International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijarbs Coden: IJARQG (USA) Volume 10, Issue 4 -2023

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2023.10.04.001

Evaluation of fungitoxicants against Septoria leaf spot of Tomato

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Abstract

Tomato (*Solanum lycopersicum* L.) is one of the most decisive and widely grown vegetable crop belonging to the family Solanaceae. Among several diseases affecting tomatoes, Septoria leaf spot has attained the status of a major disease. The disease is characterized by small, irregular spots with dark borders and grey centers measuring approximately 2mm in size which later enlarged, coalesced and formed irregular necrotic patches. The disease ultimately results in premature defoliation and consequently significant yield losses due to sunscald of fruits. Recommendations for the control of this disease include fungicide sprays, crop rotation, use of resistant cultivars and field sanitation. Various systemic, non-systemic fungitoxicants and combi-products were evaluated *in vitro* as well as on season sprays for inhibiting spore germination of the fungus and restricting disease extent. Among systemic fungicides, hexaconazole 5 EC @ 0.05% proved to be most effective in suppressing 96.42% of spore germination compared to 84.46 per cent and 78.64 per cent recorded in combi-products (captan + hexaconazole) and non-systemic fungitoxicants (captan) respectively. *In vivo* evaluation of test fungitoxicants revealed that hexaconazole 5 EC @ 0.05% (5.14%) was most effective in restricting the intensity of Septoria leaf spot disease compared to 45.36 per cent of recorded in check.

Keywords: *Solanum lycopersicum* L , Septoria leaf spot, fungitoxicants, combi-products

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crop belonging to the family Solanaceae. Tomato is a good source of vitamin A, B-complex, C, minerals, iron, phosphorus, high amount of water and low calories (Wilcox *et al.*,2003). Tomato fruit is not only a good source of lycopene, but it also contains carotenoids with a high oxygen radical scavenging and quenching capacity (Babalola *et al.*, 2010). It has high medicinal value as well; the pulp and juice is digestible, promoter of gastric secretion and blood purifier (Sameera, 2007). High values of dietary lycopene available in tomatoes have been found useful in reducing cancer, aging and arteriosclerosis (Giovannucci, 2002).

Many factors operate in successful cultivation as well as marketing quality of tomatoes, of which diseases play an important role. There are several diseases intomato caused by fungi, bacteria, viruses, nematodes and abiotic factors (Blanchard, 1992). Introduction of some high yielding varieties (HYV) of tomato have replaced most of the conventional cultivars and because of favorableagro-climatic conditions, several fungal diseases have been reported that attack the crop. Among the diseases, Septoria leaf spot is one of the most destructive foliar diseases observed in temperate regions causing spoilage of foilage, reduction in plant vigor, crop yield and market value (Gul *et al.*,2016). In attempting to check the diseases of plants, the cause of disease, life history of parasite and the circumstances which influence the establishment of parasitic relations between the pathogen and the host is usually required. Consequently, the methods aimed at controlling the disease requires application of chemicals to host surface that kills the pathogen before or after pathogen has established in the host. Keeping in view the economic importance of tomato crops and the disease in the valley, the present investigations were carried out to evaluate various fungicides against the disease.

Materials and Methods

Isolation of Pathogen

The isolation of causal agent was done by tissue bit transfer method. Leaves of tomato plants showing typical symptoms were collected from the fields of division of Vegetable Science, SKUAST-K, Wadura. The infected parts of the leaves were cut into small bits of size 2-5 mm with a sharp sterilized blade so that each diseased bit contained a portion of healthy tissue along with it. These bits were subjected to surface sterilization with 1 per cent sodium hypochloride solution for 30 seconds followed by three rinses with distilled sterilized water to remove the last trace of mercuric chloride solution. These bits were then placed on moist filter paper in a sterilized petriplate and incubated at 25°C for 24 hours to enhance symptoms of possible pathogen. The bits were later transferred aseptically to potato dextrose agar (PDA) medium in sterilized petri-plates and incubated at25±1ºC for periodic observations vis-à-vis, colony colour, texture, and sporulation. Single spore isolation as given by Jhonston, and Booth (1983) was applied to obtain axenic culture of the pathogen. The pathogenic isolate on tomato plants was identified based on morphological characteristics of somatic and reproductive structures. The pure culture of *Septoria lycopersici* was maintained on PDA slants (plate 1)at 5±1°C in the refrigerator and cultured periodically at an interval of 30 days during this study.

