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Technique for isolation of *Pythium aphanidermatum* from soil and laboratory evaluation of fungitoxicants against it

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A pointed gourd fruit is transversely cut towards any one end and such fruit was buried in moist field soil. *Pythium aphanidermatum* colonised at the cut end of the fruit. Portion of the colonized tissue was then put in water in half-submerged condition to induce mycelial growth and sporangia production in water. A bit of mycelial growth from water was used for artificial inoculation of pointed gourd/ridge gourd fruit. Pure culture of *P. aphanidermatum* was obtained when artificially inoculated diseased tissue was used for isolation in carrot extract agar medium containing carbendaxim (50 ppm) and chloramphenicol (100 ppm). Fungitoxicant was evaluated by putting colonized pointed gourd/ridge gourd tissue in aqueous solution/suspension of toxicant and observing the extent of mycelial growth and sporangia production. Copper oxychloride, Mancozeb, Thiram, Hexaconazole, Propiconazole and Ediphenphos appeared to be good inhibitor for this fungus.

Key words : *Pythium aphanidermatum,* isolation techuique, soil

INTRODUCTION

Pythium aphanidermatum, causes damping off disease (Mahmud, 1952a ; Raghunathan, 1968), root rot (Mahmud, 1952b ; Ranghunathan and Marimuthu, 1973), foot rot (Singh, 1998), fruit rot (Sharma and Asthana, 1958 ; Verma and Sengupta, 1981), rhizome rot (Kannan and Nair, 1965), and storage rot (Rao, 1966). Usually the fungus survives in soil in absence of suitable environment and host for infection. As the fungus causes various types diseases of different plant species, isolation of the fungus from diseased tissue and detection of fungus from soil becomes an important aspect for development of suitable management strategies. Selective media developed earlier for isolation of *Phytophthora* and *Pythium* sp. are costly (Taso and Ocana, 1969). In the present study attempts have been made to develop suitable techniques and cheap medium for isolation of *Pythium aphanidermatum* from soil. Method for evaluation of fungicides against this fungus was standardized.

MATERIALS AND METHODS

Methods followed in isolation *P. aphanidermatum* from soil in sequentially are as follows : Pointed gourd fruit used as bait to stimulate growth of *P. aphanidermatum* present in soil. A pointed gourd fruit is transversely cut towards any one end and such fruit is bruied in moist soil, 5-8 cm below the soil surface. The soil around the fruit is kept moist by addition of water, if necessary. The fruit undergoes partial rotting towards the cut end of the fruit within 3-5 days. If *Pythum* is present in soil around the fruit tissue, it colonizes on the cut surface, developing a thin white mycelial growth.

Colonized fruit is removed from the soil, gently washed with water to remove soil particle. Portion of colonized tissue is put in water in half-submerged condition in petridish. In next 48 hrs *Pythium* grows in water beyond the area of fruit tissue and produces hyphae and lobbed sporangium. The growth of fungus in water is confirmed through microscopic examination (the petriplate with the fruit tissue and mycelial growth is directly used for microscopic examination).

A bit of mycelial growth from water is used for inoculation of pointed gourd/ridge gourd fruit in laboratory. Pointed gourd/ridge gourd fruits were col-

lected directly from field or market, washed in running water and then surface sterilized by rubbing with cotton soaked in alcohol. Injury of 0.5 cm length and 2 mm depth was made on each fruit with the help of sterilized needle. A bit of mycelial growth from water was put into tissue of the fruit and covered with sterilized moist cotton. The inoculated fruits were kept for incubation in humid condition at 28+1^{0}C. In subsequent 3-5 days the fungus grows well over the fruit. Colonized fruit tissue is used for isolation of *Pythium* sp. in agar medium.

Selected portion of colonized fruit tissue (artificially inoculated) is surface-sterilized using 0.1% mercuric chloride, washed in sterile water and then put in agar slant containing carrot extract agar medium with chloramphenicol (100-150 ppm) and carbendazim (25-50 ppm). *Pythium* grows well in this medium.

In aqueous condition, this fungus grows well, produces abundant sporangia and the sporangia liberates zoospores. Taking the advantage of this property bioassay of fungicide has been standardized. Artificially inoculated pointed gourd/ridge gourd fruit with good mycelial growth was used as inoculum. Fungicide solutions/suspensions of different concentrations were separately taken in petridishes and small pieces of infected fruit tissue were put in petridish in half-submerged condition. Suitable control was maintained using water only. The plates were kept in room temperature or in BOD incubator at 28 + 1°C for 48 h The plates were observed under microscope to record the extent of mycelial growth in water and fungicide solution/suspension. Simultaneously extent of sporangia formation and germination of sporangia were recorded.

RESULTS AND DISCUSSION

Following procedures described above, *Pythium aphanidermaium* was successfully isolated from

Table 1 : Effect of fungicides on *P. aphanidermatum* in aqueous environment.

