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In vitro efficacy of fungicides, botanicals and bio-control agents against pomegranate scab caused by *Sphaceloma punicae*

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Abstract
Pomegranate is regarded as a fruit of paradise but the scab disease is spreading in pomegranate field and damage the crop, there is a lack of information on management of scab of pomegranate. Fungicides *viz*., thiophanate methyl, carbendazim, tebuconazole and propiconazole showed hundred per cent mycelial growth inhibition at 250, 500 and 1000 ppm. However, difenoconazole at 1000 ppm concentration showed hundred per cent inhibition of *S. punicae*. Among the contact fungicides, propineb and mancozeb were showed hundred per cent mycelial growth inhibition at 1000 ppm concentration. Among combi-products maximum inhibition of mycelial growth (100 %) was noticed in case of azoxystrobin + mancozeb, hexaconazole + zineb, fenamidone + mancozeb and carbendazim + mancozeb at all the three concentration (250, 500 and 1000 ppm) tested. Among the nine plant extracts evaluated, maximum fungal growth inhibition (73.63 %) was observed in garlic extract, which was significantly superior over all other botanicals tested. Among the fungal bio-agents tested, *Trichoderma harzianum* (Th-41) showed 83.15 per cent inhibition of the pathogen. Similarly, among the bacterial bio-agents tested, maximum inhibition was observed in *Bacillus amyloliquefaciens* (64.60 %).

Keywords: Scab, *Sphaceloma punicae*, bio-agents, botanicals

Introduction
Pomegranate is regarded as a fruit of paradise is one among the major fruit crops of arid zone. In India, it is regarded as a "vital cash crop", grown in an area of 1.93 lakh ha with the production of 17.73 lakh tons during 2014-15 (Anon., 2015) [1]. Among the different states growing pomegranate, Maharashtra is the largest producer occupying 2/3rd of total area in the country followed by Karnataka, Andhra Pradesh, Gujarat and Rajasthan. Karnataka accounts 19,040 ha area and 2.04 lakh tons production with an average productivity of 10.74 ha^{-1} in 2014-15 (Anon., 2015) [1].
The scab disease is spreading in pomegranate field and damage the crop. In recent years, there has been a major thrust on pesticide residue free organic pomegranate production. Taking the task into consideration, efficient botanicals and bio-agents need to be explored to fit into the management schedule and also there is a lack of information on management of scab of pomegranate through fungicides, but there is large number of chemicals available in the market as fungicides and their bio-efficacy and suitability was evaluated.

Material and Methods
In vitro evaluation of fungicides
The poisoned food technique was used to evaluate the efficacy of four contact, seven systemic fungicides and five combi-products were tested against *S. punicae* under *in vitro* condition. The fungicides were evaluated at three concentrations *viz*., 250, 500 and 1000 ppm. The required quantity of fungicides was added to sterilized potato dextrose medium so as to get desired concentration. Twenty ml of poisoned medium was poured to sterilized petri dishes. The test fungal disc of five mm was taken from actively growing culture and was placed on center of Petri plate. The control plate was maintained without any fungicides. Each treatment was replicated for three times. These plates were incubated at room temperature till the fungal growth reached to periphery of Petri plate in case of control plate and at the same time the colony diameter of test fungus was recorded in treatment plates. The efficacy of different fungicides was expressed as per cent inhibition of mycelial growth over control was calculated by using the formula suggested by Vincent (1947) [8].

In vitro* evaluation of botanicals against *S. punicae

The efficiency of plant extract or botanicals was tested against *S. punicae* on PDA medium by using poisoned food technique. For this, fresh plant parts (leaves/bulb) of 100 g of each as mentioned below were collected, washed with tap water and then distilled water. The fresh sample was chopped and crushed by adding sterile water of 100 ml. The crushed product was filtered through muslin cloth. The filtrate solution gave 100 per cent and which was used as stock solution. Five, ten and fifteen ml of stock solution was mixed with 95, 90 and 85 ml of PDA medium and then it was shaken for uniform mixing of plant extract. Later, the media was sterilized and allowed it to cool. Twenty ml of medium was poured into sterilized Petri plates and then fungal disc of five mm was placed at the center of Petri plate and then such plates were incubated at 27±1 ºC. The control plate was maintained on PDA medium without any plant extract. The radial growth of fungus was recorded in treatment plates when colony growth reached periphery in control plate. The per cent inhibition of mycelial growth of test fungus was calculated by using following formula suggested by Vincent (1947) [8].

