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IN VITRO STUDIES ON SYNERGISTIC ANTIFUNGAL POTENTIAL OF ESSENTIAL OILS OF *LIPPIA ALBA* AND *CARUM COPTICUM*

C. O .Samuel[1], Sandeep Chaudhary[1], Akhilesh Kumar Gupta[1], Shweta Minj[1] and P. P. Upadhyaya[2]

1. Natural Fungicide Laboratory, Department of Botany, St. Andrew's College, Gorakhpur, U.P. (India).

2. Plant pathology Laboratory, Department of Botany, D.D.U Gorakhpur University, Gorakhpur, U.P. (India).

ABSTRACT

Essential oils of Lippia alba and Carum copticum when mixed in 1: 1 ratio, besides being fungistatic to the mycelial growth of 25 fungi including mycotoxin producing strains of Aspergillus flavus and A. parasiticus, also possessed insect repellent activity and had no adverse effect on per cent seed germination and seedling growth of Cicer arietinum and Triticum aestivum.

KEYWORDS: essential oils, *Lippia alba, Carum copticum*, synergistic antifungal potential

INTRODUCTION

The compounds of organomercurial are used to control seed borne pathogens due to their penetration action (Neergaard, 1977). Most of these chemicals have become a popular target of conservationists and are treated to be one of the most vital man made pollutants (Khoshoo, 1980). During recent years many essential oils have been found as potent antifungal agents (Tripathi *et al.,* 1986; Dubey and Tripathi, 1987; Shukla and Tripathi, 1987, Mishra et al., 2003). Since such antimicrobial essential oils have penetration action, these may especially be used to control seed borne pathogens. The essential oils of *Lippia alba* and *Carum copticum* (Shweta *et al* 2012) have earlier been reported to posses antimicrobial efficacy. In the present communication findings on antifungal, insect repellent and non phytotoxic efficacy of mixture of essential oils of *Lippia alba* and *Carum copticum* are reported.

MATERIALS AND METHODS

The essential oils of fruits of *Lippia alba and Carum copticum* were collected by hydro-distillation technique using Clevenger's apparatus as described earlier (Tripathi *et al., 1986).* The water immiscible oils, thus collected, were dried over anhydrous sodium sulphate and thoroughly mixed in 1: 1 ratio to obtain the mixture of two essential oils. The mixture was tested for its antifungal activity at 0.5 x 10^3, 1.0 x 10^3 and 2.0 x 10^3 µl / l doses against mycotoxin producing strains of *Aspergillus flavus* Link (MTCC 1783) and *A. parasiticus* Speare (MTCC 2796) by the poisoned food technique (Samuel *et al*, 1999).

The range of antifungal spectrum at lethal dose was also studied by poisoned food technique (Dubey and Tripathi, 1987).

Phytotoxicity of the mixture of the two oils at lethal (0.4 x 10^3 µl / l) and hyper lethal (1.0 x 10^3 µl / l) dose was tested with respect to seed germination and seedling growth of *Cicer arietinum* and *Triticum aestivum* by the technique as described by Dubey and Tripathi (1987).

The insect repellent activity of the mixture of the two oils was assessed using an olfactometer similar to that of Read *et al.* (1970). 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 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 (Tripathi and Kumar, 1985). 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 (Gonzalex *et al.,* 1985).

RESULT AND DISCUSSION

The mixture (1:1) of the oils of *Lippia alba and Carum copticum* completely inhibited the mycelial growth of both the test fungi at a minimum dose of (0.4×10^3 □ l / l) (Table 1). At this concentration it completely inhibited the mycelial growth of *Aspergillus awamori, A. chevalieri, A. ficcum, A. fumigatus, A. nidulans, A. niger, A. ochraceus, A. sydovi, A. tamarii, Botryodiplodia theobromae, Chaetomium indicum, Cladosporium herbarum, Cochliobolus lunatus,* C. *sativus, Colletotrichum capsici, Curvularia specifera, Epicoccum nigrum, Fusarium accuminatum, F. equiseti, F. moniliforme, F. oxysporum. F. semitectum, Macrophomina phaseoli, Penicillium chrysogenum, P. citrinum* and *Sclerotium rolfsii.* It also possessed significant insect repellent activity (Table 2). The mixture at lethal (0.4×10^3 µl / l) as well as hyperlethal (1.0×10^3 µl / l) doses produced no adverse effect on seed germination and seedling growth *of C. arietinum* and *T. aestivum* (Tables 3 to 5).