Plate 1

In vitro evaluation of fungitoxicants

Twelve fungitoxicants comprising of systemic, non-systemic and their combi-products were evaluated *in vitro* at recommended concentration for their efficacy against the pathogen by adopting slide germination technique (Montgomery and Moore, 1938) as described below,

The spore suspension of the test Pathogen was prepared by dispensing a loopful of the fungal culture in sterilized distilled water and standardizing the spore concentration to give about 25 to 30 spores per low power microscopic field (10X). The recommended concentration of the twelve fungitoxicants was prepared by using sterilized distilled water and placed on the cavity slides separately. A drop of the spore suspension was placed over the fungitoxicants on the cavity slides separately. The cavity slides were placed in moist chambers prepared by lining the 90 mm diameter petri dishes from within with a double layer of moist filter paper, maintaining five replications for each treatment. The spore suspension diluted with equal volume of distilled water served as check. The slides were examined after 48 hours of incubation at 25±2°C for spore germination by taking counts at different microscopic fields on each cavity slide and per cent inhibition in spore germination was computed by using formula given by vincent (1947).

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Per cent inhibition of spore germination

C = Spore germination in control

T = Spore germination in treatment

Following are the fungitoxicants which were evaluated at given concentrations:

Fungitoxicants	Concentration (ppm)
(Systemic)	
Carbendazim 50 WP	500
Difenaconazole 25 EC	300
Flusilazole 40 EC	200
Hexaconazole 5 EC	300
(Non- Systemic)	
Mancozeb 75WP	3000
Captan 50 WP	3000
Propineb 70 WP	3000
Zineb 75 WP	3000
Combiproducts	
Carbendazim + Mancozeb 75 WP	2500
Captan +Hexaconazole 75 WP	500
Zineb + Hexaconazole 72 WP	7500
Metiram + Pyraclostrobin 60 WG	1000

In vivo evaluation of fungitoxicants

Field trial was conducted in Randomized Block Design (RBD) with 12 treatments and 5 replications during *kharif* 2018 at faculty of agriculture, SKUAST-K Wadura. Healthy "Roma" tomato seedlings were raised in nursery beds and 26 days old seedlings were transplanted into the main field with 60 cm inter-row and 45 cm intra-row spaced plots measuring 1.0 m × 1.8 m with each plot having 6 plants. All other cultural and pest control practices were followed as per recommended in SKUAST-K package of practices for tomato crops.

In all four sprays were given, the first spray was given immediately after the appearance of disease, i.e, second week of June followed by three sprays at interval of 15 days except the control where only water was sprayed. The observation on disease intensity on leaves was recorded after 20 days of the last spray i.e., third week of August by randomly picking a total of 60 leaves from each treatment. Per cent disease intensity was calculated for each treatment by adopting the Joshi's scale and formula described below. Data was subjected to statistical analysis adopting standard procedures.

In order to calculate disease intensity, the leaves showing disease symptoms were categorized using 0-5 scale given by Joshi*et al*., 2011(Plate 1) as under:

Grade value	Per cent leaf area infected
0	Leaves free from spots
1	0-5% leaf area infected and covered by spots
2	6-20% leafarea infected and covered by spots
3	21-40% leaf area infected and covered by spots
4	41-70% leaf area infected and covered by spots
5	>70% leaf area infected and covered by spots

Percent disease intensity (PDI) was calculated by using the formula:

$$\text{Per cent disease intensity} = \frac{\sum (n \times v)}{N \times G} \times 100$$

Where,

$\sum$ = Summation

n = Number of diseased leaves/twigs in each category

v = Numerical value of the category

N = Total number of leaves examined

G = Highest grade value

Results and Discussion

In vitro evaluation of fungicides

Twelve fungitoxicants were evaluated *in vitro* against *Septoria lycopersici* by slide germination technique as described in materials and methods. The data recorded after 48 hours of incubation is presented in Table 1,2 and 3

