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CULTURAL STUDIES AND MANAGEMENT OF WILT COMPLEX DISEASE OF POMEGRANATE (PUNICA GRANATUM L.)

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ABSTRACT

Pomegranate (Punica granatum L.) is one of the most Important arid zone fruit crop affected by soil born pathogen like Fusarium oxysporum Schl., Ceratocystis fimbriata and nematode which are responsible to cause wilt disease in pomegranate. Pomegranate wilt is considered to be one of the very destructive disease and causes losses about more than 5 to 10 per cent and hence the studies were undertaken on this disease with the objectives viz., isolation of causal organism, pathogenicity, cultural characteristics and management of wilt. Among the various culture media used, oat meal agar and Potato dextrose agar were excellent media for good growth of C.fimbriata and F. oxysporum Schl. fungus. The highest reduction percent of wilt incidence was observed in Tricoderma plus, neem cake and neem cake + Tricoderma plus in pot culture experiment under glass house condition.

KEY WORDS

Wilt, Complex Disease, management, Pomegranate.

INTRODUCTION:

Pomegranate (*Punica granatum* L.) belongs to family punicaceae. Pomegranate is a native of Iran and Afghanistan De Candolle (1967). Where it was first cultivated in about 2000 BC but spread to the Mediterranean countries at an early date. Now, India, Iran, China, USA, and Turkey are the five largest producers of pomegranate globally. However, in area and production India alone has about 0.125-million-hectare area under pomegranate and occupies first position in the word. In India production (000 MT) and productivity (MT/ha) is to be 743 and 6.9, 772 and 6.9, and 745 and 6.6 in the year 2011, 2012, and 2013 respectively. Pomegranate Production share major fruit crop in India (2012-13) 0.9 %. (National Horticultural Board- 2011).

The wilt disease is prevalent in Maharashtra, Karnataka, Andhra Pradesh, Gujarat, and Tamilnadu state (Jadhav and Sharma,2009). In Maharashtra, the severity of this disease has increased in past 25-30 year, especially in the Pomegranate growing areas of Sangola, Pandharpur, Baramati, Malegaon, Satana, Rahuri and Deola Tahsils. There are many views regarding the cause of disease, such some insect like pinhole boring the trunk near roots and on main roots. Nematode infestation on root, unfavourable soil condition, improper irrigation and less spacing between the plants. But whatever the source of damage to roots, wilting of Pomegranate due to infection of fungal pathogen from injured or weakened roots. If roots of partially wilted plant are observed near soil surface by splitting them, black brown streak are

observed. *Punica granatum* L.Wilt complex is caused by the association of Ceratocystis fimbriata, Fusarium oxysporum Schl.,Rhizoctonia solani and nematode.. The present investigations on wilt complex disease of pomegranate was carried out during 2014-2015 at Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri. The laboratory and glasshouse studies were

conducted at Department of Plant Pathology, P. G. I.,Mahatma Phule Krishi Vidyapeeth, Rahuri.

MATERIAL AND METHODS

The infected Root and soil sample were collected from the orchards of pomegranate from different villages in Rahuri. The root samples of partially and fully declined pomegranate plants (cv. Bhagwa and Ganesh) were collected.The fumgus was Identified as Fusarium oxysporum Schl.,Ceratocystis fimbriata on thew basis of their morphological characteristics .In order to confirm the identity of the fungus, ascospores and perithecia were observed under microscope. The Pathogenicity of the samw fungus was completed. The susceptible host was used as cv. Bhagwa.The inoculums of fungal organism were multiplied in Sand-Maize medium and utilized for further research. The pathogen C. fimbriata and Fusarium oxysporum Schl. was grown on PDA medium in Petri plates for fifteen days prior to undertake the respective experiment. All other medias were also prepared as below

Cultural studies: Growth characters of C. fimbriata and fusarium oxysporum Schl. was studies on following different media

1. Potato dextrose agar
2. Oat meal agar
3. Malt extract Agar
4. Czapeck's agar
5. Raulin's media
6. Sabourads agar
7. Sand-maize medium

