide derived from *C. flexuosus* with those of synthetic fumigants such as phosphine it was observed that the bio-pesticide was superior to the synthetic one in controlling both the storage fungi and the stored-product insects. The characteristic features including a cost benefit analysis of the formulated fumigant bio-pesticide also compared favorably with synthetic fumigants.

DISCUSSION

A perusal of the literature shows that a number of studies have been made with the essential oil of some *Cymbopogon* species against some pathogenic fungi (Arora and Pandey, 1976; Singh *et al.*, 1978; Dikshit and Husain *et al.*, 1984; Pandey *et al.*, 1996), but to our knowledge detailed studies, namely, minimum killing time, phytotoxicity levels, and minimum effective dose of the essential oil of *C. flexuosus* are reported here for the first time.

In contrast to the oils of *Raphanus* (Nehrash, 1961), *Juniperus* (Slavenas and Razinskaite, 1962), *Psoralea corylifolia* (Grover and Rao, 1978) and *Feronia elephantum* (Sharma *et al.*, 1978), which showed a narrow pesticidal spectrum, the *C. flexuosus* oil exhibited a broad pesticidal spectrum similar to the oils of *Curcuma aromatica* (Rao, 1976), *Caesulia axillaris* (Pandey *et al.,* 1982), *Nepeta hindustana* (Mishra and Dubey, 1994), *Eucalyptus citriodora* (Shukla *et al.,* 1997), and *Citrus sinensis* (Shukla *et al.,* 2000).

Earlier literature showed that generally essential oils are non phytotoxic for practical use (Dikshit *et al.*, 1979; Pandey *et al.*, 1982; Mishra and Dubey, 1994; Tiwari 1998 and Shukla *et al.*, 2000). Similarly, *C. flexuosus* oil was shown to be non phytotoxic on wheat and groundnut up to the 50 μL/mL concentration, which was the maximum concentration taken into consideration.

Most of the treatments, under *in vivo* conditions, that are highly effective in preventing fungal decay of the commodities are non-toxic to the pests *in vitro* under similar conditions. Conversely some treatments that are effective against the pests *in vitro* are completely ineffective in protecting the commodities from fungal invasions *in vivo* (Eckert, 1967). In the present study the fumigant formulation of the oil of *C. flexuosus* was tested for pesticidal activity under both *in vitro* and *in vivo* conditions and was found to be effective in protecting the wheat and groundnuts. On the basis of the above findings it can be safely said that the 'formulated fumigant bio-pesticide' involves no risk in handling, in contrast to synthetics for which many precautions are needed. Furthermore, it can be used without restriction, whereas the use of some of the synthetic fumigants such as EDB is now strictly prohibited.

This formulated bio-pesticide also possesses the important property of being an anti-feedant, and may prove to be a viable substitute to the hazardous synthetic contact pesticides, which are not only injurious to human health but also pollute the ecosphere. As such, this effective product may be recommended to agrochemical firms for exploitation as a herbal botanical protectant.

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