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Study on *Nattrassia mangiferae*, the Causal Agent of Apricot Tree Decline Disease in the Oases of South Tunisia: Biology and *in Vitro* Evaluation of Some Fungicides

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ABSTRACT

Nattrassia mangiferae (H. & P. Syd.) B. Sutton & Dyko is a wood fungus causing serious apricot decline in the oases of the South of Tunisia. In the literature there is relatively little information on the biology of *N. mangiferae*. The objective of this work was to determine the effects of temperature, pH, culture media and some fungicides on the radial growth of *N. mangiferae* on culture media, and the effect of humidity on conidial germination. *N. mangiferae* was able to grow on temperatures ranging from 20-40°C with an optimum between 30-35°C. Mycelial growth was best at pH 6. The best medium for mycelial growth was potato dextrose agar (PDA). Maximum conidial germination occurred at relative humidity higher than 90%. The systemic fungicide Benomyl (Benlate) was less effective than the non-systemic fungicides: Mancozeb (Dithane M45) and Maneb (Manèbe 80).

Keywords: chemical control, fungus, mycelial growth, *Prunus armeniaca* L., spore germination

INTRODUCTION

Apricot (*Prunus armeniaca* L.) tree decline disease incited by *Nattrassia mangiferae* was observed during the 1990s in the oases of Gafsa where it has caused the death of more than 12,500 trees. The disease has increased in importance in recent years and it is now present in all oases of the South-west of Tunisia. The fungus also causes serious diseases on several other fruit species such as plum (*Prunus domestica*), peach (*Prunus persica*), apple (*Malus domestica*), pear (*Pyrus communis* L.), jujube tree (*Zizyphus vulgaris* Lamk.), and even Russian olive (*Elaeagnus angustifolia* L.) (Namsi 2001).

This disease attacks both young and adult trees and it spreads quite fast in the field. The causal agent, *N. mangiferae* attacks the cambium and spreads downward, causing wilt, dieback, cankering or decline depending on the tree species involved. *N. mangiferae* was first described by Nattrass on apricot, plum and apple (Sutton and Dyko 1989), It was also reported from many tropical and subtropical countries (Sutton and Dyko 1989). It has a wide host range such as almond (English *et al.* 1974), vine (Granata *et al*. 1993), mango (Pandey *et al*. 1981; Lonsdale 1992), eucalyptus (Jamaluddin *et al*. 1987), cassava (Msikita *et al*. 1997) and American tree (*Arbutus menziesii*) (Elliot *et al*. 1997). The objective of the present study was to investigate the characteristics of *N. mangiferae in vitro* media culture to measure the effects of different parameters on the growth rates of *N. mangiferae*.

MATERIALS AND METHODS

Fungi and inoculation of plates

N. mangiferae was isolated from an infected branch of apricot tree in the oasis of Tozeur. Identification was confirmed by CABI (Ref 383 623). The fungus was maintained on PDA media at 27°C. For all experiments, mycelium plugs of 7 mm diam were taken from the growing margin of the colony of *N. mangiferae*. Plugs were placed in the centre of each of 90-mm Petri dish containing medium. Five replicates were used for each treatment.

Biology of *N. mangiferae*

1. Mycelial growth

Growth on different media. Mycelial growth was evaluated on 7 different media (Russel 1974): potato dextrose agar (PDA), potato sucrose agar (PSA), malt extract 2% (MEA), peptone (PA), carrot (CA), potato-carrot (PC) and Czapeck medium (CzA). These media were sterilized at 120°C for 30 min. The cultures were incubated at 27°C in the dark. The colony diameter on each dish was measured at 24, 48, 72 and 96 h after inoculation.

Effect of temperature. Effect of temperature on mycelial growth was evaluated on PDA media. Inoculated plates were sealed and incubated at 15, 20, 25, 30, 35, 40 and 45°C. There were five replicate dishes for each temperature. Colony diameters were measured after 24, 48 and 72 h of incubation.

Effect of pH. To determine the effect of pH on the colony growth of *N. mangiferae*, PDA medium was amended with HCl or NaOH to obtain a pH ranging from 5-9 before sterilization. Dishes were inoculated as described above and cultures were incubated at 35°C. Colony diameter were measured at 24 and 48 h after inoculation.

