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Pestalotia versicolor a Predominant Pathogen Associated with Decline Disease of Mango in Sub-tropical Zone of Himachal Pradesh

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Abstract

During a survey conducted in mango growing areas of four districts viz., Hamirpur, Bilaspur, Una and Kangra of Himachal Pradesh in 2013–14, the severity of mango decline ranged between 25.73% at Bairi Rajdiyan (Bilaspur) to 64.68% at Mataur (Kangra) while, its incidence ranged between 50.0% at Salauni (Hamirpur) and Nagrota Bagwan (Kangra) to 100% at various locations in all the four districts. Main symptoms observed included die back, gummosis and bark splitting. Diseased samples exhibiting different symptoms were collected and brought to laboratory for isolation and purification. Pathogenicity of the isolates was tested on young mango seedlings and pathogen isolates exhibiting similar cultural characters and sporulation were designated as I_1, I_2, I_3 and I_4, which were later identified respectively as *Phomopsis mangiferae, Pestalotia versicolor, P. versicolor* and *Colletotrichum gloeosporoides* under ITCC identification numbers 9457.14, 9458.14, 9459.14 and 9460.14. *P. versicolor* (I_2 and I_3) associated with die back, gummosis and bark splitting was observed to be prevalent in almost all the 22 locations surveyed while, *P. mangiferae* and *C. gloeosporoides* were prevalent in only 2 and 5 locations, respectively. During *in vitro* assay of fungicides, complete inhibition of *P. mangiferae* and *P. versicolor* was recorded with carbendazim as well as mancozeb and carbendazim as well as hexaconazole, respectively at all concentrations while *C. gloeosporoides* was inhibited completely by hexaconazole tested at 2000 ppm. *P. versicolor* was the predominant pathogen associated with die back, gummosis and bark splitting symptoms of decline complex and constitutes the new record from Himachal Pradesh.

Keywords: Mango decline, *Pestalotia versicolor, Phomopsis mangiferae, Colletotrichum gloeosporoides*

1. Introduction

Mango (*Mangifera indica* L.) belonging to family anacardiaceae is primarily grown in tropical and subtropical areas of India. India ranks first among mango producing countries in the world and contributes around 50% of total mango production in the world (Anonymous, 2016). In Himachal Pradesh, it is grown in the subtropical zone of the state covering an area of 39,810 ha with an annual production of 50,000 mt (Anonymous, 2014). The major factor impacting the vitality and yield of mango is susceptibility of the crop towards various diseases out of which, mango decline syndrome recognized in virtually all mango-producing regions of the world is one of the devastating problem (Anwar et al., 2012). The whole plant dies just in a few months to year (Abdul et al., 2011). The disease has worldwide distribution (Alvarez Garcia and Lopez Garcia, 1971). An increasing number of mangoes are dying from Mango Sudden Death Syndrome rather than gradual collapse or decline (Sial, 2002). Mango decline is of great economic importance, in India affecting 30–40% of plantation (Parkash and Sirvastava, 1997). Since the late nineties, mango decline or dieback disease has become one of the most severe problems (Khanzada et al., 2004a, b). In most cases, the disease has been characterized by the exudation of gum, wilting, dieback, vascular browning and death of the whole tree (Narasimhudu and Reddy, 1992; Khanzada et al., 2004a). The disease is characterized by complex symptoms like marginal leaf necrosis, die back of twigs, gummosis and vascular discolouration etc. (Khanzada et al., 2004b). Previous studies have established that *Lasiodiplodia theobromae* (Syn: *Botryodiplodia theobromae*) is a predominant causative fungus of this disease (Khanzada et al., 2004b). However, *Botryosphaeria ribis* was the first pathogen to be reported as a primary cause of mango decline (Ramos et al., 1991). In different parts of the world, association between *L. theobromae* and mango decline has been observed by many research workers (Das-Gupta and Zachariah, 1945; McSorley et al., 1981; Patial, 1988; Narasimhudu and Reddy, 1992; Sharma, 1993; Schaffer, 1994; Ramos et al., 1997; Simone, 1999; Gonztilez et al., 1999; Savant and Raut, 2000;

Al Adawi et al., 2003). In addition, *Pestalotia mangiferae, Colletotrichum gloeosporoides, Fusarium* sp., *Aspergillus* sp. and *Xanthomonas* sp. have been reported to be associated with the disease (Patial, 1988). In Himachal Pradesh, disease has been observed to appear in severe proportions resulting in huge losses as the plants ultimately die due to disease but, no significant work has been done on this disease in the state. Keeping this mind, present investigations were conducted with an objective to record the prevalence and exact symptomatology of the syndrome and finally find the most predominant pathogens associated with this disease in the state.

