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Growth kinetics and pathogenicity of *Paecilomyces fumosoroseus* isolates against *Bemisia tabaci* Grennadius (Homoptera : Aleyrodidae)

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ABSTRACT

A study was conducted using two isolates of *Paecilomyces fumosoroseus* obtained from two farming localities in Trinidad – Aranguez and Macoya. The growth kinetics and pathogenicity of both isolates were studied in the laboratory at three temperatures: 20^0C, 25^0C and 30^0C. The growth rates of the isolates differed considerably at the different temperatures and localities with both the Aranguez and Macoya isolates at 25^0C and 20^0C respectively performing optimally based on the criteria of the present study. The Aranguez isolate was the most pathogenic to *Bemisia tabaci* at 25^0C with an LC_{50} of 3.19×10^4 spores/mL causing 50% mortality (LT_{50}) in 2.39 days; while the Macoya isolate at 20^0C with an LC_{50} of 3.49×10^5 spores/mL caused 50% mortality and LT_{50} to *Bemisia tabaci* is 1.61 days. The results are discussed in relation to similar studies conducted elsewhere.

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Key words: *Paecilomyces fumosoroseus*, isolates, growth kinetics and pathogenicity, *Bemisia tabaci*

INTRODUCTION

Whiteflies of Homoptera are major pests of arable crops throughout the World. They damage crops by reducing plant vigour, facilitating growth of sooty mould on leaves and fruits and transmitting viral diseases. These pests cause substantial damage to agricultural crops worldwide. In the Caribbean, the majority of foliar insecticide applications to solanaceous crops and to a lesser extent crucifers are used to control whiteflies, particularly *Bemisia tabaci* Gennadius (Homoptera : Aleyrodidae) (Hilje, 1998, 2003). The overuse of conventional insecticides has resulted in the development of resistance in *B. tabaci* populations and increasing public concern over deleterious effects of pesticide use on human health and the environment, which have led to research into alternative strategies for control of these pests. The use of the entomopathogenic fungus, *Paecilomyces fumosoroseus* as biological control agent for *B. tabaci* has received increasing interest in many countries as part of integrated control strategies (Yeo *et al*., 2003).

The use of this entomopathogenic fungus as a biological control agent has focused on methods of conservation and augmentation (Yeo *et al*., 2003). Hoddle (1999) notes that conservation of and inoculation using *P. fumosoroseus* has the potential to enhance control of insect populations, including whiteflies, mealybugs and beetles. These strategies rely on the ability of the fungus to persist and establish in host populations to provide long-term control and not only on intrinsic virulence. The conservation and inoculation of this entomopathogenic fungus may be sufficient to suppress *B. tabaci* populations. However, when pest populations are particularly high, inundative releases may become necessary to achieve control (Yeo *et al*., 2003).

Abiotic factors affect the ability and speed with which the fungus can infect and colonize its host. In areas where temperature can be controlled (glasshouse environments) there has been some success; however, where abiotic conditions cannot be controlled there have been problems with its effectiveness in the field. A possible approach would be to select isolates that are active over a wide range of temperatures occurring under field conditions together with selection for pathogenic characteristics in laboratory bioassays. Additionally, the biological control agent should have little or no impact on natural enemies which are associated with the pest. The effect of temperature on aspects of the radial growth, virulence and pathogenicity of *P. fumosoroseus*

isolates selected for use against whiteflies on crops in Trinidad and Tobago is imperative. Quantifying these attributes will be useful in selecting the most promising isolate of *P. fumosoroseus*, to be used under large scale field situations (Yeo *et al*., 2003). The present study investigates the effect of temperature on growth kinetics and pathogenicity of two isolates of *P. fumosoroseus*.

MATERIALS AND METHODS

Fungal Isolation: Isolates of *P. fumosoroseus* were collected from eggplant (*Melongena melongena*) on farms in Aranguez and Macoya, Trinidad. Both crops were in the reproductive stage of growth with moderate infestation of *B. tabaci*. Fungal isolates were stored under aseptic conditions as first cultures at 22.0±1.0^{0}C. For each fungus, 6 sub-cultures were made on Potato Dextrose Agar (PDA) (Oxoid®, Basingstoke, UK) in 9cm petri plates and left for 4 weeks in an incubator at 22.0±1.0^{0}C for complete sporulation to occur.

