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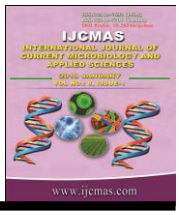

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Standardization of Isolation Methodology for Early Detection and Estimation of Major Hot Spots of *Dematophora necatrix*, Causing White Root Rot of Apple in Himachal Pradesh

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A B S T R A C T

White root rot caused by *Dematophora necatrix* is one of the most destructive soil borne diseases of apple in Himachal Pradesh. To identify the major hot spots of white root rot and to standardize the methodology for isolating *D. necatrix*, a survey was undertaken in three major apple growing districts viz., Kullu, Shimla and Kinnaur of Himachal Pradesh. Soil samples were collected from three most infected areas of each district. Three baits viz., kiwi leaf discs, apple leaf discs and avocado leaf discs were used separately to modify this technique followed by microscopic examination of infected baits. The results showed that in all infected soil samples collected from most infected sites of three districts, baits of kiwi leaf discs exhibited maximum infection (19.44%) of *D. necatrix* followed by avocado (18.89%) and apple leaf discs (8.89%). On the other hand, maximum infection of baits was observed in Ghoond area (30.00%) of district Shimla by using avocado leaf discs followed by Bhalyani area (28.33%) of district Kullu by using baits of kiwi. Whereas, minimum bait infection was recorded in Sangla area (5.00%) of district Kinnaur with apple leaf discs. Overall, the maximum mean infection (21.67%) was observed in Kilba area of district Kinnaur followed by Ghoond (20.56%) area of district Shimla and Bhalyani (19.44%) area of district Kullu. However, least mean bait infection (11.11%) was recorded in Sangla area of district Kinnaur. Therefore, keeping in view the importance of this disease, present investigation was carried out with the objective to standardize the isolation technique for quick detection and to find out the major hot spots infested by this disease.

Introduction

White root rot disease caused by *Dematophora necatrix* Hartig [*Rosellinia necatrix* (Hartig) Berl.] is one of the most destructive diseases affecting apple orchards in temperateand subtropical regions of the world. In India, the fungus has been considered as the major constraints causing extensive losses in apple production especially in Himachal Pradesh, as the state is well recognized as the apple state of country. The pathogen is a typical soil-inhabiting fungus, survive dormant in soil during unfavourable condition and cause severe losses in nurseries as well in orchards. The disease was first

recorded in 1883 from Germany (Hartig, 1883) and 1891 from France on grapes (Viala, 1891). On apple, the first occurrence of this devastating disease was observed in 1900 from Norwich, and subsequently in Canterberry by Salmon and Wormald (1913). *R. necatrix*, the perfect stage of fungus was identified as the causal organism leading to the death of apple trees in a garden at Winscombie, Sommerset (Nattrass, 1927). In India, the disease was first reported from Uttarakhand hills by Singh in 1929 (Bose and Sindhan, 1976). Agarwala (1961) observed its occurrence in apple orchard of Himachal Pradesh in a very high proportion causing severe and ultimately death of grown up plants.

The teleomorphic stage of the fungus is not known to occur in India till date. The fungus has a wide host range found associated to about 158 plant species belonging to over 45 families (Ito and Nakamura, 1984) comprising of fruit plants, forest trees and vegetable and field crops. The pathogen survives in the form of mycelium or sclerotia in the infected roots. The infection of new roots takes place by the fungal mycelium present in the sol on debris or by the contact of new plant roots with the old dead roots. The disease is more serious in water logged acidic soils. The pathogen mainly attacks the underground part of the trees. The lateral roots turn dark brown and are covered with greenish gray or white mycelial mat and with the progress of disease all the roots are attacked and fibrous root system disappears. Whitish mycelial mat like fungal growth is visible during monsoon on the affected parts. The affected plants show bronzing of the leaves and progressive decline and ultimately die within 2-3 years of infection. Management of root rot is often considered tedious because of deep seated infection. It is very difficult to make the reach of applied remedial measures up to the point of infection. Therefore, keeping in view the importance of this devastating disease on apple cultivation, the present study was conducted with the objective to standardize the technique which could help out in early detection of pathogen and also to find out the major hot spots of root rot in Himachal Pradesh.