In vitro evaluation of systemic fungicides

Perusal of data (Table 1, Fig. 1) indicated that all the test fungitoxicants significantly inhibited spore germination of *S. Lycopersici* compared to check though the fungitoxicants differed significantly from one another in their effectivity. Among the test fungitoxicants, hexaconazole 5 EC @ 500 ppm proved significantly superior to all other test fungitoxicants exhibiting a maximum of 96.42 per cent inhibition of spore germination followed by carbendazim 50 WP, difenoconazole 25 EC and flusilazole 40 EC with an inhibition of 89.80 per cent, 86.33 per cent and 85.19 per cent.

Table 1: *In vitro* efficacy of systemic fungitoxicants against the spore germination inhibition of *Septoria lycopersici*

S.no	Fungicide	Concentration (ppm)	Per cent spore germination inhibition
01	Carbendazim 50 WP	500	89.80 (71.38) *
02	Difenconazole 25 EC	300	86.33 (68.33)
03	Flusilazole 40 EC	200	85.19 (67.33)
04	Hexaconazole 5 EC	300	96.42 (79.09)
C.D ($p \leq 0.05$)			1.46

*Figures in parenthesis are Angular transformed values

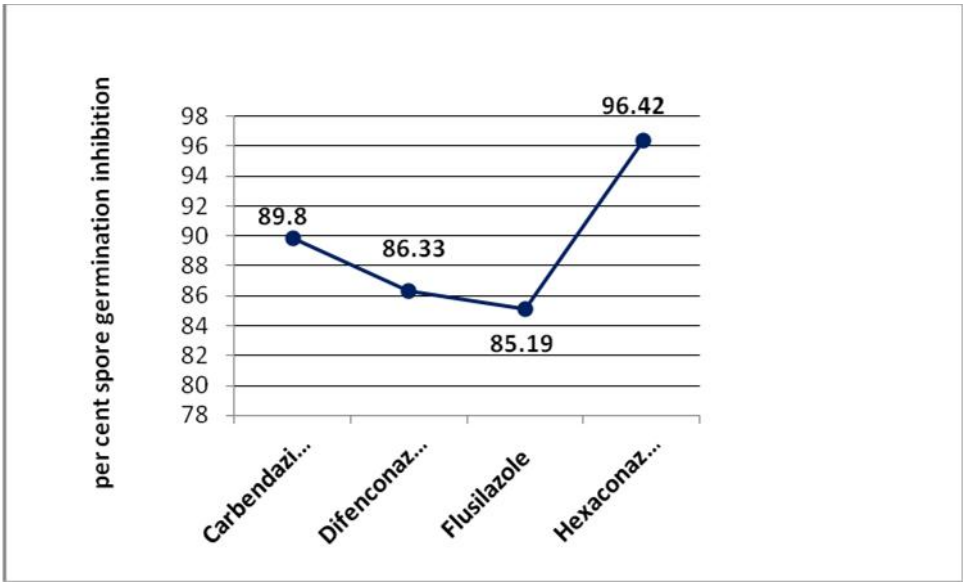

Fig. 1: *In vitro* evaluations of systemic fungitoxicants against the spore germination of *Septoria lycopersici*

In vitro evaluation of non-systemic fungicides against *Septoria lycopersici*

An insight into the data (Table 2, Fig. 2) revealed that all the test fungitoxicants significantly inhibited spore germination of *S. Lycopersici* over check. However, all the fungitoxicants differed statistically from one another. Captan 50 WP @ 3000 ppm was observed significantly moreeffective than all other fungitoxicants by exhibiting 78.64 per cent of spore germination inhibition. It was followed by mancozeb 75 WP which showed 73.94 per cent, propineb 70 WP which showed 68.14 per cent whereas least was shown by Zineb 75 WP with a minimum spore germination inhibition of 65.63 per cent at the same concentration.

