

Management of gray leaf spot of tomato caused by *Stemphylium lycopersici* under protected cultivation

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ABSTRACT

Gray leaf spot of tomato caused by *Stemphylium lycopersici* (Enjoji) W. Yamam under protected cultivation causes huge losses in the tomato growing areas of the world. Effective management of the disease through both chemical and non- chemical methods is necessary to minimize the economic losses. The present study was conducted for evaluation of cultural, biological and chemical methods of disease management. The disease was found to be very severe in March-transplanted and in closely spaced crop (45x30 cm). *In vitro* testing of bioagents viz., DMA-8 (*T. konigii*), JMA-4 (*T. harzianum*), SMA-5 (*T. harzianum*) and JMA-11 (*T. konigii*) showed inhibition of *S. lycopersici* growth, ranging from 83.3-88.2 per cent. Among the 11 fungicides tested against the pathogen *in vitro*, tebuconazole 25.9% EC and propiconazole 25% EC showed 100 per cent inhibition of mycelial growth at 250 ppm, followed by propineb 70WP at 750 ppm. Carbendazim 50 WP was observed to be the least effective. Under polyhouse condition, three sprays of tebuconazole 25.9% EC @ 0.5 ml/lt and propiconazole 25% EC @ 1ml/lt at an interval of 10 days were found most effective showing 89.3 to 88.9 per cent disease control and >200 per cent increase in the yield.

Key words: Tomato, gray leaf spot, management, spacing, *Stemphylium lycopersici*.

Tomato is a major vegetable crop that has achieved tremendous popularity over the last century and tops the list of canned vegetables. Tomatoes are a rich source of lycopene, beta-carotene, folate, potassium, vitamin C, flavonoids and vitamin E (Wilcox *et al.*, 2003). They have anticarcinogenic, cardioprotective and other health benefits (Friedman, 2013). It is grown throughout the world in open field, green houses and net houses. It is grown in mid and lower parts of Himachal Pradesh during summer and rainy seasons and fetches premium price to the growers. Protected cultivation is getting preference over open field cultivation for off-season quality production of crop. Though, protected environment provides appropriate conditions for off-season and quality production, it also provides microclimatic conditions which are suitable for the development of various diseases.

Tomato crop is prone to several fungal, bacterial and viral diseases. Gray leaf spot is one of the most destructive diseases of tomato plants and seedlings

during the rainy season under protected cultivation in Himachal Pradesh. The disease has been reported worldwide by several workers (Ellis and Gibson, 1975; Farr and Rossman, 2012; Nasehi *et al.*, 2013). It was first reported from Himachal Pradesh by Singh *et al.* (2012) in India. In the absence of proper control measures the yield losses can be very high up to 100 per cent as reported by Cedeno and Carrero (1997) from Venezuela Andes. In tomato, up to 40 per cent seedling mortality has been reported from Palampur under protected cultivation by Singh *et al.* (2012). In Himachal Pradesh, the disease is very severe from mid- June to mid- August as the environmental factors particularly the temperature and relative humidity during these months is found to be highly favourable for the development of the disease. Presently, very little information regarding the management of disease is available in India. So keeping in view the commercial importance of the crop in India and Himachal Pradesh and the magnitude of losses caused by gray leaf spot of tomato, the present study was planned to investigate strategies for effective management of the disease through chemical and non- chemical methods.

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MATERIALS AND METHODS

Non- chemical management

Effect of spacing and date of transplanting

The effect of plant density and date of transplanting on the disease development was studied by conducting an experiment under polyhouse laid in Split Plot Randomized Block Design comprising of 4 main plots (date of transplanting) and 3 sub plots (plant spacing). Twenty days old seedlings of hybrid 'Avtaar' were transplanted at three spacing viz., 45x30, 60x30 and 70x30cm (row X plant) on four transplanting dates i.e. 1st & 15th March and 15th & 31st August (2015) and the effect on the disease development was studied by recording the disease severity.

