Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2021; 10(1): 833-837
Received: 16-11-2020
Accepted: 18-12-2020

Vanama Sowmya
Master Scholar, Dept. of Plant Pathology, Institute of Agricultural Sciences, BHU, Varanasi, Uttar Pradesh, India

Ram Chandra
Professor, Department of Plant Pathology, Institute of Agricultural Sciences, BHU, Varanasi, Uttar Pradesh, India

Corresponding Author:
Vanama Sowmya
Master Scholar, Dept. of Plant Pathology, Institute of Agricultural Sciences, BHU, Varanasi, Uttar Pradesh, India

In vitro and *in vivo* efficacy of chemical fungicides against early blight of tomato (*Solanum lycopersicum* L.) incited by *Alternaria solani* (Ell. & Mart.)

Vanama Sowmya and Ram Chandra

Abstract

Early blight of tomato disease caused by *Alternaria solani* (Ell. & Mart.) is one of the most devastating diseases of tomatoes. It is a potential tomato disease that is reducing production worldwide. To date, various treatments have been adopted to minimize the disease. The present study was based on the selection of chemical fungicides against *A. solani* in tomatoes that cause blight. The *in vitro* and *in vivo* efficacy of chemical fungicides showed that minimal radial growth with a diameter of 0.51 cm gave hexaconazole, followed by thifluzamide with a radial growth of 1.18 cm. In this study, five fungicides were used at a concentration of 100 and 300 ppm. The maximum suppression of fungi was provided by hexaconazole with 94.33% at a concentration of 300 ppm, fruits 35 / plant, fruit yield 1.224 kg / plant, 15.45 kg / plot, 252.50 q / ha.

Keywords: *Alternaria solani*, tomato, fungicides

1. Introduction

Tomato (*Solanum lycopersicum* L.) is the most important vegetable in India. It is a widely used vegetable because of its high nutritional value. However, India faced a serious disease problem that significantly reduced tomato production. Early blight caused by *Alternaria solani* is one of the main diseases among tomato leaf diseases. The fruit is a real berry. Warm season harvest. Early blight disease, also known as Target Board Disease, caused by *A. solani* (Ellis & Martin), Jones, and Rout as it causes white board symptoms. It is one of the worst diseases in the world and it kills tomatoes in India both before and after harvest. Tomatoes are consumed in everyday life because they are a good source of antioxidants and also a balanced source of vitamins, i.e. A, C and E, which are necessary for metabolic activity and maintaining good human health. Olaniyi *et al*. (2010) [6]. The tomato harvest is susceptible to various fungal, bacterial, nematode and viral diseases. Among the fungal diseases, the tomato rot caused by *A. solani* Sorauer is the worst and caused great damage both qualitatively and quantitatively. Abdel-Sayed (2006) [2], Abada *et al*. (2008) [1]. Diseases are traditionally treated with chemical fungicides. The aim of this study is to compare the *in vitro* and *in vivo* efficacy of systemic chemical products in order to apply this strategy in sustainable and environmentally friendly methods to combat complex diseases.

2. Materials and Methods

The present investigation was done at the Department of Mycology & Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh during 2019-2020. The procedure and techniques applied during the course of investigations were elucidated as below.

2.1. Isolation, Purification, Identification and Maintenance of *Alternaria solani*

The leaves infected with the early disease were collected from the BHU Agricultural Research Farm in Varanasi. The leaves were examined under a microscope to confirm the presence of fungus. After confirmation of the presence of fungal spores, isolation was performed according to the standard tissue isolation method of Anurag and Ved (2019) [3]. Infected leaves with typical symptoms were cut into small pieces so that each piece contains the infected portion along with some useful portions. Then the leaf pieces were sterilized for one minute with a 0.1% mercury chloride solution. The pieces were repeatedly washed three times in sterile distilled water and transferred into sterile Petri dishes containing PDA (potato dextrose agar) and on the slopes under aseptic conditions and incubated for 2–3 minutes at room temperature

(25 ± 2 °C) for 7 days. Then the growth of fungi and sporulation were periodically observed. The purified pathogen was then identified based on its morphological and cultural properties.

