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Compatibility of *Metarhizium anisopliae* (Metsch.) Sorok. with *Ocimum sanctum* Linn. (Tulsi) (Lamiaceae) Extracts

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Abstract

The compatibility of *Metarhizium anisopliae* with *Ocimum sanctum* was studied *in vitro*. Leaves, roots, stems and seed extracts of *O. sanctum* were mixed in a Potato Dextrose Agar and Potato Dextrose Broth. *M. anisopliae* was inoculated and the mycelial dry weight and spore count were assessed. The behavior of the fungus with the extracts was similar in terms of mycelial dry weight, except for methanol extracts of leaves, ether extracts of roots, water and acetone extracts of seeds and benzene, methanol and acetone extracts of stems which reduced the mycelial dry weight of the fungal colonies. Benzene extract of leaves and methanol extract of roots of *O. sanctum* were found to be highly compatible with *M. anisopliae* whereas ether extract of roots and benzene as well as acetone extracts of stem were classified as very toxic. The results of the current study revealed that *O. sanctum* extracts did not affect the inoculum potential of *M. anisopliae* in terms of mycelial dry weight and spore count and hence *M. anisopliae* was compatible with *O. sanctum.*

Key Words: Compatibility, *Metarhizium anisopliae*, *Ocimum sanctum*, biological control.

Introduction

The production of food requests for methods that use non-chemical inputs for pest control to reduce harmful side-effects of pesticides on public health and the environment. In such production systems, pests are essentially managed by biological agents, which are considered as important factors in insect population reduction. Entomopathogenic fungus, *Metarhizium anisopliae* (Metsch.) Sorokin (Deuteromycotina: Hyphomycetes) is an important natural control agent for several pests (Borgio and Sahayaraj, 2007). Botanical pesticides are also used extensively in most agroecosystems to control insect pests. *Ocimum sanctum* (holy basil), called Tulsi in India, is ubiquitous in Indian tradition. The crude alcoholic extracts had shown antifeedant activities on *Jute semilooper*, *Anomis sabulifera* (Malik and Rafique, 1989), and mosquito repellent and toxic properties (Batta and Santhakumari, 1970; Deshmukh *et al.,* 1982); the essential oil had exhibited larvicidal activity against *C. quinquefasciatus*, *A.*

aegypti and *A. stephensi* (Pathak *et al.,* 2000). With the recent increase in the use of *O. sanctum* plant extracts in integrated pest management systems, where the entomopathogenic fungi *M. anisopliae* are also used, a study on the compatibility among them become dessessive for combined use.

Plant extracts used in agriculture might affect the action of entomopathogenic fungi in the same way, as do the chemical pesticides. The use of incompatible plant extracts may inhibit the development and reproduction of these pathogens, affecting pest control (Malo 1993, Duarte *et al.* 1992, Anderson and Roberts 1983). On the other hand, the use of selective products is an important strategy in IPM (Integrated Pest Management). In some cases, compatible products may be associated with entomopathogenic fungi, increasing control efficiency (Moino and Alves 1998; Quintela and McCoy 1998).

Reports were not available on the compatibility of *O. sanctum* extracts with *M. anisopliae*. The present study deals with the compatibility of leaves, roots, stems and seed extracts of indigenous *O. sanctum* with *M. anisopliae in-vitro*.

Materials and methods

Microorganism tested

Pure culture of *Metarhizium anisopliae* NCIM 1311 was obtained from the National Collection of Industrial Microorganism (NCIM), National Chemical Laboratory, Pune, India.

Culture media

M. anisopliae were cultured in potato dextrose agar (PDA) and potato dextrose broth (PDB) medium (HiMedia, Mumbai, India).

Preparation of plant extract

Ocimum sanctum was collected from Rajgurunagar of Pune, Maharashtra, India in March 2008. About 200g of *O. sanctum* leaves were ground in mortar and pestle with 10ml of ether. Resulting paste was collected in a conical flask and allowed to mix properly by placing in a shaker at 200 rpm for 24 hours. Then the paste was filtered through double-layered muslin cloth and the filtrate of leaves in water was used for further studies. Similar protocol was adapted to prepare leaf extracts using benzene, methanol, acetone and water. The same procedure was used to prepare the extract from other plant parts like roots, stems and seeds. All the extracts were stored at 4^{o}C for further analysis.