These media were prepared according to the laboratory procedure described by Tuite (1969), 500 ml of each media was prepared in six conical flasks separately and sterilized at 1.05 kg/cm 3 pressured for 15 minutes and poured 20ml media in each petriplate. Each medium was replicated in three times. These plates were inoculated with 5 mm diameter of the fungal disc of each pathogen i.e. Fusarium and Ceratocystis at the centre and incubated at 29 ± 1 0 C temperatures for 7 days. The observation in respect of diameter, colony growth was recorded in different day after inoculation. The diameter measured two directions at right angle to each other and average was taken. The growth characters were recorded. 34 I. Potato dextrose agar (PDA) In most of the experimental studies the

I. Potato Dextrose

Agar (PDA) used. The composition of PDA is as follows:

Potato (peeled) 200.0 g
Dextrose 20.0 g
Agar-agar 20.0 g
Distilled 1000 ml
pH 7.0

200 g of peeled potatoes were cut into small bits and boiled in distilled water and then extract were collected by filtering through muslin cloth. Dextrose 20.0 g and agar 20.0 g each were dissolved in the potato extract and the final volume were made up to 1000 ml with distilled water and sterilized in an autoclave at 121 0 c for 15-20 minutes.

II. Oat meal agar

Oat flacks 30.00 g
Agar-agar 20.00 g
Distilled water 1000 ml
pH 7.0

First oat flakes were boiled in 500 ml distilled water for thirty minutes and filtered through muslin cloth. Agar-agar was melted in 500 ml of water separately and then both the solutions were mixed thoroughly, and the volume was made up to one litre and was sterilized.

III. Malt extract agar

Malt extract 20.00 g
Agar-agar 20.00 g
Distilled water 1000 ml
pH 7.0
35

First malt were boiled in 500 ml distilled water for thirty minutes and filtered through muslin cloth. Agar- agar was melted in 500 ml of water separately and then both the solutions were mixed thoroughly, and the volume was made up to one litre and sterilized.

IV. Richard's agar

Sucrose 20.0 g
Potassium nitrate (KNO 3) 10.0 g
Dihydrogen phosphate (KH 2 PO 4) 5.0 g
Magnesium sulphate (MgS0 4 2H 2 O) 2.5 g
Ferric chloride ($FeCl_3$ .6H 2 0) 0.02 g
Agar-agar 20.0 g
Distilled water 1000.0 ml
pH 7.0

Potassium dihydrogen phosphate was dissolve separately (50ml) and mixed at the time of pouring in plates.

V. Czapeck ' s agar

Sucrose (C 6 H 12 O 6) 30.0 g
Sodium nitrate ($NaNO_3$) 2.00 g

Potassium dihydrogen phosphate (KH 2 PO 4) 1.00 g
Magnesium sulphate (MgSO 4 2H 2 O) 0.50 g
Potassium chloride (KCL) 0.50 g
Ferric Chloride (FeCl3. 6H 2 O) 0.01 g
Agar agar 20.0 g
Distilled water 1000.0 ml
pH 7.0
Agar-agar was melted in 500 ml distilled water. Remaining two ingredients in another beaker containing 500 ml distilled water 36 solutions were mixed thoroughly and the volume was made up to 1000 ml and was sterilized.
VI. Sabouraud's agar
Dextrose 40.0 g
Peptone 10.0 g
Agar-agar 20.0 g
Distilled water 1000.0 ml
pH 7.0
All the ingredients were dissolved one by one in 400 ml distilled water and agar will dissolved separately in 500 ml distilled water and mixed with the above solution and the volume will made up to one liter before sterilization.
VII Sand-Maize medium
Sand 80 g
Maize 20 g
(Used for mass multiplication of fungal pathogen).
A mixture of 80 gm sand and 20 gm crushed maize grain was taken well sterilized 250 ml flask. Sufficient water was added to the mixture to moisten it. Then the flasks were sterilized in the autoclave. per requirement. The experiment was laid out in a randomized block design with four replications and eight treatments. The experimental susceptible host was used as cv. Bhagwa. The disease was recorded as below.
1. Wilt symptoms initiates as yellowing of leaves of one or more branches and the plant appeared devitalized
2. The leaves turned pale yellow starting from lower branches/limbs and progressed upwards.
3. Partial wilting of the tree with drying and death of some branches were common symptoms.
4. In severe cases, the defoliation and complete wilting of plant within 2-3 months periods.
5. Fruit drop occurred in severe cases.
6. The entire plant wilted from top to bottom.
7. Severely infested plant by root knot nematode exbited yellowing of foliage resulting in stunted plant growth.
8. These plants produce less no of fruits or no fruits which might be due to the nematode induced nutritional deficiency.
9. In severe cases galls were predominantly found in entire root system. Brownish discoloration of vascular tissue was observed when the diseased roots were split opened. The presence of mycelium was observed in vascular bundles of cross sectioned roots (2-3mm diameter) of infected plants/trees.The symptoms observed in the present studies were more or less similar to those described by Kore and Mitkar (1993). The disease was characterized by change in colour of young leaves from dark green to light green and finally yellow.Severely infested by root knot nematode exhibited yellowing of foliage resulting in stunted plant growth. The symptoms of Ceratocystis wilt also similar to those described by Hung et al., 2003 who described the pomegranate wilt was initiated with yellowing of leaves and wilting of few branches followed by sudden death of the shrub within 3 to 4 weeks and root expressed brown to black irregular lesions.

