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Integration of soil solarization with vesicular-arbuscular mycorrhiza and *Azotobacter chrococcum* for the management of sapling wilt in mango (*Mangifera indica*)*

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Wilt of mango (*Mangifera indica* L.) caused by *Fusarium solani* (Mart.) is an important disease in the mango nurseries and up to 68% sapling mortality has been reported in Himachal Pradesh (Sharma *et al.* 1993, Raj and Gupta 1996). Soil solarization is an effective method to control soil-borne pathogens in different crops (Katan 1981). Among soil micro-organisms, vesicular arbuscular (va) mycorrhiza and *Azotobacter chrococcum* are of widespread distribution in the soils of various climatic zones and their ability to colonize major fruits and vegetables is well established (Smith 2002, Sharma *et al.* 2005, Sharma and Sharma 2006). The present investigation was therefore undertaken to study the effect of soil and root inoculation of mango saplings with native isolates of va mycorrhiza and *A.chrococcum* on the incidence of sapling wilt as well as growth of saplings.

Field experiments were conducted at Bhota in a field where nursery of mango was being grown for the last 4 years and there was high incidence of sapling wilt of mango in the nursery caused by *F. solani*. Soil samples were collected from different mango orchards of the State to isolate potent isolates of va-mycorrhiza and *A.chrococcum*. Four potent isolates, viz $AMUHF_1$ (*Glomus fesiculatum*), $AMUHF_2$ (*Glomus macrocarpum*), $AMUHF_3$ (*Glomus mosseae*) and $AMUHF_4$ (*Gigaspora* sp.) were selected on the basis of occurrence and frequency of distribution in the mango orchards. The population of the isolates was determined by spore isolation through wet sieving and decanting method (Gerdemann and Nicolson 1963). These isolates were multiplied on greengram (*Vigna radiata* L. Wilczek) in the sterilized soil in earthen pots for 3 months. These plants were then uprooted after 3 months and their roots were chopped into pieces. The inoculum of different isolates used in the field experiments contained spores of the isolate, pieces of infected chopped roots and mycelium in the pot culture soil. Two isolates of *Azotobacter chrococcum,* namely $AZUHF_1$ and $AZUHF_2$ were selected from the rhizosphere soil of the mango trees by serial dilution technique. Ten g soil from each sample was drawn and serially diluted aseptically to 10^{-3}, 10^{-4} and 10^{-5} and 1 ml of each sample was spreaded on Jensen's medium (Subba Rao 1986). Culture carrier of each isolate was prepared in 10% *gur* slurry added with gum to stick. This slurry of the culture was prepared to apply the culture to the roots.

Four isolates of va mycorrhiza and 2 isolates of *A.chrococcum* were evaluated in 14 different combinations (T_1–T_{14}) along with a control (T_{15}), i e $AMUHF_1$ (T_1), $AMUHF_2$ (T_2), $AMUHF_3$ (T_3), $AMUHF_4$ (T_4), $AZUHF_1$ × $AMUHF_0$ (T_5), $AZUHF_1$ ×$AMUHF_1$ (T_6), $AZUHF_1$ × $AMUHF_2$ (T_7), $AZUHF_1$ × $AMUHF_3$ (T_8), $AZUHF_1$ × $AMUHF_4$ (T_9), $AZUHF_2$ (T_{10}), $AZUHF_2$ × $AMUHF_1$ (T_{11}), $AZUHF_2$ × $AMUHF_2$ (T_{12}), $AZUHF_2$ × $AMUHF_3$ (T_{13}), $AZUHF_2$ × $AMUHF_4$ (T_{14}) and control (T_{15}) in solarized, sterilized and untreated plots. Soil solarization was done for 40 days during May–June 2002 and 2003 with transparent polyethylene mulch (25 µm thick). In the other treatment soil was sterilized with 5 % formalin for 10 days. The plots were 2m × 1m. Soil type was clay loam and *p*H of the soil was 6.8. The soil was having 224 kg available N, 30 kg P and 176 kg K/ha. Mango stones were grown in sterilized mixture of farmyard manure and soil and mixed in equal parts. Mango saplings were uprooted at 3–4 copper-colour leaf stage. The roots of the saplings were dipped for 1 hr in culture slurry of the 2 different isolates of *A.chrococcum* so that the bacteria could adhere on the root surface. These saplings were then planted in solarized, sterilized and untreated plots in root deep planting holes which were added with 10 g inoculum of different isolates of va mycorrhiza before planting. Fifty saplings were planted in each plot in the second week of September during 2002–03. Effect of inoculation of va mycorrhiza and *A. chrococcum* was

