

## IN VITRO STUDIES ON FUNGITOXIC AND PHYTOTOXIC PROPERTIES OF ESSENTIAL OILS OF *Carum carvi* AND *Hedychium spicatum* MIXED IN 1:1 RATIO

C.O. SAMUEL<sup>a1</sup> AND S.C. TRIPATHI<sup>b</sup>

<sup>a</sup>Department of Botany, St. Andrew's P.G. College, Gorakhpur, U.P., India

<sup>b</sup>Department of Botany, Gorakhpur University, Gorakhpur, U.P., India

### ABSTRACT

The essential oils of *Carum carvi* and *Hedychium spicatum* when tested separately against the test fungi, showed fungitoxicity. However, when the essential oil of *Carum carvi* mixed with the essential oil of *Hedychium spicatum* in 1:1 ratio, showed strong fungitoxicity for mycelia growth of 29 fungi including mycotoxin producing strains of *Aspergillus flavus* and *A. parasiticus*. The mixture also had no phytotoxicity on percent seed germination and seedling growth of *Oryza sativa* and *Triticum aestivum*.

**KEYWORDS:** Essential Oil, Fungitoxicity, Phytotoxicity

Fungi are the main infectious agents in plants, causing alterations during developmental stages including post-harvest. Fungi are ubiquitous in the environment, and infection due to fungal pathogens has become more common. There is a wide variety of fungal genera causing infection to plants and most often lead to their death. These also create quality problems related to nutritional value, organoleptic characteristics and limited shelf life (Agrios, 2004). Phytopathogenic fungi are controlled by synthetic fungicides specially organomercurials, which have more penetrating action against deep-seated seed borne pathogens (Neergaard, 1977); however, the use of these is increasingly restricted due to the harmful effects of pesticides on human health and the environment (Harris *et al.*, 2001)

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). Science such antimicrobial essential oils have penetration action, these may especially be used to control seed borne pathogens. The essential oils of *Carum carvi* and *Hedychium spicatum* have earlier been reported to possess antimicrobial efficacy (Dube, 1988). In the present communication finding on antifungal and non-phytotoxic efficacy of mixture of essential oils of *Carum carvi* and *Hedychium spicatum* are reported.

### MATERIALS AND METHODS

The essential oils of *Carum carvi* and *Hedychium spicatum* 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  $0.5 \times 10^3$ ,  $1.0 \times 10^3$  and  $2.0 \times 10^3$   $\mu\text{l/l}$  doses against mycotoxin producing strains of *Aspergillus flavus* Link (NRRL 3251) and *A. parasiticus* Speare (NRRL 2999) by the poisoned food technique (Dubey and Tripathi, 1987).

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 ( $0.4 \times 10^3$   $\mu\text{l/l}$ ) and hyper-lethal ( $1.0 \times 10^3$   $\mu\text{l/l}$ ) doses was tested with respect to seed germination and seedling growth of *Oryza sativa* and *Triticum aestivum* by the technique as described by Dubey and Tripathi (1987).

### RESULTS AND DISCUSSION

The mixture (1:1) of the two oils of *Carum carvi* and *Hedychium spicatum* completely inhibited the mycelia growth of both the test fungi at a minimum dose of  $0.4 \times 10^3$   $\mu\text{l/l}$  (Table 1).

**Table 1: Lethal Dose of 1:1 mixture of oils of *Carum carvi* and *Hedychium spicatum***

Doses ( $\mu\text{l/l}$ )	Percent mycelial inhibition	
	<i>Aspergillus flavus</i>	<i>Aspergillus parasiticus</i>
$0.5 \times 10^3$	100	100
$0.4 \times 10^3$	100	100
$0.3 \times 10^3$	88	88

At this concentration it completely inhibited the mycelia growth of *Aspergillus awamori*, *A. chevalieri*, *A. ficcum*, *A. fumigatus*, *A. nidulans*, *A. niger*, *A. ochraceus*,

<sup>1</sup>Corresponding Author

*A. sydowi*, *A. tamarii*, *Botryodiplodia*, *Theobromae*, *Chaetomium indicum*, *Cladosporium herbarum*, *Cochlibolus lunatus*, *C. sativus*, *Colletotrichum capsici*, *Curvularia specifera*, *Epicoccum nigrum*, *Fusarium oxysporum*, *F. semitectum*, *Macrophomina phaseoli*, *Penicillium chrysogenum*, *P. citrinum* and *Sclerotium*

*rofsii*. The mixture at lethal ( $0.4 \times 10^3 \mu\text{l/l}$ ) as well as hyper-lethal ( $1.0 \times 10^3 \mu\text{l/l}$ ) doses produced no adverse effect (phytotoxic effect) on seed germination and seedling growth of *Oryza sativa* and *T. aestivum* (Table 2, 3 & 4).

