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Evaluation of biocontrol agents, botanicals and fungicides against White root rot of apple caused by *D. necatrix* under pot conditions

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Evaluation of integrated management module against white root rot of apple caused by *D. necatrix* under pot conditions was checked.Evaluation of twenty three different combinations revealed that all the treatment combinations significantly lowered the disease incidence of White root rot of apple in comparison to control. The combinations *viz.,T. viride* + carbendazim, *T. viride* + (pyraclostrobin + metiram), *T. viride* + (flusilazole + carbendazim), *B. oleracea* var. *capitata* + carbendazim , *B. oleracea* var. *capitata* + (pyraclostrobin + metiram), *B. oleracea* var. *capitata* + (flusilazole + carbendazim), *T. viride* + *B. oleracea* var. *capitata* + carbendazim, *T. viride* + *B. oleracea* var. *capitata* + (pyraclostrobin + metiram), *T. viride* + *B. oleracea* var. *capitata* + (flusilazole + carbendazim), *T. harzianum* + carbendazim , *T. harzianum* + (pyraclostrobin + metiram), *T. harzianum* + (flusilazole + carbendazim), *T. harzianum* + *B. oleracea* var. *capitata* + carbendazim, *T. harzianum* + *B. oleracea* var. *capitata* + (pyraclostrobin + metiram), *T. harzianum*+ *B. oleracea* var. *capitata* + (flusilazole + carbendazim), carbendazim , pyraclostrobin + metiram and flusilazole + carbendazim were highly effective and provided complete control of the disease when applied twice at 7 days interval after soil inoculation. The combinations *T. viride* + *B. oleracea* var. *capitata*, *T. harzianum* + *B. oleracea* var. *capitata, T. viride*, *T. harzianum* and *B. oleracea* var. *capitata* were least effective showing only 11.11, 11.11, 25.92, 25.92 and 11.11 per cent disease incidence respectively. After 15 days of pathogen inoculation there was no disease incidence in any one of the treatment and disease appeared (33.33%) in *T. viride* and *T. harzianum* after 30 days of inoculation. It increased at slower rate (33.33 to 44.44 %) in *T. viride* + *B. oleracea* var. *capitata*, *T. harzianum* + *B. oleracea* var. *capitata, T. viride* and *T. harzianum* to 45 days. The rot weight of above treatments ranged between 24.47g-29.07g and the pH of the soil of all treatments ranged between 7.0-7.5. The total microbial count of treated plants lied between 10.67-14.33×10^4.

Keywords: Biocontrol agents, botanicals, *Dematophora necatrix,* fungicides, integrated management module

INTRODUCTION

Apple (*Malus X domestica* Borkh.) is one of the important fruit crops of temperate region of the world. Apple has been the staple fresh fruit in the temperate parts of the world.

Eating apples is believed to reduce the incidence of dental caries, help to control obesity and supply extra energy for the heavy exercise.Apple is believed to be the most widely grown fruit tree produced in all the continents of the world. It is grown globally in the European countries, North America, New Zealand, Australia, China and Japan. It occupies an area of 4,622,366 hectares with total production of 86,442,716 metric tonnes and productivity of 18.70 metric tonnes/hectare (Anonymous 2020). In India, apple is primarily cultivated in Jammu and Kashmir, Himachal Pradesh, hills of Uttar Pradesh and Uttarakhand. It is also cultivated to a small extend in Arunachal Pradesh, Nagaland, Punjab and Sikkim. It occupies an area of about 308,000 hectares with a total production of 2,734,000 metric tonnes and productivity of 8.87 metric tonnes/hectare (Anonymous 2020). Jammu and Kashmir is the leading state in area and production of apple with highest productivity followed by Himachal Pradesh, Uttarakhand and Arunachal Pradesh. Presently Himachal Pradesh is known as Apple Bowl of India.

