

## In Vitro Evaluation of Some Fungicides against Common Fungal Pathogen of Early Blight and Fruit Rot of Tomatoes

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### ABSTRACT

Diseases are still the main cause of reduction of yield in tomato crops in Pakistan. Early blight and fruit rot of tomatoes are the most important diseases caused by *Alternariasolani*. These diseases are responsible to cause tremendous yield loss in tomato crops in District Mansehra, Khyber Pakhtunkhwa, Pakistan. This research was initiated to isolate the causal agents of early blight and fruit rot of tomatoes and to determine the efficacy of commonly available fungicides against them. Five fungicides Helonil (Chlorothalonil), Copper Oxychloride, Antracol, Ridomil gold and Desomil platinum were evaluated in vitro against radial growth of *Alternariasolani* at six different concentrations. The results showed that Chlorothalonil has better effectiveness as compared to others followed by clipper and Antracol. The most effective dose of Helonil (Chlorothalonil) was recorded as 400 ppm with inhibition of (82.85%) followed by clipper (64.70%) at 500 ppm and Antracol (46.66%) at 1000 ppm. Least inhibition was observed in Ridomil (7.74%) and Desomil (8.57%) with concentration of 300 ppm

**KEYWORDS:** Fungicides, *Alternaria*, *Fusarium*

### 1 INTRODUCTION

More than 800 million people in developing countries have insufficient food supply and at least 10-15% of agriculture products are lost due to plant diseases (Strange and Scott, 2005). Plant diseases are caused by biotic and abiotic factors, genetic disorders and living infectious agents including various pathogens such as fungi, bacteria, viruses, viroids, phytoplasmas, nematodes, parasitic plants, and protozoan's (Agrios, 2005). As compared to other plant pathogens, phytopathogenic fungi are the most prominent parasitic organisms and can be the source of serious diseases and important yield losses in crops (Gonzalez-Fernandez and Jorrin-Novo, 2011). They are the most damaging and destructive parasites causing huge loss to plant yield. There are 20,000 species of fungal plant pathogens and about 85% of plant diseases are caused by them (Ong, 2011; Cooper, 2007).

Tomato (*Lycopersiconesculentum* Mill.) is one of the most important vegetable crops in the world. It is considered as an important cash and industrial crop in many parts of the world (Babalola *et al.*, 2010). Pakistan produced 560, 700 tons tomatoes in 2008-9. During 2008-09 total area under cultivation were 53, 400 hectares. Per hectare yield of tomatoes has been counted 10.84 tons which is very low due to several production constraints including diseases (Agric Stat, 2008-09). Early blight of Tomato caused by *Alternariasolani* became the most destructive all over the world and caused yield losses up to 80 % (Singh, 1985, Mathur and shekhawat, 1986, Chandravanshi *et al.*, 1994).

The disease of early blight was first recorded in 1882 in New Jersey, USA (Bose and Som, 1986). The fungus introduces many unwanted symptoms in tomato plants. The appearance of these symptoms depends on plant part, various stages of plant and fruit development. Usually symptoms of early blight appear on fruit, stem and foliage of tomatoes. These symptoms include early blight of leaves, stem blight and leaf spot on leaves, foot rot and collar rot on young plant stem, black rot and hard rot on fruit, and fruit drop of fruit and petioles and blossom blight on the calyx. In fruit rot foliar decay is very destructive phase which damages the fruits completely (Hawamdeh and Shabir, 2001). Leaf spot symptoms are characteristic for early blight Kemmitt (2002) reported that early symptoms on leaves become visible as small, 1-2 mm black or brown lesions. The lesions may pull out and are often surrounded by a chlorotic zone. Lesions of 10 mm have dark concentric ring so-called "bull eye". In the absence of fungicides treatment, disease incidence was about 30% in susceptible varieties (Sherf and Mecnab, 1986).

