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In vitro evaluation of fungicides, bio-control agents and plant extracts against early blight of tomato caused by *Alternaria solani* (Ellis and Martin) Jones and Grout

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ABSTRACT

The present experiment was conducted to test the efficacy of fungicides, bio-control agents and plant extracts *in vitro* against *Alternaria solani* causing early blight of tomato. Seven fungicides *viz.*, four systemic (Propiconazole, Azoxystrobin, Thiophanate methyl and Carbendazim) and three non-systemic (Mancozeb, Captan and Zineb) at four concentrations *i.e.* 50, 100, 150 and 200 ppm and seven plant extracts *viz.*, *Datura strumarium* (Jimson weed), *Allium sativum* (Garlic), *Azadirachta indica* (*Neem*), *Zingiber officinale* (Ginger), *Ocimum sanctum* (*Tulsi*), *Calotropis gigantea* (Aak) and *Eucalyptus chamadulonsis* (Eucalyptus) also at four concentrations *i.e.* 5, 10, 15 and 20 per cent were evaluated through poison food technique. Seven bio-control agents *viz.*, *Trichoderma harzianum, T. viride, T. koningii, T. hamatum, T. atroviride, Aspergillus niger* and *A. flavus* were also evaluated in this study through dual culture technique. Among the systemic fungicides, Propiconazole was proved to be highly effective and recorded cent per cent inhibition at their all concentrations while among the non-systemic, Mancozeb was proved to be effective at their all concentrations but recorded 100 per cent inhibition only at their higher concentration *i.e.* 400 ppm. Among different plant extracts used, *Azadirachta indica* (*Neem*) was significantly inhibit the mycelial growth of pathogen at all concentrations followed by *Datura strumarium* (Jimson weed) and *Calotropis gigantea* (Aak). Of all bio-control agents, highest inhibition of radial growth of test fungus was recorded in *Trichoderma harzianum* (80.37%) followed by *T. viride* (71.48%) and *T. koningii* (77.41 %). However, *T. hamatum* (27.41%) was least effective in this study.

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INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important vegetable crop of solanaceae family after potato (Pritesh and Subramanian, 2011 and Hadian *et al*., 2011). It is a second important vegetable

crop in terms of food value and ranks first in processed food product. India is one of the largest producer of tomatoes in the world, second only to China with an estimated production of 18735.91 thousand metric tonnes in 2013-14 and around 11 per cent of the total world produce of tomatoes is cultivated in India. Andhra Pradesh, Karnataka, Uttar Pradesh, Maharashtra, Haryana, Punjab, Bihar and Himachal Pradesh are the major growing states in India. There has been a gradual increase in the area under tomato while the production has been fluctuating due to various diseases and insect pest damage. There are several diseases on tomato caused by fungi, bacteria, viruses, nematodes and abiotic factors (Balanchard, 1992; Gomaa, 2001; Abdel-Sayed, 2006 and Abada *et al*., 2008). Among the fungal diseases, *Alternaria solani* (Ellis and Martin) Jones and Grout causing early blight, is the most destructive one (El-Abyad *et al*., 1993; Gomaa, 2001; Abdel-Sayed, 2006 and Abada *et al*., 2008), which resulted in great reduction in the quantity and quality of crop. The initial symptom of early blight is small dark brown spots on the lowest and oldest leaves. The tissue around the primary lesions may turns bright yellow and if lesions are numerous, the entire leaves may become necrosis and chlorotic. The spots get enlarged, they develop concentric rings which give them a bull's eye. In favourable weather conditions, disease develop, lesions can become numerous and plants defoliate, which damage the quantity and quality of tomato fruits (Kouyoumjian, 2007). The disease had resulted of 78% loss in yield of fruit caused by *Alternaria solani* (Saad *et al*., 2014). *A. solani* can infect each part of the plant (causing foliage blight, fruit lesions and stem collar rot) and can damage during all stages of plant development (Abada *et al*., 2008). So management of this disease is very necessary. Use of resistant varieties is the ultimate control of this disease. However, farmers in pursuance of high yield are inclined to cultivate some varieties which may be less resistant to disease. Also unplanned and wide use of fungicides often leads to serious environmental problems besides affecting the health of users and consumers. So, it is necessary to minimize the use of chemicals for controlling disease. Hence, the attempt has been made to evaluate some new agro chemicals, plant extracts and bio agents against *Alternaria solani*, as it is use full in short listing the effective fungicides for field experiments and also integrating the bio agents and botanicals to come up with a ecofriendly management strategy to manage the early blight of tomato. Keeping the importance of this disease in view the *in vitro* evaluation of fungicides, bio agents and botanicals was done to know their bio efficacy.

