



Compatibility of plant oils and additives with *Paecilomyces farinosus*, a potential entomopathogenic fungus

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Abstract

Addition of oils increases the infectivity of entomopathogens by enhancing conidial adhesion, prolonged persistence and infectivity against the target pests. An *in vitro* study, by following the poison food technique was conducted to determine the compatibility of plant oils and adhesive stickers in enhancing the infectivity of *Paecilomyces farinosus*, a potential entomopathogenic fungi. The compatibility of seven plant oils viz; coconut, groundnut, gingili, sunflower, neem, pongamia and castor oil and two adhesive stickers (Laboline and Triton X 100) with *P. farinosus* was investigated by measuring the radial growth of fungus at 5th, 10th and 15th day and the conidial spore yield of inoculation. The compatibility of the fungi to the oils and stickers studied varied. Among the oils and adhesives screened, groundnut, pongamia, gingili and castor oil and Triton X 100 were non-compatible with the fungus. Addition of sunflower, coconut and neem oil and Laboline enhanced the mycelia growth and sporulation of the fungus. Adding these oils and sticker with the fungi could increase their effectiveness as a bio-pesticide against the target pests.

Key words: *Paecilomyces farinosus*, plant oils, adhesive stickers, compatibility, conidiation.

Introduction

Paecilomyces farinosus, a potential entomopathogenic fungus is known to cause epizootic in insect populations under natural conditions¹⁻⁴. Augmenting the incidence level of this entomopathogen by timely, periodic application is reported to increase its effectiveness against target pests⁵⁻⁷. Creating a conducive niche for prolonged persistence of the spores without losing their viability and enhancing mycelia growth of the germinated spores are the major causative factors in successful use of entomopathogen as a bio-pesticide against the target pests of horticultural importance⁸. Addition of oils or adhesives enhances the infectivity of the entomopathogens⁹⁻¹¹. In the present study, various plant oils and adhesive stickers were screened against the entomopathogen, *P. farinosus*, that could enhance the mycelia growth and sporulation thereby increasing its effectiveness as a bio-pesticide.

Material and Methods

In the present study, seven plant oils and two adhesive stickers that are cheap and commonly available were selected to determine their effect on the mycelia growth and conidiation of the entomopathogen, *P. farinosus*. The strain IIHR-I retrieved from the sporulated cadavers of DBM larvae from the cabbage fields of Indian Institute of Horticultural Research (IIHR), Bangalore, India, was used in the present experiment.

The poisoned food technique was followed in this experiment where each 125 ml of sterilized potato dextrose agar (PDA, Hi Media Laboratories Limited, Mumbai, India) was amended with 0.2% concentration (v/v)⁹ of plant oils viz., coconut (*Cocos nucifera* Linn. Fl. Zely), groundnut (*Arachis hypogaea* L.), gingili

(*Sesamum indica* L.), sunflower (*Helianthus annuus*), neem (*Azadirachta indica* A. Juss), pongamia (*Pongamia glabra* Vent. Jard. Malm.) and castor (*Ricinus communis* L.) oil and adhesive stickers viz., Laboline (Qualigens Fine Chemicals, Glaxo SmithKline Pharmaceuticals Limited, Mumbai, India) 0.1% concentration (v/v) and Triton X-100 (Hi Media Laboratories Limited, Mumbai, India) 0.01% concentration (v/v) under aseptic condition. Unamended PDA medium served as control. The media were poured into sterilized 90 mm Petri dishes for solidification. Isolate of sporulated *Paecilomyces farinosus* IIHR-I grown on PDA for a period of two weeks was cut into 6 mm disc by using cork borer. The cut block was inverted and transferred on to the center of the plant oil + PDA-amended Petri dishes, placed lightly on the surface of PDA and was incubated at 27±1°C, 65-70% RH and a photo-cycle of 16L:8D in BOD incubator (S.K. Enterprises, Bangalore, India). Each plant oil and adhesive stickers were considered as treatment and each treatment was replicated four times on different days, ensuring reproducibility.

Determination of treatments effect on mycelia growth: The effect of oils and adhesives on the mycelia growth of the fungus was determined by measuring the radial growth of *P. farinosus* at five day intervals from 5th to 15th day after inoculation. Per cent enhancement and inhibition of mycelia growth over control was calculated using the formula given by Vincent¹². Based on the per cent mycelia growth over control, the vegetable oils and stickers were grouped as compatible or non-compatible. In compatible group, an enhancement of mycelia growth and increase in conidiation over control was recorded. In non-compatible

category inhibition of mycelia growth and reduction in conidiation over control was observed.

