Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(4): 12-13
Received: 08-05-2018
Accepted: 13-06-2018

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Isolation of pythium species from damping off affected onion rhizospheric soil, using baiting technique

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Abstract
Moist rhizospheric soil samples were collected from several spots within the same field of damping off affected onion crop field, grown in Udaipur district of Rajasthan. French bean and bottle gourd were used as bait. French bean was transversely cut into 2-3 pieces and bottle gourd was cut into several slices and buried well into the infested soil at a depth of 4-5 cm. The soil was slightly moistened by sprinkling small quantity of water. Clear fungal mycelium growth appeared after 24 hour on the bait was aseptically transferred to potato dextrose agar (PDA) media and pure cultures were prepared by using hyphal tip method.

Keywords: rhizospheric, pythium, bait and mycelia

Introduction
Pythium genus belongs to the family Pythiaceae and order Pythiales (Kirk *et al*., 2008) [1]. The genus pythium exhibits diverse and omnipresent species in it, of which many are crucial soil borne plant pathogens causing diseases of plants in field, forestry and horticultural crops. Pythium genus consists of above 200 described species depicting a wide host range and dwell in a diverse terrestrial and aquatic environment (Dick, 2001) [2]. Plant pathogenic pythium species badly affects economically important agricultural and horticultural crops and cause significant yield reduction. Pythium species are known for causing pre - and post -emergence damping -off, leading to poor crop stands and reduction in crop vigour. Pythium species can infect mature plants to induce significant yield reduction. Pythium species are in most active state, in the fields bearing high soil moisture greater than field capacity. Severity, incidence and ability of the damping -off pathogen to initiate infection were positively correlated with soil moisture for majority of pythium species (Cook, 2002) [3]. Traditionally pythium species identification has been done on the basis of morphological features. Morphology of sporangia, growth habit, homothallism vs. heterothallism, antheridia and oogonia, type of spores and size of oospores, and rate of growth on culture media are the key measures used to differentiate among pythium species (Postma *et al*., 2009 and Owen-Going *et al*., 2008) [4, 5].

Materials and Methods
Baiting method for Pythium species is carried out as a preliminary step for the isolation and identification of the pathogen for the further study. Baiting method of isolation was carried out based on Saha, *et al*., 2002 [6]. Bottle gourd and french bean were used as bait to stimulate growth of the pathogen. To prevent contamination from bacteria and other fungi and to make the fruits succulent, the fruits were dipped in a solution for 12 hrs, containing streptomycin (100 ppm) and carbendazim (500 ppm) in it. Treated fruit were cut transversely into 2-3 pieces and buried well into the infested soil sample at a depth of 4-5 cm. The soil was slightly moistened by sprinkling small quantity of water. With the minimum disturbance to the invaded pathogen, the bait fruits were carefully removed from the soil. French bean and bottle gourd fruits were separated and were kept inside air filled plastic bag and sterile petriplate under aseptic condition and kept at room temperature for 24 hours. Moistened cotton plug was kept inside the petriplates to provide humidity inside the petriplates. White cottony fungal growth appeared after 24 hour on the baits were aseptically transferred to potato dextrose agar (PDA) media and pure cultures were prepared by using hyphal tip method. The mycelial growths of fungus grown on both the baits were observed under microscope and they resembled each other. The culture was purified and maintained for further studies. The microscopic examination revealed the species as pythium.

Result and Discussion

Isolation of pythium species from the rhizospheric soils of damping of infected onion field was carried out by using bottle gourd and french bean as bait. Since the bait were pre treated with streptomycin (100 ppm) and carbendazim (500 ppm), on the same line as suggested by Saha, *et al*., 2002 [6]. Within 48 hours the bait were covered with the fluffy mycelium, free from any bacterial and fungal contamination, was pure cultured using hyphal tip method several time on Potato dextrose agar (PDA) plates and slants. Sinobas *et al*., 1999 [7] and Chamswarng, *et al*., 1991 [8] reported the baiting technique for the isolation of pythium species using carnation petals and cucumber seeds, pointed gourd and bottle gourd.

Fig 1: A. Bottle gourd and french bean with adhering infected soil, B. French bean and bottle guard kept in air filled plastic bag and petri plate with moist cotton plug, C. Mycelial growth on bottle gourd, D. Pythium species pure cultured on PDA media

Conclusion

Pythium is an important plant pathogenic fungi causes damping of vegetables, fruits and field crops drastically affecting the food production worldwide. It has ubiquitous distribution in soil. Baiting technique using bottle gourd and french bean is the simple, timely and most effective method of isolating the pythium species from soil.

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