In vitro evaluation of bioagents

To study the efficacy of bioagents against *S. lycopersici*, an experiment was conducted with four bioagents viz., two strains each of *Trichoderma harzianum* (JMA-4 and SMA-5) and *Trichoderma koningii* (DMA-8 and JMA-11) obtained from the Department of Plant Pathology, CSKHPKV, Palampur. These were tested using Dual Culture Technique (Huang and Hoes, 1976). Culture discs of 5 mm taken from the margins of seven days and four days old pure culture of the pathogen and biocontrol agents, respectively, were transferred aseptically on PDA Petri plates (90 mm diameter). These were placed on the opposite sides of the plate with the help of sterilized cork borer and needle. The disc of pathogen on the PDA served as control. The plates were incubated at 24±1°C. The linear growth of the bioagents and the pathogen from the centre of the disc towards the centre of the plates was recorded after the check plates were completely covered by the pathogen. Per cent inhibition was calculated as described by Vincent (1947).

Chemical management

In vitro evaluation of fungicides

Four non-systemic fungicides viz., Indofil M-45 (mancozeb 75 WP), Indofil Z-78 (zineb 50WP), Blitox (copper oxychloride 50 WP) & Antracol (propineb 70WP), five systemic viz., Bavistin (carbendazim 50 WP), Folicur (tebuconazole 25.9% EC), Contaf

(hexaconazole 5% EC), Tilt (propiconazole 25% EC) & Score (difenoconazole 25%EC) and two coordinated mixtures viz., Nativo-75 WG (trifloxystrobin 25% WG+ tebuconazole 50% WG) and Melody duo 66.75 WP (iprovalicarb 5.5% WP + propineb 61.25% WP) were evaluated against the pathogen through poisoned food technique at concentrations 50, 100, 250, 500, 750, 1000, 1500, 2000 & 2500 ppm.

Double strength PDA medium was prepared in distilled water and sterilized in autoclave at 1.05 kg/cm³ pressure (121.6°C) for 20 minutes. Fungicide suspensions were added separately to equal quantities of double strength PDA medium aseptically before pouring into Petri plates. These plates were then inoculated with a 5 mm diameter mycelial bit of the pathogen taken from 7 days old culture. A control treatment was also maintained without fungicide amendment. Each treatment was replicated thrice. The inoculated plates were incubated at 24±1°C. Data on radial growth of the fungus were recorded on 10 days of incubation when the control plates were fully covered with the mycelium. Per cent inhibition was calculated as described by Vincent (1947).

Fungicidal management under protected cultivation

To study the effect of fungicides as foliar spray on the management of gray leaf spot, an experiment was laid out by transplanting cultivar 'Avtaar' in polyhouse in a Randomized Block Design (RBD) and each treatment was replicated thrice. All the eleven fungicides evaluated under *in vitro* were also evaluated as foliar spray individually under polyhouse. The first spray of fungicides was given on the first appearance of disease and data on gray leaf spot severity on tomato plants were recorded using 1-9 scale (Poysa and Tu, 1993) at weekly intervals. Three sprays of each fungicide were given at 10 days intervals.

The data recorded in the present investigation were subjected to statistical analysis wherever required. The differences exhibited by the treatments in various experiments were tested for their significance by employing CRD and RBD. All the data were analyzed by using CPCS-1 and MS excel software.

RESULTS AND DISCUSSION

Non-Chemical management

Effect of spacing and date of transplanting

The data presented in Table 1 reveal that in the March (1st and 15th) transplanted crop, the disease severity was more ranging between 20.0-35.0 per cent as compared to August transplanted (15th & 31st August) crop in which the severity was recorded to be 4.3 - 10.0 per cent at different spacings (Table 1). Among dates of transplanting of each season, the disease severity was high (30.0-35.0%) on 15th March and 15th August (7.3- 10 %) transplanted crop as compared to 1st March (20.0-33.3%) and 31st August (4.3- 6.0 %) transplanted crop at different spacing.