Hawamdeh and Shabir (2001) evaluated seven fungicides against *Alternariasolani* through poison food techniques and reported that Dithane M-45 is the most effective fungicide at 1000 ppm. Haggag and

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Farghaly (2007) evaluated two fungicides, metalaxyl (Ridomil plus 50% WP) at concentrations of 12.5, 25, 50 and 75 ppm and chlorpyrifos-methyl (Reldan 50% EC) at concentrations of 25, 50, 100 and 125 ppm alone or in mixture against *A. solani* *in vitro* and reported that the mycelial growth was reduced by Chlorpyrifos-methyl followed by the two pesticides mixture and than metalaxyl, respectively. Issiakhem and Bouznad (2010) reported that difenoconazole had a better effectiveness *in vitro* than Chlorothalonil in inhibition of mycelial growth and conidial germination of *Alternariasolani* and *A. alternata*. Metaet al., (2005) tested three fungicides copper Oxychloride at 0.25%, Bavisin [carbendzim] at 0.1% and Dithane M-45 [mancozeb] at 0.25% on tomato early blight (*A. solani*) and maximum radial mycelial growth inhibition was observed under Dithane M-45 treatment (89.98%), followed by carbendazim (85.61%) and copper oxychloride (83.62%). Parvez et al., (2003) listed five fungicides Banko (chlorothalonil) 500 Sc, Score (difenoconazole) 250 EC, Acrobat M2 90/600 WP, metalaxyl+Mancozeb 72 WP and Ridomil Gold (Mancozeb+metalaxyl) 68 WP against early blight and late blight. All the fungicides much reduced disease severity comparing to control.

District Mansehra, KP, Pakistan is famous for the cultivation of various kinds of vegetables. However, fungal diseases are one of the main thorns which reduce crop yield in quality as well as in quantity. The use of fungicides in agricultural crops is a cheapest practice and their use has made it feasible to enhance crop yields and food production. During the preliminary survey conducted for present study, it has been observed that most farmers used locally available fungicides; even then the diseases could not be controlled properly. The efficacy of fungicide is not a constant phenomenon; because it is influenced by many biological and environmental factors that directly influence the metabolic activities of fungal cells. Sometimes critical concentrations are not usually effective for a long time, as the fungus can be adapted on molecules of the fungicide therefore, *In vitro* tests are very important to measure and rank the fungitoxicity of fungicides against a particular pathogen. *In vitro* evaluations of fungicides offer useful preface information regarding its efficacy against a pathogen in a shortest period of time and therefore, provide guideline for further field testing in future. The suitable concentrations recommended after *in vitro* evaluation should be utilized.

## MATERIAL AND METHODS

Diseased specimens of early blight and tomato rot were collected from tomato growing areas of Mansehra District, Pakistan. The infected tissues of leaves and fruits showing typical symptoms of early blight and fruit rot were cut in to small pieces of 1-2 mm size. These pieces were surface sterilized with 70 % ethyl alcohol solution for 60 seconds, rinsed thrice with sterile distilled water, blotted dry and incubated at 28 °C on potato dextrose agar medium for 7 days. When the colony growth appeared, it was transferred to another plate containing sterilized media through cork borer. The culture thus obtained was further purified by hyphal tip method and Single-spore Sub-culture Techniques (Haggag et al., 2007; Leslie and Summerell, 2006). The conidial morphology of the pathogens were studied and identified as *Alternariasolani* after consulting the authentic methods (Singh, 1987; David, 1991; Ellis, 1971).

Poison food technique (PFT) was used to test different concentrations (100, 200, 300, 400, 500, 1000 ppm) of the fungicides Helonil (Chlorothalonil), Copper Oxychloride, Antracol, Ridomil gold and Desomil platinum against *Alternariasolani* and *A. alternate* respectively. Fungicidal suspensions of different concentrations were prepared in six flasks by dissolving requisite quantities of each fungicide in warm media at 50 °C before pouring and shaken well. For this purpose 1 gram of fungicide was dissolved in 10 ml of distilled water for stock solution then 10 micro liter of fungicidal suspension was added to 100ml of media to form 100ppm concentration. In the same way 20, 30, 40, 50, and 100 micro liters were added to 100 ml media to form 200ppm, 300ppm, 400ppm, 500ppm, and 1000ppm concentrations respectively. Each flask containing media without fungicides was used for control purpose in further processing.