Amongst various soil borne diseases, White root rot (*Dematophora necatrix* Hartig) and Collar rot

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(*Phytophthora cactorum* (Leb. and Cohn) Schrot.) are most prevalent both under nursery and orchard conditions and other diseases like seedling blight (*Sclerotium rolfsii*), crown gall (*Agrobacterium tumefaciens* Smith and Townsend) and hairy root (*Agrobacterium rhizogenes* Conn) are most prevalent only under nursery conditions and cause huge economic losses to nurserymen and orchardists (Sharma and Gupta, 2005).White root rot is caused by *Dematophora necatrix* Hartig with its perfect stage as *Rosellinia necatrix* (Hart.) Berl. that is still unknown in India. It attacks large number of temperate fruit crops such as apple, pear, plum, almond, peach, cherry, olive and grapevine (Holevas *et al.* 2000).

In Himachal Pradesh, the disease was reported for the first time from Kotgarh area of Shimla district and the association of *Dematophora necatrix* was observed with Root rot of apple. The estimated loss due to this disease was about Rs 1.3 million which was later expected to be much more as the disease progressed and reported to occur in all apple growing regions of the country (Sharma and Sharma 2008).

MATERIALS AND METHODS

Soil treatment

Chemical sterilization of soil was done by using formalin @ 2 per cent. Soil was then covered with plastic sheet so that no air can enter inside. There was a waiting period of 20-40 days depending upon the temperature. After 35 days it was observed that there was no fume of chemical in the soil and soil was ready for pot filling.

Preparation of D. necatrix inoculum

The mass culture of *D. necatrix* was prepared on wheat grains. Initially, wheat grains were soaked in water for 12 hrs and thereafter boiled for 30 minutes. The excess water was drained off and then boiled wheat grains were supplemented with 50g sucrose, 200g sand per kg of grain and mixed thoroughly. The mixture was sterilized in autoclave at 20 lbs pressure p.s.i. and 121^{0}C for one hr in heat resistant polypropylene bags plugged with non-absorbent cotton. Sterilized grains in polypropylene bags were then inoculated with four fungal bits of two week old *D. Necatrix* culture under aseptic conditions and incubated at 25±1°C.

Raising of apple seedlings in pots

The pots of size 30×28 cm were filled with 4 kg of sterilized soil mixed with FYM and sand in the ratio of 4:1:1. After that one year old apple seedlings were planted in each pot in the month of March ,2018. The pots were watered frequently.

Inoculation of D. necatrix culture

Mass culture of *D. necatrix* was prepared as described earlier. The soil around the plant trunk was removed and 8g wheat grain culture of *D. necatrix* was put in the surrounding soil of the root and was again covered properly.

Application of effective treatments

Two soil drenching of below treatments(Table1)were given at an interval of 7 days after the inoculation. Fungicides and botanical were given as per recommended doses whereas liquid formulation of fungal antagonists were given @50ml/1kg soil.

Incubation period, symptom development and disease incidence were recorded after 15, 30 and 45 days of inoculation. After completion of experiment root weight was observed as well as per cent root infection and CFU was calculated. The pH of soil was also recorded before and after completion of experiment.

RESULTS AND DISCUSSION

Data recorded on the evaluation of twentytwo treatments (Table 2) revealed that all the treatments significantly lowered the disease incidence of white root rot in comparison to control. Nineteen treatments viz., T1, T2, T3, T5, T6, T7, T8, T9, T10, T11, T12, T13, T14, T15, T16, T19, T20, T21 and T22 were highly effective and provided complete control of the disease when applied twice at 7 days interval after inoculation. It was followed by T4 with 11.11 per cent disease incidence having incubation period 34 days respectively. T17 and T18 were the last best treatments with 25.92 per cent disease incidence having incubation period 22 and 24 days respectively.

As revealed in Table 2, only three treatments showed disease incidence *i.e.* T4, T17 and T18

Table 1: List of treatments evaluated under *in vitro* conditions against *D. necatrix* causing white root rot of apple

