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Studies on Antifungal Evaluation and Insect Repellent Efficacy of *Chenopodium Ambrosioides* Essential Oil in Combination with *Cuminum cyminum* Essential oil

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Abstract
The essential oil of *Chenopodium ambrosioides* in combination with *Cuminum cyminum* essential oil in equal ratio, showed fungitoxicity against 20 fungi including mycotoxin producing strains of *Aspergillus flavus* and *Aspergillus parasiticus*. This combination of oils also showed strong insect repellent activity.

Keywords
Essential oil, Antifungal, Insect repellent efficacy, *Chenopodium ambrosioides, Cuminum cyminum*.

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INTRODUCTION
Chemicals especially organomercurials are used to control seed borne pathogens due to their penetration action [18]. However, due to continuous use of such chemicals, several pathogens have become resistant and cause serious health problems [11]. The treatment of infected plants by traditional antimicrobial substances is also not much effective because most of such substances produce several undesirable toxic side effects to the hosts and are pollutive [12]. Most of these chemicals have now become a popular target of conservationists and are treated to be one of the most vital man made pollutants. In recent years, because of the awareness of the toxicity to man & animals and disastrous effect, the regulatory agencies have banned the use of such chemicals [15, 24, 9, 5]. This has led to an increased thrust to develop plant based and eco-friendly herbal medicines for the management of diseases. Plants are the natural sources of antimicrobial substances and have been used to control various diseases in plants and animals including humans since ancient times [19].

One of the most promising antimicrobial active constituent of plants is essential oil. It is mainly extracted from aromatic plants. Essential oils are the mixture of diverse volatile compounds and are the product of secondary metabolism [21]. During recent years many essential oils have been found as potent antifungal agents [30, 27, 1]. Since such antimicrobial essential oils have penetration action, these may especially be used to control seed borne pathogens. The essential oils of *Chenopodium ambrosioides* and *Cuminum cyminum* [16, 14, 6] have earlier been reported to possess antimicrobial efficacy. In the present communication findings on antifungal and insect repellent efficacy of mixture of essential oils of *Chenopodium ambrosioides* and *Cuminum cyminum* are reported.

MATERIALS AND METHODS

The essential oils from the leaves of *Chenopodium ambrosioides* and from the seeds of *Cuminum cyminum* were collected by hydro distillation technique using Clevenger's apparatus as described by Tripathi *et al.* [30]. The water immiscible oils, thus collected, were dried over anhydrous sodium sulphate and thoroughly mixed in equal ratio to obtain the mixture of two essential oils. The mixture was tested for its antifungal activity at 5 x 10^4 µl/l and 3 x 10^4 µl/l doses against 20 test fungi including mycotoxin producing strains of *Aspergillus flavus* Link (MTCC-277) and *A. parasiticus* speare (MTCC-2796) by disc diffusion method [2].

The insect repellent efficacy of the mixture of the two oils was assessed using an olfactometer similar to that of Read *et al.* [22]. The spongy pieces of the experimental and control arms of the Y tube of the apparatus were soaked in 0.1 ml of the mixture of oils and distilled water respectively. The apparatus was then attached to a suction pump in order to create vacuum. Twenty-five test insects *(Allacophora foveicollis* Fabr.*)* of the same age were introduced into base and of the Y tube of the olfactometer in 5 batches at interval of 6 minutes in order to avoid the mutual interference [29]. After ten minutes of introducing the insects, the number of insects present in the base, control and experimental arms of the Y tube were counted. Each experiment was repeated ten times and the average of observations was recorded. For each replication the olfactometer was washed thoroughly dried and rearranged after rotating to 180^0 to eliminate any positional bias [7].

RESULT AND DISCUSSIONS

The mixture of the oils of *Chenopodium ambrosioides* and *Cuminum cyminum* completely inhibited the mycelial growth of all the 20 test fungi viz., *Aspergillus flavus, Aspergillus fumigates*, *Aspergillus niger, Aspergillus ochraceus, Aspergillus parasiticus, Aspergillus ruber, Aspergillus terreus, Botryodiplodia theobromae*, *Chaetomium indicum, Cladosporium herbarum, Curvularia geniculate, Drechslera hawaiinse, Fusarium acuminatum, Fusarium oxysporum, Penicillium chrysogenum, Pyricularia oryzae, Pythium debaryanum, Rhizoctonia solani, Sclerotium oryzae and Sclerotium rolfsii* at a hyper-lethal dose of 5 x 10^4 µl/l. However, this mixture of the oils completely inhibited the mycelial growth of 12 test fungi at a lethal dose of 3 x 10^4 µl/l (Table: 1). The mixture of the oils also possessed significant insect repellent activity (Table: 2). The mixture of the oils at lethal (3 x 10^4 µl/l) as well as hyper-lethal (5 x 10^4 µl/l) doses produced the same effect on the insect repellent activity.