2. Spore germination

Effect of relative humidity (RH) on spore germination. The influence of moisture on spore germination was studied using a humidity chamber containing salt solutions (Trigui 1991). The humidity chamber consisted of a glass jar (1000 ml capacity, diameter 90 mm) which was capable of being tightly sealed with a metal closure. A glass cylindrical stand (45 mm in length, 80 mm diameter) was positioned upright within the chamber. This served to support glass slide. Saturated solutions of different salts were

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Research Note

Table 1 List of examined fungicides.

Fungicide name	Group of fungicide	Active ingredient	Range of effectiveness	Recommended comercial dose
Dithane M45	Dithio-carbamate	80% Mancozeb	Contact	2.5 g/l
Manèbe 80	Dithio-carbamate	80% Maneb	Contact	3 g/l
Benlate	Carbamate	50% Benomyl	Systemic	0.6 g/l

prepared the following RHs at 25°C: $SrCl_2$, 70%; NaCl, 75%; $(NH_4)_2SO_4$, 80; KCl, 85%; $BaCl_2$, 90 and H_2O, 100% (Multon *et al*. 1991). Saturated solutions of different salts were placed in jars. Each chamber was filled with 50 ml of the appropriate regulating solution.

Conidia for germination studies were obtained from 14-days-old cultures growing on PDA at 25°C. Conidia and chlamydospores were harvested by scraping the surface of the media with an inoculating loop to release the conidia from the hyphae in 50 ml of sterilized, distilled water and added to a 50 µl drop taken from conidial suspensions (2×10^5 ml^{-1}), and applied and spread on each slide. The number of conidia within each suspension was assessed using a Malassez cell. After receiving a drop of conidia the slides were air dried for 3 h in a hood and then transferred to humidity chambers containing salt solutions. All three glass slides were placed over a glass cylindrical stand. Humidity chambers were incubated at 25°C. The assessment of germination was made after 24 and 48 h of incubation. Conidia or chlamydospores were counted as germinated when germtube length equalled the width. A minimum of 200 spores (conidia and chlamydospores) were counted.

In vitro fungicide bioassay against *N. mangiferae*

Three fungicides were employed in this study. They were used at low concentrations to allow fungal growth in order to determine the lower bound of concentration which reduced or inhibited fungal growth. These are all available as commercial products: Dithane M45 (ROHM and HAAS®), Manèbe 80 (ROHM and HAAS®), Benlate (DuPont®).

The characteristics of fungicides tested are listed in **Table 1**. In order to evaluate the fungicidal effects of these 3 fungicides, 6 concentrations were tested for each: Benomyl (Benlate) at 0, 3, 30, 60, 150 and 300 $mg.L^{-1}$; Maneb (Maneb80) at 0, 24, 240, 480, 1200, 2400 $mg.L^{-1}$ and Moncozeb (Dithan M45) at 0, 20, 200, 400, 1000, 2000 $mg.L^{-1}$.

To determine the effect of each fungicide on mycelial growth, 7-cm plugs taken from the edges of actively growing PDA cultures were inverted in the centers of PDA plates amended with tested fungicides. The original concentrated fungicide was dissolved in sterile distilled water to give a stock solution. Fungicide concentrations were prepared by adding a measured quantity of stock solution to cooled (40-45°C), autoclaved (at 120°C for 30 min) PDA medium to give the desired concentration of active ingredients. Controls were represented by non-amended media.

Each treatment was replicated 5 times. The plates were incubated at 35 ± 1°C. The diameter of mycelium growth was recorded after 24, 48, 72 and 96 h of incubation.

Percent inhibition of *N. mangiferae* mycelium growth was calculated by using the following formula (Hmouni *et al*. 1996):

$$I (\%) = (1- Cn/Co) \times 100$$

where Cn = average diameter colony growth in control and Co = average diameter colony growth in treatment.

Statistical analysis

All experiments were conducted twice. Data were analysed using analysis of variance with M-STAT software. Treatment means were separated using the Neuman-Keuls test at $P = 0.05$. For comparison, only the original data of treatment means are presented.