Mycelial dry weight assessment

Five mm disc of 72 hours old *M. anisopliae* was inoculated into sterile PDB. 500 µl of plant extract (0.05%) was added into seeded PDB and incubated at room temperature for 7 days. Controls were amended with respective solvents. After a week, the mycelial mat was taken with sterile spatula, placed in sterile dishes containing filter paper. The initial weight of the paper was recorded. The Petri dishes were kept in hot air oven at 50°C for 30 minutes and the final weight of the fungal mat along with the filter paper was recorded immediately. The difference between the final and initial weight was considered as dry weight of mycelium.

Spore production assessment

500 µl of plant extract (0.05%) was added to sterile PDA at 45± 5^0 C and poured into sterile Petri dishes. After solidification of the medium, *M. anisopliae* was inoculated and incubated at room temperature for 7 days. After incubation, a central colony disk (5mm) was placed in a test tube and the conidia were suspended in 10 ml of sterile water containing 0.02% Tween 20 and quantified using a Neubauer chamber.

Statistical analysis

Data were subjected to descriptive statistics using STATISTICA/w 5.0.

Compatibility calculation: Compatibility was calculated according to Alves *et al.* (1998), as follows:

$$T = \frac{2\,0\,(MDW) + 8\,0\,(SC)}{100}$$

In this model, values for vegetative growth (MDW) and sporulation (SC) are given in relation to the control (100%). Where T= 0 to 30 = very toxic; 31 to 45 = toxic; 46 to 60 = moderately toxic; 60 - 90 =compatible; > 90 = highly compatible.

Results and Discussion

Leaves, roots, stems and seed extracts of *O. sanctum* did not affect conidial production regardless of the ether, benzene, methanol, acetone and water solvents used (Table 1). The behavior of the fungus with the extracts was similar in terms of mycelial dry weight, except for methanol extracts of leaves, ether extracts of roots, water and acetone extracts of seeds and benzene, methanol and acetone extracts of stems which reduced the mycelial dry weight of the fungal colonies (Table 1).

Table 1 Mycelial dry weight (mean±SD) and spore count (mean±SD) of *Metarhizium anisopliae* NCIM 1311 with different solvent extracts of various parts of *Ocimum sanctum.*

Name of the plant part	Solvent	MDW in gm (mean ± SE)	SC in conidia/ml (mean ± SE)
Leaves	Ether	0.0607 ± 0.001	$1.80x10^9 \pm 5.75x10^4$
	Benzene	0.0772 ± 0.013	$1.77x10^9 \pm 5.86x10^3$
	Methanol	0.0472 ± 0.013	$6.15x10^8 \pm 2.29x10^4$
	Water	0.0695 ± 0.004	$9.34x10^8 \pm 1.14x10^3$
	Acetone	0.0212 ± 0.041	$5.32\ x10^8 \pm 2.29x10^2$
Roots	Ether	0.0433 ± 0.003	$3.64x10^8 \pm 3.25x10^4$
	Benzene	0.0624 ± 0.021	$4.69x10^8 \pm 1.87x\ 10^4$
	Methanol	0.0734 ± 0.005	$9.57x10^8 \pm 3.18x10^3$
	Water	0.0803 ± 0.021	$1.02\times10^9 \pm 2.06x10^3$
	Acetone	0.0510 ± 0.013	$1.10x10^9 \pm 2.10x10^4$

Seeds	Ether	0.0603±0.0012	$1.82\times10^{9}\pm 1.07\text{x } 10^{4}$
	Benzene	0.0549±0.0045	$1.41\times10^{9}\pm 2.14\text{x}10^{4}$
	Methanol	0.0643±0.0011	$4.15\times10^{8}\pm 2.26\text{x}10^{4}$
	Water	0.0448±0.0017	$1.03\times10^{9}\pm 1.43\text{x}10^{3}$
	Acetone	0.0446±0.0024	$5.06\times10^{8}\pm 1.48\text{x}10^{3}$
Stems	Ether	0.0626 ± 0.005	$1.42\text{x}10^{9} \pm 7.49\text{x}10^{3}$
	Benzene	0.0494 ± 0.013	$2.09\text{x}10^{8} \pm 2.97\text{x}10^{4}$
	Methanol	0.0458 ± 0.005	$7.40\text{x}10^{8}\pm 2.40\text{x}10^{3}$
	Water	0.0626 ± 0.004	$8.04\text{x}10^{8} \pm 3.87\text{x}10^{2}$
	Acetone	0.0470 ± 0.020	$5.08\text{x}10^{8} \pm 1.77\text{x}10^{3}$
Control	Ether	0.0802 ± 0.006	$3.11\text{x}10^{9}\pm 2.88\text{x}10^{2}$
	Benzene	0.0799 ± 0.003	$1.87\text{x}10^{9} \pm 3.01\text{x}10^{4}$
	Methanol	0.0812 ± 0.001	$1.05\text{x}10^{9}\pm 4.45\text{x}10^{3}$
	Water	0.0839 ± 0.007	$3.77\text{x}10^{9} \pm 3.88\text{x}10^{4}$
	Acetone	0.0842 ± 0.002	$2.57\text{x}10^{9} \pm 2.26\text{x}10^{4}$