The effect of 3 spacings *i.e.* 45x30, 60x30 and 70x30 cm on the development of disease was also observed on the crop transplanted at different dates. The observations showed that the maximum disease was observed when the crop was transplanted at 45x30 cm (row x plant) as compared to 60x30 and 70x30 cm. Maximum disease severity *i.e.* 33.3 and 35.0 per cent was observed in 1st and 15th March transplanted crop at 45x30 cm followed by 24.0 and 32.0 % per cent at 60x30 cm being minimum *i.e.* (20 & 30.3%) at 70x30 cm spacing, respectively. In August transplanted crop also, maximum disease severity (6 & 10%) was observed on 15th and 31st

August transplanted crop at 45x30 cm spacing followed by 60x30 cm (8.6 & 4.6 %) and 70x30cm (7.3 & 4.3%) spacing, respectively.

It can be concluded from the data that the gray leaf spot was very severe in March transplanted crop as the environment was more favourable in June- July for the disease development. The disease seems to be of no importance for tomato crop transplanted in August. However, seedlings transplanted in August were severely infected with pathogen before transplanting and even death of seedling was observed after transplanting. The plant spacing had a definite impact on the disease development. In narrow spacing (45x30cm), high disease severity was observed as compare to wider spaced crop (70x30cm).

In the present investigation, high disease severity was observed at closely spaced crop and these results are in conformity with those of Jakhar *et al.* (1994). They also observed that the disease incidence caused by *Stemphylium* sp. was influenced by sowing date and spacing. It was highest 52.2 per cent when the crop sown on 30th September at a spacing of 45 x30 cm and lowest when sown on 30th October at 60x 45 cm spacing. Kim *et al.* (2004) also found that gray leaf spot in pepper often occurred during seedling stage and the disease is checked after transplanting in the late April to early May, probably due to relatively low temperature. They observed severe disease in July

Table 1. Effect of spacing and planting dates on the development of gray leaf spot of tomato

Month	Date	Spacing (cm)	Disease severity (%)	
March	1 st	45x30	33.3	
		60x30	24.0	
		70x30	20.0	
	15 th	45x30	35.0	
		60x30	32.0	
		70x30	30.0	
August	15 th	45x30	10.0	
		60x30	8.6	
		70x30	7.3	
	31 st	45x30	6.0	
		60x30	4.6	
		70x30	4.3	
	CD (p=0.05)		A=1.74 B=1.51 AxB=3.02	

Table 2. *In vitro* evaluation of bioagents against *Stemphylium lycopersici*

Bioagent	Mycelial growth (mm)	Per cent mycelial inhibition(mm)
<i>T. harzianum</i>		
JMA-4	11.6	87.1
SMA-5	13.0	85.5
<i>T. koningii</i>		
DMA-8	10.6	88.2
JMA-11	15.0	83.3
Control	90.0	
CD (p=0.05)	1.3	

Average of five replications

and August due to increase in temperature, which is contrary to our findings.

***In vitro* evaluation of bioagents**

Data presented in Table 2 revealed that all four bioagents (two of each *Trichoderma harzianum* and *Trichoderma koningii*) gave good inhibition of pathogen ranging from 83.3- 88.2 per cent. DMA-8 (*T. koningii*) and JMA-4 (*T. harzianum*) gave 88.2 and 87.1 per cent mycelial inhibition, respectively, followed by SMA-5 (*T. harzianum*) i.e. 85.5 per cent. Minimum mycelial inhibition (83.3%) was given by JMA-11 (*T. koningii*). The present observations are in agreement with those of Kumar *et al.* (2012) They found that *T. harzianum* inhibited 81.2 per cent growth of *Stemphylium* sp. Shahnaz *et al.* (2013) used biocontrol agents for the control of leaf blight of onion and found lesser disease incidence with *T. harzianum* as compared to *T. viride*.