Table 2:*In vitro* efficacy of non-systemic fungitoxicants against spore germination inhibition of *Septoria lycopersici*

S.no	Fungicide	Concentration (ppm)	Per cent spore germination inhibition
01	Mancozeb 75 WP	3000	73.94 (59.30)
02	Captan 50 WP	3000	78.64 (62.47)
03	Propineb 70 WP	3000	68.14 (55.64)
04	Zineb 75 WP	3000	65.63 (53.95)
C.D ($p \leq 0.05$)			1.51

*Figures in parenthesis are angular transformed values

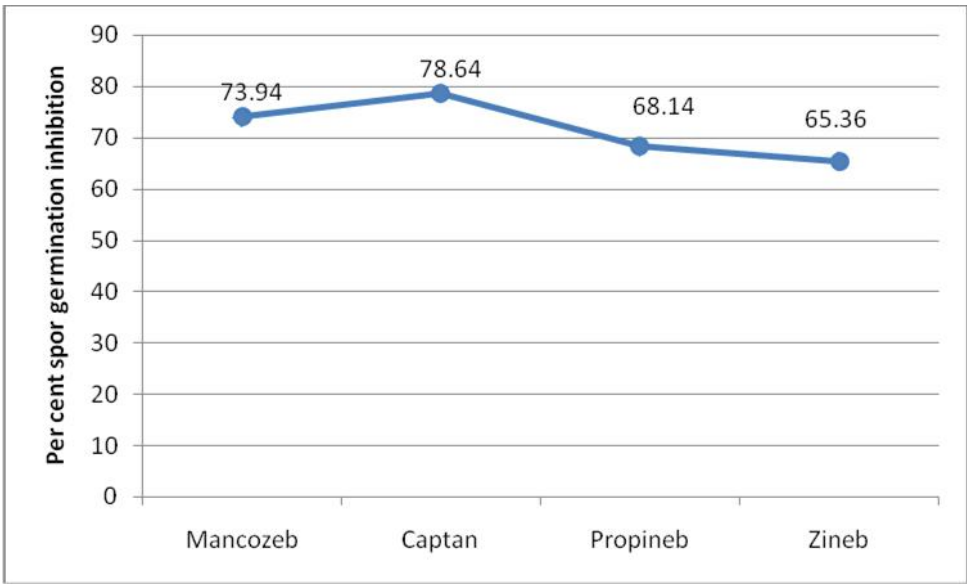

Fig. 2: *In vitro* evaluation of non-systemic fungitoxicants against the spore germination of *Septoria lycopersici*

In vitro evaluation of combi-products against spore germination of *Septoria lycopersici*

Analysis of data (Table 3, Fig. 3) revealed that all the test fungitoxicants significantly inhibited spore germination of *S. Lycopersici* compared to check though the fungitoxicants differed significantly from one another in their effectivity. Among the combi-products evaluated, captan + hexaconazole 75 WP proved significantly superior to all other combi-products exhibiting a maximum of 84 46 per cent inhibition of spore germination followed by carbendazim + mancozeb 75 WP (82.16 %), zineb + hexaconazole 72 WP (80.87%) and Metiram + pyraclostrobin 60 WG (79.03%).

Table 3:***In vitro*** **efficacy of Combi-products against spore germination inhibition of** ***Septoria lycopersici***

S.no	Fungicide	Concentration (ppm)	Per cent spore germination inhibition
01	Carbendazim + Mancozeb 75 WP	2500	82.16 (65.02) *
02	Captan + Hexaconazole 75 WP	500	84.46 (66.78)
03	Zineb + Hexaconazole 72 WP	7500	80.87 (64.06)
04	Metiram + Pyraclostrobin 60 WG	1000	79.03 (62.75)
	C.D (p≤0.05)		1.63

