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Effects of Some Abiotic Factors on Mycelial Growth Rates of *Isaria fumosorosea* Wize and Laboratory Evaluation against *Planococcus citri* (Risso)

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ABSTRACT

Studies were carried out in Plant Protection Research Institute, Sharkia branch during 2012-2013 years to determine the effects of different temperatures (10, 15, 20, 25, 30, 35 and 40°C), pH values (2, 4, 6, 7, 8, 10 and 12) and incubation periods (2, 4, 6, 7, 8, 10, 12 and 14 day) on *Isaria fumosorosea* Wize mycelial growth rates as a biological control agent of *Planococcus citri* (Risso). The toxicity of *I. fumosorosea* spores suspension on citrus mealybug, *P. citri* under laboratory conditions was studied. The optimal environmental conditions for the mycelial growth rate of *I. fumosorosea* were obtained at 25°C, in pH 5.8, for 7 days incubation period. The LC_{50} value was 5×10^{6} spores mL^{-1} and LC_{90} was 4×10^{13} spores mL^{-1}.

Key words: *Isaria fumosorosea*, temperatures, pH values, incubation periods, efficacy, *Planococcus citri*

INTRODUCTION

The entomopathogenic fungi, *Isaria farinosa* and *Isaria fumosorosea* were known as *Paecilomyces farinosus* and *Paecilomyces fumosoroseus*, for more than 30 years. Both fungi have a worldwide distribution and a relatively wide host range. While, *I. farinosa* currently is of minor importance in research and as biocontrol agent, *I. fumosorosea* is regarded as a species complex and various strains are successfully used for biocontrol of several pest insects (Zimmermann, 2008). *Paecilomyces* spp., a common insect borne filamentous fungus belongs to Hyphomycetes of Deuteromycota and has been reported to cause diseases in a wide species of insects, occasionally resulting in natural epizootics (Altre *et al*., 1999; Cantone and Vandenberg, 1999; Lacey *et al*., 1999; Nam *et al*., 2000). Panyasiri *et al*. (2007) founded that five strains of *I. fumosorosea* isolated from a Coleopteran, an unkown host and white flies, were highly active against thrips, *Ceratothripoides claratris* (80-93% mortality), moderate activity against *Bemisia tabaci* (37-77% mortality) and less active against mealybug, *Pseudococcus cyrptus* (10-43% mortality). Also, Demirci *et al*. (2011) reported that the entomopathogen *I. farinose* may be used as a biocontrol agent against citrus mealybug, *Planococcus citri*.

Fungal spores are living organisms and their viability diminishes with time depending on environmental conditions. Temperature is considered as one of the important factors affecting the natural activity of parasitic fungi. The rapidity of mycelial development and evaluation of infection depend on temperature. In general, the optimum growth and germination rates on artificial media vary around 25°C for *P. fumosoroseus* (MacLeod, 1963; Kalvish, 1974; Ekbom, 1979; Fang *et al*.,

1985; Moore *et al*., 2000). *Paecilomyces fumosoroseus* mycelial growth has been expedited gradually in proportion to the rise of temperature and was the most suitable at 25°C. Even though the mycelial growth of *P. fumosoroseus* was favorable at the range of 20-25°C and had been expedited in proportion to the rise of temperature, the mycelial growth appeared to be suppressed at the temperature higher than 30°C. Similarly, there was slow growth at 15 and 35°C (Shim *et al*., 2003b).

The pH is considered as one of the important factors affecting the natural activity of fungi. Choi *et al*. (1999) reported that mycelial growth of *P. japonica* was optimal at pH 7. Shim *et al*. (2003a) also reported that *P. sinclairii* showed maximal mycelial growth at pH 8. The mycelial growth and density of *P. fumosoroseus* was almost identical in the range of pH 6-9. The pH values suitable for a favorable growth of *P. fumosoroseus* were obtained in the range of pH 6-9 (Shim *et al*., 2003b).

Incubation period considered as one of the important factors affecting the natural activity of parasitic fungi. Ghareeb (2009) indicated that fungal growth of *Paecilomyces violaceae* increased rapidly during the linear growth phase. The optimum biomass production by tested fungi was achieved at the end of 5 days of growth periods. However, biomass yield gradually decreased during the longer period of the culture (7-12 days). Growth rate was gradually dependant on the duration of fermentation period.