About 20 ml of sterilized medium was poured in each 9 cm sterilized petridish. After solidification, the plates were inoculated by placing 5 mm discs of 10 days old cultures of fungal isolates. The mycelium disk was taken by cork borer. Three replicated plates were used for each concentration of all fungicides. Three replicated PDA plates received no fungicides and served as control. Colony growth was measured (mm) in two directions from the back side perpendicular to each other, taking the value of growth as the mean of two measures. Percent inhibition of radial growth was computed based on colony diameter on control plate using the following formula of Nene and Thapliyal (1993):

$$\% \text{ Inhibition} = \frac{C-T}{C} \times 100$$

Where,

C= Colony Growth (mm) of control plate

Y= Colony Growth (mm) of fungicide treated plate

Data were analyzed by using MSTAT- C program as described by Khan et al, (2007).

## RESULTS AND DISCUSSION

*Alternariasolani* was isolated from infected leaves and fruit rot of tomato plants (Fig 1) by standard tissue isolation techniques. The leaves of infected plants showing typical early blight symptoms while the fruits were contaminated with circular concentric rings.

The taxonomic and morphological studies were made to confirm the pathogen and following micro and macro observations were made. The morphology of spores under microscope revealed that the spores were singly straight or slightly flexuous oblong or muriform or ellipsoidal tapering to beak, pale or olivaceous brown, with 8-10 transverse and 0-4 longitudinal septa. The beaks were found flexuous, pale and sometime segmented (Fig 1). The morphological feature of the isolated fungal strain coincides with the characteristic of *Alternariasolani* (Ellis and Martin, Jones and Grout, 1971). Thus the pathogen causing early blight of tomato and fruit rot has been identified as *A. solani* as described by Common Wealth Mycological institute, Kew, Surrey, England (Ellis, 1971). A set of pure culture of *A. solani* (Fig 1) was also sent to PMAS Arid Agriculture University Rawalpindi for further confirmation and it was confirmed that the species is *A. solani*.

The results of *in vitro* evaluation of fungicides against *A. solani* showed that there was a significant difference among fungicides in inhibiting the growth of *A. solani* (Table 1). Helonil (Chlorothalonil) was observed as superior fungicide at all concentrations over other treatments. The most effective dose of Helonil (Chlorothalonil) was recorded at 400 ppm with inhibition of (82.85%) followed by clipper (64.70%) at 500 ppm and Antracol (46.66%) at 1000 ppm. Least inhibition was observed in Ridomil (7.74%) and Desomil (8.57%) with concentration of 300 ppm.

All the fungicides showed variable response in inhibiting the colony growth of the phytopathogen according to their nature and specificity at different concentrations. In District Mansehra mostly farmers use the commonly available fungicides Ridomil gold, Desomil platinum and Antracol as the traders recommend these for broad spectrum. However, our finding shows that these fungicides are least effective against selected pathogens in District Mansehra. The variation in the action of fungicides with reference to concentration and fungal strain indicated that none of the fungicide is uniformly effective with a single concentration. According to our finding the traders must recommend chlorothalonil because of its effectiveness against early blight and fruit rot.

The above results are congruent with the finding of Hawamdeh and Shabir (2011). Braithwaite *et al.*, (1996) recommended that chlorothalonil was the best fungicides against *Alternaria* species *in vivo*. Rehman *et al.* (2011) evaluated Chlorothalonil against *B. theobromae* *in vitro* and reported that it is the second most effective fungicide after Carbendazim showing colony diameter of 1.59 cm at 100 ppm. Ashoka (2005) also listed chlorothalonil among the most effective fungicides. The current effective concentration should be checked in field for further confirmation. The present investigation will be very useful for the local growers in controlling different fungal diseases.