2.2. Evaluation of chemical fungicides

Two concentrations of newly marketed fungicides were used for *in vitro* and *in vivo* testing, which are found to be effective against *Alternaria solani*. Five fungicides were selected to test against *Alternaria solani* under *in vitro* conditions. Selected fungicides were Azoxystrobin, Thifluzamide, Probineb, Difenconazole, Pyraclostrobin.

Table 1: Quantity of chemical required to make 1 gm of a.i:

Fungicide Name	Quantity of Fungicide taken (for 1 gm a.i in grams)	Amount of Sterilized Distilled water(ml)
Azoxystrobin	4.34	100
Thifluzamide	4.16	100
Probineb	1.42	100
Difenconazole	4.02	100
Pyraclostrobin	4.89	100

2.2.1. Observation recorded: The radial growth of the fungus on the poisoned media was recorded at the time of mycelium growth, which in the control reached 9 cm. The rate of inhibition of fungal mycelium growth by fungicides *in vitro* was calculated using the formula described by Vincent (1927) [10].

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

Where,

I = Per cent reduction in growth of *A. solani*

C = Radial growth of fungus (mm) in control

T = Radial growth of fungus (mm) in treatment

2.3. *In vivo* evaluation of fungicides against *Alternaria solani*:

Randomized block design was followed to evaluate the fungicidal efficiency under field conditions of tomato.

2.3.1 Observation recorded

2.3.1.1 Disease intensity

Ten plants were selected randomly in each treatment and observation on severity of the disease on the foliage was recorded at 15, 30, 45, 60, 75 and 90 days after first spray using 0-5 disease rating scale (Pandey *et al.*, 2003) [7]. Percent Disease Index (PDI) was calculated using the following formula.

$$\%\text{infectiomn/PDI} = \frac{\text{Sum of all disease rating}}{\text{Total no. of rating}} \times \frac{100}{\text{Maximum disease grade}}$$

Table 2: Disease rating scale (Datar and Mayee, 1986)

Grade	Symptoms
0	Free from infection
1	< 10% surface area covering leaf, stem and fruit infected by early blight
2	11-25% foliage of plant covered with a few isolated spot
3	Many spots coalesced on the leaves, covering 26-50% surface area of plant
4	51-75% area of plants infected, fruits and also infect at peduncle and defoliation and blightening started. Sunken lesions with prominent concentric ring on stem, petioles and fruits
5	> 75% area of plant part blighted, severe lesion on stem and fruit rotting on peduncle end

3. Results and Discussion

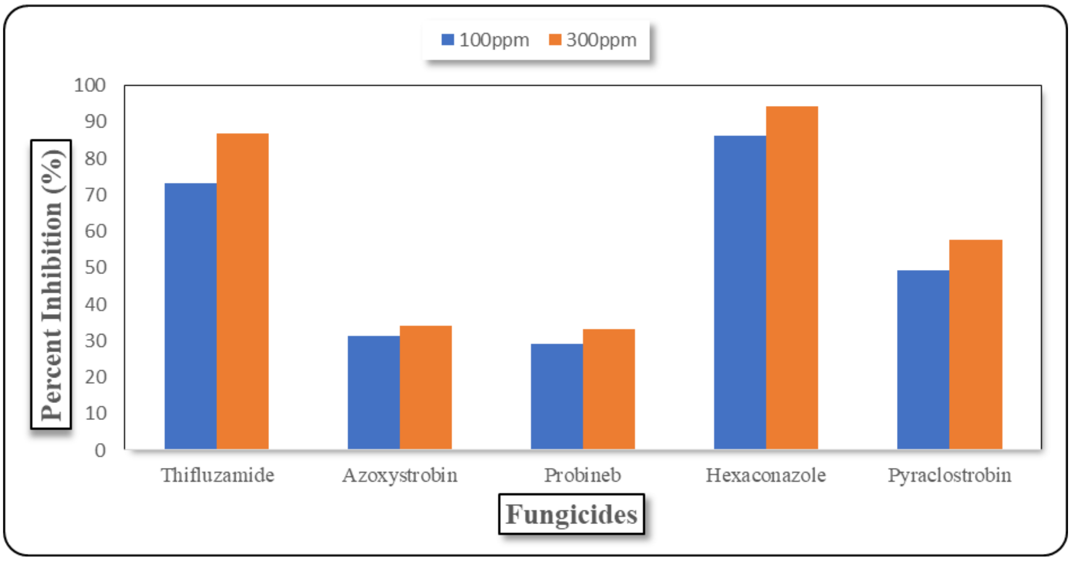
3.1. *In vitro* evaluation of chemical fungicides against *A. solani*