**Table 2: Effect of 1:1 mixture of oils of *Carum carvi* and *Hedychium spicatum* on seed germination of *Oryza sativa* and *Triticum aestivum***

Period (days)	Soaking period / % seed germination											
	<i>Oryza sativa</i>						<i>T.aestivum</i>					
	6 h			12 h			6 h			12 h		
	C	T <sub>1</sub>	T <sub>2</sub>	C	T <sub>1</sub>	T <sub>2</sub>	C	T <sub>1</sub>	T <sub>2</sub>	C	T <sub>1</sub>	T <sub>2</sub>
2	80	86	67	73	73	60	70	70	70	63	63	55
3	87	87	87	82	82	82	80	80	80	70	70	57
4	90	90	90	86	86	86	89	89	89	82	82	82
5	93	93	93	90	90	90	90	90	90	90	90	90
6	95	95	95	92	92	90	92	90	90	90	90	90
7	95	95	95	92	92	92	90	90	90	90	90	90
Value of t	1.113*			1.422*			1.110*			1.478*		
C : Control ; T <sub>1</sub> : Treatment at $0.4 \times 10^3 \mu\text{l/l}$ ; T <sub>2</sub> : Treatment at $1.0 \times 10^3 \mu\text{l/l}$ ; *Insignificant at 5% level												

**Table 3: Effect of 1:1 mixture of oils of *Carum carvi* and *Hedychium spicatum* radical length of *Oryza sativa* and *Triticum aestivum***

Period (days)	Soaking period / Radicle length (mm)											
	<i>Oryza sativa</i>						<i>T.aestivum</i>					
	6 h			12 h			6 h			12 h		
	C	T <sub>1</sub>	T <sub>2</sub>	C	T <sub>1</sub>	T <sub>2</sub>	C	T <sub>1</sub>	T <sub>2</sub>	C	T <sub>1</sub>	T <sub>2</sub>
2	23	22	16	14	11	4	18	7	5	15	4	1
3	43	35	30	33	15	12	30	20	24	18	12	18
4	53	42	27	31	25	25	45	33	30	30	32	20
5	68	63	35	43	43	34	50	45	45	46	46	26
6	72	68	48	52	48	42	67	65	58	59	49	30
7	80	72	68	63	50	51	70	70	68	68	63	33
Value of t	0.186*	0.923*		0.134 *	0.976*		0.151 *	0.752*		0.263*	0.411*	
C : Control ; T <sub>1</sub> : Treatment at $0.4 \times 10^3 \mu\text{l/l}$ ; T <sub>2</sub> : Treatment at $1.0 \times 10^3 \mu\text{l/l}$ ; *Insignificant at 5% level												

**Table 4: Effect of 1:1 mixture of oils of *Carum carvi* and *Hedychium spicatum* plumule length of *Oryza sativa* and *Triticum aestivum***

Period (days)	Soaking period / Plumule length (mm)											
	<i>Oryza sativa</i>						<i>T.aestivum</i>					
	6 h			12 h			6 h			12 h		
	C	T <sub>1</sub>	T <sub>2</sub>	C	T <sub>1</sub>	T <sub>2</sub>	C	T <sub>1</sub>	T <sub>2</sub>	C	T <sub>1</sub>	T <sub>2</sub>
2	0.0	0.0	0.0	0.0	0.0	0.0	4	0.0	0.0	4	0.0	0.0
3	0.0	0.0	0.0	0.0	0.0	0.0	23	2	0.0	12	12	0.0
4	5	5	0.0	5	5	0.0	38	28	27	30	13	16
5	8	7	6	7	7	3	42	32	29	45	42	20
6	23	20	18	14	14	8	53	43	32	59	59	30
7	34	30	25	16	16	15	70	55	35	76	70	68
Value of t	0.038* 0.052*			0.038* 0.058*			0.474* 0.501*			0.083* 2.411*		
C : Control ; T <sub>1</sub> : Treatment at 0.4 x 10 <sup>3</sup> µl / l ; T <sub>2</sub> : Treatment at 1.0 x 10 <sup>3</sup> µl / l ; *Insignificant at 5% level												