*Short note

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observed on the incidence of sapling wilt, shoot and root length, number of spores of the mycorrhiza and colonization of the roots by va-mycorrhiza. The va mycorrhizal colonization was assessed by sampling roots during November–December. Root system was carefully separated from the soil mass by washing it gently with tap water and cleaned tertiary roots were stained according to the method described by Phillips and Haymann (1970). A set of stained root samples was observed under microscope for the presence of vesicles, arbuscules and sporocarps of the va mycorrhizal fungi. Per cent root colonization was observed in accordance with Gridline intersect method (Giovannetti and Mosse 1980). The population of va mycorrhizal fungi in the rhizosphere soil was determined by spore isolation through wet sieving and decanting method (Gerdemann and Nicolson 1963) and identified to the genus level under a tri-nocular biological microscope (Leica DMLB) attached with a digital camera and measurement was done with image analysis software system. Spores isolated were counted and identified by different synoptic keys (Morton 1988).

Mulching with transparent polyethylene resulted in 47.8 and 48.4° C average maximum soil temperature in the solarized plots during 2002–03 that was 11.6 and 11.9° C higher in comparison with unsolarized plots respectively (Table 1). The average of maximum daily air temperature during 2002 was 37.3° C and 37.6° C in 2003. In earlier studies, transparent polyethylene mulch (25 µm thick) was found most effective in increasing the average maximum soil temperature (Katan 1981).

Different combinations of va-mycorrhiza and *A. chrococcum* were found effective in reducing the incidence

Table 1 Effect of solarization on soil temperature at various soil depths

Treatment	Depth (cm)	Maximum soil temperature (°C) during 1 May–9 June			
		2002		2003	
		Average	Range	Average	Range
Solarized*	10	47.8	32–52	48.4	33–53
Unsolarized		36.2	25–39	36.5	26–40
Solarized	30	37.9	27–43	37.7	26–44
Unsolarized		34.8	24–38	35.2	24–39
Air temperature		37.3	27–42	37.6	25–43

*Plots covered with transparent polyethylene mulch (25 µm)

of sapling wilt of mango in comparison to control both in solarized and sterilized soil (Table 3). However, performance of different combinations was found best in solarized soil than in sterilized soil. Inoculation of saplings with $AMUHF_4$ isolate of va-mycorrhiza and $AZUHF_1$ isolate of *A.chrococcum* and then their planting in solarized soil was found most effective with no incidence of sapling wilt in

Table 2 Effect of soil inoculation of vesicular-arbuscular mycorrhiza on root colonization and spore density in soil

Treatment	Number of spores/ 100 g of soil		Root colonization (%)	
	2002	2003	2002	2003
Soil solarization	367	361	33.40 (35.28)	35.40 (36.51)
Soil sterilization	263	258	22.60 (28.37)	23.80 (29.16)
Untreated	62	58	9.80 (18.15)	10.40 (18.77)
CD (*P*=0.05)	13.41	11.74	2.70	2.28

*Figures in parentheses are angular-transformed values

comparison to 32.33 % in control. Soil solarization with transparent polyethylene mulch has been reported to be effective in reducing the incidence of pigeonpea wilt, wilt and root rot in apple saplings, mango wilt and many soil-borne diseases in different crops (Sharma and Sharma 2006, Gade *et al.* 2007).

Soil application and root inoculation of different isolates of va-mycorrhiza and *A. chrococcum* in different combinations resulted in the reduction of the incidence of sapling wilt in different soil treatments including control (Table 3). However, the performance of different isolates of va-mycorrhiza and. *A chrococcum* was best in plots treated with soil solarization in comparison to sterilized and untreated plots. Treatment combination T_9 of $AZUHF_1$ isolate of *A. chrococcum* and $AMUHF_4$ (*G. vesiculatum*) of va-mycorrhiza was found most effective and recorded no incidence of the sapling wilt in solarized and sterilized plots in comparison with 32.33 % in control (T_{15}) during 2003–04 (Table 3). Inoculation of va-mycorrhiza has been reported to reduce the incidence of white root rot (*Dematophora necatrix*) of apple and (Bharat and Bhardwaj 2001). Inoculation of va-mycorrhiza in roots of guava also resulted in reduction of wilt caused by *Fusarium oxysporum* f.sp. *psidii* (Srivastava *et al.* 2001). Different workers have reported number of factors, like lignification of mycorrhizal roots, increased respiration, increased production of arginine and isoflavonoids, imparting resistance against soil-borne pathogens (Morandi 1996). *A. chrococcum* also had a synergistic effect in reducing the incidence of sapling wilt as it is evident from the treatments T_1 and T_6, T_4 and T_9, T_3 and T_8. Application of *A.chrococcum* in soil has been reported to reduce the incidence of root rots caused by *Rhizoctonia solani* and *Sclerotium rolfsii* (Mahmoud and Mahmoud 1999). Seed bacterization of cauliflower with *A. chrococcum*, followed by soil application of the culture has also been reported to reduce the incidence of black rot caused by *Xanthomonas campestris* pv.*campestris* (Beura *et al.* 2006). Soil sterilization with chloropicrin or fungicide application