MATERIAL AND METHODS

The present study was carried out under laboratory conditions during *Rabi* season 2016-2017 at Department Of Plant Protection, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh. *In vitro* evaluation of fungicides and plant extracts against *Alternaria solani* (Ellis and Martin) Jones and Grout were carried out through poison food technique (Nene and Thapliyal, 1979) and bio-control agents were evaluated through dual culture (Cherif and Benhamou, 1990).

Isolation of pathogen and preparation of pure culture :

The pathogen was isolated from infected leaves of tomato plants by following single spore isolation methods. Pure cultures of *A. solani* was maintained on PDA slants for further investigations.

In-vitro efficacy of fungicides and plant extracts on mycelial growth of *Alternaria solani* :

Relative efficacy of 7 fungicides on mycelial growth inhibition of *A. solani* was studied *in vitro*, using poison food technique (Nene and Thapliyal, 1979). In this experiment, three non-systemic fungicide (Mancozeb, Captan and Zineb) and four systemic fungicides (Propiconazole, Azoxystrobin, Thiophanate methyl and Carbendazim) were used for their efficacy at 4 different concentrations *i.e.* 100, 200, 300 and 400 ppm.

Efficacy of 7 plants extracts *i.e.Datura strumarium* (Jimson weed), *Allium sativum* (Garlic), *Azadirachta indica* (*Neem*), *Zingiber officinale* (Ginger), *Ocimum sanctum* (*Tulsi*), *Calotropis gigantea* (Aak) and *Eucalyptus chamadulonsis* (Eucalyptus) were evaluated in fresh forms at four different concentrations *viz*., 5, 10, 15 and 20 per cent by employing food poison technique against *A. solani*. Selected plants were collected from the surrounding areas of Aligarh Muslim University, Aligarh and washed thoroughly with tap water and air dried. One hundred grams of plant tissue was grind using pestle and mortar by adding equal amount (100 ml) of sterilized distilled water (1: 1, w/v). The pulverized mass was squeezed through the cheese cloth

and the extracts were centrifuged at 10000 rpm for 5-10 minutes. The supernatant was filtered through Millipore filters (45µm) using vacuum pump assembly under aseptic conditions.

For both fungicides and plant extracts 5 mm mycelial disc was placed at the center. Suitable check was maintained without addition of fungicides or plant extracts. Nine days old 5 mm mycelial disc of *Alternaria solani* was placed in the centre of petriplates and incubated at 25 ±1ºC. The observation on the radial growth diameter was made the check Petri plates were fully covered with test fungus. Each treatment was replicated thrice with a suitable control. The efficacy of fungus in each treatment and average of three replications was calculated. The per cent inhibition in mycelial growth (T) over control (check) was calculated by using following formula:

$$\text{Per cent inhibition (\%)} = \frac{C - T}{C} x100$$

where, C = Colony growth diameter (mm) of fungus in check.

T = Colony growth diameter (mm) of fungus in treatment.

***In-vitro* efficacy of bio-control agents on mycelial growth of *Alternaria solani* :**

Bio-efficacy of seven BCAs namely *T. harzianum*, *T. viride*, *T. koningii*, *T. hamatum*, *T. atroviride*, *Aspergillus niger* and *A. flavus* were evaluated, *in vitro,* against *Alternaria solani* yielded from tomato plants. Some antagonist fungal species were isolated from experimental field of Department of Plant Protection, Aligarh Muslim University, Aligarh and identified the species on the basis of the microscopic characteristics and some of antagonist procured from Indian Type Culture Collection, IARI, New Delhi.