Among the fungicides tested, hexaconazole and tyfluzamide were found to be very effective against the pathogen. The maximum inhibition was observed with these fungicides at 94.33% and 86.80% at a concentration of 300 ppm. This result was followed by the pyraclostrobin fungicide, which was also effective by 57% at 300 ppm. The lowest suppression of the pathogen was found with the Probineb fungicide with an inhibition rate of only 33.33% at 300 ppm and 31.35% at 100 ppm. Therefore, in this experiment, hexaconosol and tifluzamide were recognized as the best fungicides even at lower concentrations, and pyraclostrobin also controls 50% of the infection.

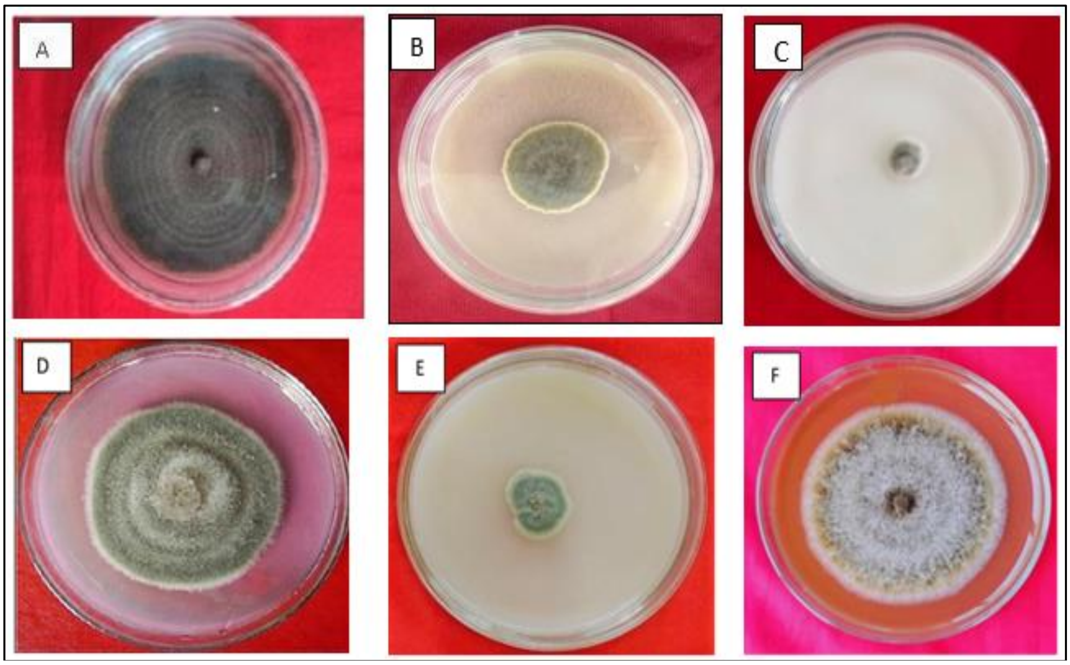
The current results are similar to those of Yadav *et al*. (2018) [11] carried out *in vitro* and *in vivo*. A similar result was also obtained by Sreenivasulu *et al*. (2019) [8] received.

Table 3*:* *In vitro* evaluation of different fungicides on mycelial growth of *Alternaria solani*

		Radial growth (cm)		Percent Inhibition (%)	
S. No.	Fungicides	100ppm	300ppm	100ppm	300ppm
1	Thifluzamide	2.42	1.18	73.11	86.80
2	Azoxystrobin	6.18	5.94	31.35	34.22
3	Probineb	6.37	6.00	29.21	33.33
4	Hexaconazole	1.23	0.51	86.33	94.33
5	Pyraclostrobin	4.56	3.87	49.33	57.83
	Control	9.00	9.00	0.00	0.00
		Fungicides	Concentration	Fungicides X Concentration	
	SEm ±	0.146	0.092	0.207	
	CD at 5%	0.434	0.124	0.614	



Graph 1: *In vitro* evaluation of different fungicides on mycelial growth of *Alternaria solani*



A-Control, B-Pyraclostrobin, C-Hexaconazole, D-Azoxystrobin, E-Thifluzamide, F-Probineb