RESULTS AND DISCUSSION

Biology of *N. mangiferae*

1. Growth on different media

The radial growth of *N. mangiferae* mycelia was significantly affected by culture medium (**Table 2**). In general, PDA and Czapek medium were most favorable for radial growth of mycelium. Colony on PDA reached the edge of the plates after 72 h, which indicates that PDA was the best growth medium for *N. mangiferae.* The poorest growth was recorded on CPA medium, where the fungus had very limited radial growth (38 mm).

Table 2 Mycelial growth of *Nattrassia mangiferae* on seven media at 35°C in the dark after 72 hr of incubation.

Media[b]	Colony diameter (cm) [a]
PDA	8.30 a
CzA	7.80 a
SPA	5.80 c
CA	6.80 b
CPA	3.80 e
PA	5.18 d
MEA	4.68 d

[a] Measured colony diameter - mycelial plug 7 mm diameter
[b] PDA, potato dextrose agar; Cz, Czapek ; SPA, sacharose potato agar; CA, carrot agar; CPA, carrot potato agar; P, peptone; MEA, malt extract agar (2%).

2. Effect of temperature

In general, mycelial growth in response to temperature was quite similar for the different periods of incubation (**Fig. 1**). Mycelial growth was observed at temperatures ranging from 20 to 40°C. Radial growth rate increased with temperature up to 30°C, and then decreased as temperature increased. Optimal radial growth occurred between 30 and 35°C for all incubation periods. At 35°C, colonies on PDA reached the edge of the plates after 48 h and the maximum growth was at 35°C. In general, mycelial growth was reduced when temperature was < 30°C or > 35°.

These results differ from those of Davison (1972) who reported that optimum growth of *N. mangiferae* on culture medium was at 25°C. According to Pandey *et al*. (1981), in natural conditions, the development of the fungus is optimal at a temperature ranging from 30 to 35°C.

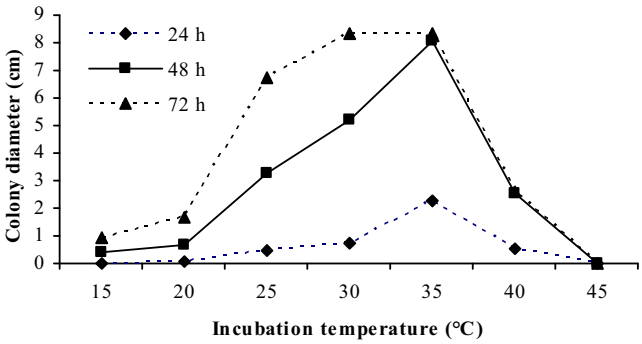

Fig. 1 Effect of temperature on radial mycelial growth of *Nattrassia mangiferae*.

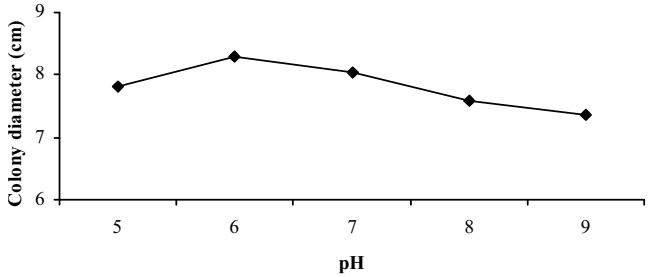

Fig. 2 Effect of pH on radial mycelial growth of *Nattrassia mangiferae*.

Table 3 Effect of relative humidity on *Nattrassia mangiferae* spore germination after 24 h incubation at 25°C.

RH (%)	Percent spore germination [a]	
	Conidia	Chlamydospores
70	22.33 a	16.00 a
80	24.33a	20.33 a
85	33.00 a	23.00 a
90	35.33 b	31.66 b
100	54.33 c	64.33 c

[a] Each value is the average of three replicates of 200 spores each.

2. Effect of pH

N. mangiferae grows on wide pH range (5-9). The optimum pH was 6 with an average colony diameter of 5.7-6.2 cm diameter at 48 h after inoculation. A notable decline of growth occurred when pH increased from 6 to 9 (**Fig. 2**).

4. Effect of RH on spore germination

At 25°C, the germination of conidia increased with RH. Optimum germination was at a RH > 85% (**Table 3**). The same results were obtained for the germination of chlamydospores (**Table 3**). These results confirm the work of Pandey *et al*. (1981) who reported that development of *N. mangiferae* is favored by high RH (70-90%).