TREATMENTS	CONCENTRATION (%)
T. viride+ carbendazim 50 WP	5×10^4 CFU+.00625
T. viride+ (pyraclostrobin 5% + metiram 55% WG)	5×10^4 CFU+0.0125
T. viride+ (flusilazole 12.5% + carbendazim 25% EC)	5×10^4 CFU+0.00625
T. viride+ *B. oleracea* var. *capitata*	5×10^4 CFU+10
B. oleracea var. *capitata* + carbendazim 50 WP	10+0.00625
B. oleracea var. *capitata* + (pyraclostrobin 5% + metiram 55% WG)	10+0.0125
B. oleracea var. *capitata* + (flusilazole 12.5% + carbendazim 25% EC)	10+0.00625
T. viride+ carbendazim 50 WP + *B. oleracea var. capitata*	5×10^4 CFU+0.00625+10
T. viride + (pyraclostrobin 5% + metiram 55% WG) + *B. oleracea* var. *capitata*	5×10^4 CFU+ 0.0125+10
T. viride+ (flusilazole 12.5% + carbendazim 25% EC) + *B. oleracea* var. *capitata*	5×10^4 CFU+ 0.00625+10
T. harzianum+ carbendazim 50 WP	4×10^4 CFU+0.00625
T. harzianum+ (pyraclostrobin 5% + metiram 55% WG)	4×10^4 CFU+0.0125
T. harzianum+ (flusilazole 12.5% + carbendazim 25% EC)	4×10^4 CFU+0.00625
T. harzianum+ carbendazim 50 WP + *B. oleracea* var. *capitata*	4×10^4 CFU+0.00625+10
T. harzianum+ (pyraclostrobin 5% + metiram 55% WG)+. *B. oleracea* var. *capitata*	4×10^4 CFU+ 0.0125+10
T. harzianum+ (flusilazole 12.5% + carbendazim 25% EC) + *B. oleracea* var. *capitata*	4×10^4 CFU+ 0.00625+10
T. viride	5×10^4 CFU
T. harzianum	4×10^4 CFU
Carbendazim 50 WP	0.00625
Pyraclostrobin 5% + metiram 55% WG	0.0125
Flusilazole 12.5% + carbendazim 25% EC	0.00625
B. oleracea var. *capitata*	10
Control	

Table 2: Evaluation of biocontrol agents, botanicals and fungicides in potconditions

Treatments	Incubation Period (Days)	Disease incidence (%) after days of inoculation			Mean %	Root Weight (g)	% increase in root weight	CFU/g soil× 10^4	pH
		15	**30**	**45**					
T1	0	0(1.00)	0(1.00)	0(1.00)	0(1.00)	25.17	25.08(5.10)	12.00	7.4
T2	0	0(1.00)	0(1.00)	0(1.00)	0(1.00)	25.40	25.63(5.15)	11.00	7.5
T3	0	0(1.00)	0(1.00)	0(1.00)	0(1.00)	25.70	26.61(5.23)	10.67	7.5
T4	34	0(1.00)	0(1.00)	33.33(5.03)	11.11(2.34)	24.47	22.96(4.88)	14.33	7.0
T5	0	0(1.00)	0(1.00)	0(1.00)	0(1.00)	24.20	24.04(4.79)	10.67	7.2
T6	0	0(1.00)	0(1.00)	0(1.00)	0(1.00)	28.13	32.66(5.77)	10.33	7.4
T7	0	0(1.00)	0(1.00)	0(1.00)	0(1.00)	28.17	33.05(5.83)	11.00	7.5
T8	0	0(1.00)	0(1.00)	0(1.00)	0(1.00)	28.57	34.02(5.91)	12.33	7.2
T9	0	0(1.00)	0(1.00)	0(1.00)	0(1.00)	29.07	35.06(5.99)	13.33	7.5
T10	0	0(1.00)	0(1.00)	0(1.00)	0(1.00)	26.17	27.61(5.31)	11.00	7.2
T11	0	0(1.00)	0(1.00)	0(1.00)	0(1.00)	26.93	30.00(5.56)	10.33	7.5
T12	0	0(1.00)	0(1.00)	0(1.00)	0(1.00)	26.03	27.66(5.35)	13.00	7.3
T13	0	0(1.00)	0(1.00)	0(1.00)	0(1.00)	26.53	28.64(5.42)	11.67	7.4
T14	0	0(1.00)	0(1.00)	0(1.00)	0(1.00)	27.63	31.60(5.69)	12.67	7.0
T15	0	0(1.00)	0(1.00)	0(1.00)	0(1.00)	28.00	32.33(5.75)	11.00	7.1
T16	0	0(1.00)	0(1.00)	0(1.00)	0(1.00)	29.17	35.09(5.98)	10.33	7.3
T17	22	0(1.00)	33.33(5.03)	44.44(5.64)	25.92(3.89)	23.10	18.15(4.32)	14.33	7.0
T18	24	0(1.00)	33.33(5.03)	44.44(5.64)	25.92(3.89)	23.53	19.56(4.47)	13.67	7.1
T19	0	0(1.00)	0(1.00)	0(1.00)	0(1.00)	25.83	26.61(5.19)	11.67	7.3
T20	0	0(1.00)	0(1.00)	0(1.00)	0(1.00)	27.27	30.96(5.65)	11.00	7.1
T21	0	0(1.00)	0(1.00)	0(1.00)	0(1.00)	28.07	32.86(5.82)	10.67	7.2
T22	31	0(1.00)	0(1.00)	33.33(5.03)	11.11(2.34)	24.13	21.87(4.77)	9.67	7.3
Control	14	33.33(5.03)	66.66(8.04)	100(10.05)	66.66(8.04)	18.83		6.67	6.7
$C.D._{0.05}$		1.9				2.82	0.96	2.69	