The effect of mixture i.e., 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 efficacy 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 (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 (Chatwal, 1983) therefore, molecular rearrangement 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 oils of *Carum carvi* in its individual capacity exhibited toxicity against the test fungi at 2.0x10<sup>3</sup>µl/l dose while that of *Hedychium spicatum* at 0.5x10<sup>3</sup>µl/l dose (Dube, 1988). However, the lethal dose of the mixture was found to be 0.4x10<sup>3</sup>µl/l there by indicating the phenomenon of synergism of potentiation. According to Scardavi (1966) such a synergism leads to the arithmetical sum of the activity exercised by the individual chemical.

Interestingly the oils of *Carum carvi* and *Hedychium spicatum* in their individual capacity produced phytotoxic effect on seed germination and

seedling growth of *Oryza sativa* 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 *Oryza sativa* and *T. aestivum* indicating there by 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 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.

## REFERENCES

- Agrios G.N., 2004. Losses caused by plant diseases. Plant Pathology. Elsevier, Oxford, UK. p. 29-45.
- Chatwal G.R., 1983. The chemistry of organic natural products Vol. II. Himalaya Publishing House, Bombay, India.
- Chaurasia S.C. and Vyas K.M., 1977. In vitro effect of some volatile oils against *Phytophthora parasiticavar .piperina*. J. Res. Indian Med. Yoga Homeop., **12**:139-142.
- Dube S., 1988. Antifungal, physicochemical, phytotoxic and insect repellent properties of some essential oils. Ph.D. Thesis, University of Gorakhpur, Gorakhpur, India.
- Dubey P. and Tripathi S.C., 1987. Studies on antifungal physic-chemical and phytotoxic properties of the

- essential oil of *Piper betle*. J.PI. Dis. Prot., **94**(3): 235-241.
- Gonzalez J.W., Mathews R.W. and Mathews J.R., 1985. Asex pheromone in male of *Melittobia australica* and *M.femorata* (Hymenoptera: Eulophide). Florida Entomologist, **68**: 279-286.
- Gruzdyev G.S., Zinchenko V.A., Kalinin and Slotsov R.I., 1983. The chemical protection of plants. Gruzdyev, G.S. (ed), MIR Publishers Moscow. Pp. 237-239.
- Haagen Smit A.J., 1948. The chemistry, origin and function of essential oils in plant life. In: The Essential oils (Rep. ed.) Vol. I. Guenther, R. (ed). Robert E. Krieger Publishing Co. Huntington, York, pp. 17-83.
- Harris C.A., Renfrew M.J. and Woolridge M.W., 2001. Assessing the risk of pesticide residues to consumers: recent and future developments. Food Additives and Contamination, **18**: 1124-1129.
- Jain S.R. and Jain M.R., 1974. Antifungal studies on some indigenous volatile oils and their combination. Planta Medica, **22**: 136-139.
- Khoshoo T.N., 1980. Environmental priorities in India and sustainable development. 73<sup>rd</sup> Session Indian Science Congress, New Delhi.
- Neergaard P., 1977. Seed Pathology Vol. I. The McMillan press Ltd. London and Basingstoke.
- Pandey D.K., Chandra H., Tripathi N.N. and Dixit S.N., 1983. Fungitoxicity of some higher plants with special reference to the synergistic activity amongst volatile fungitoxicants. Phytopath. Z., **106**: 226-232.
- Read D.P., Feency P.P. and Root R.B., 1970. Habitat selection by the aphid parasite *Diaerctiellarapae* (Hymenoptera: Braconidae) and the hyper - parasite *Charpis brassicae* (Hymenoptera : Cynipidae). Can. J. Ent., **102**: 1567-1578.
- Roberts H.A., 1982. Weed Control Handbook Principles. Blackwell Scientific Publications. London.
- Scardavi A., 1966. Synergism among fungicides. Ann. Rev. Phytopath., **1**: 335-348.
- Shukla H.S. and Tripathi S.C., 1987. Studies on physico-chemical, phytotoxic and fungitoxic properties of essential of *Foeniculum vulgare* Mill. Beitr. Pflanzen, **62**: 149-158.