2. Materials and Methods

Five to six locations comprising of two to four orchards at each location were surveyed in four districts viz., Hamirpur, Bilaspur, Una and Kangra of Himachal Pradesh during the year 2013–14. Data on disease severity (%), disease incidence (%) and type of symptoms observed were recorded. Disease severity was recorded on 0–4 scale (Chester, 1950) and per cent disease index calculated further by using the formula given by McKinney (1923). Diseased samples exhibiting different type of symptoms were collected and brought to laboratory for isolation. The associated pathogens were isolated on potato dextrose agar (PDA) medium and further purified and maintained on PDA slants. Pathogens exhibiting similar cultural characters and sporulation were designated with same number.

For pathogenicity trials, mango plants were planted in the pots and a piece of 2 cm length was removed from the bark of the plants with the help of a sterilized scalpel to create a wound. The wound was swabbed with spirit and moistened with sterile distilled water and a culture bit of isolated pathogen was tied on it with the help of cello tape. The un-inoculated plants were simply covered with cello tape to record the pattern of wound healing. The pots were covered with polythene bags internally moistened with sterile water to maintain the optimum RH and incubated at room temperature. The polythene bags were sprayed internally with sterile water daily and inoculated plants were observed for symptom development, if any. Data were recorded in terms of incubation period. Those isolates, for which Koch's postulates were proved, were maintained for further studies. The pure cultures of these isolates were sent to Indian Type Culture Collection (ITCC), New Delhi for identification.

After affirming the identity of the pathogens, a trial was conducted to evaluate different microflora isolated from rhizosphere of healthy mango plants and various fungicides *in vitro* against all the pathogens identified. For the isolation of associated microflora, 10 g to 20 g soil was taken from 5 cm deep layer of rhizosphere having healthy plants of mango at experimental farm, Neri. Soil was sieved properly and 10 g soil was dissolved in 100 ml of sterilized distilled water and shaken well and allowed to stand overnight. The supernatant thus obtained was serially diluted up to a dilution level of 10^{-9} and from each dilution; 1 ml solution was plated on agar plates (PDA for fungal isolations and NA for bacterial and actinomycetes isolations). The fungal, bacterial and actinomycetes colonies thus isolated were purified and maintained in the agar slants. Isolated microflora was tested *in vitro* by dual culture method for their antagonistic activities, if any against the associated pathogens and observations were recorded in terms of inhibition zone diameter (mm). Simultaneously, six systemic and non systemic fungicides viz., Hexaconazole, Azoxystrobin, Captan+hexaconazole, Copper oxychloride, Mancozeb and Carbendazim were tested *in vitro* against the pathogenic isolates by poisoned food technique and observations were recorded in terms of radial mycelial growth (mm) of the fungus. Each treatment was replicated four times. Fungus grown on medium devoid of any microflora/fungicide served as control. Further, inhibition (%) in radial growth of fungus in various treatments w.r.t. control was calculated.

3. Results and Discussion

In all, mango orchards at 21 locations of four districts were surveyed and data on disease severity (%), disease incidence (%) and type of symptoms observed were recorded. Data thus recorded have been presented in Table 1. It is evident from the Table that the disease severity at different locations ranged between 25.73% at Bairi Rajdiyan in district Bilaspur to 64.68% at Mataur in district Kangra while, disease incidence ranged between 50.0% at Salauni in district Hamirpur and Nagrota Bagwan in district Kangra to 100% at various locations in all the four districts. Main symptoms observed in different orchards included die back, gummosis and bark splitting (Plate 1) however; at one location i.e. Bhota in district Hamirpur, partial or complete wilting of the trees was also observed. Association of such symptoms with decline of mango have earlier been reported by Narasimhudu and Reddy (1992), Khanzada et al. (2004a and b); Masood et al. (2011) and Anonymous (2015). Mango decline syndrome has been reported to display diverse symptoms including dieback of terminal shoots with or without accompanying defoliation, gummosis on branches, and scaffold limbs, vascular discoloration, marginal chlorosis and necrosis of leaves, foliar deficiencies and root degeneration (Ploetz et al., 1996; Schaffer, 1994).