The antagonistic activity of these bio-control agents were studied on *A. solani* by dual culture technique (Cherif and Benhamou, 1990). On Petri dishes with PDA and placing equidistantly a disk (5 mm in diameter) with mycelium of the pathogen and on the other side of petri dish, a disk of the mycelium of the same diameter of bio-control agents under study. The inoculated plates were incubated at 25± 1ºC until the growth of control treatment (with only plant pathogen disk) covered the Petri dish.

$$\text{Per cent inhibition (\%)} = \frac{C - T}{C} x100$$

where, C = Growth of the phytopathogen in the absence of the antagonist.

T = Growth of the phytopathogen in the presence of the antagonist.

In all experiments, test and control plates were set up in three replicates and average thereof used for the analysis.

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under the following heads:

***In-vitro* efficacy of fungicides on mycelial growth of *Alternaria solani* :**

The result presented in Table 1 revealed that all systemic and non-systemic fungicides used in this experiment at different concentrations significantly inhibit the mycelial growth of *A. solani* causing tomato early blight.The results showed that the increase concentrations of each fungicides resulted a proportionate reduction in mycelial growth of *A. solani*. The efficacy of each fungicides was found to show descending trend in growth inhibition from higher to lower concentration *i.e.* 100 ppm, 200 ppm, 300 ppm and 400 ppm. However, maximum growth inhibition was recorded at 400 ppm. Among the systemic fungicides, Propiconazole was proved to be highly effective and caused cent per cent inhibition of mycelial growth of pathogen at all concentrations. This was followed by Carbendazim and Azoxystrobin which recorded 34.07, 48.15, 64.07, 81.48 per cent and 18.52, 28.88, 40.37 and 50.37 per cent inhibition of *A. solani* at 100, 200, 300 and 400 ppm concentrations, respectively (Table 1). Thiophanate methyl was not so much effective as compare to other fungicides at their all concentrations which recorded only 35.93 per cent inhibition at their higher concentration (400 ppm). While amongst the non-systemic, Mancozeb was superior to other two fungicides at all concentrations but recorded 100 per cent inhibition only at 400 ppm (Table 1). This was followed by Zineb and Captan which recorded 23.33, 35.55, 51.85, 64.82 per cent and 21.48, 31.11, 40.74 and 54.07 per cent inhibition of radial growth of pathogen at 100, 200, 300 and 400 ppm concentrations, respectively (Table 1). Thus, it is clear from this study that Propiconazole (systemic) and Mancozeb (non-systemic) proved to be most effective at their all four concentrations against *A.*

solani. The results were in conformity with Herle and Kamanna (2014) where propiconazole was found to be effective in inhibiting the mycelial growth of *A. solani*. The present finding also confirms the reports of several earlier workers like Chethana *et al*. (2012) and Gondal *et al*. (2012) who reported the efficiency of Mancozeb on growth inhibition of *A. solani* from tomato crop.

In-vitro efficacy of plant extracts on mycelial growth of *Alternaria solani* :

Seven plant species were selected and evaluated for the antimicrobial activity against *A. solani*. All the leaf extracts of tested plants at 5 per cent, 10 per cent, 15 per cent and 20 per cent concentrations were effective in inhibiting the mycelial growth of *A. solani*, when compared to the control. The results showed that the increase concentrations of each botanicals resulted a proportionate reduction in radial growth of *A. solani*. The results revealed that, the plant extracts were effective at 20 per cent than 5 per cent, 10 per cent and 15 per cent concentrations. Among the seven plant extracts evaluated, *Azadirachta indica* (*Neem*) at 20