Chemical management

***In vitro* evaluation of fungicides**

The efficacy of 11 non systemic, systemic and coordinated fungicides against *S. lycopersici* between 50-2500 ppm concentrations were evaluated (Table 3). At 50 ppm, Folicur and Tilt were found highly effective with 82.78 and 78.33 per cent mycelial inhibition, respectively, as compared to control. However, Bavistin was found least effective with 7.56 per cent mycelial inhibition at 50 ppm. Both Folicur and Tilt provided complete mycelial inhibition at 250 ppm, whereas at 250 ppm Melody duo, Indofil Z-78 and Bavistin showed 52.67, 41.89 and 13.56 per cent mycelial inhibition, respectively. Other fungicides at 250 ppm inhibited mycelial growth between 78.89 to

83.11 per cent as compared to control. All the other fungicides except Score, Melody duo and Bavistin gave complete mycelial inhibition at 2000 ppm. Melody duo and Bavistin did not inhibit complete mycelial growth of *S. lycopersici* even at 2500 ppm and gave only 84.6 and 41.1 per cent mycelial inhibition, respectively. From the study, it was clearly evident that Folicur, Tilt and Antracol were highly effective, whereas Bavistin and Melody duo were least effective against the pathogen.

Kumar *et al.* (2011) evaluated 10 fungicides viz., Benomyl, Mancozeb, Vitavax, Companion, Topsin M, Captan, Thiram, Apron and Zineb for their efficacy by using poisoned food technique against *Stemphylium botryosum* and found that all tested fungicides inhibited the growth of pathogen under *in vitro* conditions.

Fungicidal management under protected cultivation

Under protected cultivation Folicur was most effective giving 89.3 per cent disease control with 202 per cent increase in the yield, which was followed by Tilt and Antracol providing 88.9 and 86.3 per cent control and 200 and 164 per cent increase in the yield, respectively, over the check. Contaf, Indofil M-45, Blitox, Indofil Z-78 and Nativo were also found effective having 81.3 to 85.9 per cent disease control and 110 to 162 per cent increase in yield over control (Table 4).

Score and Melody duo also provided 79.2 and 78.7 per cent control with 74 and 67 per cent increase in yield, respectively, as compare to control. Among all the tested fungicides, Bavistin was found least effective with 75.2 per cent control and 63 per cent

Table 3. In vitro evaluation of fungicides against *Stemphylium lycopersici* causing gray leaf spot of tomato

Fungicide	Mycelial growth at different concentrations (µg/ml)										Per cent inhibition of mycelial growth at different concentrations (µg/ml)									
	50	100	250	500	750	1000	1500	2000	2500		50	100	250	500	750	1000	1500	2000	2500	
Indofil M-45	34.0	24.5	15.0 (1.58)	12.3 (1.49)	9.0 (1.38)	07.1 (1.30)	06.3 (1.28)	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)	62.22	72.77	83.33	86.66	90.00	91.89	93.00	100.00	100.00	100.00
Indofil Z-78	80.1	61.8	52.3 (2.49)	48.2 (2.41)	42.0 (2.28)	38.1 (2.19)	33.0 (2.07)	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)	11.00	31.33	41.89	46.44	53.33	57.67	63.33	100.00	100.00	100.00
Blitox	75.8	67.5	19.8 (1.70)	12.5 (1.53)	09.7 (1.42)	08.0 (1.34)	02.7 (1.12)	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)	12.78	25.00	78.89	86.11	89.22	91.11	97.00	100.00	100.00	100.00
Antracol	65.2	51.3	37.3 (2.17)	15.7 (1.60)	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)	27.56	43.00	58.56	82.56	100.00	100.00	100.00	100.00	100.00	100.00
Bavistin	83.2	80.5	77.8 (2.96)	73.5 (2.89)	69.5 (2.81)	68.1 (2.79)	65.0 (2.73)	53.0 (2.50)	53.0 (2.50)	53.0 (2.50)	7.56	10.56	13.56	18.33	22.78	24.33	27.78	28.89	41.11	41.11
Contaf	28.7	21.2	16.0 (1.61)	17.3 (1.65)	13.2 (1.52)	09.7 (2.81)	01.5 (1.07)	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)	68.11	76.44	81.11	80.77	85.33	89.22	98.33	100.00	100.00	100.00
Folicur	15.5	5.5	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)	82.78	88.33	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Tilt	19.5	10.6	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)	78.33	88.22	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Score	21.5	18.6	15.5 (1.59)	14.1 (1.55)	12.7 (1.50)	11.0 (1.44)	08.3 (1.35)	01.8 (1.08)	0.0 (1.00)	0.0 (1.00)	76.11	79.33	82.78	84.33	85.89	87.78	90.78	98.00	100.00	100.00
Nativo	24.5	18.2	15.2 (1.58)	12.2 (1.48)	10.5 (1.43)	08.3 (1.35)	05.8 (1.25)	0.0 (1.00)	0.0 (1.00)	0.0 (1.00)	73.11	79.78	83.11	86.44	88.33	90.78	93.56	100.00	100.00	100.00
Melody duo	65.7	56.8	42.6 (2.29)	36.8 (2.16)	31.5 (2.03)	28.1 (1.95)	25.1 (1.95)	19.3 (1.71)	13.8 (1.54)	13.8 (1.54)	27.00	36.89	52.67	56.11	65.00	68.78	71.11	78.56	84.66	84.66
Control	90.0	90.0	90.0 (3.16)	90.0 (3.16)	90.0 (3.16)	90.0 (3.16)	90.0 (3.16)	90.0 (3.16)	90.0 (3.16)	90.0 (3.16)										
CD (p=0.05)	1.9	1.2	3.2	3.3	4.4	2.2	3.6	2.3	2.5	2.5										