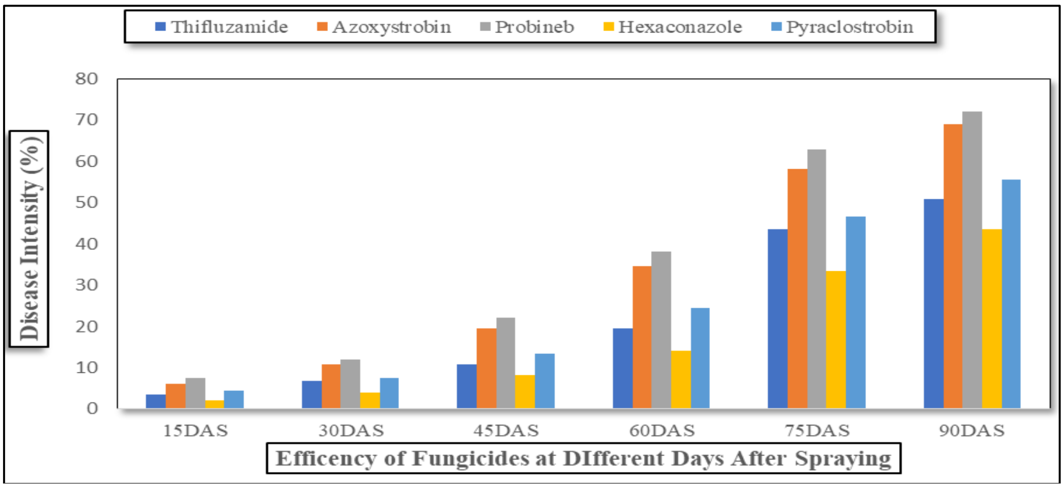
3.2. *In vivo* evaluation of different fungicides against *Alternaria solani* and its impact on yield

Similar fungicides Hexaconazole (Contaf), Azoxystrobin (One star), Thifluzamide (Matsuri), Probineb (Antracol), and Pyraclostrobin (Insignia) were used at 300ppm concentration to determine their efficiency under field conditions. The results obtained were quite similar to that of lab conditions.

Table 4: Evaluation of fungicides on Disease intensity of *Alternaria solani* under field condition

S. No.	Treatments at 300ppm concentration	Disease Intensity (%)					
		15DAS	30DAS	45DAS	60DAS	75DAS	90DAS
1	Thifluzamide	3.43 (5.85)	6.77 (8.60)	10.87 (13.29)	19.53 (23.94)	43.53 (36.94)	50.87 (43.13)
2	Azoxystrobin	6.10 (8.45)	10.77 (13.28)	19.43 (23.40)	34.67 (28.79)	58.10 (50.48)	68.87 (60.29)
3	Probineb	7.43 (10.72)	12.10 (15.47)	22.10 (26.70)	38.20 (32.18)	62.77 (54.85)	72.10 (63.61)
4	Hexaconazole	2.10 (3.44)	4.10 (6.02)	8.20 (10.86)	14.10 (17.75)	33.43 (39.21)	43.43 (36.84)
5	Pyraclostrobin	4.40 (6.09)	7.43 (9.72)	13.43 (16.66)	24.57 (28.95)	46.50 (40.81)	55.63 (48.18)
6	Control	10.77 (14.28)	16.77 (20.09)	38.57 (44.21)	52.10 (45.21)	73.53 (64.96)	83.43 (74.13)
SEm ±		0.260	0.514	0.837	1.074	2.558	2.451
CD at 5%		0.831	1.639	2.672	3.429	8.163	7.824

