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Prevalence and management of leaf spot and dry fruit rot (*Coniella granati*) of pomegranate

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ABSTRACT

Leaf spot and dry fruit rot in pomegranates caused by *Coniella granati* (Sacc) Petrak and Sydow have emerged as a new threat in Himachal Pradesh. Survey reports indicated that per cent disease index (PDI) varied between 11.8 to 25.1 on leaves and 12.6 to 39.8 on fruits. In vitro evaluation revealed that systemic fungicides viz difenoconazole (Score 25 EC), hexaconazole (Contaf 5 EC), carbendazim (Benfil 50 WP), propiconazole (Tilt 25 EC), pyraclostrobin (Insignia 20 WG), kresoxim methyl (Ergon 44.30 w/w) and flusilazole (Governor 40 EC) at 50 ppm, combi-products namely carbendazim (12%) + mancozeb (63%) (Companion 75 WP), hexaconazole (5%) + captan (70%) (Taqat 75 WP), metiram (55%) + pyraclostrobin (5%) (Cabriotop 60 WG), carbendazim (25%) + iprodione (25%) (Quintal 50 WP), metiram (Sanit 70 WG), captan (Captafol 50 WP) and copper oxychloride (Maincop 50 WP) at 250 ppm concentration provided complete inhibition of fungal growth. Similarly out of eight bio-resources vermiwash and mixture of cow urine + *Melia azadirach* + *Vitex negundu* + *Artimesia roxburghiana* + *Juglans regia* + *Azadirachta indica* in equal proportion at 5 per cent concentration also completely inhibited the fungal growth. Among five biocontrol agents *Bacillus subtilis* and *Trichoderma harzianum* gave maximum growth inhibition of *C granati* under in vitro conditions. Field evaluation for two consecutive years (2012-13) revealed that propiconazole (0.05%) and carbendazim (0.05%) were highly effective providing 89.9 and 90.7 per cent and 89.5 and 90.3 per cent disease control on leaves and fruits respectively whereas hexaconazole + captan (0.1%), pyraclostrobin (0.05%) and difenoconazole (0.02%) were next best fungicides in order.

Keywords: Bio-control agents; bio-resources; *Coniella granati*; fungicides; pomegranate

INTRODUCTION

Pomegranate, *Punica granatum* has been introduced in Himachal Pradesh for its cultivation under the umbrella of diversification program of different fruit crops since last ten years. The area under this crop has increased by about 2.5 times since 2003-2004 (475 ha) and has been growing over 113000 hectares with annual production of 745000 tons (Anon

2012-13). Number of pathogens attacking leaves, fruits, stems, branches and roots of pomegranate have been reported (Khosla and Bhardwaj 2013). Amongst these dry fruit rot and leaf spot caused by *C granati* has been appearing in moderate to severe form since its first appearance in 1998 in Himachal Pradesh (Sharma 1998). Scrutiny of literature indicated that extremely scanty research work has been done in India on the disease. Therefore studies were conducted to record the incidence in major pomegranate growing districts of Himachal Pradesh and to develop management practices to control it.

MATERIAL and METHODS

Prevalence: Periodical survey of different pomegranate growing areas of Himachal Pradesh was conducted during June to August. At each location fifty plants were randomly selected to record the incidence and severity of the disease. The incidence of disease on leaves and fruits of pomegranate was recorded by the standard formula:

$$\text{Diseases incidence (\%)} = \frac{\text{Number of diseased leaves or fruits}}{\text{Total number of leaves or fruits}} \times 100$$

Disease severity on leaves and fruits was recorded by following 0-5 scale, where 0= no infection, 1= 1 to 5 per cent, 2= 5.1 to 15 per cent, 3= 15.1 to 25 per cent, 4= 25.1 to 50 per cent, and 5= >50 per cent of leaf/fruit area infected. Per cent disease index (PDI) was calculated according to the formula:

$$\text{PDI (\%)} = \frac{\text{Sum of all the disease ratings}}{\text{Total number of ratings} \times \text{maximum disease grade}} \times 100$$