In vitro* evaluation of bio-agents against *S. punicae

Six fungal bio agents and seven bacterial bio agents (Table 2) were tested against *S. punicae* by dual culture technique. In order to get fresh and active growing bio agents as well as test organism, these were cultured on potato dextrose agar medium.

Sterilized and cooled potato dextrose agar medium of twenty mL was poured into sterilized Petri plates. After solidification, the mycelial disc of test fungus was inoculated at one end of Petri plate and antagonistic fungus was placed opposite to it on the other end where as in case of bacterial bio-agents, antagonistic bacteria was streaked at one end of the petri plate and the test fungus placed at the opposite end. A control plate was also maintained where in test fungal disc was placed at the center of medium without any bio agents. Each treatment was replicated for three times and incubated at 27±1 ºC. The observation in treatment plates were recorded when fungal growth reaches periphery of Petri plate in the control plate. The inhibition zone between test organism and antagonistic microorganism was measured and compared with control. The per cent inhibition of growth of the pathogen was calculated by using the formula suggested by Vincent (1947) [8].

Gopal *et al*. (2014) [2] reported that benomyl, carbendazim, thiophanate methyl, trifloxystrobin, azoxystrobin, trifloxystrobin, and pyraclostrobin plus oil were the best chemicals for scab control. *Elsinoe fawcetti* was effectively controlled by spraying of copper-based fungicides like Macuprax (0.3%) or Burcop (0.3%).