RESULTS AND DISCUSSION

The results on the symptomatology, isolation of pathogen, cultural studies and management of wilt disease are and discussed. The cottony growth was observed on solid PDA plate and pure culture was maintained on PDA at 28±10 C. Sub-culturing was done at every fortnight interval. Identification of the pathogenic fungi the fungus isolates were identified as C. fimbriata and Fusarium oxysporum Schl. at the department of Plant Pathology and Microbiology, PGI, MPKV, Rahuri and was confirmed on the basis of morphological characters. The fungi C. fimbriata was identified based on morphological characters mentioned below plant and brownish discoloration of vascular tissues of roots. And subsequently complete wilting ofplants was noticed. The yellowing of leaves started at 90 days of planting and complete wilting observed within 120 days of planting. However, control plants did not show the wilting up to 120 days of planting. The Bhagwa variety was tested for pathogenicity and found susceptible to the Fusarium oxysporum Schl. and C. fimbriata fungus pathogen. Reisolation. The fungus was reisolated from the roots of infected seedling of pomegranate. The reisolated fungal culture was found similar to that of originally used for inoculation. The results are in close agreement with

kore and Mitkar (1993) and Chavhan (1999) who isolated Fusarium solani from the roots and stem of wilted pomegranate seedlings and prove the pathogenicity of Fusarium solani causing dry root rot of pomegranate by soil inoculation method. Cultural studies of Ceratocystis fimbriata on different solid media at 5, 10 and 16 days after inoculation. The growth of Ceratocystis fimbriata fungus in different solid medias like Oat meal agar, Potato dextrose agar, Malt agar,46Sabouraud's agar, Czapeck's (dox) agar and Raulin's media were presented in (Table 2). The cultural characteristics of Ceratocystis fimbriata were studied in different media at room temperature 27 ± 1 0 C as described in "Material and methods". The radial growth of the fungus were recorded, when the maximum growth was attained on any one of the tested media. The mean colony diameter of Ceratocystis fimbriata was recorded on different media like Oat meal agar, Potato dextrose agar, Malt agar, Sabouraud's agar, Czapeck's (dox) agar and Raulin's at different 5, 10 and 16 day of inoculation. The significant maximum mean colony (9 cm) was recordedon Oat meal agar as compare rest of media followed by Potato dextroseagar (8.6 cm), Malt agar (7.2 cm), Sabouraud's agar (6.7 cm), Czapeck's agar (6.3 cm) and Raulin's media (0 cm) (Plate 8). Therefore, the result revealed that Oat meal agar and Potato dextrose agar were excellent media for growth of C. fimbriata fungus.

The growth of Fusarium oxysporum Schl. fungus on different solid medias like Oat meal agar, Potato dextrose agar, Maltagar, Sabouraud's agar, Czapeck's (dox) agar and Raulin's media were recored and presented in (Table 3). The diversity in cultural character of Fusarium oxysporum Schl. were studied on different media at room temperature 27 ± 1 0 C as described in "Material and methods". The radial growth of the fungus were recorded, when the maximum growth was attained on any one of the tested media. The mean colony diameter of Fusarium oxysporum Schl. was recorded on different media like Oat meal agar, Potato dextrose agar, Malt agar, Sabouraud's agar, Czapeck's (dox) agar and Raulin'sat different 3, 7 and 10 day of inoculation .The significant maximum mean colony (9 cm) was recorded on Oat meal agar as compare rest of the media which was followed by 48 Potato Dextrose Agar (6 cm), Czapeck's agar (5.5 cm), Sabouraud's agar (5.3 cm), Malt agar (5 cm) and Raulin's medium (1.1 cm) (Plate 9). Therefore, the result revealed that Oat meal agar and Potato dextrose agar were excellent media for growth of Fusarium oxysporum Schl. fungus.