Table 3 Effect of root inoculation of mango saplings with vesicular-arbuscular mycorrhiza and *A. chrococcum* on incidence of sapling wilt in different soil treatments during 2003–04

Treatment	Disease incidence (%)								
	2003			2004			Pooled		
	Solarized 1.33	Sterilized 4.0	Control 17.33	Solarized 2.66	Sterilized 4.0	Control 18.67	Solarized 2.00	Sterilized 4.00	Control 20.00
$AMUHF_1$	(1.41)	(2.23)	(4.27)	(1.82)	(2.23)	(4.42)	(1.66)	(2.20)	(4.65)
$AMUHF_2$	1.33	4.0 1	8.67.	1.33	2.66	18.67	1.53	3.33	13.33
	(1.41)	(2.23)	(4.42)	(1.41)	(1.82)	(4.42)	(1.49)	(1.96)	(3.77)
$AMUHF_3$	1.33	4.0	16.00	1.33	2.66	22.67	1.33	3.33	15.33
	(1.41)	(2.23)	(4.10)	(1.41)	(1.82)	(4.86)	(1.49)	(2.07)	(4.04)
$AMUHF_4$	0	4.0	13.33	1.33	2.66	18.00	1.33	3.33	12.33
	(1.0)	(2.23)	(3.77)	(1.41)	(1.82)	(4.35)	(1.49)	(2.07)	(3.65)
$AZUHF_1$	2.66	5.33	20.00	1.33	2.66	20.00	1.33	2.66	14.00
	(1.82)	(2.49)	(4.57)	(1.41)	(1.82)	(4.57)	(1.41)	(1.41)	(3.87)
$AZUHF_1 \times AMUHF_1$	0	1.33	12.00	1.33	1.33	12.00	0.66	1.33	8.66
	(1.0)	(1.41)	(3.57)	(1.41)	(1.41)	(3.57)	(1.24)	(1.41)	(3.09)
$AZUHF_1 \times AMUHF_2$	0	2.66	9.33	1.33	1.33	9.33	0.66	2.00	8.66
	(1.0)	(1.66)	(3.20)	(1.41)	(1.41)	(3.20)	(1.24)	(1.54)	(3.08)
$AZUHF_1 \times AMUHF_3$	0	1.33	8.00	0	1.33	13.33	0	1.33	9.67
	(1.0)	(1.41)	(2.94)	(1.0)	(1.41)	(3.77)	(1.00)	(1.41)	(3.26)
$AZUHF_1 \times AMUHF_4$	0	0	5.33	0	0	8.0	0	0	6.67
	(1.0)	(1.0)	(2.49)	(1.0)	(1.0)	(2.94)	(1.00)	(1.00)	(2.76)
$AZUHF_2$	2.66	4.0	24.0	2.66	4.0	24.0	1.33	2.00	17.33
	(1.82)	(2.23)	(4.98)	(1.82)	(2.23)	(4.98)	(1.48)	(1.66)	(4.27)
$AZUHF_2 \times AMUHF_1$	1.33	1.33	9.33	1.33	1.33	9.33	0.67	2.00	9.33
	(1.41)	(1.41)	(3.20)	(1.41)	(1.41)	(3.20)	(1.24)	(1.66)	(3.21)
$AZUHF_2 \times AMUHF_2$	0	1.33	12.0	0	1.33	12.0	0.66	1.33	13.33
	(1.0)	(1.41)	(3.60)	(1.0)	(1.41)	(3.60)	(1.24)	(1.49)	(3.76)
$AZUHF_2 \times AMUHF_3$	0	2.66	13.33	0	2.66	13.33	0.66	2.00	14.00
	(1.0)	(1.82)	(3.77)	(1.0)	(1.82)	(3.77)	(1.24)	(1.66)	(3.87)
$AZUHF_2 \times AMUHF_4$	0	1.33	8.0	0	1.33	9.33	0.66	1.33	12.67
	(1.0)	(1.41)	(2.94)	(1.0)	(1.41)	(3.20)	(1.24)	(3.49)	(3.69)
Control	4.0	6.67	32.0	5.33	6.67	3267	3.33	4.66	32.33
	(2.07)	(2.74)	(5.73)	(2.49)	(2.74)	(5.79)	(2.06)	(2.32)	(5.77)
CD (P=0.05) Treatment (root inoculation)		0.20			0.24			0.38	
Soil treatment		0.46			0.55			0.17	
Treatment × soil treatment			0.81			0.96			0.66