Materials and Methods

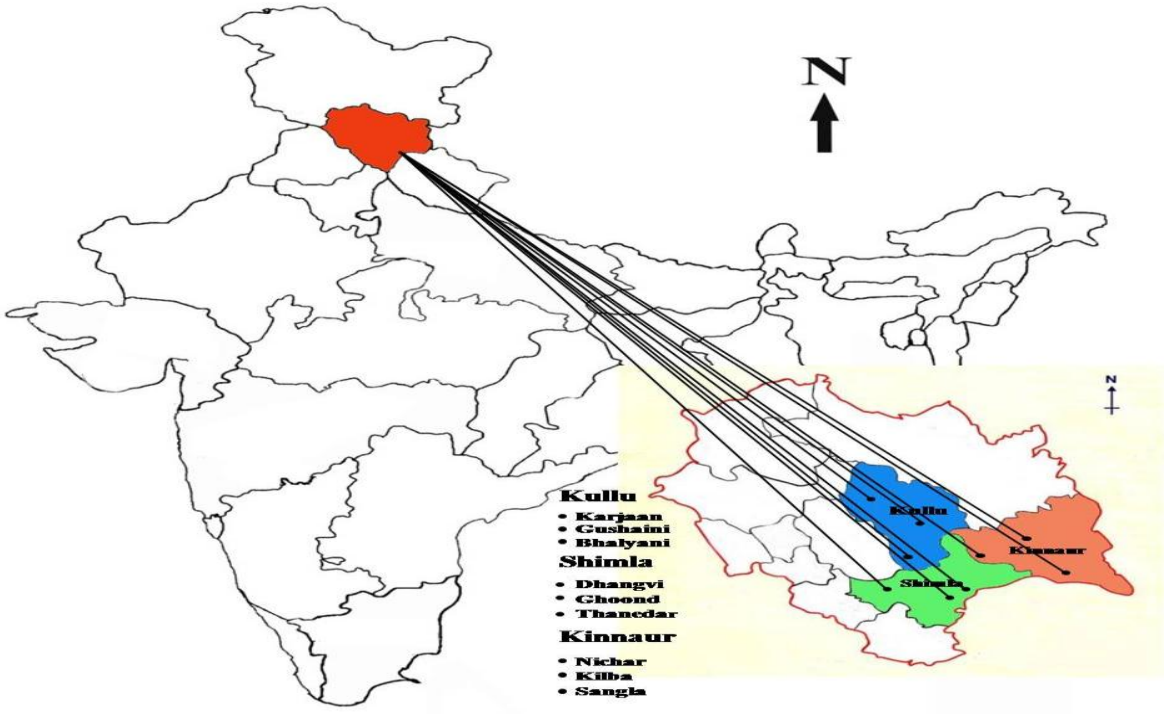
Undoubtedly, many researchers had developed several techniques over the period of time for estimation of *R. necatrix* population keeping in view the importance of pathogen. Notably, advancement in molecular techniques alleviated some of the issues associated with the detection and estimation. Although, these modern techniques seems to be rapid and highly sensitive in detecting *R. necatrix* by employing real time scorpion-PCR, but are too expensive. However, the development of a quantitative detection method will facilitate studies to determine inoculum threshold levels, and to ascertain some still unclear epidemiological aspects that are necessary for the development of white root rot disease due to non-sporulating nature of the fungus *D. necatrix* as compared to its teleomorph *R. necatrix* (Schena and Ippolito, 2003).

For assessment of *R. necatrix*, trapping technique using avocado leaf discs was described earlier by Sztejnberg *et al.,* (1987) but has proven laborious, time consuming and non realible due to its inconsistency in the effective and quick isolation of pathogen from soil. These constraints have encouraged the search for alternative approaches. Therefore, technique was further modified and standardized for ease in estimation of *D. necatrix* causing root rot of apple. The baiting step of existing technique was replaced with adding up different baits followed by microscopic examination. Each treatment was replicated thrice in completely randomized design (CRD). Different steps for estimation

of *D. necatrix* population from soil are described as below:

Soil collection

To know the prevalence of white root disease a survey was conducted in three major apple growing district viz., Kullu, Shimla and Kinnaur of Himachal Pradesh. After conducting survey, samples of soils near infected tree basin of depth 20-30 cm were collected in polypropylene bags from three most infected locations in each district.



Soil sieving: Partially dried soil was sieved by passing through 2 to 2.5 mm sieve.

Air drying: Sieved soil samples were air dried in shade at room temperature for 24 hours on sterilized filter paper.

Mix plating: The soil samples were thoroughly mixed, of which 200 g of soil was taken out and added to the 20 cm diameter glass Petri plate.

Baiting: Petri plates were then embedded with twenty baits such as avocado leaf discs, kiwi leaf discs and apple leaf discs (1.5 cm each) separately.

These leaf discs were placed in close contact with the infected soil and plates were then covered immediately.

Moistening: Soil in Petri plates was moistened with sterile distilled water using atomizer. The plates were further moistened at three days interval or depending upon moisture level of soil.

Incubation: Covered plates were then incubated at room temperature (20-25 ^{o}C) for 15 days to allow the pathogen to infect and grow on the leaf discs. The incubated plates were kept as such until more or less whitish layer of mycelium appeared on respective leaf discs.

Microscopic examination: Finally, mycelium from infected or colonized leaf discs were taken out by scratching with sterilized needle and placed on clean glass slide for microscopic examination.