Results and Discussion

Evaluation of systemic fungicides against S. punicae

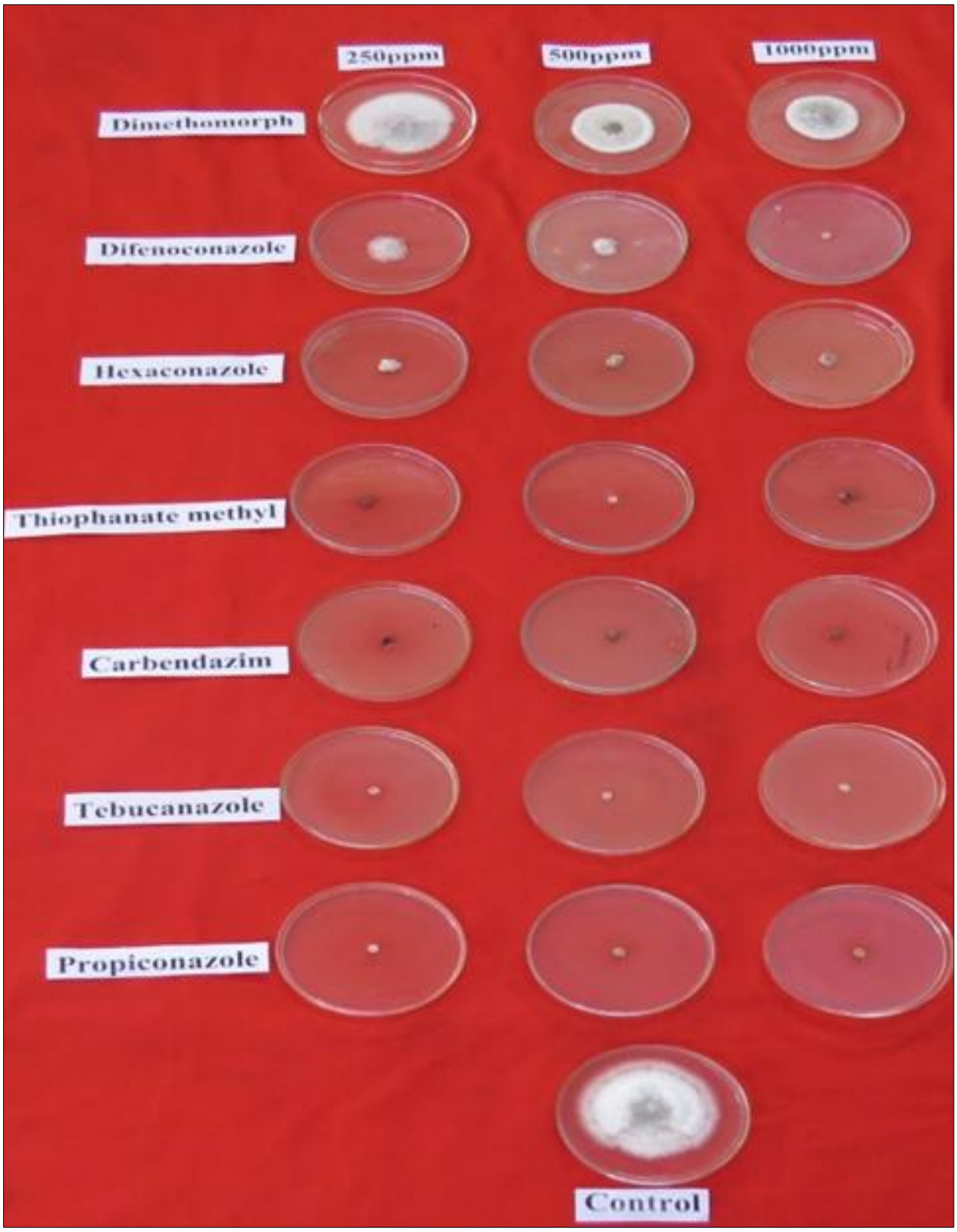
Seven systemic fungicides were tested at three different concentrations (250, 500 and 1000 ppm) for their efficacy in inhibition of *S. punicae*. The inhibition of mycelial growth of *S. punicae* was recorded and per cent inhibition was calculated and presented in Table 1 and Plate 1

Among seven systemic fungicides were tested, hundred per cent inhibition of *S. punicae* was recorded in thiophanate methyl, carbendazim, tebuconazole and propiconazole. Out of the three different concentrations (250, 500 and 1000 ppm) tested, 1000 ppm resulted in significantly highest inhibition of 92.35 per cent followed by 500 ppm and 250 ppm with 88.52 and 84.14 per cent inhibition respectively.

Table 1: *In vitro* evaluation of systemic fungicides against *S. punicae*

Sl. No.	Fungicides	Per cent inhibition over control			
		Concentration (ppm)			
	Common name	250	500	1000	Mean
1.	Dimethomorph	18.42 (25.4)*	42.68 (40.79)	53.7 (47.13)	38.26 (37.77)
2.	Thiophanate methyl	100 (90.00)	100 (90.00)	100(90.00)	100(90.00)
3.	Carbendazim	100(90.00)	100(90.00)	100(90.00)	100(90.00)
4.	Hexaconazole	89.36(70.97)	91.47(73.02)	92.82(74.46)	91.21(72.82)
5.	Tebuconazole	100(90.00)	100(90.00)	100(90.00)	100(90.00)
6.	Difenoconazole	81.26(64.35)	85.56(67.67)	100(90.00)	88.94(74.00)
7.	Propiconazole	100(90.00)	100(90.00)	100(90.00)	100(90.00)
	Mean	84.14 (74.38)	88.52 (77.34)	92.35 (81.64)	
		Fungicides (F)	Concentration (C)	Interactions (F×C)	
	S.Em ±	0.57	0.38	0.99	
	C.D. @ 1%	1.15	0.75	1.99	

* The values in the parenthesis are arcsine transformed values



T1. Dimethomorph, T2. Difenoconazole, T3. Hexaconazole, T4. Thiophanate methyl T5. Carbendazim, T6. Tebuconazole, T7. Propiconazole

Plate 1: *In vitro* evaluation of systemic fungicides against *S. punicae*

In vitro* evaluation of contact and combi-product fungicides against *S. punicae

The inhibition of mycelial growth of *S. punicae* by four contact and five combi-product fungicides at three different concentrations (250, 500 and 1000 ppm) was recorded and presented in Table 2, Plate 2a and 2b.

