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COMPATIBILITY OF NATIVE POTENTIAL ANTAGONISTS WITH FUNGICIDES AND ESSENTIAL OILS AGAINST *CORTICIUM SALMONICOLOR*, BERK & BR.THE INCITANT OF PINK CANKER IN APPLE

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ABSTRACT
Seventeen native antagonists of fungal, bacterial and actinomycetes origin were isolated from phylloplane of pink canker apple branches collected from different regions of Himachal Pradesh. There *in vitro* evaluation revealed that seven antagonists comprising four fungal, two bacterial and one actinomycetes origin were quite effective against the pink canker (*Corticium salmonicolor*). Further compatibility studies of these antagonists with effective fungicides and plant oils indicated that five antagonist's viz., *T. harzianum*, *T. viride*. *Pseudomonas* sp. *B. subtilis* and *Actinomycetes* were compatible with commonly recommended fungicide viz., zineb + hexaconazole (Avtar). Another effective fungicide hexaconazole (0.05%) was compatible with *T. hamatum* but incompatible with *T. harzianum* (86.10%), *T. koningi* (42.57%) and *T. viride* (17.34%). Azoxystrobin (0.05%) was compatible with *T. koningi, T. harzianum, Pseudomonas* sp. *B. subtilis* and *Actinomycetes* sp. Among effective plant oils three viz., *Brassica juncea* var. *cunefolia, Olea europea* and *Azadirachta indica* @0.05 per cent were compatible with all effective antagonists under *in vitro* conditions. These potential native antagonists can further be incorporated in IDM programme to develop suitable management strategies to combat pink canker in apple.

INTRODUCTION

Apple (*Malus x domestica* Borkh.) is one of the most important cash crops of hilly states of India including Himachal Pradesh with an annual productivity of 7-9 tonnes per hectare. It is quite low in comparison to that of other apple growing countries (30-35 tonnes/ ha) like China, New Zealand, US and European countries (Sharma, 2014). Out of various reasons attributed to low productivity, diseases are one of the most important contributing factors. Among diseases, *Corticium salmonicolor* is most predominant and destructive and has emerged as one of the major constraint in its successful cultivation. It occurs in moderate to severe form (9.88 - 51.84 %) in different regions of Himachal Pradesh (Prashad, 2013). Pink canker causes blight, die-back or cankerous symptoms on twigs and branches leading to untimely death of the entire tree (Gupta and Agarwala, 1973; Sharma and Bhardwaj, 2002; Sharma and Ram, 2010). Though some fungicides have been recommended to manage this disease but these are cost prohibitive, environmental hazardous and moreover results in development of resistance strains of pathogen. Now-a-days biocontrol agents, essential oils and products of plant origin have been recognized to possess antibacterial and antifungal compounds to mange different diseases; however scanty information on their inter compatibility as well as with effective fungicides is available (Bagwan, 2010). These non-chemical methods to control plant pathogens are the most important alternatives over chemicals and can be exploited within the frame work of integrated disease management. The present study is therefore undertaken to isolate and identify native potential antagonists of *C. salmonicolor*. Further, potential biocontrol agents are evaluated for their compatibility with already tested fungicides and essential oils (Prashad, 2013), so as to identify the effective and economic management inputs to develop integrated disease management practice to manage the disease.

MATERIALS AND METHODS

Isolation and identification of potential bioagents against *C. salmonicolor*

The microflora (fungi, bacteria and actinomycetes) that were isolated from the infected as well as uninfected branches and twigs of apple were subjected to identification keys based on the cultural and morphological characters (Gilman, 1957; Rifai, 1969 and Sarbhoy *et al.*, 1975). Bacterial and actinomycetes were identified based on morphological and

cultural characters (Schaad, 1980). Fungal, identification of potential bioagents was also confirmed by sending thecultures to National Centre for Fungal Taxonomy (NCFT) New Delhi and effective bacterial antagonists were identified by their molecular characterization.