*Figures in parenthesis are angular transformed values

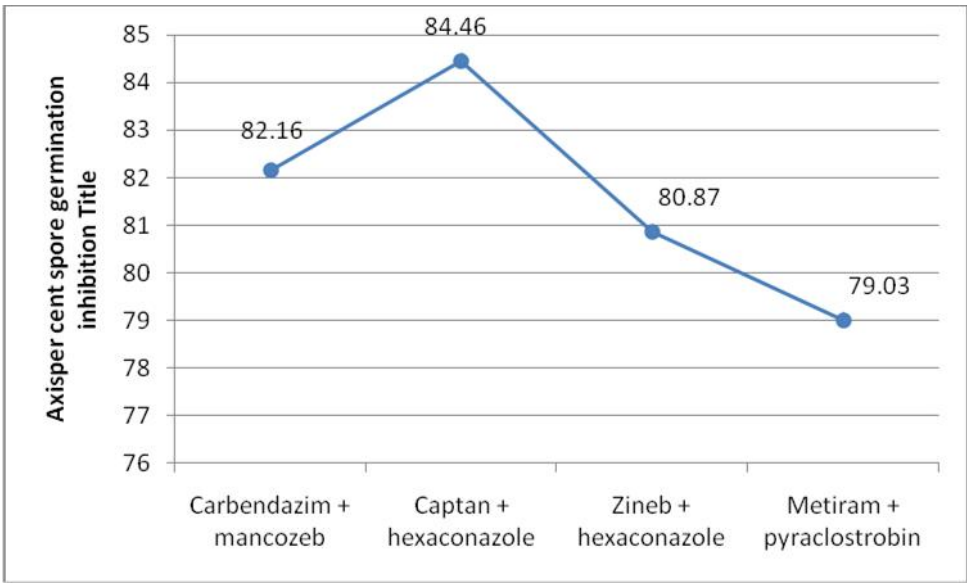

Fig. 3: ***In vitro*** **evaluatiuon of combi-products against the spore germination of** ***Septoria lycopersici***

In vivo evaluation of fungitoxicants

The individual effect of foliar sprays of different systemic, non-systemic fungitoxicants or their combinations on intensity of Septoria leaf spot of tomato on cultivar Roma was evaluated under natural conditions at best concentrations during 2018 growing season at FoA, Wadura. The data recorded after 4th spray is presented in Table 4.

Analysis of the data revealed that all the test fungitoxicants applied as foliar sprays proved significantly superior over check in reducing the intensity of Septoria leaf spot disease on tomatoes. However, the magnitude of reduction varied varied from fungitoxicant to fungitoxicant. Perusal data indicated that minimum disease intensity of 5.14 per cent was observed in plants treated with hexaconazole 5 EC @ 0.05 per cent with 88.66 per cent disease over control.

Carbendazim 50 WP @ 0.05 per cent was the next best fungitoxicant exhibiting disease intensity and per cent disease over control of 8.11 per cent and 82.12 per cent respectively. Difenconazole 25 EC @ 0.03 per cent and flusilazole 40 EC @ 0.02 per cent exhibited 73.10 per cent and 67.72 per cent of disease over control.

Among non-systemic fungitoxicants, captan 50 WP @ 0.3 per cent exhibited 28.22 per cent of disease intensity followed by mancozeb (28.71%) and propineb (32.58%). Zineb was the least effective fungitoxicant and statistically inferior to all other test fungitoxicants by exhibiting 36.86 per cent disease intensity.

Among combi-products, maximum disease Control was observed in captan + hexaconazole 75 WP @ 0.05 per cent treated plants exhibiting 16.98 per cent disease intensity and 62.57 per cent disease over control respectively, followed by carbendazim + mancozeb 75 WP (18.76%), Zineb + hexaconazole 72 WP (21.35%) and metiram + pyraclostrobin 60 WG (25.61%).

It was also observed that all the test fungitoxicants effectively reduced the disease intensity and were superior to check by exhibiting 5.14 to 36.86 per cent of disease intensity compared to 45.36 per cent recorded in check. Furthermore, all the test fungitoxicants were statistically at par with one another and per cent disease over control ranged from 88.66 per cent in hexaconazole 5 EC to 18.74 per cent in zineb 75 WP.