Testing of different bio-agents and organic fertilizers against wilt complex disease of pomegranate .The wilting symptoms were observed in pomegranate plants after 120 days of inoculation and per cent wilting data was recorded and presented The results presented in (Table 3) indicated that the application of Tricoderma plus (T 1), Neem cake (T 6) and Neem cake +Tricoderma plus (T 7) native antagonist through seedling dip and soil application was found most effective in suppressing wilt complex disease and resulted in highest reduction wilt disease i.e. i 75 % control of wilt disease as compare to control (T 8). The next best bio agent and bio fertilizer was Vermicompost (T 3), Vermicompost + Tricoderma plus (T 5), and FYM + Tricoderma plus (T 4) resulted in reduction of wilt incidence 50 % i.e. 50 % control of wilt disease followed by 75 % wilt of incidence in T 2 treatment.

The present study clearly indicated that the bioagent and biofertilisers like Tricoderma plus, Neem cake and neem cake +Tricoderma plus are highly effective and Vermicompost, Vermicompost +Tricoderma plus and FYM + Tricoderma plus the moderately effective against wilt complex disease of pomegranate.

Cultural characters of Ceratocystis fimbriata were studied on different media. Among the solid culture media tried for characterization, the significant maximum mean colony (9 cm) was recorded on Oat meal agar as compare rest of media which followed by Potato dextrose agar (8.6 cm), Malt agar (7.2 cm), Sabouraud's agar (6.7 cm), Czapeck's agar (6.3 cm) and minimum growth was recorded in Raulin's medium (0 cm). Similar observations were made by many research workers on C. paradoxa (Sastry et al., 1989 and Kiryu, 1939). There was no much difference in the measurements of colony diameter taken five days after inoculation on different media. However, the difference was substantial recorded at 10 and 16 days after inoculation on different media. A comparison of Oat meal agar, potato dextrose agar, Malt agar and Sabouraud's agar at sixteen days after inoculation revealed that the presence of fungal bit in the medium almost doubled the diameter of fungal colony. Similar results were reported by (Yadahalli, 2005) in C. paradoxa. Cultural characters of F. oxysporum Schl. Were studied on different media. Among the solid culture media tried for characterization,67 the

significant maximum mean colony (9 cm) was recorded on Oat meal agar as compare rest of the media which followed by Potato Dextrose Agar (6 cm), Czapeck's agar (5.5 cm), Sabouraud's agar (5.3 cm), Malt agar (5cm) and Raulin's medium (1.1 cm). This result agreement with McCulloch (1944), Jhamarica (1972) and Kulkarni (2006) who reported similar results in cultural studies of F. oxysporum f. sp. Gladioli, F. oxysporum f.sp. Niveum and F. oxysporum f.sp. gladioli respectively. There was no much difference in the measurements of colony diameter taken five days after inoculation on different media. However, the difference was ubstantial recorded at 5 and 7 days after inoculation on different media. Comparison fungal growth Oat meal agar, potato dextrose agar, Czapeck's agar and Malt agar after seven days of inoculation were also recorded.

Table 1. Mean colony diameter of Ceratocystis fimbriata on different solid media at 5, 10 and 16 days after inoculation *:

Sr. No.	Medium	Mean colony diameter in (cm*) (Days after inoculation= DAI)			Mean
1	Oat Agar	2.8	5.1	9.0	5.6
2	PDA	2.63	4.7	8.6	5.3
3	Malt Agar	2.2	4.16	7.2	4.5
4	Sabouraud's agar	1.9	3.2	6.7	3.9
5	Czapek's (dox) agar	1.8	3.4	6.3	3.8
6	Raulin's media	00	00	00	00
	Source				
	SE ±	0.28	0.14	0.047	
	CD @ 5 %	0.88	0.44	0.145	