Diseased samples from the orchards were brought to the laboratory and associated pathogens were isolated and purified. On the basis of cultural characters, sporulation pattern and type of symptoms produced by them, similar isolates were given the same number and in all, four isolates were purified and maintained. These isolates were designated as I_1, I_2, I_3 and I_4 (Plate 2 and Table 1). Isolates I_2 associated with gummosis and bark splitting symptoms and I_3 associated with die back were observed to be most prevalent in maximum locations surveyed.

All the four isolates tested for their pathogenicity proved

Table 1: Prevalence of mango decline disease in different areas of Himacahal Pradesh

District	Location	Disease severity (%)	Disease incidence (%)	Pathogen isolate (In)	Symptoms observed
Bilaspur	Bairi Rajdiyan	25.73	100.0	I_2 and I_3	Die back and gummosis
	Bairi	53.11	100.0	I_2, I_3 and I_4	Die back, gummosis and bark splitting
	Badsai	49.98	100.0	I_2 and I_3	Die back and gummosis
	Nihari	50.34	100.0	I_2, I_3 and I_4	Die back, gummosis and bark splitting
	Dangaar	43.22	98.0	I_1 and I_3	Die back, gummosis and bark splitting
Hamirpur	Neri	28.18	83.52	I_2 and I_3	Die back and gummosis
	Bhota	35.92	100.0	I_2 and I_3	Die back, gummosis and wilting
	Jalari	43.22	100.0	I_2, I_3 and I_4	Die back and gummosis
	Mehre – Barsar	42.18	85.16	I_2 and I_3	Die back, gummosis and bark splitting
	Salauni	32.02	50.0	I_2 and I_3	Die back and gummosis
	Dera Parol	28.63	75.0	I_1, I_2 and I_3	Die back and gummosis
Una	Lathiyani	39.80	75.0	I_2 and I_3	Die back, gummosis and bark splitting
	Bangana	30.46	75.0	I_2 and I_3	Die back, gummosis and bark splitting
	Thana Kalan	30.20	100.0	I_2 and I_3	Die back and gummosis
	Gagret	36.47	100.0	I_2 and I_3	Die back and gummosis
	Amb	35.62	78.0	I_2 and I_3	Die back, gummosis and bark splitting
Kangra	Dehra	39.06	100.0	I_2, I_3 and I_4	Die back and gummosis
	Nagrota Suriyan	39.57	75.0	I_2 and I_3	Die back and gummosis
	Jachh	42.18	95.0	I_2 and I_3	Die back, gummosis and bark splitting
	Mataur	64.68	100.0	I_2 and I_3	Die back and gummosis
	Nagrota Bagwan	49.9	50.0	I_2, I_3 and I_4	Die back, gummosis and bark splitting
	Jwalaji	36.78	90.0	I_2 and I_3	Die back, gummosis and bark splitting

I_1: Die Back isolate; I_2: Gummosis and bark splitting isolate; I_3: Die back isolate; I_4:Die back isolate

pathogenic and produced symptoms within 15 to 20 days (Table 2). Incubation periods of 15, 18, 18 and 20 days were recorded in case of isolate I_1, I_2, I_3 and I_4, respectively. Initially the symptoms appeared as dark discolouration of the tissue at the spot of inoculation which further increased and resulted in necrosis of the affected tissue. The same pathogens were re-isolated from the inoculated symptomatic tissues as the original ones, thus proving the Koch's postulates. The cultures were sent to ITCC, New Delhi from where the same were identified as *Phomopsis mangiferae, Pestalotia versicolor, P. versicolor* and *Colletotrichum gloeosporoides* under ITCC identification numbers 9457.14, 9458.14, 9459.14 and 9460.14, respectively for I_1, I_2, I_3 and I_4. *Botryosphaeria ribis* was the first pathogen to be reported as a primary cause of mango decline (Ramos et al., 1991). Many additional fungi have been associated with symptomatic tissues exhibiting bud necrosis, tip die-back, gummosis and vascular discoloration including: *Alternaria alternata, Cladosporium sp., Colletotrichum gloeosporioides, Dothiorella dominicana, Fusarium* spp., *Lasiodiplodia theobromae, Penicillium* sp., *Pestalotiopsis* sp. and *Phomopsis* spp. in different parts of the world (Ploetz et al., 1996). Sharma (1993) established *Botryodiplodia theobromae* as a primary cause of die back of mango and *C. gloeosporiodies* (*Glomerella cingulata*), *Pestalotia mangiferae*, P*homa* sp., *Sclerotium (Corticium) rolfsii*, *Rhizoctonia solani,*