Among four contact fungicides tested, highest mycelial growth inhibition was observed in propineb (57.41 %) and least in chlorothalonil (32.03 %). Among seven combi-products tested, hundred per cent inhibition of *S. punicae* was recorded in azoxystrobin + mancozeb, hexaconazole + zineb, fenamidone + mancozeb and carbendazim + mancozeb whereas, least observed in hexaconazole + captan (72.46 %). Out of the three different concentrations (250, 500 and 1000 ppm) tested, 1000 ppm resulted in significantly highest inhibition of 88.28 per cent followed by 500 ppm and 250 ppm with 69.50 and 63.56 per cent inhibition respectively.

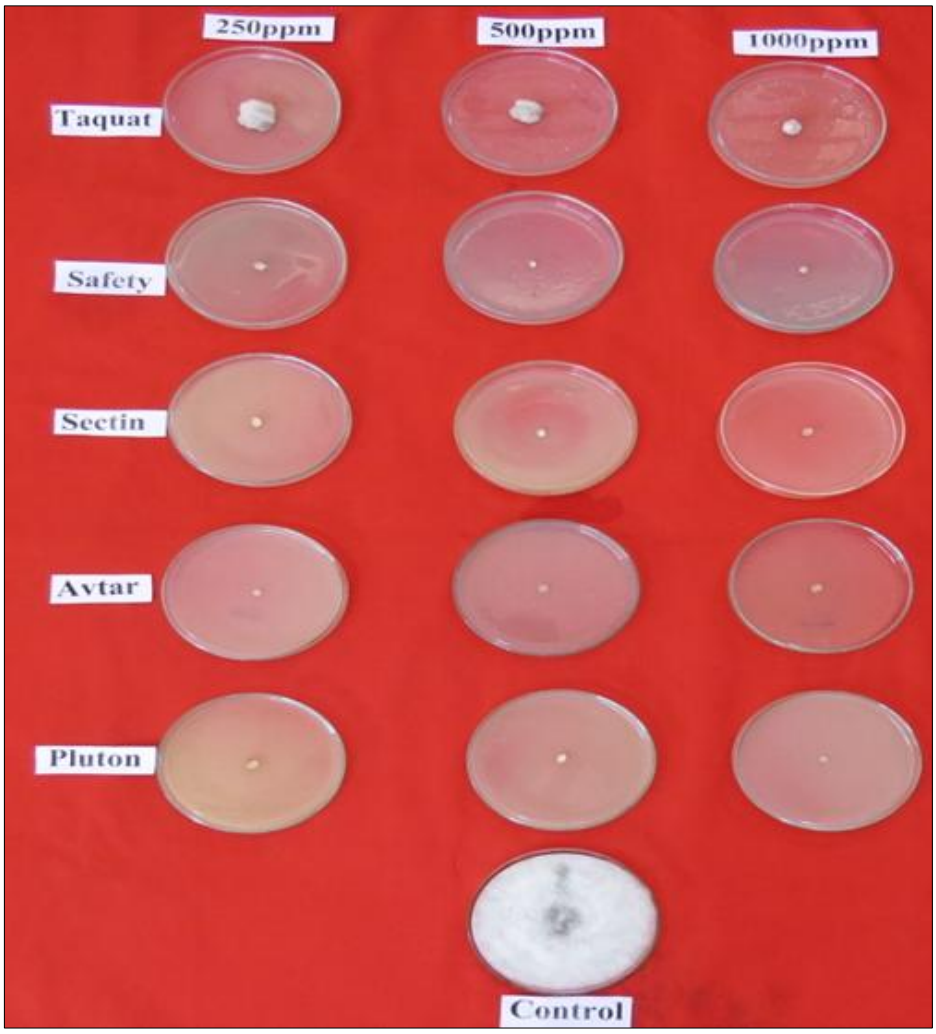
Table 2: *In vitro* evaluation of contact and combi-product fungicides against *S. punicae*