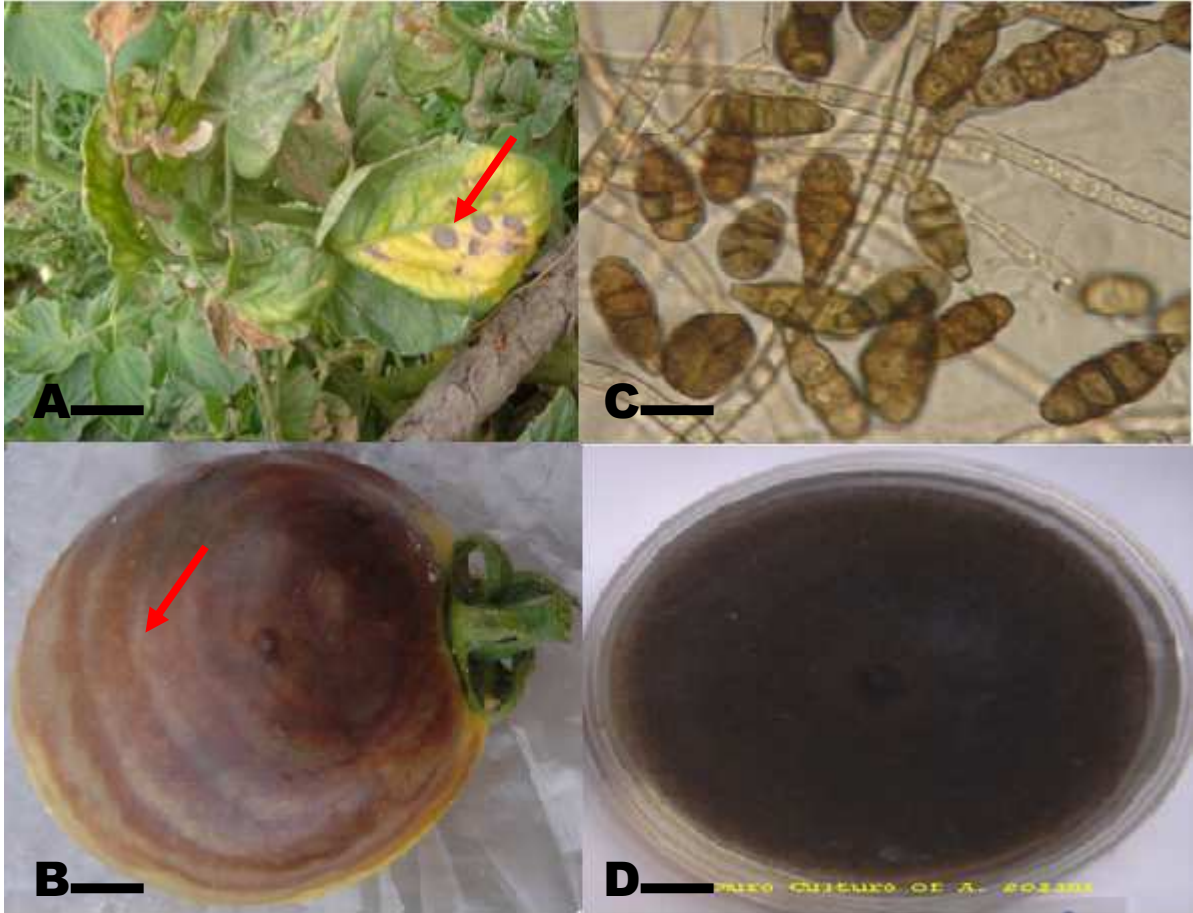
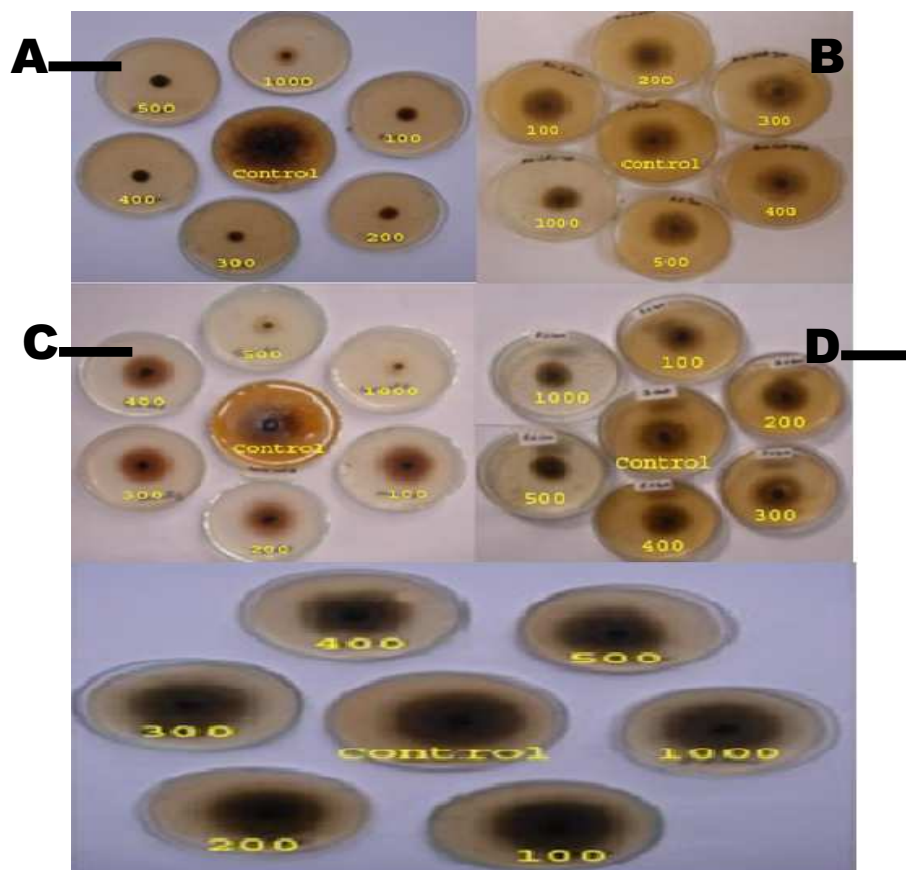