The present study aimed to determine the effects of some abiotic factors such as temperature, pH values and incubation periods on growth rate of entomopathogenic fungi, *Isaria fumosorosea* and its effectiveness on citrus mealybug, *Planococcus citri* under laboratory conditions.

MATERIALS AND METHODS

Studies were carried out in Plant Protection Research Institute, Sharkia Branch during 2012-2013 years to determine the effects of some abiotic factors such as temperature, pH values and incubation periods on growth rates of entomopathogenic fungi, *Isaria fumosorosea* and the pathogenisty against citrus mealybug, *P. citri*.

Isolation, identification and maintenance of fungal culture stock: *Isaria fumosorosea* was isolated from naturally infected *Ceroplastes floridensis* (Soft scale insect) on lemon trees at Sharkia Governorate in 2012 and identified using ribosomal DNA sequences data according to Abd-Elsalam *et al*. (2003). The fungal isolates used in present study were descendant of pure single slant culture. The stock culture of fungal isolates were maintained on Czapek-Dox's and potato dextrose then stored at 4.0°C with transfer at monthly intervals slant (Smith and Onions, 1983).

Effects of some abiotic factors: Each treatment was carried out in triplicates and the results obtained throughout this study were the arithmetic average of at least two experiments.

Temperature: In this experiment, Sterilized Czapek-Dox's liquid medium adjusted at pH 6 were inoculated with equal amounts of the fungal spores and incubated at the following temperature 10, 15, 20, 25, 30, 35 and 40°C. At the end of incubation period after 7 days, cultures were harvested for determining the mycelial dry weights.

pH values: The basal medium was adjusted to following pH values, 2, 4, 6, 7, 8, 10 and 12 by addition of varying amounts of 1 N HCl and NaOH using pH electrode. The inoculated flasks were incubated at 25°C for 7 days after which, mycelial dry weights were determined.

Incubation periods: Sterilized Czapek-Dox's liquid media was inoculated with culture of *I. fumosorosea*, incubated at 25°C and the experiment was extended for 14 days. Dry weight was determined respectively every 48 h.

Preparation of inocula: Inoculum was prepared by agitating a slant of the fungal isolates (7 days old fungal culture) with 10 mL sterile distilled water using an inoculated needle. One milliliter of the spores suspension was then used as standard inocula.

Cultivation: Erlenmeyer conical flasks (250 mL capacity) for each treatment, each one containing 50 mL of fermented medium, were used in the present study. The pH of the medium adjusted to 6. The flasks were plugged with cotton wool and sterilized at 121°C for 20 min. Each flask, after being cooled was inoculated with 1 mL spore suspension (standard inoculum) under aseptic conditions. The culture flasks were then incubated at 25°C in incubator.

Determination of mycelial dry weight: At the end of the required incubation period, the culture flasks were filtered through preweighted Whatman No. 1 filter papers. The filter papers were washed twice with distilled water, dried in an electric oven at 80°C and left in desiccator to attain room temperature. The filter papers were then weighted at regular intervals till the two successive weights were the same.

Laboratory evaluation

Culture of *Planococcus citri*: The citrus mealybug, *P. citri* was reared on Squash fruit (*Cucurbita pepo*) under laboratory conditions 25±1°C, 65±5 RH% and 12 h photoperiod. Baby Sun Rose leaves, *Aptenia cordifolia* were preferred for the laboratory evaluation in order that its leaves can survive for a long period after detached. *Planococcus citri* ovisacs were taken by a fine brush and located on *A. cordifolia* leaves. The leaves were incubated under the same laboratory conditions 25±1°C, 65±5 RH% and 12 h photoperiod. After 48 h from ovisac hatching 5 leaves were puked up and put in a plastic petri plates on filter papers that saturated daily with water.

Fungal inocula: Spores of fungal isolates were harvested by rinsing with sterilized 0.005% Tween 80 from 7 day old culture (PDA media grown at 25±1°C for *I. fumosorose*a isolates). The suspensions were filtered through cheesecloth to reduce mycelium clumping. The spores were counted in the suspensions using a haemocytometer. The concentrations were adjusted to 1×10^5, 1×10^6, 1×10^7 and 1×10^8.