Effect of temperature on *in vitro* radial colony growth : Forty-eight PDA plates using sterile petri dishes were prepared for each isolate. Petri plate edges were sealed with Parafilm® and incubated in separate incubators at 20^{0}C, 25^{0}C and 30^{0}C respectively. Eight replicates were prepared for each isolate-temperature combination. The radial growth was recorded every 48 hours using two cardinal diameters that were previously drawn on the bottom of each dish. The experiment continued for 45 days or until the fungal colony covered the petri dish. For each *P. fumosoroseus* replicate at each temperature, the colony radial growth rate, K_r (μm h^{-1}), was estimated from the slope of the linear regression of colony radius on time. The values of the slopes were compared to determine differences in growth rates at different temperatures (Yeo *et al.,* 2003).

Effect of temperature on pathogenicity of *P. fumosoroseus*: Spores from four plates of each isolate stored at 20^{0}C, 25^{0}C and 30^{0}C were removed using a scalpel, transferred to a 100mL beaker and labeled according to the location and temperature. One drop of Tween 80® was added to each beaker and then placed on a magnetic stirrer for 20 minutes at room temperature to ensure that the spores were well suspended. Twenty μl of the dissociated spore suspension was removed using a micropipette and spore density determined using a Neubauer haemocytometer. Six serial dilutions (10^4, 10^5, 10^6, 10^7, 10^8, 10^9 spores/mL) were made from the stock suspension. The control comprised 20mL of water and 0.5mL of Tween 80®. Cabbage leaves were obtained and cut into 4 cm in diameter discs. These discs were washed in 500mL of distilled water, 0.5% bleach and finally rinsed in 500mL of sterile water and left to air dry in a laminar flow chamber. For each concentration a total of five leaves were dipped and air dried in an Envair® laminar flow cabinet (Model HLF/4/B). Leaves were placed in labeled petri dishes containing a minimum of ten *B. tabaci* adults. Dishes were sealed around the circumference with Parafilm® and stored at ambient temperature. Adult mortality was recorded on a daily basis. The LC_{50} and LT_{50} were calculated for *P. fumosoroseus* at each temperature using probit analysis (EPA Probit Analysis Ver. 1.4) and values compared.

RESULTS AND DISCUSSION

Biological control is an attractive component of Integrated Pest Management programmes since it helps to reduce the use of pesticides that cause resistance, pollute the environment and are becoming too expensive, especially for resource poor farmers in developing countries. Laboratory testing of two isolates of *P. fumosoroseus* from Aranguez and Macoya, Trinidad was done to determine the most promising isolate for management of *B. tabaci*. The radial diameter of colonies increased between Days 2 to 35 post-inoculation for both the Aranguez and Macoya isolates of *P. fumosoroseus* (Figs. 1 and 2).

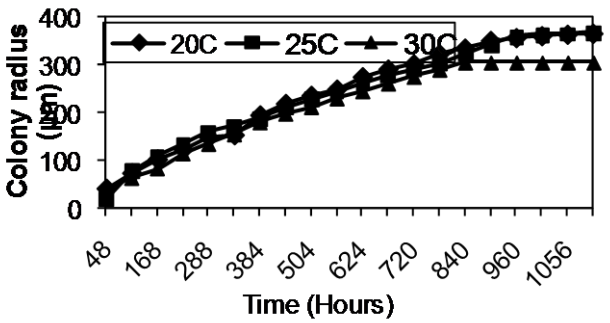

Fig. 1. Effect of temperature on colony growth of *Paecilomyces fumosoroseus* from Macoya, Trinidad on Potato Dextrose Agar

Table 1. Summary of regression equations and time taken for 50% germination (GT_{50}) of *Paecilomyces fumoso-roseus* at three temperatures and two locations in Trinidad.