Evaluation of native potential antagonists against *C. salmonicolor*

Seventeen antagonists including fungi viz., Tv_1 (Mandi), Tv_2 (Kotgarh)and Tv_3 (Haripurdhar) isolates of *Trichoderma viride;* TH_1 (Kotgarh) and TH_2 (Mandi) of *Trichoderma harzianum, Trichoderma koningi,* Th_1 (Kullu) and Th_2 (Haripurdhar) isolates of *Trichoderma hamatum, Aspergillus* sp.,*Verticillium* sp.P_1 (Kotgarh)of *Penicillium* sp., and *Aspergillus versicolor* and five bacteria *i.e.* BS_1 (Kotgarh), BS_2 (Kullu) and BS_3 (Mandi) of *Bacillus subtilis*, *Pseudomonas* sp. and *Pseudomonas aeruginosa* and an actinomycetes were isolated and tested under *in vitro* conditions by dual culture technique and streak plate method (Huang and Hoes, 1976; Utkhede and Rahe, 1983).

Culture bit of 5 mm size of each of the antagonist and pathogen taken from the margin of vigorously growing cultures were transferred aseptically to solidified PDA in Petriplates (90 mm) on opposite sides facing each other. The distance between the inoculation point of test pathogen and the antagonist was kept 5 cm apart for formation of inhibition zone, if any. The petriplates containing culture bits of pathogen alone served as control. Each treatment was replicated four times and incubated at 25 ± 1ºC. The colony diameter of the pathogen was recorded after 7 days of incubationperiod when plates were filled with fungal growth and expressed as percent inhibition after comparison with control which was calculated according to the formula given by Vincent (1947).

In case of bacteria and actinomycetes, the antagonistic activity was studied by streak plate method (Utkhede and Rahe, 1983).The petriplates containing sterilized solidified nutrient agar medium were streaked with bacterial and actinomycetes both sides of the 5 mm size culture bit of the pathogen placed in the centre of the plates with 48 hours old colonies of bacteria as well as actinomycetes., only pathogen was inoculated and replications remained the same in each case as discussed above. Data on zone of inhibition as well as the per cent inhibition of the radial growth of fungus, if any was calculated (Vincent 1947).

Compatibility of fungicides with biocontrol agents

Compatibility of fungicides viz., Amistar (azoxystrobin), Score (difenaconazole), Contaf (hexaconazole), Tilt (propiconazole), Punch (flusilazole), Bavistin (carbendazim), Saaf (carbendazim + mancozeb), Quintal (carbendazim + iprodione), Taqat (contaf + captan), Cabrio Top (pyraclostrobin + metiram) and Avtar (zineb + hexaconazole)were evaluated at their recommended dose for their compatibility with effective biocontrol agents under *in vitro* conditions following poisoned food technique (Nene and Thapliyal, 2011). A double strength potato dextrose agar (PDA) medium was prepared by doubling the amount of constituents except water. PDA was sterilized at15 lb pressurepsi for 20 minutes in an autoclave. An equal amount of the test double strength fungicide was added separately in double strength medium in different flasks, shaken well, aseptically poured in Petri plates separately and were allowed to solidify. A small culture bit of 5 mm size of antagonists was cut with a sterile cork borer and picked up with the help of a sterile inoculating needle and was placed in the centre of each Petriplates under aseptic conditions in a laminar air flow chamber. Petri plates without fungicide in the medium served as control. Whereas, bacterial antagonists were evaluated for their compatibility with antagonists by streaking them on nutrient agar amended with fungicides. A bacterial antagonist that grows on the medium considered compatible. Each concentration of fungicides was replicated three times and the Petriplates were incubated at $25 + 1$ºC for the period of 7 days until the mycelial growth in control fully covered the medium in Petriplates.

Compatibility of plant oils with biocontrol agents under *in vitro* condition

Compatibility of plant oils viz., *Brassica junceavar. cunefolia,Ocimum sanctum,Azadirachta indica, Cymbopogon citratus,Eugenia caryophyllata*and *Olea europea*were evaluated at 5 per cent concentration to know their compatibility with effective biocontrol agents using poisoned food technique. A double strength potato dextrose agar (PDA) medium was prepared by doubling the amount of constituents except water. PDA was sterilized at15 lb pressurepsi for 20 minutes in an autoclave. An equal amount of the test double strength plant oil was added separately in double strength medium in different flasks, shaken well, aseptically poured in Petri plate separately and were allowed to solidify. In order to dissolve plant oil in medium, small amount of solvent *i.e.* ethyl alcohol (10 ml) was added in the medium.Small culture bit of 5 mm size of antagonists was cut with a sterile cork borer and picked up with the help of a sterile inoculating needle and were placed in the centre of each Petriplates under aseptic conditions in a laminar air flow chamber. Petri plates without plant oils in the mediumserved as control. Whereas, bacterial antagonists were evaluated for their compatibility with antagonists by streaking them on nutrient agar amended with plant oils. Each concentration of plant oils was replicated thrice and the Petriplates were incubated at $25 + 1$ºC for the period of 7 days until the mycelial growth in control fully covered the medium in petriplates.