Determination of treatments effect on conidiation: The conidia were scraped on 15th day from the surface of the mycelium covering the Petri plates using a sterile spatula, grounded gently in a mortar and pestle with 50 ml of sterile water. The solution was filtered through a sterile muslin, centrifuged at 3000x g for 15 min. at 4°C, washed with 15 ml cold sterile distilled water and re-suspended in 10 ml cold sterile distilled water. By using an improved Neubauer double-ruled haemo-cytometer and phase contrast microscope at a magnification of 600x, the spore yield was recorded and the results were expressed in number of spores per millilitre to determine the cumulative effect.

Data analysis: The experiment was conducted in a controlled randomized design (CRD). The data obtained on mycelia growth was pooled from all four replicates and subjected to one-way ANOVA. The treatment means were compared using critical difference (CD) at p=0.05.

Results and Discussion

The compatibility of different plant oils and adhesive stickers on *P. farinosus* under *in vitro* condition is presented in Tables 1 to 3. The data indicated varied compatibility of the fungus with different plant oils and adhesives studied.

Effect of plant oils and adhesives on mycelia growth: On the 5th day, a maximum colony diameter (3.33 cm) of the fungus was recorded in sunflower oil-amended PDA followed by coconut and pongamia oil-amended (3.03 cm) (Table 1). Castor and neem oil-amended recorded radial mycelia growth (2.8 and 2.6 cm), which was higher compared to control (2.37 cm diameter). The mycelia growth in groundnut-amended media was at par to control. Addition of gingili oil with the fungus resulted in 1.33 cm diameter mycelia growth that was lower compared to control. Inclusion of the adhesives, Laboline and Triton X, with the fungus recorded mycelia growth (2.07 and 2.03 cm diameter) that was also lower compared to control.

On 10th day, a colony diameter of 4.57 cm radial growth of the fungus was recorded in castor oil-amended media, followed by 4.33, 4.30, 4.23 and 4.03 cm in sunflower, neem, coconut and

pongamia oil-amended media respectively that were significantly higher compared to control (3.4 cm). There was no increase in mycelia growth in gingili oil-amended from 5th to 10th day. In Laboline and Triton X-100 treatments radial growth of the fungus (1.97 and 1.67 cm) was lower compared to control.

On 15th day, the maximum mycelia growth in terms of cumulative growth of colony diameter (6.23 cm) was recorded in neem oil treatment followed by castor (5.9 cm), coconut (5.37 cm) and pongamia oil treatments (5.43 cm). Mycelia growth in sunflower oil and groundnut oil-amended (4.53 cm and 3.57 cm) was lower than control (5.23 cm). Least growth of the fungus was recorded in gingili oil treatment. The adhesives Laboline and Triton X-100 recorded 4.97 and 4.17 cm cumulative growth of the mycelia by 15th day of inoculation.

The compatibility of the fungus with the plant oils and adhesives varied with time. On 5th day, the maximum rate of mycelia growth was found in sunflower oil-amended media at the rate of 0.27 cm/day that decreased to 0.19 and 0.17 cm/day by 10th and 15th day respectively (Table 2). Castor oil-amended media recorded the highest rate of mycelia growth on 10th day (0.2 cm/day). Neem oil treatment was observed to have a uniform rate of growth from 5th to 15th day of inoculation. The minimum rate of mycelia growth was found in gingili oil treatment for 5th, 10th and 15th day at the rate of 0.07, 0.04 and 0.03 cm/day, respectively. The rate of growth of the fungus was maximum on the 5th day in sunflower, coconut and pongamia oil-amended, while in castor and neem treatments the maximum mycelia growth was observed between 10th and 15th day of inoculation.

Effect of plant oils and adhesives on conidiation: Addition of plant oils and adhesives resulted in variation in the spore yield. Sunflower oil treatment recorded the maximum spore yield of 15x10⁶ conidial spores/ml followed by 12x10⁶ and 8.2x10⁶ conidial spores/ml in coconut oil and neem oil treatment as against 7x10⁶ conidial spores/ml in control (Table 3). Least conidial spore production was recorded in gingili oil treatment (0.93x10⁶ conidial spores/ml). Among the adhesives, Laboline recorded 3.30 spores/ml, followed by Triton X-100 that recorded 0.5 x10⁶ conidial spores/ml.