PDI data at an early stage of the disease were recorded periodically at intervals of 15 days after spraying. After 15 days of spraying, the treatment showed minimal disease intensity (2.10%). Concentration of hexaconazole at 300 ppm was at the level of tifluzamide, pyraclostrobin, azoxystrobin and probineb and was significantly lower than in the control. While 30 days after spraying, the minimum disease intensity (4.10%) was recorded for pyraclostrobin, tyfluzamide was at the same level and significantly lower than with other treatments. After 45 days of spraying, a minimum disease intensity (8.20%) was observed with hexaconazole treatment. This was at the level of tifluzamide treatment, with the disease intensity being 10.87%. The same trend was observed 60 days after spraying. Within 75 days of the spray treatment, the hexaconazole was found to be significantly higher than the rest of the treatments, which showed a minimum disease intensity of 33.43%. In the case of the 90-day spray, treatment with hexaconazole showed a minimum disease intensity (43.43%), which was equivalent to tifluzamide and significantly decreased compared to other treatments. At the same time, the maximum disease intensity of 83.43% was recorded on the control card. Similar results were obtained by Sukrutha and Kamanna (2014) [9], who reported that fungicides in combination with zineb 68% + hexaconazole 4% (21.50% PDI) showed that it was comparable to tebuconazole 25% EC (22%). PDI) and captan 70% + hexaconazole 5% (24.66% PDI).



Graph 2: Evaluation of fungicides on intensity of *Alternaria solani* in field condition

3.3. Number of tomato fruits and Yield

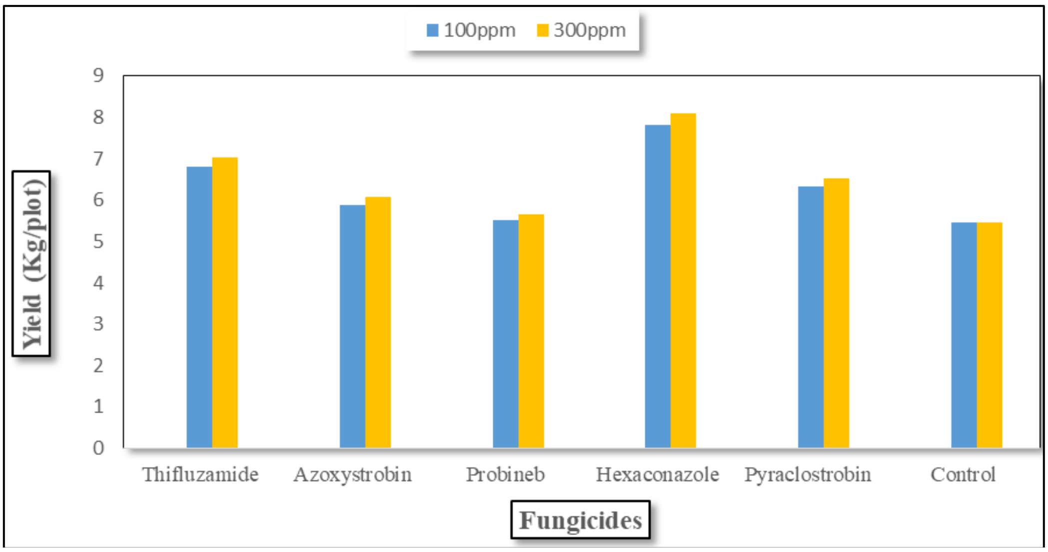
All fungicidal treatments recorded significantly a greater number of fruits and yield in comparison to control. Hexaconazole was proved to be best followed by Thifluzamide, Pyraclostrobin, Azoxystrobin and Probineb in reducing disease and increasing yield.

3.3.1. Number of Fruits

Highest number of fruits (35 plant^{-1}) was recorded in the treatment Hexaconazole which was at par with Thifluzamide (32.81 plant^{-1}) and Pyraclostrobin (30.54 plant^{-1}) and significantly higher over rest of treatments. In control plot it was recorded lowest (20.27 plant^{-1}).

3.3.2 Fruit yield

Maximum fruit yield (1.224 Kg plant^{-1}) (15.45 Kg/plot) and (252.50 q ha^{-1}) was recorded in treatment Hexaconazole followed by Thifluzamide (1.127 Kg plant^{-1}), (14.61 Kg/plot) and Pyraclostrobin (1.066 Kg plant^{-1}). While, least fruit yield per plant was recorded in control plot (0.708 Kg plant^{-1}), (10.46 Kg/plot) and (196.50 q ha^{-1}).



Graph 3: Evaluation of different fungicides against *Alternaria solani* under field conditions and their impact on yield

The result obtained from this experiment was confirmative with the findings of Ganie *et al*., (2013) [5]. He reported that Hexaconazole at 5% EC was most effective irrespective of concentration and exhibited fungal growth inhibition. Similarly, Yadav *et al.*, (2018) [11] who reported that Hexaconazole at its highest concentration showed most effective and exhibited maximum mean mycelia growth inhibition and high yield.