Table 4: Effect of different fungicides on intensity of Septoria leaf spot of tomato

S.no	Fungicide	Concentration (%)	Per cent disease intensity	Per disease over control
01	Carbendazim 50WP	0.05	8.11 (2.84) *	82.12
02	Difenconazole 25 EC	0.03	12.20 (3.49)	73.10
03	Flusilazole 40 EC	0.02	14.64 (3.82)	67.72
04	Hexaconazole 5 EC	0.03	5.14 (2.26)	88.66
05	Mancozeb 75 WP	0.3	29.71 (5.45)	34.50
06	Captan 50 WP	0.3	28.22 (5.31)	37.79
07	Propineb 70 WP	0.3	32.58 (5.70)	28.17
08	Zineb 75 WP	0.3	36.86 (6.07)	18.74
09	Carbendazim + Mancozeb 75 WP	0.25	18.76 (4.33)	58.66
10	Captan + Hexaconazole 75 WP	0.05	16.98 (4.12)	62.57
11	Zineb + Hexaconazole 72 WP	0.75	21.35 (4.62)	52.93
12	Metiram + Pyraclostrobin 60 WG	0.1	25.61 (5.06)	43.54
13	Control		45.36 (6.73)	
C.D			2.01	

*Figures in parenthesis are square transformed values

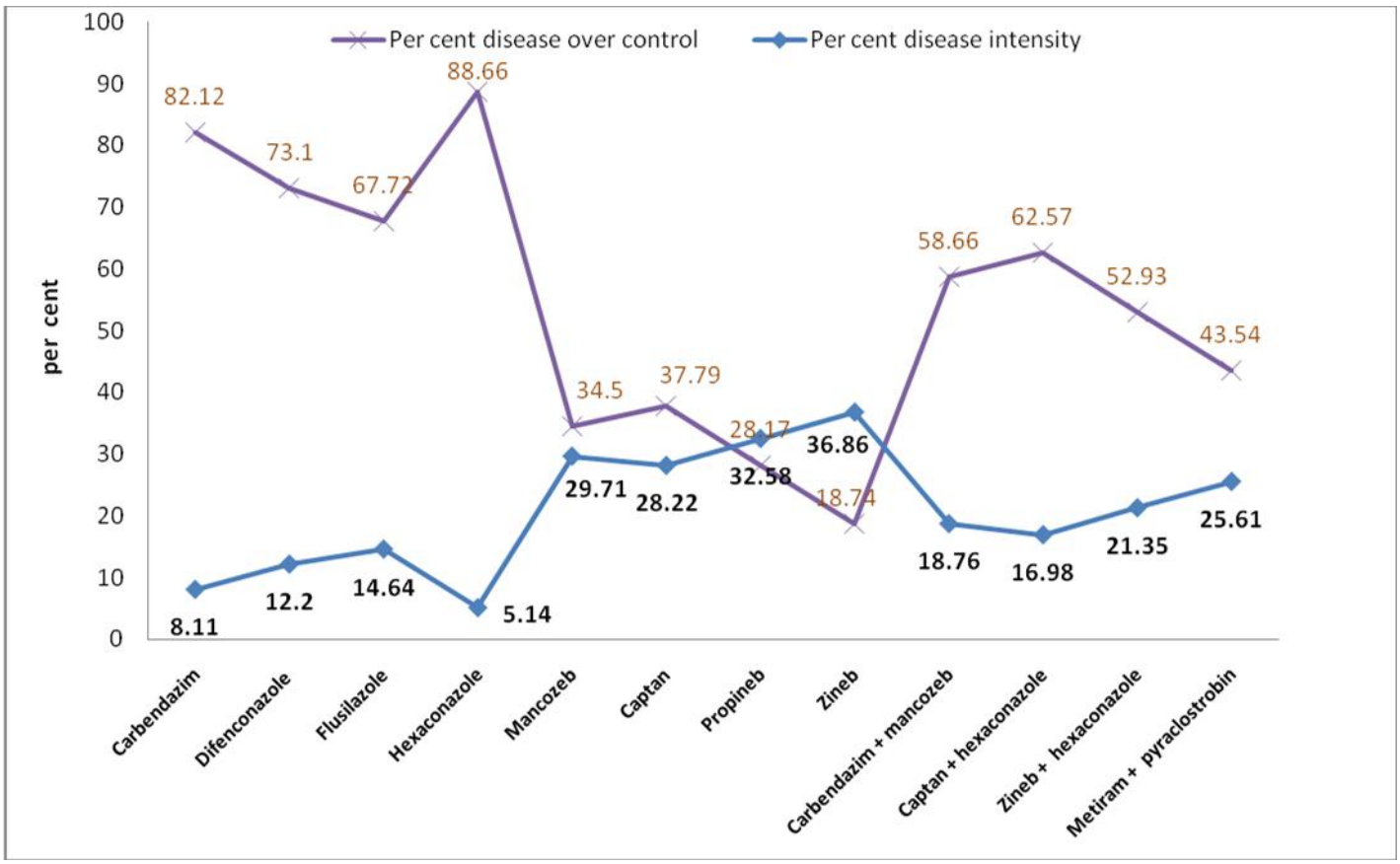

Fig. 4: Effect of different fungicides on intensity of Septoria leaf spot disease under field conditions

Evaluation of fungitoxicants *in vitro* is a handy tool to screen a large number of fungitoxicants. In the present study, the laboratory evaluation of 12 fungitoxicants by slide germination technique revealed that all the fungitoxicants could inhibit the spore germination of test pathogen. It was noticed that most effective among systemic fungitoxicants, hexaconazole 5 EC @ 500 ppm inhibited 96.42 per cent of spore germination over check followed by carbendazim 50 WP (89.80%), difenconazole 25 EC (86.33%) and flusilazole 40 EC (85.19%). Similar inferences were drawn by Lartaud and Lipatoff (1980) and Kashyap (2013).

Among non-systemic fungitoxicants, captan 50 WP @ 3000 ppm (78.64%) was found to be effective in inhibiting the spore germination *in vitro*. This was followed by mancozeb 75 WP (73.94%) and propineb 70 WP (68.14%). The least inhibition of spore germination was shown by Zineb 75 WP (65.36%). The results obtained are in accordance with the results obtained by many workers like Ahmad and Ahmad (2000), Govardhan (2001), Mohan *et al.* (2016) and Anwer*et al* (2017) who reported, mancozeb and captan were most effective chemicals in inhibiting the growth of *Septoria lycopersici.*