Statistical analysis

Data recorded for percent mycelial growth inhibition were calculated for each experiment. Later data were subjected to statistical analysis following the method of variance described by Gomez and Gomez (1983). Least significant difference (LSD) at 5% level was calculated to determine significant differences between treatments.

RESULTS AND DISCUSSION

Isolation and identification of native potential antagonists

Major four potential *Trichoderma* sp. were isolated from cankerous portion of apple twigs or branches and were identified as *Trichoderma koningi* with yellowish colony, dull to dark green long slender, without sterile hyphae, phialides are ellipsoidal, conidiophores in compact tuft. Conidiophores of *T. hamatum* appear as whitish green, long thick, often sterile hyphae, phialospores small green to large in size. Whereas Conidiophores of *T. viride* seems yellowish growth with

Table 1: *In vitro* efficacy of native biocontrol agents against *C. salmonicolor*

Antagonist	Mycelial growth (mm)	Growth inhibition (%)	Zone of inhibition (mm)
Trichoderma viride (TV_1)	27.90	76.00 (60.65)	-
Trichoderma koningi	13.00	82.62 (65.33)	-
Trichoderma harzianum (TH_1)	27.50	63.47 (52.79)	-
Trichoderma harzianum (TH_2)	18.50	75.31 (60.19)	-
Trichoderma hamatum (Th_1)	16.50	78.02 (62.06)	-
Trichoderma hamatum (Th_2)	17.16	77.15 (61.50)	-
Bacillus subtilis (BS_1)	36.05	51.95 (46.10)	12.30
Trichoderma viride (TV_2)	19.70	73.82 (59.21)	-
Trichoderma viride (TV_3)	18.00	62.82 (52.41)	-
Pseudomonas sp.	33.66	55.17 (47.95)	15.70
Verticillium sp.	33.96	54.72 (47.69)	8.40
Bacillus subtilis (BS_3)	35.44	52.75 (46.56)	7.10
Actinomycetes sp.	18.33	75.58 (60.36)	14.20
Penicilluim sp. (P_1)	40.33	46.21 (42.81)	-
Bacillus subtilis (BS_2)	40.83	45.56 (42.43)	6.61
Pseudomonas aeruginosa	31.16	58.44 (49.86)	9.83
Aspergillus versicolor	44.35	40.92 (39.74)	13.40
Control	75.00	0.00 (0.00)	75.00
$CD_{0.05}$		2.400	

compact tuft, side branches long and slender, phialides not crowded.However, conidiophores of *T. harzianum* were long and slender, phialides not crowded and globose. Further, the identity of *Trichoderma* sp. was confirmed from NCFT, New Delhi and assigned ID no. as *Trichoderma koningi* (ID no-5216.12), *Trichoderma hamatum* (ID no-5213.12), *Trichoderma harzianum* (ID no-5212.12), *Trichoderma viride* (ID no-5214.12) whereas, new sp. of *Aspergillus i.e. Aspergillus versicolor* was also isolated and identified as white to yellow tan, pale green or pink colour colony surface. Based on morphological and cultural studies, PGPRs were also identified. *Pseudomonas* sp.(BS_2) was identified as gram negative rod shaped bacteria, produces fluorescent pigment (blue), grows on King's (Proteose peptone ,3 (Difco) - 20g, $K_2HPO_4.3H_2O$ – 1.5 g, $MgSO_4.7H_2O$ – 1.5 g, Agar -15 g, Glycerol– 15 mL), nutrient agar ((Beef extract- 3 g , peptone- 5 g, NaCl-5 g, Agar - agar-15-20 g, distilled water - 1000 ml) medium within 24hr whereas, *Pseudomonas aeruginosa* (BS_3)was identified as gram - ve rod shaped, produces chocolate brown pigment, growth within 24hr at 30 °C. *Bacillus* sp. was identified as gram +ve rodshaped, creamy white/light brown colony colour, mucoid, grows on NA/ PDA medium within 24h, anaerobic (Schaad, 1980).