The compatibility of the entomogenous fungus, *P. farinosus*, to different plant oils and adhesive stickers is varied and based on the interaction and final spore production, that ultimately

Table 1. Effect of different plant oils and adhesive stickers on the mycelial growth of *Paecilomyces farinosus* and per cent growth of mycelium over control.

Treatment	Colony diameter in 'cm'			Per cent growth over the control (cumulative)		
	5 th day	10 th day	15 th day	5 th day	10 th day	15 th day
Coconut oil	3.03 ^b	4.23 ^{de}	5.37 ^{de}	27.85	24.41	2.68
Groundnut oil	2.37 ^d	3.40 ^{fg}	3.57 ⁱ	0.00	0.00	-31.74
Gingili oil	1.33 ^f	1.33 ⁱ	1.40 ^j	-43.88	-60.88	-73.23
Sunflower oil	3.33 ^a	4.33 ^{bcd}	4.83 ^g	40.51	27.35	-7.65
Neem oil	2.60 ^c	4.30 ^{cd}	6.23 ^a	9.71	26.47	19.12
Pongamia oil	3.03 ^b	4.03 ^e	5.43 ^{cd}	27.85	18.53	3.82
Castor oil	2.80 ^{bc}	4.57 ^a	5.90 ^b	18.14	34.41	12.27
Laboline	2.07 ^e	3.37 ^g	4.17 ^h	-12.66	-0.88	-20.27
Triton-X	2.03 ^e	2.97 ^h	4.97 ^{fg}	-14.35	-12.65	-4.97
Control	2.37 ^d	3.40 ^{fg}	5.23 ^e	-	-	-
CD=0.05	0.24	0.20	0.19	-	-	-

* Mean of 4 replications Means in the same column with letters in common are not significantly different.

Table 2. Growth rate of *Paecilomyces farinosus* in various plant oils and adhesives.

Treatment	Growth rate 'cm ² /day (cumulative)		
	Upto 5 th day	Upto 10 th day	Upto 15 th day
Coconut oil	0.24	0.18	0.16
Groundnut oil	0.18	0.14	0.10
Gingili oil	0.07	0.04	0.03
Sunflower oil	0.27	0.19	0.14
Neem oil	0.20	0.19	0.19
Pongamia oil	0.24	0.17	0.16
Castor oil	0.22	0.20	0.18
Laboline	0.15	0.15	0.12
Triton-X	0.14	0.12	0.15
Control	0.18	0.14	0.15

Table 3. Influence of different plant oils and adhesive stickers on conidiation of *Paecilomyces farinosus*.

Treatment	Conidia (x10 ⁶ /ml) #
Coconut oil	12.0 (3.46) ^b
Groundnut oil	6.50 (2.55) ^e
Gingili oil	1.80 (1.34) ^g
Sunflower oil	15.0 (3.87) ^a
Neem oil	8.20 (2.86) ^c
Pongamia oil	1.20 (1.09) ^h
Castor oil	0.93 (0.96) ⁱ
Laboline	3.30 (1.82) ^f
Triton-x	0.50 (0.71) ^j
Control	7.00 (2.65) ^{de}
CD (p=0.05)	0.10

*Figures in parenthesis are transformed square root values.

Mean of 4 replications

Means in the same column with letters in common are not significantly different.

determines the availability of the inoculum in subsequent generation. Among the plant oils screened, addition of coconut, neem and sunflower oil was compatible with the fungus in relation to mycelia growth and conidiation. Oils such as pongamia and castor were non-compatible with the fungus. These oils though enhanced mycelia growth, caused a significant reduction in sporulation. Addition of gingili and groundnut oil was also non-compatible with the fungus that even after 15 days of association produced -73.23 and 31.74 % inhibition of mycelia growth over control (Table 2). Among the adhesives screened, Laboline was found to be better in relation to mycelia growth and conidiation than Triton X-100.

Based on the present study, it could be suggested that addition of plant oils such as coconut, neem and sunflower oil could accelerate mycelia growth and sporulation of the entomogenous fungi *P. farinosus* thereby enhancing its infectivity against target pests. The results of the current study would form a key factor in our future research programmes that aims to carry out pathogenicity tests and formulate *P. farinosus* as a potential bio-pesticide for augmentative biological control of many economically important horticultural crop pests.

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