MDW - Mycelial dry weight, SC – Spore count

When the data concerning mycelial dry weight and spore count were submitted to the formula for the determination of T (Table 2), benzene extract of leaves and methanol extract of roots were found to be highly compatible; ether extract of leaves, ether and benzene extracts of seeds and methanol extracts of stems were compatible (Table 2). Ether extract of roots and benzene as well as acetone extracts of stem were classified as very toxic for *M. anisopliae* (Table 2).

Table 2 "T" values and compatibility classification of various parts of *Ocimum sanctum* with different solvent extracts on *Metarhizium anisopliae* NCIM 1311.

Name of the plant part	**Solvent**	**Values of "T"[1]**	**Classification[2]**
Leaves	Ether	61.44	C
	Benzene	95.04	HC
	Methanol	58.47	MT
	Water	36.37	T
	Acetone	31.59	T
Roots	Ether	20.16	VT
	Benzene	35.67	T
	Methanol	90.98	HC
	Water	41.58	T
	Acetone	46.35	MT
Seeds	Ether	61.84	C
	Benzene	74.06	C
	Methanol	47.44	MT
	Water	32.52	T
	Acetone	26.34	VT
	Ether	52.13	MT

Stems	Benzene	21.30	VT
	Methanol	67.67	C
	Water	31.98	T
	Acetone	26.97	VT

[1]Alves *et al.* (1998); [2] HC = highly compatible, C = compatible, MT = moderately toxic, T = toxic, VT = very toxic.

The formula proposed by Alves *et al.* (1998) represented in an appropriate way the toxic effect on the entomopathogenic fungi *in vitro*. Thus, when the treatment is compatible *in vitro*, there are strong evidences of its selectivity under field conditions. However, a high toxicity *in vitro* does not always mean that the same will happen in the field (Alves *et al.* 1998), but rather indicates only the possibility of the occurrence of damage of this nature. In addition, under field conditions, vegetative growth inhibition may not be a good indication of fungicidal effects such as spore viability (Loria *et al.* 1983). Under field condition, compatibility germination should be considered as the most important factor (Malo 1993; Anderson and Roberts 1983) due to the fact that pathogens infect insects through conidia germination by ingestion or contact. The survival of inoculum of the entomopathogenic fungi in the field is made by conidia. In the beginning of the epizootic, the conidia are responsible for the first disease focuses (Alves and Lecuona, 1998).

Information about compatibility among *M. anisopliae* and plant extracts used in pest control is scarce. Aguda *et al.* (1986) and Gonzalez *et al.* (1996) verified the negative effect caused by neem on *M. anisopliae* germination and conidiogenesis. According to the model of Alves *et al.* (1998), neem oil was moderately toxic for *M. anisopliae*. However, compatibility of chemical pesticides with this mycopathogen have been studied by Fargues (1975) and Anderson *et al*. (1989), Mohammed *et al*. (1987); Castinerias *et al*. (1991). Hassan and Charnely (1989) revealed the in-consistent interaction between fungus and insecticides. Li and Holdam (1994) observed chlorinated hydrocarbon insecticides as more deleterious than other insecticide groups to the mycopathogen. They observed extremely detrimental effect of chlorpyriphos, tempephos and malathion to mycelial growth and sporulation of *M. anisopliae*, while carbamate insecticides like carbofuran, methomyl and oxamyl were moderately toxic.

The results of the current study revealed that *O. sanctum* extracts did not affect the inoculum potential of *M. anisopliae* in terms of mycelial dry weight and spore count and hence *M. anisopliae* was compatible with *O. sanctum.* Field studies with the application of *O. sanctum* and *M. anisopliae* together with pests could provide extra information to that obtained by this study to help in the development of IPM strategies in agriculture.

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