Table–1. Fungitoxicity of mixture of oils of *Chenopodium ambrosioides* and *Cuminum cyminum*

Fungi Tested	Percent Mycelial Inhibition	
	3 x 10^4 µl/l dose	5 x 10^4 µl/l dose
Aspergillus flavus Link.	100	100
Aspergillus fumigates Fres.	100	100
Aspergillus niger Van Tiegh.	92	100
Aspergillus ochraceus Withelm	100	100
Aspergillus parasiticus Speare	100	100
Aspergillus ruber Bremer Thom & Raper	88	100
Aspergillus terreus Thom.	100	100
Botryodiplodia theobromae Patomillard	89	100
Chaetomium indicum Corba	100	100
Cladosporium herbarum (pers.) Link.	87	100
Curvularia geniculata Boedijin	100	100
Drechslera hawaiinse (Bugn.) (Subram. & Jain)	100	100
Fusarium acuminatum Ellis & Everhart	100	100
Fusarium oxysporum Schlecht	85	100
Penicillium chrysogenum Thom.	100	100
Pyricularia oryzae Cavara	90	100
Pythium debaryanum R. Hessse	100	100
Rhizoctonia solani Kuhn.	100	100
Sclerotium oryzae Catt.	84	100
Sclerotium rolfsii Saccardo	86	100

Table–2. Response of test insect (*Allacophora foveicollis* Fabr) to the mixture of oils of *Chenopodium ambrosioides* and *Cuminum cyminum*

Number of insects in the base arm (Non – reactive)	Number of insects in the control arm (Repelled)	Number of insects in the experimental arm (Attracted)
4	19	5
3	17	0
3	23	3
2	19	0
3	20	1
5	13	0
4	19	1
5	23	0
2	18	5
2	22	2
Total 33	193	17
Mean 3.3	19.3	1.7

The phenomenon of synergism among fungicides and weedicides has been studied by a number of workers [23, 8, 17]. However, there is paucity of information on synergistic antifungal efficacy of essential oils barring some reports [13, 4, 20]. According to Scardavi [25] three types of synergism viz., (a) Additional synergism (b) Synergism of potentiation (c) Synergism of degradation may be expected when two or more fungi toxicants are mixed together. Chemically essential oils contain unsaturated hydrocarbons and such hydrocarbons are unstable and have a tendency to attain stability [3]. Therefore, molecular rearrangement is very much possible if essential oils containing unsaturated hydrocarbons are mixed together. Literature supports that essential oils undergo molecular rearrangement leading to shifting of the connection between the carbon atoms [10]. This view gets strengthened from our observations as the essential oil of *Chenopodium ambrosioides* in its individual capacity exhibited toxicity against the test fungi at 7 x 10^4 µl/l dose while that of *Cuminum cyminum* at 9 x 10^4 µl/l dose (Dube, 1981). However, the lethal dose of the mixture was found to be 3 x 10^4 µl/l, thereby indicating the phenomenon of synergism of potentiation. According to Scardavi (1966) such a synergism leads to enhance the fungitoxic action of the participating chemicals which is superior to the arithmetical sum of the activity exercised by the individual chemical. This further supports that when two essentials are mixed together molecular rearrangement occurs and this molecular rearrangement in present study proved fruitful for utilizing these two essential oils in armamentarium of plant protection.

CONCLUSION

Chemical treatment is the cheapest method to control several diseases caused by fungal phytopathogens. Besides being economical, even for capital weak small farmers, it required relatively small quantity of chemical and simple way of application. Unfortunately, most of the chemicals used to control fungal phytopathogens belong to organomercurials group which do not easily degrade in the soil environment and also produce cumulative toxicity due to mercury accumulation in food chain. The non-mercurial fungicides prove ineffective for deep seated infections and also interfere with the development of root hairs. Their toxicity to N_2 fixing bacteria in soil and irritative action on skin and mucous membrane of operators are additional disadvantages. They are proved to be pollutive and carcinogenic. The alternative choice, therefore, would be the use of natural fungicides for controlling fungal phytopathogens, which are advocated to be non-toxic to humans, biodegradable and non-pollutive. Many synthetic chemicals are also being used for their insect repellent properties, but their wide use has created several safety and health issues to humans and the environment.

Higher plants release volatile substances which keep the air remarkably free from fungal phytopathogens and insects. Essential oils are important in this connection, because they entail special promise for use as fumigants. These being lipophilic, can easily penetrate deeper, unbarred by selective permeability of cell membrane. Essential oils also show favourable toxicity to humans because its constituents are already used in several products for human consumption. These are ecofriendly, biodegradable and plant based natural products, therefore if essential oils are used as crop protectant or as insect repellent, will not pose any threat to humans and the environment. Thus, essential oils extracted from higher plants could be considered as alternatives to synthetic pesticides.

Thus, the antifungal and insect repellent properties of essential oil of *Chenopodium ambrosioides* in combination with *Cuminum cyminum* essential oil offer the prospect of using them as natural pesticides and they can have market niches with a commercial value.

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