Sl. No.	Fungicides	Percent inhibition			
		Concentration (ppm)			
	Common name	250	500	1000	Mean
Contact fungicides					
1	Chlorothalonil	18.58(25.53)*	32.14(34.53)	45.39(42.35)	32.03(34.13)
2	Captan	41.74(40.25)	43.94(41.52)	62.56(52.28)	49.41(44.68)
3	Propineb	32.65(34.85)	39.59(38.99)	100(90.00)	57.41(54.61)
4	Mancozeb	23.08(28.71)	35.27(36.43)	100(90.00)	52.78(51.71)
Combi-product fungicides					
5	Azoxystrobin 11.5% + Mancozeb 30% WP	100(90.00)	100(90.00)	100(90.00)	100(90.00)
6	Hexaconazole 4%+ Zineb 68% WP	100(90.00)	100(90.00)	100(90.00)	100(90.00)
7	Fenamidone 10% + Mancozeb 50% WG	100(90.00)	100(90.00)	100(90.00)	100(90.00)
8	Hexaconazole 5%+ Captan 70% WP	56.03(48.47)	74.65(59.79)	86.70(68.63)	72.46(58.96)
9	Carbendazim 12% + Mancozeb 63% WP	100(90.00)	100(90.00)	100(90.00)	100(90.00)
	Mean	63.56(59.75)	69.50(63.46)	88.28(78.13)	
			Fungicides (F)	Concentration (C)	Interactions (F×C)
	S.Em ±		0.39	0.22	0.67
	CD at 1%		0.76	0.44	1.32

* The values in the parenthesis are arcsine transformed values

T1. Chlorothalonil, T2. Captan,
T3. Propineb, T4. Mancozeb

Plate 2a: *In vitro* evaluation of contact fungicides against *S. punicae*



T1. Hexaconazole + Captan, T2. Carbendazim + Mancozeb, T3. Fenamidon + Mancozeb, T4. Zineb + Hexaconazole, T5. Azoxystrobin + Mancozeb

Plate 2b: *In vitro* evaluation of combi-product fungicides against *S. punicae*

In vitro* evaluation of botanicals against *S. punicae

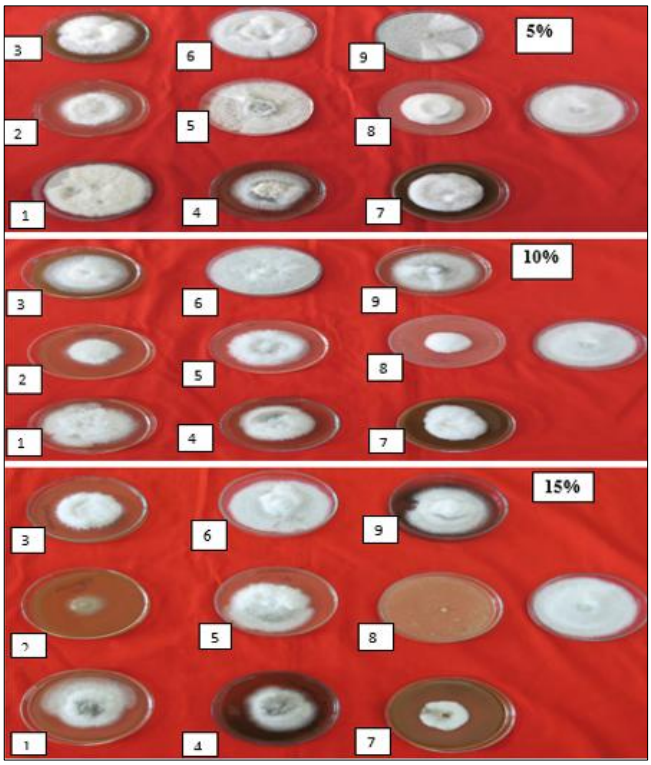
Nine botanical extracts were tested at three different concentrations for their efficacy in inhibition of *S. punicae* by poison food technique under *in vitro* condition. The zone of inhibition was recorded and per cent inhibition of growth of fungus was calculated and the results are presented in Table 3 and Plate 3