In vitro evaluation of combi-products revealed that maximum inhibition of spore germination was observed in case of captan + hexaconazole @ 500 ppm (84.46%). Next best combi-product was carbendazim + mancozeb @ 2500 ppm (82.16%) followed by zineb + hexaconazole 72 WP @ 7500 ppm (80.87%). The least spore germination inhibition effect was observed in case of metiram + pyraclostrobin 60 WG @ 1000 ppm (79.03%). Similar results were shown by Kashyap (2013).

Evaluation of fungitoxicants, as spray in the field against Septoria leaf spot has revealed that all fungitoxicants, at best concentration recorded *in vitro*, were significantly effective in reducing the disease. The overall disease intensity in fungitoxicants treated plants ranged from 5.14 per cent to 36.86 per cent against 45.36 per recorded in check.

Among fungitoxicants tested, hexaconazole 5 EC proved significantly superior in suppressing the disease, least disease intensity of 5.14 per cent was recorded in plants sprayed with hexaconazole 5 EC at four different stages, against 45.36 per cent recorded in check. These observations are supported by the findings of Kashyap (2013), kirpop*et al.* (2007) and Mohan *et al.* (2016).

Conclusion

In the light of present investigations, it was deduced that the disease is very much prone in dense cropping conditions, the seedlings should be planted at proper spacing followed by staking. The fungitoxicant sprays at four different stages of tomato seemed to be essential for disease management. The fungitoxicant hexaconazole 5 EC @ 0.05 % and carbendazim 50 WP proved the most effective against test pathogen.

Author contributions: All the authors contributed equally to the manuscript. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement: Not applicable.

Funding: No funds, grants and other support was received.

Conflicts of Interest: The authors declare no conflict of interest. Financial interests: The authors declare they have no financial interests.

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Quick Response Code	Subject: Agricultural Sciences
DOI:10.22192/ijarbs.2023.10.04.001	

How to cite this article:
Aabida Abas and Sabiha Ashraf. (2023). Evaluation of fungitoxicants against Septoria leaf spot of Tomato. Int. J. Adv. Res. Biol. Sci. 10(4): 1-12.
DOI: http://dx.doi.org/10.22192/ijarbs.2023.10.04.001