In vitro evaluation of fungicides against *N. mangiferae*

All fungicides inhibited the linear growth of mycelium to different degrees. Results in **Table 4** indicate that all concentrations of tested fungicides against the linear growth of *N. mangiferae* had an inhibitory effect on mycelial growth. Their effects increased with increasing fungicide concentrations. Benomyl at 300 mg.L^{-1} resulted in a high reduction (96.96%). Maneb and Mancozeb at 240 and 200 mg.L^{-1}, respectively resulted in complete reduction. Maneb and Dithan were equally effective at 2400 and 2000 mg.L^{-1} respectively, were used (i.e. the recommended concentrations). Benomyl limited mycelial growth and conidia formation of *N. mangiferae*.

Table 4 Percentage of inhibition of mycelial growth of *Nattrassia mangiferae* on potato dextrose agar media amended with different concentrations of Benomyl, Maneb and Mancozeb.

	Ingredient active in medium (mg.L^{-1})	Inhibition (%)
Benomyl	300	96.96
	150	95.57
	60	93.64
	30	92.25
	3	1.80
Maneb	2400	100.0
	1200	100.0
	480	100.0
	240	100.0
	24	45.2
Mancozeb	2000	100.0
	1000	100.0
	400	100.0
	200	100.0
	20	63.8
Control	0	0

REFERENCES

Davison AD (1972) Factors affecting development of Madrone canker. *Plant Diseases Report* **56**, 50-52

Elliott M, Edmonds RL, Tuff LJ (1997) Role of stress in the development of *Arbutus* canker (*Nattrassia mangiferae*). *Phytopathology* **87 (27)** Publication no. P-1997-0192-AMA

English H, Davis JR, Devay JE (1974) Relationship of *Botryosphaeria dothidea* and *Hendersonula toruloidea* to a canker disease of almond. *Phytopathology* **65**, 114-122

Ennamsi A, Zouba A, Triki MA (2003) Dépérissement de l'abricotier dans les oasis tunisiennes. *Phytoma* **562**, 18-21

Namsi A (2001) Etiologie et bio-écologie de la maladie du dépérissement de l'abricotier (*Prunus armeniaca* L.) dans les oasis du sud-ouest tunisien. MSc, Ecole Supérieure d'Horticulture et d'Elevage de chott-mariam, Tunisie, 53 pp

Granata G, Sidoti A (1993) Nuova grave malattia della vite causata da *Nattrassia toruloidea*. *Informatore Fitopatologica* **44**, 4-6

Hmouni A, Hajlaoui MR, Mlaiki A (1996) Resistance de *Botrytis cinerea* aux benzimidazoles et aux dicarboximides dans les cultures abritées de tomate en Tunisie. *Bulletin OEPP* **26**, 697-705

Jamaluddin K, Soni K, Dadwal VS (1987) Some noteworthy diseases of *Eucalyptus* in Madhay Pradesh. *Indian Journal of Forestry* **10**, 55-57

Lonsdale JH (1992) Etiology and control of some mango blossom diseases in Transvaal. MSc thesis, University of Pretoria, South Africa, 103 pp

Msikita W, Yaninek JS, Abounou M, Baimey H, Fagbemissi R (1997) First report of *Nattrassia mangiferae* rot and stem rot of cassava in West Africa. *Plant Disease* **81**, 1332

Multon JL, Bizot H, Martin G (1991) Techniques d'analyse et de contrôle dans les industries agroalimentaires. In: *Analyse des Constituants Alimentaires (Vol IV) Mesure de l'eau Adsorbée dans les Aliments*, Lavoisier-TEC & DOC, France, pp 1-60

Pandey RS, Bhargava SN, Shukla DN, Khati DVS (1981) A new leaf spot disease of mango. *Plant Disease* **65**, 441-442

Russel BS (1974) *Mycology Guidebook*, Mycology Guidebook Committee, Mycological Society of America, University of Washington Press, Seattle and London, pp 651-692

Sutton B, Dyko BJ (1989) Revision of *Hendersonula*. *Mycological Research* **93**, 466-488

Trigui A (1991) Recherches sur la septoriose du pistachier causée par *Septoria pistacina* Allesh. *Annales INRAT* **64 (6)**, 32 pp