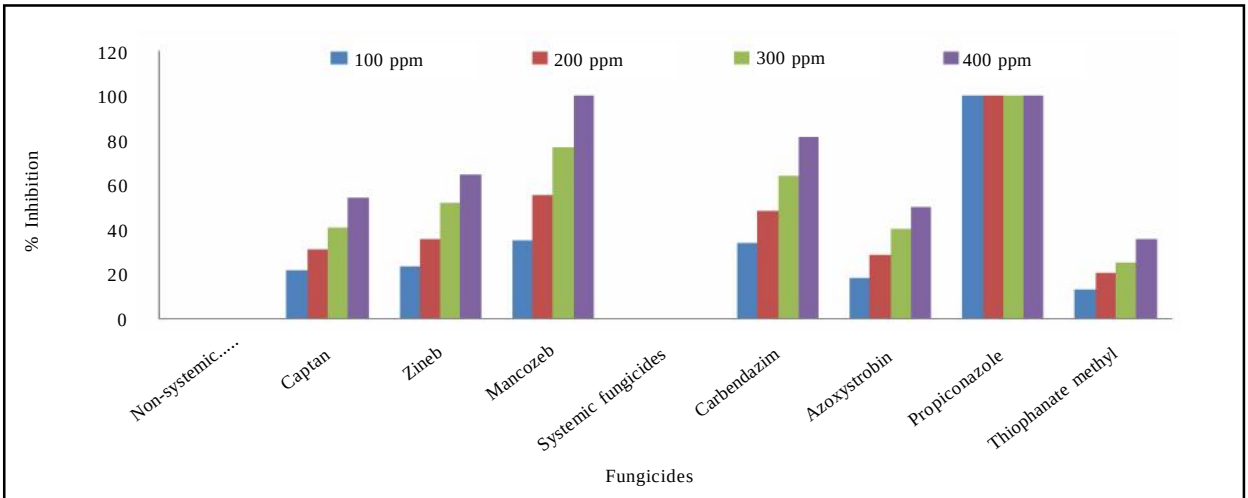

Fig. 1 : Effect of fungicieds on per cent inhibition of mycelial growth of *Alternaria solani*

Table 1: *In-vitro* efficacy of fungicides on mycelial growth of *Alternaria solani*

Fungicides/ concentrations	100 ppm		200 ppm		300 ppm		400 ppm	
	Mycelial growth (mm)	% inhibition	Mycelial growth (mm)	% inhibition	Mycelial growth (mm)	% inhibition	Mycelial growth (mm)	% inhibition
Non-systemic								
Captan	70.66 (57.18)	21.48	62.00 (51.92)	31.11	53.33 (46.89)	40.74	41.33 (39.99)	54.07
Zineb	69.00 (56.14)	23.33	58.00 (49.58)	35.55	43.33 (41.15)	51.85	31.66 (34.23)	64.82
Mancozeb	58.66 (49.97)	34.82	40.33 (39.41)	55.18	20.33 (26.79)	77.04	00.00 (00.00)	100.0
Systemic								
Carbendazim	59.33 (50.35)	34.07	46.66 (43.07)	48.15	32.33 (34.64)	64.07	16.66 (24.08)	81.48
Azoxystrobin	73.33 (58.88)	18.52	64.00 (53.11)	28.88	53.66 (47.08)	40.37	54.66 (47.65)	50.37
Propiconazole	00.00 (00.00)	100.0	00.00 (00.00)	100.0	00.00 (00.00)	100.0	00.00 (00.00)	100.0
Topsin-M	78.33 (62.33)	12.96	71.66 (57.81)	20.37	67.33 (55.12)	25.18	57.66 (49.39)	35.93
Check	90.00 (71.53)		90.00 (71.53)		90.00 (71.53)		90.00 (71.53)	
C.D. (P=0.05)	0.92		0.80		0.62		0.50	
S.E.±	0.30		0.26		0.20		0.16	
S.E. (d)	0.43		0.37		0.29		0.23	
C.V.	0.93		0.91		0.83		0.86	

Figures in parentheses are the arcsin √ per cent transformed values * Each value is an average of 3 replicates

per cent concentration was found to be best in inhibiting the mycelial growth of *A. solani* (80.37%) and found significantly superior over all the other extracts, followed by *Datura strumarium* (69.63%), *Calotropis gigantea* (67.04%), *Ocimum sanctum* (60.74%) and *Eucalyptus chamadulonsis* (57.77%) at 20 per cent (Table 2). The least inhibition of mycelial growth of *A. solani* was recorded in *Allium sativum* (47.41%) followed by *Zingiber officinale* (39.63%) at 20 per cent concentration. The results revealed that all of the tested plant extracts at given concentration inhibited the growth of pathogens (Table 2). Various plant extracts were found effective against *A. solani* have been reported by several workers (Yanar *et al*., 2011 and Khafari *et al*., 2014).