Experimental design: Experimental studies were applied on three replicates for each concentration and the same for control. Each replicate contains 5 leaves of *A. cordifolia* every leaf has 5 individuals of *P. citri* larval instar. Leaves sprayed with 2 mL of spores suspension and the control was treated with 2 mL of 0.005% Tween 80 only. The treatments and control were incubated for 7 days under laboratory conditions 25±1°C, 65±5 RH% and 12 h photoperiod. Larval mortality was observed after 1, 3, 5 and 7 days. The LC_{50}, LC_{90} and slope values were calculated after 7 days according to Finny (1971).

RESULTS AND DISCUSSION

Effects of temperature: Data given in Table 1 showed that there was gradual increase in mycelial growth rates of *I. fumosorosea* under temperatures of 10, 15, 20 and 25°C with values of 0.02, 0.06, 0.23 and 0.47 g/100 mL, respectively. After that mycelial growth rates were decreased with values of 0.45, 0.14 and 0.05 under temperatures of 30, 35 and 40°C. The optimal temperature for mycelial growth was obtained at 25°C with value of 0.47 g/100 mL. This result was agreed with those given by MacLeod (1963), Kalvish (1974), Ekbom (1979), Fang *et al.* (1985), Moore *et al.* (2000) and Shim *et al.* (2003b) who reported that *P. fumosoroseus* mycelial growth has been expedited gradually in proportion to the rise of temperature and the optimum temperature for growth and sporulation were ranged between 20-25°C.

Effects of pH values: Data given in Table 2, clarified that there were seven levels of pH that were adjusted at 2, 4, 6, 7, 8, 10 and 12. The mycelial dry weight of *I. fumosorosea* reached values of 0.07, 0.17, 0.09, 0.10, 0.13, 0.07 and 0.07 g/100 mL, respectively. The suitable pH final value for mycelial dry weight was obtained at pH 5.8 with value of 0.17 g/100 mL. This result was agreed with this given by Shim *et al.* (2003b) who reported that the suitable pH values for a favorable growth of *P. fumosoroseus* were obtained in the range of pH 6-9.

Effects of incubation periods: Data given in Table 3 showed that the optimum incubation period for mycelial growth of *I. fumosorosea* was obtained after 7 days of incubation with value of 0.45 g/100 mL.

This result was agreed with Ghareeb (2009) who reported that *P. violaceae* biomass increased rapidly during the linear growth phase. The optimum biomass production by tested fungi was achieved at the end of 5 days of growth periods. The yield gradually decreased during the longer period of the culture (7-12 days). Growth rate was gradually dependant on the duration of fermentation period.

Table 1: Effect of different temperatures on growth rates of *Isaria fumosorosea* after 7 days

Temperatures (°C)	Mycelial dry weight (g/100 mL)
10	0.02
15	0.06
20	0.23
25	0.47
30	0.45
35	0.14
40	0.05

Table 2: Effect of different levels of pH values on growth rates of *Isaria fumosorosea* after 7 days

Mycelial dry weight (g/100 mL)	pH values final	pH values initial
0.07	2.2	2
0.17	5.8	4
0.09	5.3	6
0.10	6.4	7
0.13	6.4	8
0.07	6.5	10
0.07	6.9	12

Table 3: Effect of different incubation periods on growth rates of *Isaria fumosorosea* under laboratory conditions 25±1°C,65%±5 RH% and 12 h photoperiod

Incubation periods (days)	Mycelial dry weight (g/100 mL)
2	0.07
4	0.09
6	0.35
7	0.45
8	0.43
10	0.42
12	0.39
14	0.36

Table 4: Mortality percentages of larval instars of *Planococcus citri* after application with different concentrations of *Isaria fumosorosea* spores suspension under laboratory conditions (25±1°C, 65%±5 RH% and 12 h photoperiod)

	Mortality percentages of larval instars of *Planococcus citri* per 75 individuals											
	After 1 day			After 3 days			After 5 days			After 7 days		
Concentrations	Life	Dead	Mortality (%)	Life	Dead	Mortality (%)	Life	Dead	Mortality (%)	Life	Dead	Mortality (%)
1×10^5 spores mL^{-1}	74	1	1.33	69	6	8.00	62	13	17.33	48	27	36.00
1×10^6 spores mL^{-1}	73	2	2.67	66	9	12.00	60	15	20.00	39	36	48.00
1×10^7 spores mL^{-1}	72	3	4.00	65	10	13.33	55	20	36.36	38	37	49.33
1×10^8 spores mL^{-1}	72	3	4.00	62	13	17.33	39	36	48.00	30	45	60.00