Molecular analysis and amplification of 16sDNA of *Bacillus* sp. was achieved by isolating DNA and was further amplified by PCR. Phylogenetic trees were constructed for each bacterium.There were three clusters viz., cluster I, II and III. Cluster I is further subdivided in to cluster I. a and I. b. Cluster I. a. consisted of *Bacillus subtilis,* Kotgarh isolate (BS_1) and JQ927217 *Bacillus subtilis* strain AZ01, whereas cluster I. b. consisted of only AB734701*Bacillus subtilis*. However cluster II and III consisted of HM154526*Bacillus subtilis* and HE17022*Bacillus subtilis*aprN which were found distant relatives of Kotgarh isolate of*Bacillus subtilis* (Fig. 1).

The phylogenetic analysis and sequence comparison of BS_2 isolate revealed its identity as*Pseudomonas* sp., The BS_2sequence had been submitted to NCBI with its GenBank Accession number KF564924. Based on phylogenetic analysis, three clusters were constructed, cluster i., consisted of only DQ095204 *Pseudomonas fluorescens*whereas, GQ228659 uncultured *Pseudomonas* sp. in cluster ii. BS_2 isolate of bacterium was closest to GQ228659 uncultured *Pseudomonas* sp. in cluster ii with highest sequence similarities. Cluster iii. consisted of CT573326*Pseudomonas entomophila* and further sub-divided in to cluster iii a and b. CP003588 *Pseudomonas putida* ND and CP000712 *Pseudomonas putida* F1 grouped in cluster iii .a and b., which were distant relative to Kullu isolate of *Pseudomonas* sp.

Antagonistic activity of native potential bioagents by dual culture technique

The perusal of data (Table 1.) revealed that all biocontrol agents either of fungal, bacterial and actinomycetes in origin inhibited the mycelial growth of C. *salmonicolor* to various extents over control.However, maximum inhibition of mycelial growth was observed in *Trichoderma koningi* (82.62 %) followed by Th_1 of *T. hamatum* (78.02 %) and Th_2 of *T. hamatum* (77.15 %) which were statistically at par with each other.All other isolates of *Trichoderma* sp. inhibited the growth of *C. salmonicolor* to greater extent in comparison to other fungal flora while minimum per cent inhibition was recorded with *Aspergillus versicolor* (40.92%).Amongst bacterial and actinomycetes sp. *Pseudomonas* sp. provided maximum inhibition zone (15.70 mm) followed by *Actinomycetes* sp. (14.20 mm) which was statistically at par with each other. Among *Bacillus* sp.BS_1 (Kotgarh) isolate of *Bacillus subtilis* caused maximum inhibition zone of 12.3 mm as compared to others. Amongst fungal biocontrol agents only *Aspergillus versicolor* and *Verticillium* sp. provided maximum inhibition zone of 13.4 and 8.40 mm respectively. These results are in consonance with the findings of Jollands (1983) who found that *Trichoderma* spp. were antagonistic to *C. salmonicolor* infecting rubber and oil palm. Jansen (2005) noticed parasitic fungi (*Gliocladium* spp., *Trichoderma* spp., and *Verticillium* sp.) showed antagonistic properties against *C. salmonicolor* affecting coffee production. Similarly, Sharma (2005) reported maximum (92.50%) wound healing of apple trees when treated with *Trichoderma viride*

Table 2: Compatibility of effective fungicides with biocontrol agents under *in vitro* conditions