Fungicide	Stages of fungal growth	Concentrations of fungicides (g or ml/liter) 1.0	0.5	0.25	0.125
Blitox 50w (Copper oxychloride 50%, WP)	Mycelial growth	No hyphal growth	Poor deformed hyphae	Profuse hyphal growth	Profuse hyphal growth
	Sporangia formation	Nil	Absent	Few sporangia	Few sparangia
	Sporangia formation	Nil	Nil	Absent	Absent
Dithane M-45 (Mancozel 75%, WP)	Mycelial growth	No hyphal growth	No hyphal growth	No hyphal growth	Very poor hyphal growth
	Sporangia formation	Nil	Nil	Nil	Nil
	Sporangial germination	Nil	Nil	Nil	Nil
Thiram (Thiram 75% WS)	Mycelial growth	No hyphal growth	No hyphal growth	Poor growth	Huge hyphal growth
	Sporangia formation	Nil	Nil	Absent	Absent
	Sporangial germination	Nil	Nil	Nil	Nil
Hinosan (Ediphenphos 50%, EC)	Mycelial growth	No hyphal growth	No hyphal growth	No hyphal growth	No hyphal growth
	Sporangia formation	Nil	Nil	Nil	Nil
	Sporangial germination	Nil	Nil	Nil	Nil
Calixin (Tridemorph 80% EC)	Mycelial growth	Very poor, short eformed hypha	Poor deformed hypha	Very poor, short deformed hypha	Huge hyphal growth
	Sporangia formation	Absent	Absent	Absent	Absent
	Sporangial germination	Nil	Nil	Nil	Nil
Bavistin (Carbendazim 50%, WP)	Mycelial growth	Hyphal growth present	Huge hyphal growth	Huge hyphal growth	Huge hyphal growth
	Sporangia formation	Sporangia present	Abundant sporangia	Abundant sporangia	Abundant sporangia
	Sporangial germination	Absent	Present	Present	Present
Contaf (Hexaconazole 5%, EC)	Mycelial growth	No hyphal growth	No hyphal growth	No hyphal growth	No hyphal growth
	Sporangia formation	Nil	Nil	Nil	Nil
	Sporangial germination	Nil	Nil	Nil	Nil
Tilt (Propiconazole 25%, EC)	Mycelial growth	No hyphal growth	No hyphal growth	No hyphal growth	Very poor hyphal growth
	Sporangia formation	Nil	Nil	Nil	Absent
	Sporangial germination	Nil	Nil	Nil	Nil
Control	Mycelial growth	Huge hyphal growth			
	Sporangia formation	Many sporangia present			
	Sporangial germination	Many sporangia release zoospores			

soil of infested field of ginger, papaya and pointed gourd. The fungus thus isolated from ginger, papaya and pointed gourd field was found to cause rhizome rot of ginger, foot rot of papaya and fruit rot of pointed gourd respectively, on artificial inoculation.

For isolation of the fungus from naturally diseased tissue, a piece of infected tissue was put in water to stimulate mycelial growth and formation of sporangia in water. After confirmation of the identity of the fungus, the mycelial bit was used for inoculation in pointed gourd or ridge gourd fruits. Further step would be followed as described earlier.

The fungus, *Pythium aphanidermatum* was maintained in carrot extract agar medium (Carrot 200 g, Agar agar 20 g and Water 1000 ml). This fungus grew well and produced abundant sporangia in this medium. It also produced oospores later on the same medium.

Among the eight fungicides tested (Table 1) against *P. aphanidermatum* in aqueous condition, copper oxychloride, mancozeb, thiram, haxaconazole, propiconazole and ediphenphos inhibited hyphal growth, sporangia formation upto concentration of 0.25 g/ml per liter. Tridemorph had adverse effect on hyphal growth upto concentration of 0.25 ml per liter. Carbendazim had no effect on hyphal growth and sporangia formation. This is an easy method for evaluation of fungicide compare to poison food technique. It also provides information about effect of fungicide on the hyphal growth, sporangia formation and germination of the sporangia simultaneously.

REFERENCES

Kannan, K. and Nair, K.P.V. (1965). *Zingiber officinale* (ginger) in Kerala *Madras Agric.* J. **52** : 168-176.

Mahmud, K. A. (1952a). Root rot of maize by *Pythium aphanidermatum* (Eds) Fitz. *Sci. & Cult.* **17** : 339.

Mahmud, K. A. (1952b). *Pythium* damping off of brinjal seedling. *Sci. & Cult.* **18** : 149-150.

Raghunathan, V. (1968). Damping off of green gram, cauliflower, daincha, ragi and clusterbean. *Indian Phytopathology.* **21** : 456-457.

Ranganathan, K. and Marimuthu, T. (1973). A new root rot of safflower in India, *Sci. & Cult.* **39** : 354-355.

Rao, V.G. (1966). An account of the market and storage diseases of fruits and vegetables in Bombay. Maharastra, *Mycopath. et Mycol. Appl.* **28** : 165-176.

Sharma, O. P. and Asthana R. P. (1958). Fruit rot disease of *Momordica dioica* Roxb. due to *Pythium aphanidermatum* (Eds.) Fitz. *Sci. & Cult.* **23** : 435.

Singh, R. S. (1998). Foot fot of papaya. In *Plant Diseases*, Oxford and IBH Publishing Co. Pvt. Ltd. pp 179-181.

Taso, P. H. and Ocana, G. (1969). Selective isolation of species of *Phytophthora* from natural soil on an improved antibiotic medium. *Nature* **223** : 636-638.

Verma. R. N. and Sengupta, T. K. (1981). A new *Phythium* fruit rot of bhat karela. *Indian Phytopathology* **34** : 518.

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