Among the nine plant extracts, maximum per cent inhibition of mycelial growth was observed in garlic (73.63 %). The other botanicals showed inhibition ranging from 3.83 to 50.89 per cent and least observed in onion (3.83 %). Among the three different concentrations (5, 10 and 15 %) tested, 15 per cent concentration resulted in significantly highest inhibition of 44.81 per cent followed by 10 and 5 per cent with 31.67 and 20.67 per cent inhibition, respectively.

Table 3: *In vitro* evaluation of botanicals against *S. punicae*

Sl. No	Botanicals	Per cent inhibition over control			
		5 Per cent	10 Per cent	15 Per cent	Mean
1	Jatropa	14.49(22.32) *	30.37(33.43)	35.69(36.69)	26.85(30.80)
2	Glyricidia	29.79(33.08)	52.37(46.36)	70.51(57.12)	50.89(45.52)
3	Simarouba	22.75(28.48)	24.99(29.98)	44.61(41.91)	30.78(33.45)
4	Pongamia	27.56(31.67)	29.45(32.87)	44.67(41.94)	33.89(35.49)
5	Subabul	0.00(0.00)	20.83(27.15)	26.05(30.68)	15.62(19.27)
6	Onion	0.00(0.00)	0.00(0.00)	11.51(19.81)	3.83(6.60)
7	Neem	33.30(35.24)	50.70(45.40)	53.34(46.92)	45.78(42.52)
8	Garlic	58.26(49.76)	62.64(52.33)	100(90.00)	73.63(64.03)
9	Lantana	0.00(0.00)	13.77(21.72)	16.92(24.29)	10.23(15.33)
	Mean	20.67(22.27)	31.67(32.13)	44.81(43.25)	
		Botanicals (B)	Concentration(C)	Interactions (B×C)	
S.Em ±		0.85	0.49	1.47	
CD at 1%		1.67	0.96	2.89	

* The values in the parenthesis are arcsine transformed values



1. Jatropa, 2. Glyricidia, 3. Simarouba, 4. Pongamia, 5. Subabul, 6. Onion, 7. Neem, 8. Garlic, 9. Lantana

Plate 3: *In vitro* evaluation of botanicals against *S. punicae*

In vitro evaluation of bio-agents against *S. punicae*

Efficacy of bacterial and fungal bio-agents was studied under *in vitro* condition by dual culture method and the results are presented in Table 4, Plate 4a and 4b.

There was a significant difference among tested bio-agents. *Trichoderma harzianum* and *T. viride* isolates *viz.*, Th-14, Th-41, Th-55, Th-B_5, Th-B_2 and Tv-1 were tested against *S. punicae*. Among these fungal bio-agents, Th-14 showed 83.15 per cent inhibition, which was significantly superior over other bio-agents, followed by Th-41 (78.85 %) isolate. The least inhibition was recorded in Th-B_2 (56.28 %). Among the seven bacterial bio-agents tested, maximum inhibition was recorded in *Bacillus amyloliquefaciens* (64.60 %) and least inhibition was recorded in *B. pumillus* (21.50 %).