Location/ Temperature (^{0}C)	Regression equation	GT_{50} (hours) (95% confidence interval)[1]	S.E of GT_{50}
Macoya			
20	Y = 2.24x - 0.15	199.47 (198.79, 200.15)^a	1.002
25	Y = 2.15x - 0.49	353.73 (254.67, 491.33)^b	1.183
30	Y = 1.68x + 0.59	421.38 (266.05, 667.39)^b	1.264
Aranguez			
20	Y = 2.31x - 0.35	207.98 (207.29, 208.67)^c	1.002
25	Y = 2.45x - 0.86	246.51 (164.84, 368.66)^{ab}	1.228
30	Y = 0.88x + 1.84	4.08 x 10^3 (1.41 x 10^3 , 1.18 x 10^4)^d	1.717

[1]Values followed by the same letter are not significantly different (P>0.05) from each other based on Tukey-Kramer Multiple Comparisons test

The results indicated that there was no significant difference (P>0.05) between growth rates in isolates from Macoya at 25^{0}C and 30^{0}C. However, for the Aranguez isolate, the GT_{50} was significantly lower (P>0.05) at 30^{0}C than at both 20 and 25^{0}C (Table 1). Isolates from both Aranguez (GT_{50} = 207.98h) and Macoya (GT_{50} = 199.47h) grew fastest at 20^{0}C compared with isolates at other temperatures (Table 1). These results were similar to those obtained by several authors using *Paecilomyces* spp. Carillo-tolerance was a specific characteristic of an isolate from a particular species.

Fargues and Bon (2004) and Hassani *et al.* (2000) conclude that optimum growth of *P. fumosoroseus* occurred between 20 and 25^{0}C. Hassani *et al.* (2000) also indicate that after 16h at 25, 30 and 35^{0}C conidial germination was 98.3, 79.6 and 0% respectively, indicating that germination decreased with increasing temperature; a trend which was also observed with both Aranguez and Macoya isolates (Figure 3).

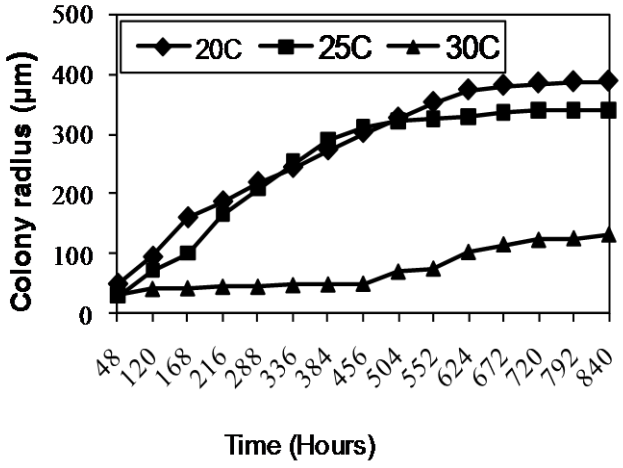

Fig. 2. Effect of temperature on colony growth of *Paecilomyces fumosoroseus* from Aranguez, Trinidad on Potato Dextrose Agar

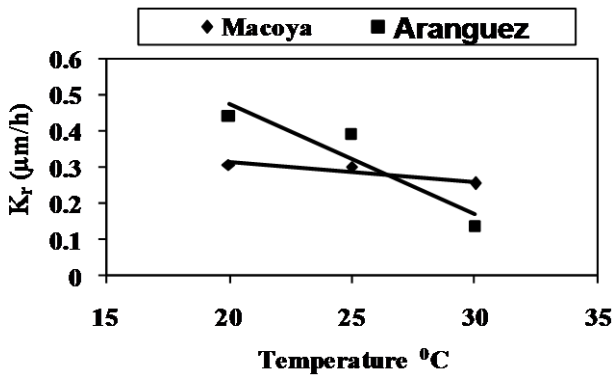

Figure 3. Comparison of K_r values for isolates of *Paecilomyces fumoso-roseus* at three temperatures at Aranguez (y = -0.0308x + 1.0943; R^2 = 0.8698) and Macoya (y = -0.0052x + 0.4199; R^2 = 0.8278)

Pérez *et al.* (2013) investigated thermo-tolerance of two isolates of *Isaria fumosorosea* (= *Paecilomyces fumosoroseus*) and concluded that ideal growth ccurred between 20 and 28^{0}C and that thermo-

When the pathogenicity of both isolates were compared, *P. fumosoroseus* Aranguez isolate at 25^{0}C had the lowest LC_{50} value, however this was not significantly different (P>0.05) from either the Aranguez isolate at 30^{0}C or the Macoya isolate at 20^{0}C (Table 1).