Fungicide	Conc.(%)	Mycelial Growth Inhibition (%)								Mean
		Trichoderma harzianum	*Trichoderma viride*	*Trichoderma koningi*	*Trichoderma hamatum*	*Aspergillus versicolor*	*Pseudomonas* sp.	*Bacillus subtilis* (BS_1)	*Actinomycetes* sp.	
Avtar (zineb + hexaconazole)	0.25	0.00(0.00)	0.00(0.00)	49.41(44.60)	91.17(72.69)	65.68(54.13)	0.00(0.00)	0.00(0.00)	0.00(0.00)	25.78(21.43)
Saaf (carbendazim + mancozeb)	0.25	100(89.96)	100(89.96)	100(89.96)	100(89.96)	100(89.96)	0.00(0.00)	0(0.00)	0(0.00)	62.50(56.22)
Taqat (captan + hexaconazole)	0.20	80.00(63.41)	77.50(61.66)	70.81(58.15)	100.0(89.96)	100.0(89.96)	0.00(0.00)	0.00(0.00)	0.00(0.00)	53.54(45.39)
Cabrio Top (pyraclostrobin + metiram)	0.10	93.46(75.15)	84.55(66.83)	100.0(89.96)	100.0(89.96)	100.0(89.96)	0.00(0.00)	0.00(0.00)	0.00(0.00)	59.76(51.48)
Tilt (propiconazole)*	0.05	100.0(89.96)	35.11(36.32)	67.18(55.03)	96.61(79.37)	82.09(64.96)	0.00(0.00)	0.00(0.00)	0.00(0.00)	47.62(40.71)
Contaf (hexaconazole)	0.05	86.10(68.09)	17.34(24.59)	42.57(40.71)	0.00(0.00)	49.49(44.69)	0.00(0.00)	0.00(0.00)	0.00(0.00)	24.44(22.26)
Governor (flusilazol)	0.05	81.73(64.67)	31.40(34.06)	71.20(57.52)	29.20(32.70)	100.0(89.96)	0.00(0.00)	0.00(0.00)	0.00(0.00)	39.19(34.86)
Bavistin (carbendazim)	0.05	96.93(80.00)	100.0(89.96)	100.0(89.96)	100.0(89.96)	100.0(89.96)	0.00(0.00)	0.00(0.00)	0.00(0.00)	62.12(54.98)
Score (difenaconazole)	0.025	97.36(80.64)	24.46(29.62)	88.20(69.88)	35.19(36.37)	100.0(89.96)	0.00(0.00)	0.00(0.00)	0.00(0.00)	43.15(38.31)
Amistar (azoxystrobin)*	0.05	0.00(0.00)	39.79(39.09)	0.00(0.00)	39.78(39.08)	56.81(48.90)	0.00(0.00)	0.00(0.00)	0.00(0.00)	17.05(15.88)
Quintal (iprodione + carbendazim)	0.15	86.70(68.59)	97.96(81.78)	92.48(74.06)	100.0(89.96)	100.0(89.96)	0.00(0.00)	0.00(0.00)	0.00(0.00)	59.64(50.54)
Control	-	75(59.98)	75(59.98)	75(59.98)	75(59.98)	75(59.98)	75(59.98)	75(59.98)	75(59.98)	
Mean		74.77(61.86)	56.93(50.35)	71.40(60.89)	72.25(64.55)	45.58(39.80)	46.42(40.40)	6.25(5.00)	6.25(5.00)	
$CD_{0.05}$		Fungicides			1.011					
		Antagonists			0.862					
		Fungicides x Antagonists			2.859					

Table 3: Compatibility of *in vitro* effective plant oils with biocontrol agents

Plant oil	Conc.(%)	Mycelial Growth Inhibition (%)								Mean
		Trichoderma harzianum	*Trichoderma viride*	*Trichoderma koningi*	*Trichoderma hamatum*	*Aspergillus versicolor*	*Pseudomonas* sp.	*Bacillus subtilis* (BS_1)	*Actinomycetes* sp.	
Brassica juncea var. *cunefolia*Roxb.	0.05	0.000.00	0.000.00	0.000.00	0.000.00	0.000.00	0.000.00	0.000.00	0.000.00	0.000.00
Ocimum sanctum L.	0.05	0.000.00	0.000.00	0.000.00	0.000.00	45.29(42.28)	0.000.00	0.000.00	0.000.00	5.62(5.28)
Azadirachta indica A. Juss.	0.05	0.000.00	0.000.00	0.000.00	0.000.00	0.000.00	0.000.00	0.000.00	0.000.00	0.000.00
Cymbopogoncitratus (DC) Stapf.	0.05	89.57(71.18)	27.73(31.76)	96.55(79.32)	83.58(66.08)	90.29(71.84)	0.000.00	0.000.00	0.000.00	48.46(40.20)
Eugenia caryophyllata L.	0.05	0.000.00	14.10(22.04)	0.000.00	0.000.00	0.000.00	0.000.00	0.000.00	0.000.00	1.76(2.75)
Olea europea L.	0.05	0.000.00	0.000.00	0.000.00	0.000.00	0.000.00	0.000.00	0.000.00	0.000.00	0.000.00
Control	-	75(59.98)	75(59.98)	75(59.98)	75(59.98)	75(59.98)	75(59.98)	75(59.98)	75(59.98)	
$CD_{0.05}$		Essential oils			0.247					
		Antagonists			0.285					
		Oils x Antagonists			0.697					

followed by 87.50 and 85.00 per cent with *T. longibrachiatum* and *T. harzianum* respectively,whereas, 12.50 per cent was noticed in control.