Table 4: *In vitro* evaluation of bio-agents against *S. punicae*

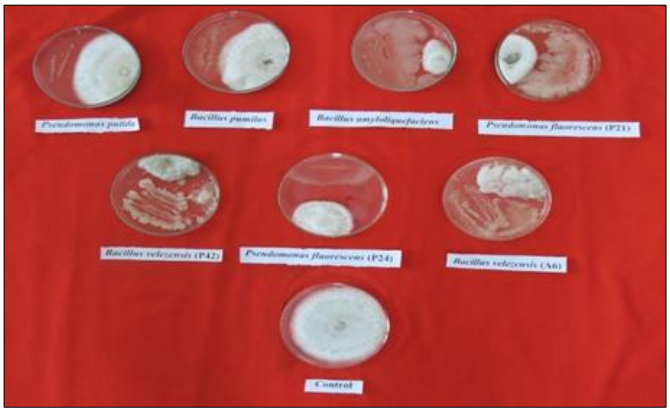
Sl. No.	Bio agents	Per cent inhibition #
1	*Trichoderma harzianum* (Th-14)	83.15 (65.77)*
2	*T. harzianum* (Th-41)	78.85 (62.62)
3	*T. harzianum* (Th-55)	66.19 (54.44)
4	*T. harzianum* (Th-B5)	75.60 (60.39)
5	*T. harzianum* (Th-B_2)	56.28 (48.60)
6	*T. viride* (Tv-1)	67.51 (55.25)
7	*P. putida*	25.42 (30.27)
8	*B. pumillus*	21.50 (27.62)
9	*B. amyloliquefaciens*	64.60 (53.48)
10	*P. fluorescens* (P24)	49.62 (44.78)
11	*P. fluorescens* (P21)	44.49 (41.83)
12	*B. velezensis* (42)	59.14 (50.26)
13	*B. velezensis* (A6)	41.34 (40.01)
	Mean	56.43(48.87)
	S.Em ±	0.32
	CD at 1%	1.25

Mean of two replication

*The values in the parenthesis are arcsine transformed values

1. Th-41, 2. Th-14, 3. Tv1, 4. Th-55, 5. Th-B2, 6. Th-B5

Plate 4a: *In vitro* evaluation of fungal bio-agents against *S. punicae*



1. *P. putida*, 2. *B. pumilus*, 3. *B. amyloliquefaciens*, 4. *P. fluorescens* (P21), 5. *B. velezensis* (P42), 6. *P. fluorescens* (P24), 7. *B. velezensis* (A6)

Plate 4b: *In vitro* evaluation of bacterial bio-agents against *S. punicae*

Discussion

Pomegranate is an important commercial fruit crop of India, it is affected by many diseases caused by various pathogens, resulted in poor quality and quantity of fruits. The present study was carried out with reference to find effective management strategies for control of the scab of pomegranate. The systemic fungicides *viz.*, thiophanate methyl, carbendazim, tebuconazole and propiconazole showed hundred per cent mycelial growth inhibition at 250, 500 and 1000 ppm. However, difenoconazole at 1000 ppm concentration showed hundred per cent inhibition of *S. punicae* results were similar with Minutolo *et al.* (2016) [4]. Among the contact fungicides, propineb and mancozeb were showed hundred per cent mycelial growth inhibition at 1000 ppm concentration, among combi-products maximum inhibition of mycelial growth (100 %) was noticed in case of azoxystrobin + mancozeb, hexaconazole + zineb, fenamidone + mancozeb and carbendazim + mancozeb at all the three concentration (250, 500 and 1000 ppm) tested results were similar with Sharma (2010) [6]. Among the nine plant extracts evaluated, maximum fungal growth inhibition (73.63 %) was observed in garlic extract, which was significantly superior over all other botanicals tested results were similar with Gopal *et al.*, (2014) [2]. Among the fungal bio-agents tested, *Trichoderma harzianum* (Th-41) showed 83.15 per cent inhibition of the pathogen. Similarly among the bacterial bio-agents tested, maximum inhibition was observed in *Bacillus amyloliquefaciens* (64.60%) results were similar with Sumartini, 2014 [7].

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