Table 2. Comparison of LC_{50} and LT_{50} analyses for *B. tabaci* at Macoya and Aranguez, Trinidad and at three temperatures.

Location	Temperature (^{0}C)	Probit equation	LC_{50} (spores/ml) (95% Confidence interval)[1]	χ^2	S.E. of LC_{50}
LC_{50} Parameters					
Aranguez	20	Y=0.15x + 3.99	6.44×10^6 (8.93×10^5, 4.65×10^7)[a]	1.879	2.741
	25	Y=-0.21x + 5.95	3.19×10^4 (1.85×10^3, 5.48×10^5)[b]	9.457	9.457
	30	Y=0.23x + 3.54	2.19×10^6 (2.00×10^5, 2.39×10^7)[ab]	0.751	0.234
Macoya	20	Y=-0.06x + 5.36	3.49×10^5 (2.60×10^3, 4.68×10^5)[b]	0.833	0.082
	25	Y=-0.28x + 7.44	5.32×10^8 (4.69×10^7, 6.02×10^9)[c]	7.357	0.289
	30	Y=-0.12x + 6.20	1.09×10^7 (1.09×10^7, 2.75×10^{12})[ac]	6.505	0.042
LT_{50} (days) (95% Confidence interval)[1]				χ^2	S.E. of LT_{50}
Aranguez	20	Y=2.05x + 4.18	2.52 (2.22, 2.86)[a]	2.723	1.066
	25	Y=2.37x + 4.10	2.39 (1.85, 3.08)[ab]	0.258	1.140
	30	Y=2.17x + 3.58	4.53 (3.41, 6.01)[b]	4.77	1.156
Macoya	20	Y=1.62x + 4.66	1.61 (1.31, 1.98)[b]	6.59	1.111
	25	Y=1.13x + 4.55	2.48 (1.57, 3.92)[ab]	0.876	1.262
	30	Y=1.63x + 4.55	1.89 (1.33, 2.68)[a]	0.898	1.197

[1]Values followed by the same letter are not significantly different (P>0.05) from each other based on Tukey-Kramer Multiple Comparisons test

The shortest lethal time for 50% mortality occurred with Macoya isolate at 20^{0}C (LT_{50} = 1.61 days) which also had the fastest germination time, but was not significantly different (P>0.05) from either the Aranguez isolate at 25^{0}C or the Macoya isolate at 25^{0}C (Table 2). Thungrabeab *et al.* (2006) determined the efficacy of five entomopathogenic fungi (two isolates of *Beauveria bassiana* (Bb 4591 and Bb 5335), two isolates of *Metarhizium anisopliae* var. *anisopliae* (Ma 6079 and Ma 7965) as well as one isolate of *Paecilomyces fumosoroseus* (Pfu 5338) against *Thrips tabaci* and concluded that efficacy varied depending on the *temperature* and the fungal isolate. This is supported in the present study as the LC_{50} for *P. fumoso-roseus* generally increased with increasing temperature in both the Aranguez and Macoya isolates. A similar relationship was also found by Yeo *et al* (2003) in their study of fungal isolates against aphids. The range of effective concentrations obtained in the present study was also similar to those obtained by Wu *et al* (2007) for *B. tabaci*. In their study they determined that the most effective concentrations for *B. tabaci* control were in the range 10^5 to 10^8 spores /mL. Both isolates appear to be narrowly thermo-tolerant with optimum growth and pathogenic abilities occurring between 20 - 25^{0}C and can thus be recommended for use in management of *B. tabaci* at cooler locations in the country.

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