Table 2: Incubation period and identification of various isolates associated with mango decline disease collected from different areas of Himachal Pradesh

Isolate	IP	Identification	ITCC IN
Die back isolate I_1	15	*Phomopsis mangiferae*	9457.14
Die back isolate I_2	18	*Pestalotia versicolor*	9458.14
Gummosis and bark splitting isolate I_3	18	*Pestalotia versicolor*	9459.14
Die back isolate I_4	20	*Colletotrichum gloeosporoides*	9460.14

IP: Incubation period (days); ITCC IN: ITCC Identification number

Diplodia sp. and *F. solani* were also reported pathogenic. *Ceratocystis fimbricata* has also been isolated from vascular bundles of declining trees and is considered to be one of the contributing factors of mango decline (Wyk et al., 2005; Fateh et al., 2006). In India, *Botryodiplodia theobromae* Pat., (synonyms: *Lasiodiplodia theobromae)* has been reported an important pathogen that induces decline symptoms on mango. It has induced a serious dieback disease of mango in India (Verma and Singh, 1970), in Salvador as well as in Egypt (Acuna and Waite, 1977). It was found associated with trunk canker disease of mango in Indonesia (Muller, 1940) and Malaysia (Lim and Khoo, 1985) and also caused gummosis and dieback of mango in Puerto Rico (Alvarez Garcia and Lopez Garcia, 1971). Narasimhudu and Reddy (1992) also isolated *B. theobromae* from mango trees severely affected by gummosis and confirmed its pathogenicity. Surprisingly, during present investigations, *B. theobromae* could not be isolated from even a single sample and *Pestalotia versicolor* having meager reports in the literature regarding its association with this disease, was found to be predominant pathogen in the region. This shift in the pathogen population might be due to global climate change during the last few years. However, association of *P. versicolor* with mango decline disease constitutes a new record from Himachal Pradesh, India.