In-vitro efficacy of bio-control agents on mycelial growth of *Alternaria solani* :

Seven bio-control agents were selected to check their efficacy against *A. solani* through dual

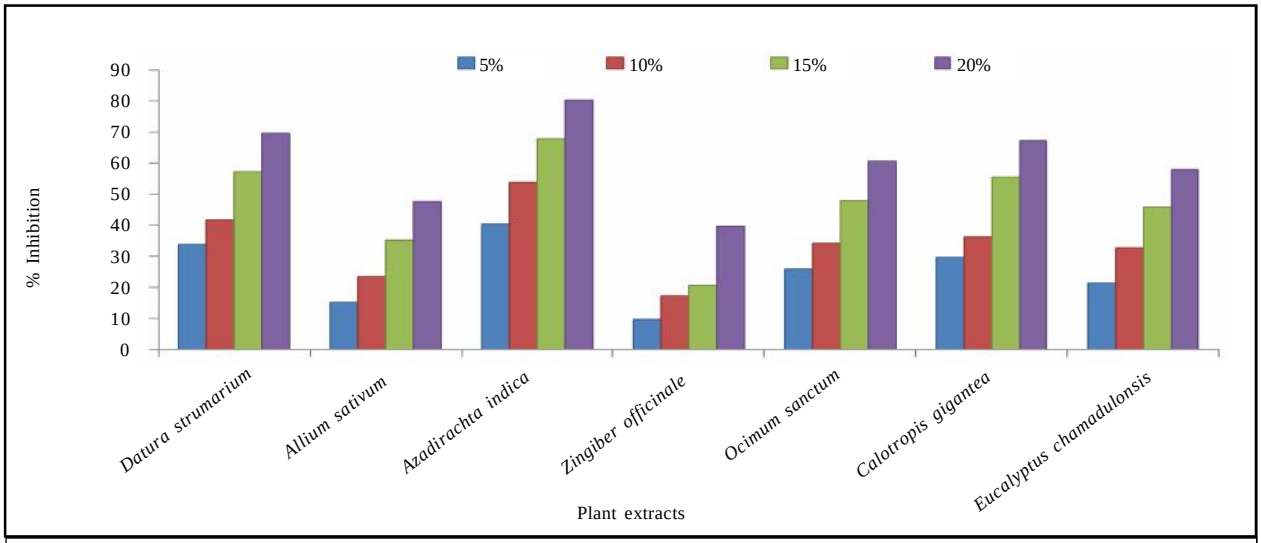

Fig. 2 : Effect of fungicieds on per cent inhibition of mycelial growth of *Alternaria solani*

Table 2: *In-vitro* efficacy of plant extracts on mycelial growth of *Alternaria solani*

Plant extracts	Concentrations (%)							
	5%		10%		15%		20%	
	Mycelial growth (mm)	% inhibition	Mycelial growth (mm)	% inhibition	Mycelial growth (mm)	% inhibition	Mycelial growth (mm)	% inhibition
Datura strumarium (Jimson weed)	59.66(50.55)	33.71	52.33 (46.31)	41.85	38.66 (38.43)	57.04	27.33 (31.50)	69.63
Allium sativum (Garlic)	76.33 (60.87)	15.18	69.00 (56.14)	23.33	58.33 (49.77)	35.18	47.33 (43.45)	47.41
Azadirachta indica (*Neem*)	53.66 (47.08)	40.37	41.33 (40.18)	53.71	29.00 (32.56)	67.77	17.66 (24.84)	80.37
Zingiber officinale (Ginger)	81.33 (64.38)	9.63	74.33 (59.54)	17.41	63.33 (52.71)	20.63	54.33 (47.46)	39.63
Ocimum sanctum (*Tulsi*)	66.66 (54.71)	25.93	59.33 (50.35)	34.07	47.00 (43.26)	47.77	35.33 (36.45)	60.74
Calotropis gigantean (Aak)	63.33 (52.71)	29.63	57.33 (49.19)	36.30	40.00 (39.21)	55.55	29.66 (32.98)	67.04
Eucalyptus chamadulonsis (Eucalyptus)	70.66 (57.18)	21.48	60.66 (51.13)	32.60	48.66 (44.21)	45.93	38.00 (38.04)	57.77
Check	90.00 (71.53)		90.00 (71.53)		90.00 (71.53)		90.00 (71.53)	
C.D. (P=0.05)	0.93		0.75		0.76		0.55	
S.E. ±	0.31		0.25		0.25		0.18	
S. E. (d)	0.43		0.35		0.35		0.25	
C.V	0.93		0.82		0.94		0.77	