Per cent infection of baits was calculated as given below:

$$\text{Infection of baits (\%)} = \frac{\text{Number of infected baits}}{\text{Total number of baits}} \times 100$$

Results and Discussion

In the present investigation, attempts were made to standardize isolation methodology for early detection and also to determine the major hot spots of white root rot in Himachal Pradesh. The perusal of data (Table 1) reveals that in all infected soil samples collected from infected sites of three districts, baits of kiwi leaf discs showed maximum mean infection (19.44%) of *D. necatrix* followed by avocado (18.89%) and apple leaf discs (8.89%). Trapping nature of kiwi leaf disc may be attributed to the highly hairy character of leaves which can easily trap the pathogen from soil. Avocado leaf discs also showed good colonization behavior but cannot be employed due to its non availability in the state (Fig. 1).

Table.1 In vitro evaluation of different leaf baits for the estimation of infection and hot spots of white root rot pathogen of apple in Himachal Pradesh

District	Sites	Infection of baits (%)			Mean (%)
		Kiwi leaf discs	Apple leaf discs	Avocado leaf discs	
Kullu	Karjaan	25.00 (29.91)	8.33 (16.59)	13.33 (21.33)	**15.56 (22.61)**
	Gushaini	15.00 (22.59)	10.00 (18.43)	16.67 (24.04)	**13.89 (21.68)**
	Bhalyani	28.33 (32.08)	8.33 (16.59)	21.67 (27.70)	**19.44 (25.46)**
Shimla	Dhangvi	16.67 (24.04)	6.67 (14.75)	20.00 (26.44)	**14.44 (21.74)**
	Ghoond	23.33 (28.84)	8.33 (16.59)	30.00 (33.15)	**20.56 (26.19)**
	Thanedar	15.00 (22.59)	5.00 (12.92)	13.33 (23.85)	**11.11 (19.78)**
Kinnaur	Nichaar	16.67 (24.04)	6.67 (14.75)	15.00 (22.78)	**12.78 (20.52)**
	Sangla	15.00 (22.59)	5.00 (12.92)	16.67 (21.14)	**12.22 (18.88)**
	Kilba	20.00 (26.44)	21.67 (27.58)	23.33 (28.77)	**21.67 (27.60)**
Mean		**19.44 (25.90)**	**8.89 (16.79)**	**18.89 (25.46)**	
$CD_{0.05}$	Sites (S)= 2.97 Leaf discs (LD)=1.71, Sites (S) X Leaf discs (LD) = 5.14				

Figures in the table are arc sign transformed values

Fig.1 Plates showing the infection of *D. necatrix* on different leaf baits (kiwi, apple and avocado; left to right) in soil samples collected from major white root rot infected zones of Himachal Pradesh

Kullu **Shimla**

Kinnaur

Maximum infection of baits was observed in Ghoond area (30.00%) of district Shimla by using avocado leaf discs followed by Bhalyani area (28.33%) of district Kullu by using baits of kiwi. Whereas, minimum bait infection was recorded in Sangla area (5.00 %) of district Kinnaur with apple leaf discs. Overall, the maximum mean infection (21.67 %) was observed in Kilba area of district Kinnaur followed by Ghoond (20.56%) area of district Shimla and Bhalyani (19.44%) area of district Kullu. However, least mean bait infection (11.11%) was recorded in Sangla area of Kinnaur district.

The leaf disc colonization method for trapping *Rosellinia bunodes* and *R. necatrix* from diseased cocoa roots and avocado roots has been utilized earlier by various workers for assessment of inoculums levels of this pathogen (Freeman *et al.,* 1986; Freeman and Sztejnberg, 1992). Baits like avocado leaf disc (Sztejnberg *et al.,* 1983), twigs of *Populus sieboldii* (Ito and Nakamura, 1984) have also been tried earlier for isolation of *Rosellinia* sp. from soil. Eguchi *et al.,* (2009) have also established that baits of mulberry can be used for detecting *Rosellinia necatrix* at an early stage of infection from naturally infested sick soil in apple and pear orchards in Japan.

In conclusion, opportunistic soil borne pathogens such as *D. necatrix* (teleomorph *Rosellinia* spp.) are notoriously difficult to control once they manifest themselves. The bait twig method facilitated quicker diagnosis of *D. necatrix* during the early stages of the infection and can be employed to know the major hotspots of disease and subsequent selection of control measures. Moreover, the detection of *D. necatrix* infection on roots before aerial symptoms are observed would facilitate the application of control strategies during the early stages of the infection, avoiding tree death and further disease spread, which are major problems to overcome when managing white root rot disease. Baiting followed by microscopic examination has several advantages over direct plating technique because in baiting technique large quantity of soil can be tested even when pathogen are present in low population density. In addition, microscopic examination of mycelium from baits made identification easy and more confirmatory. From the aforesaid findings, it is deduced that Ghoond and Dhangvi in district Shimla, Bhalyani and Karjaan in district Kullu whereas, Kilba and Nichaar in district Kinnaur are the major hot spots of root rot disease. In addition, kiwi leaf discs, avocado leaf discs (if available) and to small extent apple leaf discs could be employed for quick detection and estimation of the major hot spots of root rot infecting areas in the state as well as country.

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