In vitro evaluation of different management inputs

Fungicides: Fourteen fungicides were evaluated using poisoned food technique against *C granati* (Falck 1907). Systemic fungicides viz difenoconazole (Score 25 EC), hexaconazole (Contaf 5 EC), carbendazim (Benfil 50 WP), propiconazole (Tilt 25 EC), pyraclostrobin (Insignia 20 WG), kresoxim methyl (Ergon 44.3 w/w) and flusilazole (Governor 40 EC) were tested at 50, 100, 150 and 200 ppm concentrations whereas combi-products including carbendazim (12%) + mancozeb (63%) (Companion 75 WP), hexaconazole (5%) + captan (70%) as Taqat 75 WP, metiram (55%) + pyraclostrobin (5%) (Cabriotop 60 WG), carbendazim (25%) + iprodione (25%) (Quintal 50 WP) and

non-systemic fungicides captan (Captaf 50 WP), copper oxychloride (Maincop 50 WP) and metiram (Sanit 70 WG) were evaluated at 250, 500, 750 and 1000 ppm concentrations. Three replications were maintained for each treatment. The radial growth of the colony was recorded when maximum growth was observed in control and per cent inhibition was calculated by using the formula given by Vincent (1947).

Bio-resources: Eight bio-resources viz *Melia azadirach (*Darek), *Aloe barbadensis* (Aloe), *Murraya exotica* (Gandla), *Artimesia roxburghiana* (Artimesia), *Vitex negundo* (Bana), *Juglans regia* (walnut leaves), vermiwash and a mixture of cow urine + *Melia azadirach* + *Vitex negundo* + *Artemisia roxburghiana* + *Juglans regia* + *Azadirachta indica* (1:1:1:1:1:1) were tested at 5, 10 and 15 per cent concentrations. To prepare the water extract fresh leaves and seeds (200 g) of each plant were taken, washed under tap water and ground for 5 minutes in blender by adding small quantity of sterilized warm distilled water. After grinding 200 ml distilled water was added and homogenized in orbital shaker at 2000 rpm for half an hour to get 100 per cent extract of plant parts. The plant material was then filtered through double-layered muslin cloth. Sterilization of the extracts was done in autoclave at 5 psi pressure for one hour for three consecutive days and the extracts were kept in refrigerator for further use. The per cent growth inhibition for each treatment was calculated.

Bio-control agents: Three different species of fungal antagonist and two of bacteria were procured from the Department of Plant Pathology, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan, HP. Fungal antagonists were evaluated for their antagonistic activities against *C granati* by dual culture method (Denis and Webster 1971) whereas efficacy of bacterial antagonists was carried out by streak plate method (Utkhede and Rahe 1983). The experiment was laid out in CRD and each treatment was replicated thrice. The colony diameter of test fungus was recorded till the control plates achieved full growth of the test fungus and the per cent inhibition was calculated by the formula given by Vincent (1947).

Evaluation of fungicides under field conditions: Fourteen in vitro evaluated fungicides referred above were further evaluated under field conditions in pomegranate block of Department of Fruit Science, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan, HP for two consecutive years (2012-13). The experiment was laid out in randomized block design and each treatment was replicated thrice. Spray was initiated with first appearance of disease followed by another spray at an interval of 15 days. Three untreated trees were kept as control.

Observations on disease severity (on leaves and fruits) were recorded after 20 days of the last application by following the 0-5 disease rating scale as described above and PDI was also calculated separately for each treatment..

RESULTS and DISCUSSION

Prevalence: Perusal of data (Table 1) reveals that average incidence of disease on leaves and fruits of two years (2012-2013) varied from 36.6 to 54.9 and 38.7 to 56.4 per cent respectively whereas its PDI varied from 11.8 to 25.1 on leaves and 12.6 to 39.8 per cent on fruits at various locations in three major pomegranate growing districts of the state. Maximum disease incidence and PDI on leaves and fruits was recorded at Nauni in district Solan and minimum at Bajaura in district Kullu. It was due to occurrence of favourable climatic conditions as well as availability of higher amount of initial inoculum of the pathogen. Sharma and Tegta (2011) have also reported that incidence of dry fruit rot varied from 0.55 to 22.7 per cent at various locations in HP.

In vitro evaluation of fungicides: Among the seven systemic fungicides carbendazim, propiconazole, difenoconazole, flusilazole and hexaconazole were the most effective and completely inhibited the mycelial growth of the fungus at minimum test concentration of 50 ppm. Ergon was the next best fungicide with 72.0 per cent inhibition followed by Insignia (69.1 %). Among combi and non-systemic fungicides mancozeb + carbendazim, hexaconazole + captan, metiram + pyraclostrobin, carbendazim + iprodione and metiram were the most effective and completely inhibited the mycelial growth of the fungus at 50 ppm concentration followed by Captan with 95.5 per cent inhibition. Copper oxychloride proved the least effective fungicide. Lukose and Singh (1997) found that thiophanate methyl (0.1%) and mancozeb (0.2%) were effective against three fruit rot/spot pathogens viz *C gloeosporioides, C granati* and *Pseudocercospora granati*. Thakur (2009) found that carbendazim, thiophanate methyl, hexaconazole, mancozeb and mancozeb + carbendazim gave 100 per cent inhibition of *Coniella granati* under in vitro conditions. Tegta (2008) also recorded similar inferences against *C granati* under in vitro conditions.