The figures in parentheses are square root transformed values

Table 4. Fungicidal management of gray leaf spot of tomato under protected cultivation

Fungicide	Dose (g or ml/lit)	Per cent disease severity	Per cent disease control	Yield (kg/plant)	Per cent yield increase
Indofil M-45	2.5	11.6	84.5	1.65	137.0
Indofil Z-78	2.5	11.0	85.3	1.37	116.4
Blitox	3.0	11.0	85.3	1.33	110.1
Antracol	2.5	10.3	86.3	1.45	164.0
Bavistin	1.0	18.6	75.2	1.03	63.0
Folicur	0.5	8.0	89.3	1.91	202.0
Contaf	1.0	10.6	85.9	1.50	162.0
Tilt	1.0	8.3	88.9	1.90	200.0
Score	0.5	15.6	79.2	1.10	74.0
Nativo	2.5	14.0	81.3	1.67	129.0
Melody duo	2.5	16.0	78.7	1.06	67.0
Control		75.0	-	0.63	-
CD(p=0.05)		3.19	-	0.19	-

increase in yield over check. The disease severity in the control was 75 per cent with yield of 630 g/plant. All eleven fungicides except Bavistin and Melody duo provided good disease control *i.e.* 80 per cent with high yield increase (more than 110 per cent) over the control. So, these fungicides can be recommended for the effective management of the disease under protected cultivation.

Sardar (2005) found that Tilt is very effective against *Stemphylium* blight of lentil. Khosla *et al.* (2007) evaluated the efficacy of seven fungicides alone or in combination against *Stemphylium* blight of garlic and Score was found best followed by Folicur in controlling the disease. Devlash (2011) evaluated 8 fungicides *viz.*, Bavistin, Indofil M-45, Companion, Zineb + Hexaconazole, Score, Contaf, Indofil Z-78 and Antracol and found that Score and Contaf were highly effective. Mancozeb showed very less disease control of *Stemphylium* blight of onion. Shahnaz *et al.* (2013) also found that mancozeb (0.25%) was most effective in managing foliar blights of onion including gray blight caused by *Stemphylium vesicarium* and it was at par with hexaconazole (0.06%). It is concluded from the study that systemic fungicides were more effective in controlling gray leaf spot disease of tomato as compared to non systemic fungicides.

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