Compatibility of native potential antagonists with different fungicides by poisoned food technique

Compatibility of commonly used fungicides viz., Amistar (azoxystrobin), Score (difenaconazole), Contaf (hexaconazole),Tilt (propiconazole), Punch (flusilazole), Bavistin (carbendazim), Saaf (carbendazim + mancozeb), Quintal (carbendazim+ iprodione), Taqat (contaf + captan), Cabrio Top (pyraclostrobin+ metiram) and Avtar (zineb + hexaconazole) revealed that fungicides viz., Avtar (zineb + hexaconazole) provided compatible reaction with *Trichoderma harzianum* (TH_1),*Trichoderma viride* (Tv_1),*Pseudomonas* sp., *Bacillus subtilis* (BS_1) and *actinomycetes* sp., when evaluated at 0.25 per cent, though inhibited mycelial growth of *T. koningi* (49.41%), *T. hamatum* (91.17%) and *Aspergillus versicolor* (65.68%) respectively. These results supports the findings of Gupta and Sharma (2004) who revealed *T. harzianum* exhibited compatible, moderately compatible and incompatible reactions with Captan, Vitavax and Bavistin respectively. Similarly, Basha *et al.* (2010) reported complete inhibition of *Trichoderma* spp. with carbendazim and propiconazole at 50 ppm and 25 ppm concentration while compatible with bacterial antagonist. Similar trend was also reported by earlier workers (Bhat and Srivastava, 2003). However, Strobilurin fungicide viz., Amistar (azoxystrobin) @ 0.025 per cent was found compatible with *T. harzianum* (TH_1),*T. koningi, Pseudomonas* sp.,*Bacillus subtilis* (BS_1)and *actinomycetes* sp., although inhibited mycelium of (Tv_1,Mandi) isolate of *T. viride* (39.79%),*T. hamatum*(39.78%) and *Aspergillus versicolor* (56.81%) respectively. These results supports the findings of Anand *et al.,* (2009) who reported *Pseudomonas fluorescens* (Pf1) to be compatible with azoxystrobin at different concentrations viz., 100, 150, 200, 250 and 300 ppm revealed that it was compatible with all the concentrations of azoxystrobin tested and the growth of the bacterium was unaffected even at the maximum concentration of 300ppm.

Combiproduct fungicides like, Saaf (carbendazim + mancozeb),Taqat (captan + hexaconazole),Cabrio Top (pyraclostrobin + metiram), Tilt (propiconazole),Contaf (hexaconazole), Governor (flusilazol), Bavistin (carbendazim), Score (difenaconazole) and Quintal (iprodione + carbendazim)at their recommended dose found compatible with bacterial and actinomycetes sp., but resulted significant mycelial inhibition of *Trichoderma* spp. Karunanithi and Usman (1999) reported that copper oxychloride supported the survival and competitive saprophytic ability of *T. viride* while captan and carbendazim caused marked reduction. Bavistin (carbendazim) either alone or in combination i.e. Saaf (carbendazim + mancozeb) exhibited cent per cent mycelial inhibition of *T. viride* (Tv_1), *T. koningi, T. hamatum* and *Aspergillus versicolor* except *T. harzianum* (96.93%).These results corroborate the findings of Chakrabarty *et al*., (2013) who showed Calixin, Bavistin and Mancozeb @0.3% completely inhibited the growth of *Trichoderma* spp. whereas Captan and Blitox-50 showed 61.67 and 41.89 per cent inhibition over control respectively. Basha *et al*. (2010) isolated bacteria from phylloplane of mango (PB_{28}) was more compatible with thiophanate-methyl (96.07%) and propiconazole (88.05%) at 50 ppm and 25 ppm respectively followed by mancozeb, carbendazim, copper oxychloride and propioconazole. However, *Trichoderma* sp. showed varying degree of compatibility with propiconazole and hexaconazole at 500 $^{\mu l}$/L. Pallavi *et al*. (2012) proved that *Pseudomonas* and *Bacillus* sp., showed higher antagonistic activity against grey blight pathogen were tolerant with selected fungicides (hexaconazole and carbendazim) under *in vitro* condition. The same result was previously reported by Malathi *et al*. (2002). *Trichoderma viride* was not compatible with Dithane, Bavistin and Ridomil in any level of selected concentration (Tapwal *et al.,* 2012). Deepthi (2013) isolated bacterial isolate GRE 9 was more compatible with mancozeb followed by carbendazim, Copper oxychloride. Similar observations were made by Vidyasekaran and Muthamilan (1995) and reported that carbendazim was not inhibitory to *P. fluorescens*.