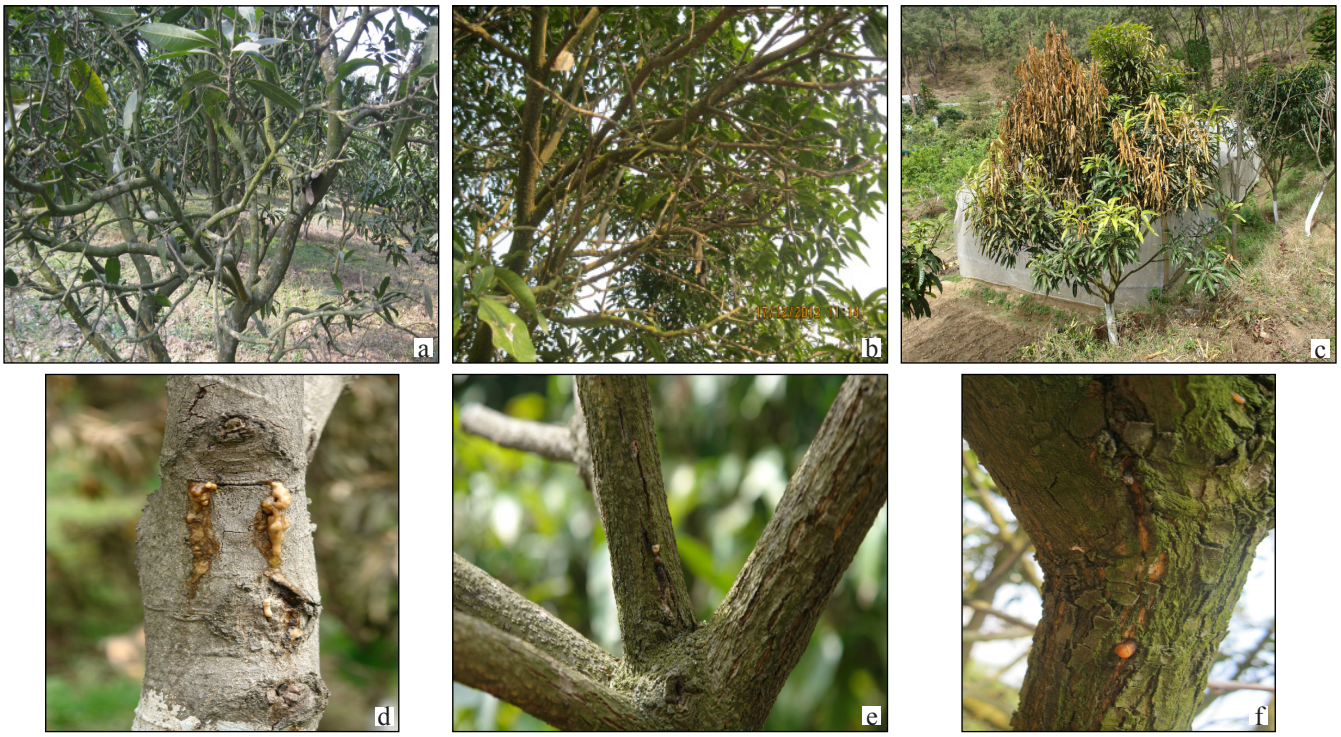
As far as *in vitro* studies were concerned, three fungi, one bacterium and one actinomycete species were isolated in all from the healthy rhizosphere of mango plants. When tested *in vitro* for their antagonistic activity against different pathogenic isolates, none of these were found to exhibit antagonistic activity against any of the three pathogens. As far as fungicide evaluation was concerned, different fungicides tested inhibited the radial growth of three pathogens to variable extents significantly. The analyzed data thus obtained have been presented in Table 3 and Figure 1. It is evident from the Table that irrespective of the pathogens and concentrations involved; carbendazim exhibited significantly maximum (96.91%) inhibition of radial mycelial growth of the pathogens followed by hexaconazole (95.68%), captan+hexaconazole (90.27%) and copper oxychloride (87.03%). Azoxystrobin proved to be significantly least effective in inhibiting the growth of these pathogens (78.75%). Among the three pathogens under test, irrespective of the fungicides and concentrations used, *P. mangiferae* (90.64%) and *P. versicolor* (90.19%) were inhibited to statistically equal level while, *C. gloeosporoides* was inhibited to significantly minimum

Table 3: Effect of fungicides on the growth inhibition of three pathogens associated with mango decline disease

Fungicide	Inhibition (%) in Radial growth of fungus at concentration (ppm)												Overall mean (fungicides)
	Phomopsis mangiferae				Pestalotia versicolor				Colletotrichum gloeosporoides				
	50	100	150	200	50	100	150	200	50	100	150	200	
Hexaconazole (Hexaconazol)	84.32	87.5	92.84	100	100	100	100	100	92.14	94.08	97.33	100.0	95.68b
Azoxystrobin (Azoxystrobin)	66.16	76.12	80.33	87.5	68.14	74.26	81.91	88.30	72.16	78.28	82.33	89.50	78.75f
Captan+hexaconazole (Captan+Hexaconazol)	84.96	90.69	93.35	95.34	85.27	92.33	95.28	98.64	80.33	85.18	88.62	92.34	90.27c
Copper oxychloride* (Copper oxychloride)	74.69	77.59	88.39	94.61	96.36	100	100	100	70.36	76.85	80.33	85.25	87.03d
Mancozeb* (Mancozeb)	100	100	100	100	63.69	68.59	72.39	79.61	58.79	65.35	71.38	78.32	79.84e
Carbendazim (Carbendazim)	100.0	100.0	100	100	100	100	100	100	85.35	88.56	92.67	96.38	96.91a
Overall mean (Pathogens)	90.64[a]	90.19[a]	83.41[b]										
Overall mean (Concentrations)	82.37[d]	86.41[c]	89.89[b]	93.65[a]									