Table 5: Evaluation of different fungicides against *Alternaria solani* under field conditions and their impact on yield

S. No	Fungicides	No. of Fruits/ plant		Yield (Kg/plant)		Yield (Kg/plot) 2 X 2 m^2		Calculated yield (q/ha) on plot basis		% Increase in yield	
		100 ppm	300 ppm	100ppm	300ppm	100 ppm	300 ppm	100ppm	300ppm	100 ppm	300 ppm
1	Thifluzamide	30.45	32.81	1.052	1.124	14.41	14.61	240.25	243.50	27.41	28.40
2	Azoxystrobin	25.85	27.32	0.901	0.956	13.00	13.18	216.75	219.75	19.53	20.63
3	Probineb	20.47	24.02	0.714	0.838	12.30	12.53	205.10	207.25	14.95	16.25
4	Hexaconazole	32.33	35.00	1.129	1.224	15.05	15.45	250.75	252.50	40.49	42.29
5	Pyraclostrobin	28.72	30.54	0.991	1.066	14.00	14.22	233.50	235.00	25.28	26.44
6	Control	20.27	20.27	0.708	0.708	10.46	10.46	196.50	196.50		
SEm ±		0.446	1.608								
CD at 5%		1.423	5.132								

4. Conclusion

Therefore, in this experiment, hexaconazole and tifluzamide were found to be the best fungicides even at lower concentrations, and pyraclostrobin also controls 50% of infection at higher concentrations. Therefore, in the present study it can be determined that systemic fungicides can effectively suppress both pre- and post-infectious activity and inhibit sporulation and or limit the spread of early putrefactive pathogens. It is also found to not only fight late blight, but also increase the yield of tomatoes.

5. References

1. Sabada KA, Mostafa SH, Hillal MR. Effect of some chemical salts on suppressing the infection by early blight disease of tomato. Egyptian Journal of Basic and Applied Sciences 2008;23:47-58.
2. Abdel-Sayed MHF. Pathological, physiological and molecular variations among isolates of *Alternaria solani* the causal of tomato early blight disease. (Doctoral dissertation, Ph. D. Thesis, Faculty of Agriculture, Cairo University 2006.
3. Anurag S, Ved R. Management of Early Blight of Potato by Using Different Bioagents as Tuber Dressing and its Effect on Germination and Growth. International Journal of Current Microbiology and Applied Sciences 2019;8(06):1965-1970.
4. Datar VV, Mayee CD. Chemical management of early blight of tomato. Journal of Maharashtra Agriculture University 1986;10:278-280.
5. Ganie SA, Ghani MY, Nissar Q, Rehman SU. Bio-efficacy of plant extracts and biocontrol agents against *Alternaria solani*. African Journal of Microbiology Research 2013;7(23):4397-4402.
6. Olaniyi JO, Akanbi WB, Adejumo TA, Akande OG. Growth, fruit yield and nutritional quality of tomato varieties. African Journal of Food Science 2010;4:398-402.
7. Pandey KK, Pandey PK, Kallo G, Banerjee MK. Resistance to early blight of tomato with respect to various parameters of disease epidemics. Journal of General Plant Pathology 2003;69:364-371.
8. Sreenivasulu R, Reddy MSP, Tomar DS, Sanjay SSM, Reddy BB. Managing of Early Blight of Tomato Caused by *Alternaria solani* through Fungicides and Bioagents. International Journal of Current Microbiology and Applied Sciences 2019;8(6):1442-1452.
9. Sukrutha HG, Kamanna BC. *In Vitro* and *In vivo* Evaluation of Fungicides against Early blight of Potato caused by *Alternaria solani* (Ellis and Martin) Jones and Grout. Plant Archives 2014;14(2):971-975.
10. Vincent JM. Distortion of fungal hyphae in presence of certain inhibitors. Nature 1927;159:850.
11. Yadav VK, Kumar V, Arghya M. Evaluation of fungicides, Bio-control agents and plant extracts against early blight of potato caused by *Alternaria solani*. International Journal of Chemical Studies 2018;6(1):1227-1230.
12. NC G, Kulkarni GU, Datta B, Shyadambi R. Correlation coefficient studies for fruit yield and its components in tomato (*Solanum lycopersicum* L.).