Compatibility of plant oils with biocontrol agents

Compatibility of potential biocontrol agents with six plant oils viz., *Brassica juncea* var. *cunefolia, Ocimum sanctum, Azadirachta indica, Cymbopogon citratus, Eugenia caryophyllata* and *Olea europea* were evaluated at 5 per cent concentration which revealed that *Brassica junceavar. cunefolia, Azadirachta indica* and *Olea europea* @ 0.05 per cent found compatible with all fungal and bacterial antagonists. These results support the finding of Bagwan (2010) and Goswami *et al*. (2006) who reported *Trichoderma harzianum* and *T. viride* to be compatible with neem oil (5%), neem leaves extract (10%), wild sorghum leaves extract (10%), neemcake, castor cake and mustard cake extract (10%) enhanced the growth of *Trichoderma* sp. While, *Ocimum sanctum* was found to inhibit only mycelial growth of *Aspergillus versicolor* (45.29%) and compatible with rest of *T. viride, T. harzianum, T koningi* and *T. hamatum*.These results support the earlier findings of Kumar (2007) who found compatibility of aqueous extract of *Ocimum* with *T. harzianum* evenat 5 per cent concentration. Similarly, *Eugenia caryophyllata* inhibited mycelial growth of *Trichoderma viride* (14.10%).*Cymbopogon citratus* was found to inhibit *Trichoderma hamatum* (83.58%), *Trichoderma harzianum* (89.57%), *Aspergillus versicolor* (90.29%), *Trichoderma koningi* (96.55%) and *Trichoderma viride* (27.73%) respectively, but found compatible with bacterial antagonists.These results corroborate the findings of Kumar (2007) who reported compatibility of *Pseudomonas fluorescens* with aqueous extract of *Ocimum*, *Clerodendron, Bougainvillea* and *Pongemia* sp.

Recently, the scientific interests in to the compatibility of fungicides, essential oils with biocontrol agents have been increasing. Biocontrol have been used along with fungicides of no toxic effect on antagonists (Papavizas and Lumsden, 1980). The present investigations were therefore made in connection with the effect of commonly used fungicides and essential oils on mycelial growth of biocontrol agents and their efficacy against *C. salmonicolor* under *in vitro* conditions. Each three species of *Trichoderma* exhibited maximum mycelial growth inhibition as compared to other species. *Pseudomonas, Bacillus* sp. and *Actinomycetes* sp. showed

higher antagonistic effect against pink canker pathogen under *in vitro*. Similar results were already reported by Sharma, (2005).

Avtar (zineb + hexaconazole) provided compatible reaction with *Trichoderma harzianum*, *Trichoderma viride*, *Pseudomonas* sp., *Bacillus subtilis* and *actinomycetes* sp. Azoxystrobin was also compatible with *T. harzianum*, *T. koningi*, *Pseudomonas* sp., *Bacillus subtilis* and *actinomycetes* sp. *Pseudomonas* sp. and *Bacillus subtilis* did not show any deleterious effect when mixed with azoxystrobin even at a concentration higher than 250 ppm. This might be due to azoxystrobin being an inhibitor of electron from cytochrome b to c in mitochondria (Hewitt, 1998; Sendhil Vel *et al*., 2004). However combiproduct viz., Saaf (carbendazim + mancozeb), Taqat (captan + hexaconazole), Cabrio Top (pyraclostrobin + metiram), Tilt (propiconazole), Contaf (hexaconazole), Governor (flusilazol), Bavistin (carbendazim), Score (difenaconazole) and Quintal (iprodione + carbendazim) at their recommended dose found compatible with bacterial and actinomycetes sp. Among plant oils, *Brassica juncea* var. *cunefolia, Azadirachta indica* and *Olea europea* @ 0.05 per cent were compatible with all fungal and bacterial antagonists. These results are in consonance with the finding of several workers (Goswami *et al*., 2006; Bagwan, 2010).

Thus, potential native fungal and bacterial antagonists which would benefit apple industry by use of biological products to replace chemicals are extremely important in future. It is therefore quite clear that standardization of material preparation for fungicidal tolerant bioagents is urgently required for successful multiplication and further their efficacy in field. This approach might presumably become good and effective for integrated disease management strategies to combat with pink canker in apple.

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