In vitro evaluation of bio-resources: Data (Table 2) reveal that among the eight different bio-resources vermiwash and a mixture of cow urine + *Melia azadirach* + *Vitex negundo* + *Artemisia roxburghiana* + *Juglans regia* + *Azadirachta indica* in equal amount provided complete inhibition of *C granat* followed by water extracts of *J regia* (66.6%) and *V negundo* (34.2%). Minimum inhibition was observed in *M azadirach* (4.5%). *A roxburghiana, Murraya exotica* and *Aloe barbadensis* extracts were less inhibitory.

Table 1. Prevalence of *Coniella granati* leaf spot and dry fruit rot on pomegranate in three districts of Himachal Pradesh (pooled data of 2012 and 2013)

District	Localion	Disease incidence (%) on		Disease severity (%) on	
		Fruits	Leaves	Fruits	Leaves
Kullu	Bajaura	38.8	36.8	12.6	11.8
	Jarad	41.1	39.0	22.1	19.0
	Hurla	39.0	36.6	16.5	15.2
	Thras	40.8	38.1	18.3	15.4
	Mean	40.0	37.6	17.4	15.4
Mandi	Nagwain	39.5	38.7	15.0	13.4
	Panarasa	38.7	37.8	14.1	13.2
	Mean	39.1	38.3	14.6	13.3
Solan	Nauni	56.4	54.9	39.8	25.1
	Deothi	53.6	52.7	35.9	21.5
	Mean	55.0	53.8	37.9	23.3

Table 2. In vitro evaluation of bio-resources

Bio-resource	Diametric mycelial growth (mm) at different concentrations (%)			Mean	Growth inhibition (%)
	5	10	15		
Darek	86.2	86.0	85.8	85.9	4.5 (12.2)
Artimesia	84.8	65.0	62.5	70.8	21.4 (26.4)
Gandla	80.9	78.9	78.4	79.4	11.8 (20.0)
Aloe	83.1	76.1	49.8	69.7	22.6 (27.0)
Bana	67.5	65.4	44.9	59.3	34.2 (35.5)
Walnut leaves	82.2	7.9	0.0	30.1	66.6 (59.9)
Vermiwash	0.0	0.0	0.0	0.0	100.0 (89.9)
Cowurine + Darek + Bana + Artemisia + Walnut + Kadu (1:1:1:1:1:1)	0.0	0.0	0.0	0.0	100.0 (89.9)
Control	90.0	90.0	90.0	90.0	
Mean	63.9	52.2	45.7	53.9	

Figures in parentheses are arc sin transformed values

$CD_{0.05}$

Bioresource= 0.195, Concentration= 0.112, Bioresource × concentration= 0.337

Sharma and Tegta (2011) found that pre-inoculation treatment with plant extracts of Darek *(Melia azadirach)*, Dedonia *(Dedonia ifenoc)*, lantana (*Lantana camara)* and peppermint *(Mentha piperita)* provided significant control of fruit rot of pomegranate caused by *C granati*. The fungi-toxicity of the plant extracts can be attributed to presence of phenolics and related compounds that suppress the growth of advancing pathogen (Natarajan and Lalithakumari 1987, Nawwar et al 1994). Cong Jun et al (2005) observed that extracts from stems and leaves of tomato with six different kinds of solvents exhibited strong fungistatic effect against *Coniella diplodiella* and completely inhibited the fungal growth.

In vitro evaluation of bio-control agents: It is evident from data (Table 3) that *Bacillus subtilis* and *Trichoderma harzianum* provided maximum inhibition of fungus to the tune of 71.3 and 70.7 per cent respectively and were statistically at par with each other. It was followed by *Pseudomonas flourescense* (68.6%), *T viride* (64.7%) and *T hamatum* (63.3%). Sesan et al (2003) reported *T viride* to be the most effective antagonist against *C diplodiella* causing white rot of grapes followed by *Verticillium tenerum, Coniothyrium minitans* (isolates IVT, C15, C18 and CR), *Epicoccum nigrum* (isolates Ep.5, Ep.6, Ep.3 and Ep.4) and *Gliocladium roseum* (isolates GI.1 and GI.2). Fungal antagonists viz *Trichoderma virens, T harzianum, T hamatum* and *T polysporum* inhibited mycelial growth of *C granati* under in vitro conditions (Sharma and Tegta 2011).