Figures in parentheses are arc-sine transformed values; $AZUHF_1$ and $AZUHF_2$ (isolates of *Azotobacter chrococcum*); $AMUHF_1$ (*Glomus fesiculatum*), $AMUHF_2$ (*G. macrocarpum*), $AMUHF_3$ (*G.mosseae*) and $AMUHF_4$ (*Gigaspora* sp.)

is also reported to increase va-mycorrhizal colonization (Kandula *et al.* 2006). In the present study also, lower incidence of sapling wilt in solarized soil may be due to higher spore count of the va-mycorrhiza resulting in higher root colonization in comparison with sterilized and untreated plots (Table 2). In solarized and sterilized soil, there is very less competition from soil-borne pathogens and other micro-organisms in soil and in addition root exudates released by the plants also provide idle conditions for the higher growth of the va-mycorrhizal fungi. In solarized plots, numbers of spores/100 g of soil were 367 and 359 during 2002–03, with root colonization of 33.4 and 35.1% respectively. In earlier studies also, higher spore count of va-mycorrhizal fungi has been found to result in higher root colonization and lower incidence of the root rot in apple (Bhardwaj *et al.* 2000, Bharat and Bhardwaj 2001).

Different treatment combinations of isolates of va-mycorrhiza and *A. chrococcum* also resulted in increased shoot and root length in different soil treatments including control (Table 4). In general, all the treatments (T_1 to T_{14}) resulted in higher shoot and root length in solarized soil in comparison with sterilized and untreated soil. Inoculation of saplings with $AMUHF_4$ isolate of va-mycorrhiza and $AZUHF_1$ isolate of *A.chrococcum* and then their planting in solarized soil was found most effective with 107.2–123.1% increase in shoot length and 79.7–85.2% increase in root

Table 4 Effect of root inoculation of mango saplings with vesicular-arbuscular mycorrhiza and *A. chrococcum* on shoot and root length in different soil treatments during 2003–04

Treatment	Shoot length (cm)									Root length (cm)								
	2003			2004			Pooled			2003			2004			Pooled		
	Solarized	Sterilized	Control	Solarized	Sterilized	Control	Solarized	Sterilized	Control	Solarized	Sterilized	Control	Solarized	Sterilized	Control	Solarized	Sterilized	Control
$AMUHF_1$	44.50	43.17	36.33	42.50	41.07	33.6	43.50	42.12	34.97	11.17	10.67	9.0	12.8	12.07	10.30	11.98	11.37	9.65
$AMUHF_2$	46.50	44.67	36.65	40.67	40.83	36.37	43.58	42.75	36.35	11.00	10.36	9.16	12.47	12.10	10.60	11.73	11.38	9.88
$AMUHF_3$	46.33	43.67	37.33	39.97	40.87	36.87	43.15	42.27	37.10	10.67	10.50	8.95	11.90	11.33	10.87	11.28	10.92	9.85
$AMUHF_4$	47.83	45.75	40.00	41.30	41.17	36.30	44.57	43.50	38.15	11.50	11.00	9.60	12.27	11.97	10.83	11.88	11.48	9.92
$AZUHF_1$	42.54	39.60	34.20	40.93	41.03	36.20	44.13	43.10	37.27	10.07	9.77	8.87	12.07	11.77	10.87	11.70	11.30	9.93
$AZUHF_1$ × $AMUHF_1$	49.17	47.30	41.53	59.77	57.30	41.53	54.47	53.40	41.60	13.00	12.17	933	16.50	15.17	10.93	14.48	14.33	10.13
$AZUHF_1$× $AMUHF_2$	49.85	47.66	41.64	64.43	58.63	42.50	56.80	52.98	41.83	13.17	12.24	9.94	16.87	16.10	11.33	14.72	14.32	10.42
$AZUHF_1$ × $AMUHF_3$	51.10	48.97	41.70	61.10	58.97	40.70	55.13	53.15	40.35	14.20	13.07	10.00	16.20	15.07	11.00	14.68	14.03	10.08
$AZUHF_1$ × $AMUHF_4$	58.33	53.67	42.00	67.70	61.43	44.47	67.02	62.55	43.73	15.80	14.17	10.17	17.83	16.30	11.73	16.02	15.55	10.95
$AZUHF_2$	44.50	40.93	36.33	44.50	40.93	36.33	45.50	42.80	36.50	11.40	10.07	8.53	12.40	11.07	10.53	11.95	10.95	9.68
$AZUHF_2$ × $AMUHF_1$	52.00	49.13	40.23	64.13	59.13	42.23	57.07	53.73	41.37	12.87	11.67	9.33	16.07	15.53	10.80	14.10	13.87	10.07
$AZUHF_2$ × $AMUHF_2$	51.83	48.83	39.33	64.13	59.13	40.77	57.48	53.98	40.05	12.50	11.67	9.50	15.50	15.13	10.73	14.00	13.73	10.12
$AZUHF_2$ × $AMUHF_3$	51.17	49.23	39.50	63.37	59.70	39.57	57.27	54.43	39.53	12.65	11.45	9.33	16.50	15.93	11.00	14.50	13.63	10.17
$AZUHF_2$ × $AMUHF_4$	65.24	58.36	43.21	64.43	59.33	40.23	60.30	56.42	40.95	16.85	14.95	10.50	15.80	14.95	10.97	14.15	13.58	10.23
Control	39.17	37.00	29.67	39.93	38.47	30.40	39.55	37.73	30.03	10.83	10.13	8.16	11.83	10.13	9.16	11.90	10.93	8.65
CD (P=0.05) Treatment (root inoculation)		0.98		0.75				1.29			0.28			0.34			0.52	
Soil treatment		2.21		1.64				0.58			0.63			0.76			0.23	
Treatment × soil treatment		3.83		2.93				2.24			1.09			1.32			0.89	