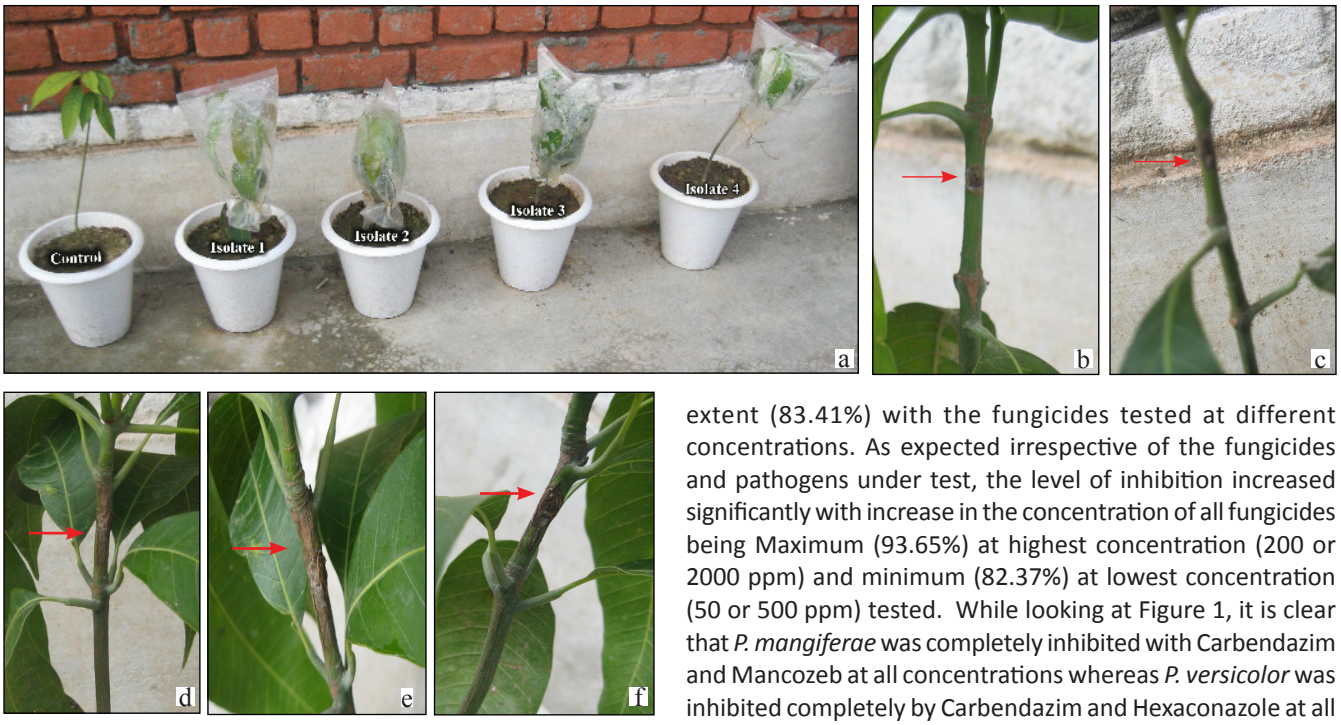
	S E	CD (p>0.05)
Fungicides	0.25	0.71
Pathogens	0.18	0.50
Concentrations	0.21	0.58
Fungicides×Pathogens×Concentrations	0.88	2.45

*Concentrations tested were 500,1000, 1500 and 2000 ppm



a and b: Die back; c: Wilting; d: Gummosis; e: Bark splitting; f: Gum oozing out of split bark