Figures in parentheses are the arcsin √ per cent transfored values *Each value is an average of 3 replicates

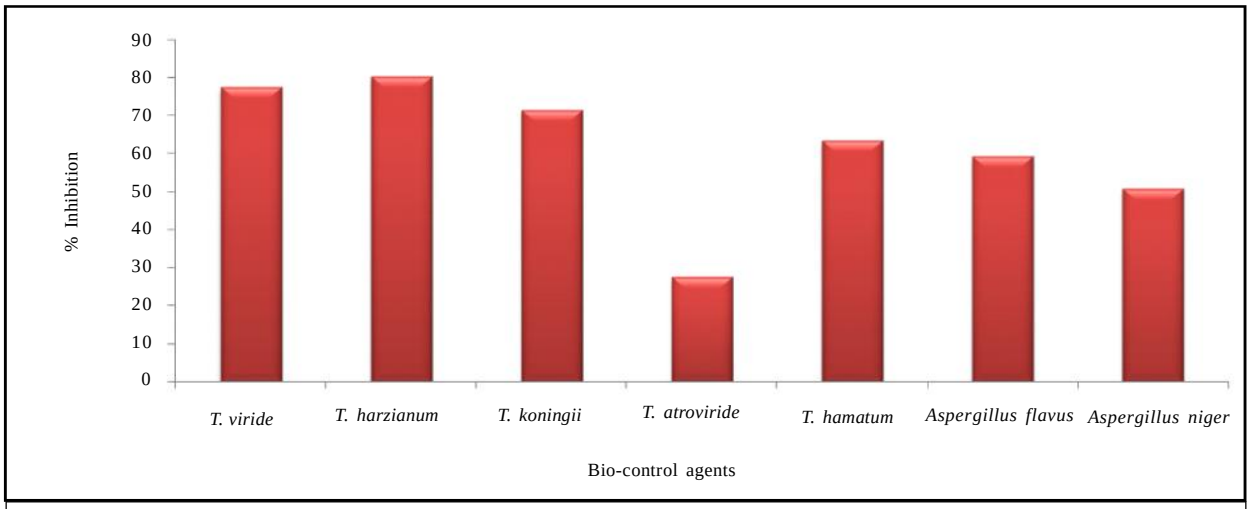

Fig. 3 : Effect of bio-control agents on per cent inhibition of mycelial growth of *Alternaria solani*

Table 3 : *In-vitro* efficacy of bio-control agents on mycelial growth of *Alternaria solani*

BCAs	Mycelial growth (mm)		% Inhibition
	Trichoderma spp.	*Alternaria solani*	
Trichoderma viride	69.66	20.33 (26.79)	77.41
T. harzianum	72.66	17.66 (24.84)	80.37
T. koningii	64.33	25.66 (30.41)	71.48
T. hamatum	34.66	65.33 (53.90)	27.41
T. atroviride	57.00	33.00 (35.04)	63.33
Aspergillus niger	53.66	36.33 (37.05)	59.33
Aspergillus flavus	45.66	44.33 (41.72)	50.74
Check		90.00 (71.53)	
C.D. (P=0.05)		0.79	
S.E.±		0.26	
S.E. (D)		0.37	
C.V.		1.12	

Figures in parentheses are the arcsin √ per cent transformed values * Each value is an average of 3 replicates

culture technique. The results of the experiment are presented in Table 3. In this experiment, *Trichoderma harzianum* was found to be effective and recorded highest inhibition (80.37%) of the mycelial growth of the pathogen followed by *T. viride* (77.41%), *T. koningii* (71.48%) (Table 3). While *T. atroviride* recorded 63.33 per cent inhibition of pathogen. However, *T. hamatum* was least effective and inhibited 27.41 per cent mycelial growth of *A. solani*. Similarly, Ganie *et al*. (2013) also reported the effectiveness of mycelial inhibition of *A. solani* by *T. harzianum* (71.85%), which was followed by *T. viride* (65.93%) and *T. virens* (58.65%). The effectiveness of *Trichoderma harzianum* ISO-1, *T. harzianum* ISO-2 and *T. piluliferum* against *A. solani* was also reported by Thakur and Harsh, 2014.