*Figures in parentheses are square root transformed value

after 34, 22 and 24 days. Affected trees show declining symptoms. Above ground symptoms of the disease include progressive decline in the vigour of the plant, leaves show incurved margin, change of the color and reduction in size.After examination of underground plant parts, it was found that the fibrous roots were rottened, turned dark brown and covered with subtle layer of white mycelium (Fig.1). Similar symptoms have also been reported by various workers (Sharma *et al*. 2005).

Sharma (2004) reported that carbendazim in combination with *Enterobactor aerogenes* exhibited more than 92 per cent disease control when applied as pre-inoculation to the pathogen under pot conditions.

The perusals of data in Table 2 indicated that root weight was significantly higher in all the treatments when comparison with respective non-treated inoculated soil. Among treatments, combination of fungal antagonist + botanical + fungicides were highly effective and maximum root weight was observed. Six treatments *viz.,* T16 (29.17g), T9 (29.07g), T8 (28.57g), T15 (28.00g), T14 (27.63g)) and T10 (26.17g) had maximum root weight respectively while it was minimum in non-treated inoculated soil (18.83g). Same results were reported by Sharma (2000) that apple seedlings planted in healthy soil have measureable increase in shoot length, root length, shoot weight and root weight in comparison with white root infected soil. Same results were also observed under the present study.

It is evident from the data that there was not much change in the pH of soil before and after completion of experiment (before 7.4) but in case of control plant which was inoculated with *D. necatrix* had 6.7.

The individual effect as well as combined effect of fungicides, biocontrol agents and botanicals on microbial population was also studied under field conditions. Data (Table 2) showed that total microbial population increased significantly in all the treatments as compared to control. Maximum

microbial population in soil was found in T4 (14.33×10^4 CFU), T17 (14.33×10^4 CFU) and T16 (13.67×10^4 CFU) per gram of soil as compared to population in control (6.67×10^4 CFU). Similarly, Huang *et al.* (2012) reported that microbial communities play an important role in soil nutrient cycling and there was significant higher biomass and lower bulk density in the soils of higher yield orchards than that in low yield orchards. Van

Fig.1: Symptomatology of white root rot on apple seedlings A- Bronzing and wilting in diseased apple seedling; B- Healthy apple seedling; C- Bronzing and wilting in diseased apple seedling; D- Healthy roots

Burggen *et al.* (2006) stated that in healthy soil, the level of microbial diversity and activity were high, so that soil borne diseases faces more competitors and antagonists. The regular addition of soil organic matter increase level of microbial activity, increase nutrient cycling, increase microbial diversity and enhance natural disease suppression. However, the analysis of microbial communities showed that this result seemed to be caused by a different ratio among biocontrol agents' populations, rather than by a consistent reduction of fungal root pathogen. This data provided evidence that microbial population should be more in healthy soil. Similar results were also observed under present study.

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