Field evaluation of fungicides: Data (Table 4) indicate that two consecutive sprays of propiconazole (0.05%) at an interval of 15 days with the first appearance of target disease were highly effective and provided 89.9 and 90.7 per cent disease control on leaves and fruits respectively. The next best fungicides were carbendazim, hexaconazole + Captan, flusilazole and Captan in order. Metiram provided 84.2 and 85.4 per cent whereas mancozeb + carbendazim provided 81.4 and 83.5 per cent disease control on leaves and fruits respectively. Pyraclostrobin, difenoconazole and copper oxychloride were less effective fungicides. Combi-product fungicides viz carbendazim + mancozeb or benomyl + mancozeb have also been demonstrative to be effective in controlling leaf and fruit spots caused by *Cercospora punicae, Coniella granati, C lythracearum, C gloeosporioides (Gloeosporium cingulata), A alternata* and *D rostrata*. Single application with carbendazim (0.1%) proved to be as effective as the combi-product fungicides application (Gaikwad 2000). Sesan and Stefan (2003) reported that fungicides viz vinclozolin, carbendazim and tebuconazole were quite effective against *Coniella diplodiella* causing white rot of grapes under filed conditions. Further Sharma and Tegta (2011) observed that

Table 3. In vitro evaluation of bio-control agents against *C granati*

Biocontrol agent	Growth inhibition (%)
Trichoderma harzianum	70.7 (57.2)
T hamatum	63.3 (52.7)
Bacillus subtilis	71.3 (57.6)
Pseudomonas flourescens	68.6 (55.9)
T viride	64.7 (53.5)
$CD_{0.05}$	2.751

Table 4. Field efficacy of fungicides in controlling dry fruit rot of pomegranate during 2012-13

Fungicide	Dose (%)	Leaves		Fruits	
		Disease index (%)	Disease control (%)	Disease index (%)	Disease control (%)
Benfil (carbendazim)	0.05	3.4 (10.6)	89.5	4.1 (11.7)	90.3
Insignia (pyraclostrobin)	0.05	17.2 (24.4)	46.6	21.4 (27.3)	49.8
Companion (mancozeb + carbendazim)	0.25	5.9 (14.1)	81.4	7.0 (14.9)	83.5
Captan	0.3	14.5 (21.9)	55.1	18.8 (25.1)	55.7
Copper oxychloride	0.3	20.2 (26.5)	37.3	25.8 (30.3)	39.3
Taqat (hexaconazole+captan)	0.1	3.4 (10.4)	89.4	4.2 (11.5)	90.1
Cabriotop (metiram + pyraclostrobin)	0.1	4.7 (12.4)	85.3	5.5 (13.0)	87.1
Quintal (carbendazim+iprodione)	0.15	4.8 (12.6)	84.9	5.8 (13.5)	86.5
Sanit (metiram)	0.25	5.1 (12.8)	84.2	6.2 (14.0)	85.4
Contaf (hexaconazole)	0.05	6.0 (13.8)	81.2	7.6 (15.3)	82.1
Score (difenoconazole)	0.02	4.8 (12.4)	85.0	6.0 (13.8)	85.9
Ergon (kresoxim methyl)	0.05	14.7 (22.2)	54.3	18.2 (24.8)	57.1
Tilt (propiconazole)	0.05	3.2 (10.2)	89.9	3.9 (11.1)	90.7
Governor (flusilazole)	0.01	5.4 (13.5)	83.1	6.9 (14.8)	83.9
Control		32.2 (34.3)		42.5 (40.6)	
$CD_{0.05}$		2.549		0.833	

Figures in parentheses are arc sin transformed values

pre-harvest sprays with carbendazim among systemic fungicide and Indofil M-45 among non-systemics were most effective against *C granati* in pomegranate.

CONCLUSION

From the foregoing results it is concluded that pomegranate is highly susceptible to leaf spot and fruit rot caused by *Coniella granati*. Survey of different pomegranate growing areas of Himachal Pradesh revealed that the disease severity varied from 11.8 to 25.1 per cent on leaves and 12.6 to 39.8 per cent on fruits. Sprays with Tilt, Benfil, Governor, Taqat and Captan provided reasonably good control of the disease under natural field conditions. Therefore they can be recommended to pomegranate growers for controlling the disease.

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