REFERENCES

Abada, K. A., Mostafa, S. H. and Mervat, R. (2008). Effect of some chemical salts on suppressing the infection by early blight disease of tomato. *Egyptian J. Appl. Sci.*, **23**: 47–58.

Abdel-Sayed, M. H. F. (2006). Pathological, physiological and molecular variations among isolates of *Alternaria solani* the causal of tomato early blight disease: 181pp.

Balanchard, D. (1992). *A colour atlas of tomato diseases.* Wolfe Publication Limited, Book House, London. p.298.

Cherif, M. and Benhamou, N. (1990). Cytochemical aspects of chitin breakdown during the parasitic action of *Trichoderma*

spp. on *Fusarium oxysporum* f. sp. *radicis-lycopersici*. *Phytopathology*, **80**: 1406-1412.

Chethana, B.S.,Ganeshan, G. , Rao, A.S. and Bellishree, K. (2012). *In vitro* evaluation of plant extracts, bioagents and fungicides against *Alternaria porri* (Ellis) Cif. Causing purple blotch disease of onion. *Pest Mgmt. Hort. Ecosyst.*, **18** (2): 194-198.

El-Abyad, M. S., El-Sayad, M. A., El-Shanshoury, A. R. and El-Sabbagh S. M. (1993). Towards the biological control of fungal and bacterial diseases of tomato using antagonistic *Streptomyces* spp. *Plant Soil*, **149**:185–95.

Ganie, S. A., Ghani, M. Y., Qazi Nissar and Shabir-u-Rehman (2013). Bioefficacy of plant extracts and biocontrol agents against *Alternaria solani*. *African J. Microbiol. Res.*, **7**(34): 4397 - 4402.

Gomaa, A. M. I. (2001). Pathological studies on early blight of tomato. M.Sc. Thesis., Fac. Agric., Cairo University.

Gondal, A. S., Ijaj, M., Riaj, K. and Khan, A. R. (2012). Effect of different doses of fungicide (Mancozeb) against *Alternaria* leaf blight of tomato in tunnel. *J. Plant Pathol Microb.*, **3**: 125.

Hadian, S., Rahnama, K., Jamali, S. and Eskandari, A. (2011). Comparing neem extract with chemical control on *Fusarium oxysporum* and *Meloidogyne incognita* complex of tomato. *Adv. Environ. Biol*., **5**(8): 2052-2057.

Herle, G. S. and Kamanna, B.C. (2014). *In vitro* and *in vivo* evaluation of fungicides against early blight of potato caused by *Alternaria solani* (Ellis and Martin) *Jones & Grout.*, **14**. 971-975.

Khafari, A.S., Bahraminejad, S. and Abbasi, S.(2014). Evaluation of the Anti- *Alternaria solani* activity of *Allium hirtifolium Boiss. Pak. J. Bot.*, **46**(2): 741-747.

Kouyoumjian, R. E. (2007). Comparison of compost tea and biological fungicides for control of early blight in organic heirloom tomato production. M.Sc. Thesis, Clemson University, South Carolina 16-37pp.

Nene, Y.L. and Thapliyal, P.N. (1979). *Fungicides in plant disease control*. 2nd Ed. Oxford and IBH Pub. Co., New Delhi, India.

Pritesh, P. and Subramanian, R.B. (2011). PCR based method for testing Fusarium wilt resistance of tomato. *African J. Basic & Appl. Sci*., **3**(5): 222-227.

Saad, A.S.A., Kadous, E.A., Tayeb, E.H., Massoud, M.A., Ahmed S.M. and Abou El- Ela, A.S.A. (2014). The inhibitory effect of some antioxidants and fungicides on the growth of *Alternaria solani* and *Fusarium solani* in vitro. *Middle east J. Agric. Res*., **3**(2): 123- 134.

Thakur, S. and Harsh, N.S.K. (2014). Phylloplane fungi as biocontrol agent against Alternaria leaf spot disease of (Akarkara) Spilanthes oleracea. *Bioscience Discovery*, **5**(2):139-144.

Yanar, Y. A. Gokce, Kadioglu, H. Cam and Whalon, M. (2011). *In vitro* antifungal evaluation of various plant extracts against early blight disease (*Alternaria solani*) of potato. *African J.Biotechnol*., **10**(42): 8291- 8295.