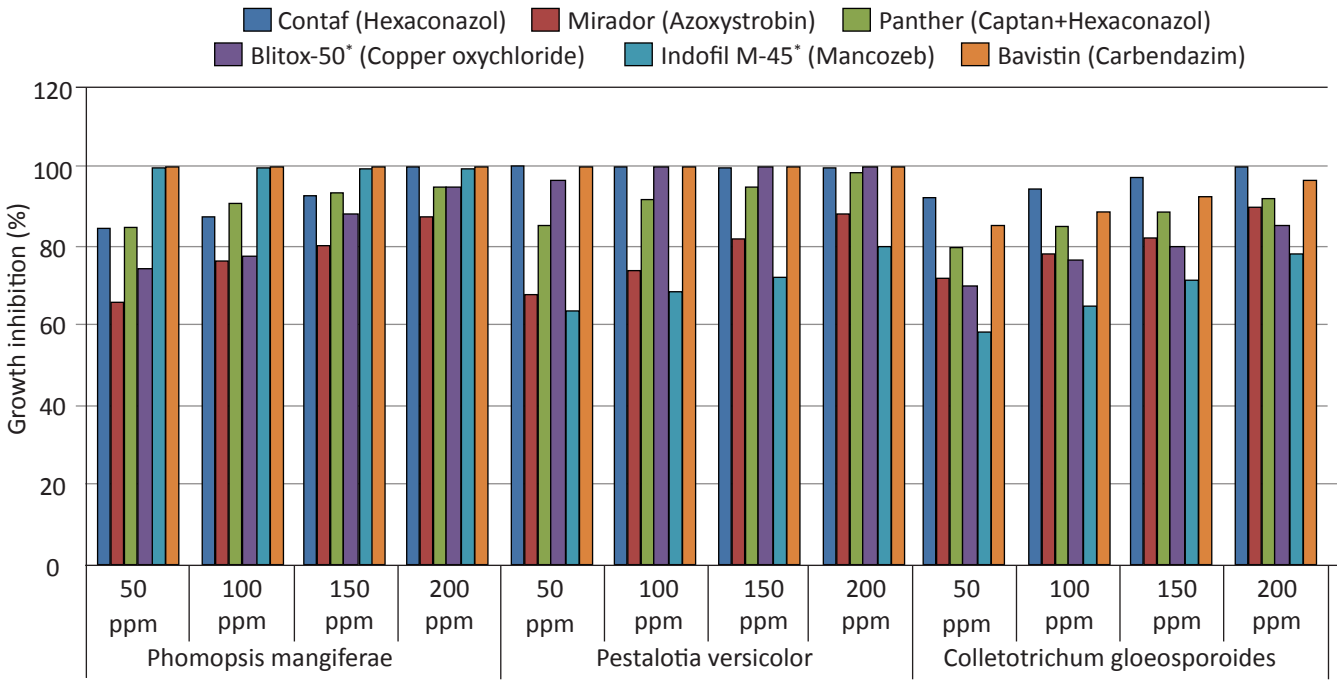
Plate 1: Various symptoms of mango decline observed in sub tropical zone of Himachal Pradesh



a: Inoculated plants; b: Uninoculated plant; c: Symptom development by isolate 1; d: Symptom development by isolate 2 ; e: Symptom development by isolate 3; f: Symptom development by isolate 4

Plate 2: Pathogenicity trial of different isolates on mango plants

extent (83.41%) with the fungicides tested at different concentrations. As expected irrespective of the fungicides and pathogens under test, the level of inhibition increased significantly with increase in the concentration of all fungicides being Maximum (93.65%) at highest concentration (200 or 2000 ppm) and minimum (82.37%) at lowest concentration (50 or 500 ppm) tested. While looking at Figure 1, it is clear that *P. mangiferae* was completely inhibited with Carbendazim and Mancozeb at all concentrations whereas *P. versicolor* was inhibited completely by Carbendazim and Hexaconazole at all concentrations and Blitox–50 at 1000, 1500 and 2000 ppm. The efficacy of carbendazim, Dithane M-45, hexaconazol and Copper oxychloride has earlier been reported against different *Phomopsis* spp. and *Pestalotia* spp. by various workers (Ponmurugan et al., 2006; Das et al., 2014; Rahman et al., 2013). However against *C. gloeosporoides,* Hexaconazole



*Concentrations used were 500, 1000, 1500 and 2000 ppm

Figure 1: Radial growth inhibition of different pathogens associated with mango decline disease as influenced by different fungicides

proved to be the best which could inhibit its radial mycelial growth completely at 2000 ppm. Carbendazim proved to be the second best fungicide against *C. gloeosporoides.* Efficacy of carbendazim and different triazoles including hexaconazole has also been reported against *C. gloeosporoides* by Jagtap et al. (2015); Tasiwal et al. (2009).

4. Conclusion

Mango decline is getting serious in Sub tropical regions of Himachal Pradesh. It is a syndrome including symptoms like die back, gummosis, bark splitting and wilting. During present studies, instead of *B. theobromae, P. versicolor* has been observed to be predominantly associated pathogen which constitutes a new record from Himachal Pradesh. Fungicides like Carbendazim, Hexaconazole, Blitox–50 and Mancozeb have been found to be effective against the associated pathogens under *in vitro* conditions and need further testing.

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