The phenomenon of synergism among fungicides and weedicides has been studied by a number of workers (Roberts, 1982; Gruzdyev *et al., 1983).* However, there is paucity of information on synergistic antifungal potential of essential oils barring some reports (Jain and Jain, 1974; Chaurasia and Vyas, 1977 and Pandey *et al.,* 1983). According to Scardavi (1966) three types of synergism viz. (a) additional synergism (b) Synergism of potentiation and (c) Synergism of degradation may be expected when two or more antifungal potential plants are mixed together. Chemically essential oils contain unsaturated hydrocarbons and such hydrocarbons are unstable and have a tendency to attain stability (Chatwal, 1983). 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 (Haagen Smith, 1948). This view gets strengthened from our observations as the essential oil *of Lippia alba* in its individual capacity exhibited toxicity against the test fungi at 2.0×10^3 □µl / l dose while that of *Carum copticum* at 0.5×10^3 □µl /l dose (Shweta *et al* 2012)), However, the lethal dose of the mixture was found to be (0.4×10^3 µl / l) thereby indicating the potential of synergism. According to Scardavi (1966) such a synergism leads to enhance the antifungal

potential action of the participating chemicals which is superior to the arithmetical sum of the activity exercised by the individual chemical.

Interestingly the oils of *Lippia alba* and *Carum copticum* in their individual capacity produced phytotoxic effect on seed and seedling growth of *C. arietinum* and *T. aestivum* (Dube, 1988). However when the two phytotoxic essential oils were mixed in 1:1 ratio the combination produced no adverse effect on seed germination and seedling growth of *Ciceer arietinum* and *Triticum aestivum* indicating thereby that a synergism of degradation contrary to the theory of potentiation was observed as far as phytotoxic efficacy of the oils is concerned. This further supports that when two essential oils 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.

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Table 1. Lethal Dose of 1:1 Mixture of essential oils of *Lippia alba* and *Carum copticum*		
Doses (µl / l)	Percent mycelial inhibition	
	A. Flavus	*A. parasiticus*
0.5×10^3	100	100
0.4×10^3	100	100
0.3×10^3	89	89

Table 2. Response of test insect (*Allacophora Foveicollis* Fabr) to the mixture of essential oils of *Lippia alba* and *Carum copticum*		
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)
2	18	5
4	20	1
6	13	6
3	17	5
2	23	0
6	19	0
5	20	0
5	19	1
3	22	0
2	23	0

Table 3. Effect of 1:1 mixture of essential oils of *Lippia alba* and *Carum copticum* on seed germination of *Cicer arietinum* and *Triticum aestivum*

Period (days)	Soaking period / % seed germination											
	Cicer arietinum						*Triticum aestivum*					
	6 hours			12 hours			6 hours			12 hours		
	C	T_1	T_2	C	T_1	T_2	C	T_1	T_2	C	T_1	T_2
2	81	87	68	72	73	61	69	70	70	62	63	54
3	82	87	85	82	83	82	80	80	80	70	71	56
4	88	91	89	86	86	85	88	89	89	82	82	82
5	91	92	90	91	90	89	90	90	90	90	89	90
6	89	95	95	92	92	90	93	90	90	90	88	90
7	92	94	92	93	92	91	90	91	90	90	89	90

C : Control T_1 : Treatment at 0.4 x 10^3µl / l dose T_2 : Treatment at 1.0 x 10^3µl / l dose

Table 4. Effect of 1:1 mixture of essential oils of *Lippia alba* and *Carum copticum* on radicle length of *Cicer arietinum* and *Triticum aestivum*

Period (days)	Soaking period / radicle length (mm)											
	Cicer arietinum						*Triticum aestivum*					
	6 hours			12 hours			6 hours			12 hours		
	C	T_1	T_2	C	T_1	T_2	C	T_1	T_2	C	T_1	T_2
2	22	21	15	14	11	4	18	10	8	18	4	1
3	44	34	29	33	15	12	31	19	24	19	17	11
4	51	40	23	32	22	20	42	34	30	29	25	21
5	62	60	31	42	40	31	51	42	40	41	40	25
6	71	66	42	50	43	40	69	60	52	52	45	31
7	81	70	64	61	54	49	72	70	65	60	55	34

C : Control T_1 : Treatment at 0.4 x 10^3µl / l dose T_2 : Treatment at 1.0 x 10^3µl / l dose

Table 5. Effect of 1:1 mixture of essential oils of *Lippia alba* and *Carum copticum* on plumule length of *Cicer arietinum* and *Triticum aestivum*

Period (days)	Soaking period / plumule length (mm)											
	Cicer arietinum						*Triticum aestivum*					
	6 hours			12 hours			6 hours			12 hours		
	C	T_1	T_2	C	T_1	T_2	C	T_1	T_2	C	T_1	T_2
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3	0.0	0.0
3	0.0	0.0	0.0	0.0	0.0	0.0	23	2	0.0	11	11	0.0
4	5	5	0.0	5	6	0.0	32	27	26	29	22	15
5	8	7	6	7	10	3	41	31	28	46	43	22
6	22	21	16	13	16	8	54	42	30	49	49	31
7	33	29	24	18	17	15	69	54	32	71	68	62

C : Control T_1 : Treatment at 0.4 x 10^3 µl / l dose T_2 : Treatment at 1.0 x 10^3 µl / l dose