Table 2. Mean colony diameter of Fusarium oxysporum Schl. on different solid media at 3, 5 and 7 days after inoculation

Sr. No.	Medium	Mean colony diameter in (cm*) (Days after inoculation= DAI)			Mean
1	Potato dextrose agar	2.5	4.6	6.0	4.3
2	Oat Meal Agar	3.3	6.9	9.0	6.4
3	Sabouraud's agar	1.7	3.8	5.3	3.6
4	Czapek's (dox) agar	2.0	4.0	5.5	3.8
5	Malt agar	1.8	3.8	5.0	3.5
6	Raulin's media	00	0.8	1.1	0.63
Source					
	SE ±	0.078	0.070	0.066	
	CD @ 5 %	0.240	0.21	0.20	

Table 3. Testing of different bio-agent and organic fertilizers against wilt complex

Sr. No.	Treatments	No. of plant wilting				Total No. of Plant wilting	% disease Incidence
		R 1	R 2	R 3	R 4		
1	T 1	1	0	0	0	1	25
2	T 2	0	1	1	1	3	75
3	T 3	1	0	1	0	2	50
4	T 4	1	0	0	1	2	50
5	T 5	1	0	1	0	2	50
6	T 6	0	1	0	0	1	25
7	T 7	0	1	0	0	1	25
8	T 8	1	1	1	1	4	100

All pots were inoculated with combination mixture of C.fimbriata + F. Oxysporum + Meloidogyne incognita.

T 1: Tricoderma plus (10 g/seedling).

T 2: FYM (300gm/seedling)

T 3: Vermicompost (50 g/seedling)

T 4: FYM + Tricoderma plus (300 g + 10 g)/seedling

T 5: Vermicompost + Tricoderma plus (50 g +10 g)/seedling

T 6: Neem cake (250 g/seedling)

T 7: Neem cake + Tricoderma plus (250 g + 10 g) seedling

T 8: Untreated (control).

The highest percent reduction of wilt disease incidence was recorded in Tricoderma plus, neem cake and neem cake + Tricoderma plus. The next best bioagent and bio fertiliser combination were Vermicompost followed by Vermicompost + Tricoderma plus and FYM + Tricoderma plus and FYM. The data of pot culture study not only confirm observations of in vitro studies here in. but also confirmed the results obtained by research worker like Haseeb (2003). Leeman et al (1991), Meena and Mathur (2003), Nakkerean (1992)

The results are in agreement with Nakkeeran (1992) who reported that neem cake amendment to soil reduced the pigeon pea root rot and wilt incidence resulting in increased yield. The results are also in agreement with Sharon et al. (2001) who reported that the fungal species belonging to the genus Tricodermaare worldwide in occurrence and easily isolated from soil. The potential of Tricoderma species as a biocontrol agent against various plant diseases has been reported by many research workers in different pathogen-host systems. These results are also agreement with Mandhare et al.(1996) who reported that good control fusarium sp. of pomegranate was obtained by Coen + Tricoderma viridia and Tricoderma viridia alone.

The pathogen isolated from Pomegranate (punica granatum) infected roots wilt was identified as Ceratocystis fimbriata and Fusarium oxysporum Schl. The fungus Ceratocystis and Fusarium mostly infect root portion and disease which characterized as per symptoms i.e., change in colour of young leaves from dark green to light green and finally become yellow. In advanced stage leaves dry, fall down and finally entire plant wilt and die. The pathogenicity of the Ceratocystis fimbriata and Fusariumoxysporum Schl. was proved by soil inoculation method.5. Among the six medias Oat meal agar and Potato dextrose agar media were excellent for growth of Ceratocystis fimbriata fungus. Among the six media Oat meal agar and Potato dextrose agar were excellent medias for growth of Fusarium oxysporum Schl. fungus. Bhagwa, Mridula, Arakta and G-137 varieties of Pomegranate under study were found to be susceptible to wilt complex disease of pomegranate. The different bio fertilisers and bio agents viz., Tricoderma plus,neem cake and neem cake + Tricoderma plus were found to be effective for control of Ceratocystis fimbriata + Fusariumoxysporum Schl. + Nematode in pot culture experiment, under glass house condition.

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