$AZUHF_1$ and $AZUHF_2$ (isolates of *Azotobacter chrococcum*); $AMUHF_1$ (*Glomus fesiculatum*), $AMUHF_2$ (*G. macrocarpum*), $AMUHF_3$ (*G.mosseae*) and $AMUHF_4$ (*Gigaspora* sp.)

length. Shalby and Mohamed (2005) reported that soil solarization reduced the incidence of wilt and root rot of strawberry and also increased growth and yield of the crop. Soil solarization has been reported to support higher growth and yield in different crops including nursery of fruits and vegetables (Patel 2001, Raj 2004). The mechanism for explaining increased growth responses and yield in plants has been attributed to chemical factors (like release of nutrients and other growth factors, nullification of toxins) and biological factors (elimination of minor or unknown pathogens) and stimulation of beneficial micro-organisms (Stevens *et al.* 2003).Va mycorrhizal fungi have been reported to have better growth of saplings in sterilized and infected soil in apple, citrus, peach and many field crops (Bharat and Bhardwaj 2001, Sharma *et al.* 2005). Higher growth in plants inoculated with va mycorrhiza has been attributed to increased uptake of phosphorus, other minerals and water (Smith and Gianinazzi-Pearson 1988). Similarly, application of *A. chrococcum* has also been reported to increase different growth characters of the plants (Sharma *et al.* 2005).

SUMMARY

A study was conducted during 2002–04 at Nauni and Bhota of Himachal Pradesh to find out the effect of root inoculation of saplings of mango (*Mangifera indica* L.) with native isolates of vesicular-arbuscular mycorrhiza and *Azotobacter chrococcum* in solarized and sterilized soil on the incidence of sapling wilt caused by *Fusarium solani* (Mart.) and growth of the saplings. Mango saplings were inoculated with 14 different combinations of 4 native isolates of va-mycorrhiza, i e $AMUHF_1$ (*Glomus fesiculatum*), $AMUHF_2$ (*G. macrocarpum*), $AMUHF_3$ (*G. mosseae*) and $AMUHF_4$ (*Gigaspora* sp.) and 2 native isolates of *A. chrococcum* ($AZUHF_1$ and $AZUHF_2$) and grown in soil solarized with transparent polyethylene mulch (25μm thick) for 40 days in summer months and also in soil sterilized with 5% formalin. Inoculation of saplings with $AMUHF_4$ isolate of va-mycorrhiza and $AZUHF_1$ isolate of *A.chrococcum* and then their planting in solarized soil was found most effective with no incidence of sapling wilt in comparison to 32.33% in control accompanied with 107.2–123.1% increase in shoot length and 79.7–85.2% increase in root length.

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