Table 5: Lethal concentration (LC_{25-99}) of *Isaria fumosorosea* spores per mL after 7 days of application against larval instar of *Planococcus citri* under laboratory conditions 25±1°C, 65%±5 RH% and 12 h photoperiod

	Concentration of *Isaria fumosorosea* spores per mL			
Lethal concentration	Concentration	Lower limit	Upper limit	Slope
LC_{25}	1275	240	41619	0.186
LC_{50}	5.0×10^6	675330	1×10^8	
LC_{75}	2.0×10^{10}	4×10^8	1×10^{19}	
LC_{90}	4.0×10^{13}	4×10^{10}	3×10^{29}	
LC_{95}	3.6×10^{15}	6×10^{11}	7×10^{35}	
LC_{99}	1.7×10^{19}	9×10^{13}	4×10^{47}	

Laboratory evaluation: Data given in Table 4 showed the efficacy of *I. fumosorosea* spores suspension on larval instars of citrus mealybug, *P. citri*, after application with different concentrations of *I. fumosorosea* under laboratory conditions 25±1°C, 65±5 RH% and 12 h photoperiod. The concentrations were adjusted to 1×10^5, 1×10^6, 1×10^7 and 1×10^8 spores mL^{-1}. Mortality percentages after 7 days of application showed 36.00, 48.00, 49.33 and 60.00%, respectively.

Data given in Table 5 clarified the LC_{50} and LC_{90} values of *I. fumosorosea* spores/mL after 7 days of application on larval instars of citrus mealybug, *P. citri*. The obtained results revealed that LC_{50}: 5×10^6 spores mL^{-1} and LC_{90}: 4×10^{13} spores mL^{-1} (Fig. 1).

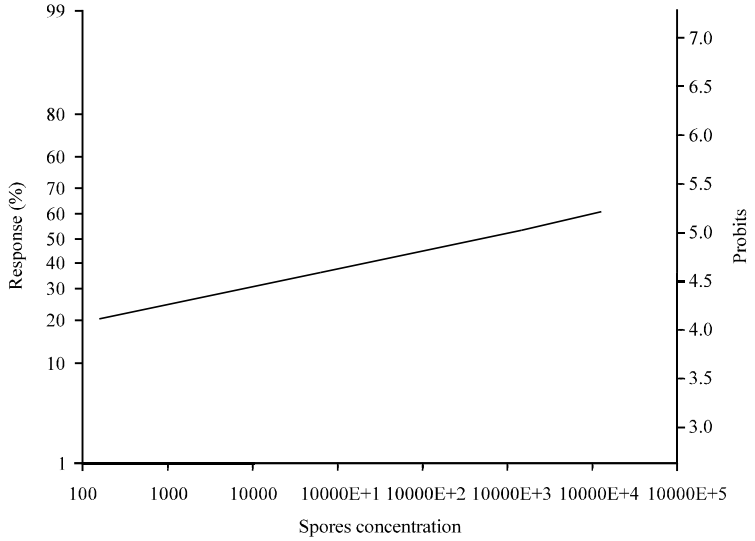

Fig. 1: Concentration mortality probit line of *Isaria fumosorosea* spores per mL on larval instars of *Planococcus citri* under laboratory conditions 25±1°C, 65±5% RH and 12 h photoperiod after 7 days

This result was agreed with that of Panyasiri *et al.* (2007) who found that *I. fumosorosea* has moderate activity against *B. tabaci* (37-77% mortality) and less active against mealybug, *P. cyrptus* (10-43% mortality) and Demirci *et al.* (2011) who recorded that entomopathogenic fungi, *I. farinosa* used as biocontrol agent against mealybug, *P. citri* causing 89.39% mortality ovisac, 84.07% mortality in second larval stage, 84.53% mortality in adult females and 78.71% mortality in first larval stage at 1×10^{8} conidia mL^{-1} inoculm concentration.

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