Fig. 1. A. Symptoms of early blight on tomato leaves, B. fruit rot of tomato, C. Spores of *A. solani* in LM, D. pure culture of *A. solani*

Table. 1. Radial colony Growth along with %inhibition of *Alternariasolani* with fungicides

Concentration (ppm)	Fungicides									
	Helonil (Chlorothalonil)		Clipper (Copper Oxychloride)		Antracol (Proponib)		Ridomile		Desomil	
	Radial growth (mm)*	Inhibition (%)	Radial growth (mm)	Inhibition (%)	Radial growth (mm)	Inhibition (%)	Radial growth (mm)	Inhibition (%)	Radial growth (mm)	Inhibition (%)
100	17.67 b	74.75	55.00 b	19.11	39.67 b	20.66	39.67 b	7.74	55.50 d	20.71
200	14.33 c	80.00	48.33 c	29.41	37.67 c	24.66	38.00 c	11.62	56.67 d	19.04
300	13.00 d	81.42	40.00 d	41.17	34.67 d	30.66	39.67 b	7.74	64.00 b	8.57
400	12.00 d	82.85	42.00 d	38.23	34.67 d	30.66	35.67 d	17.04	59.67 c	14.75
500	12.50 d	82.14	24.00 e	64.70	29.67 e	40.66	34.77 e	19.13	60.33 c	13.81
1000	18.33 b	74.28	25.00 e	63.23	26.67 f	46.66	34.33 e	20.16	57.33 d	18.1
Control (Untreated)	70.00	0.00	68.00	0.00	50.00	0.00	43.00	0.00	70	0.00
LSD (P=0.05)	1.233		3.351		1.372		0.9175		1.933	

\*Mean values followed by different letters in the same column are significantly different from one another at 0.05 level of significance



**Fig. 2. Inhibitory effects of different fungicides on radial growth of *A. solani* in vitro.**

**A.** Helonil (chlorothalonil) against *A. solani*, **B.** Clipper (copper oxychloride) against *A. solani*, **C.** Antracol against *A. solani*, **D.** Ridomil against *A. solani*, **E.** Desomil against *A. solani*

#### REFERENCES

1. Agriculture statistics of Pakistan, 2008-09. Govt of Pakistan, Area, production of fruit, vegetables and Condiments. Ministry of Food and Agriculture (Economic wing) 2008- 2000. 84 p.
2. Agrios, G.N. 2005. Plant Pathology, 5th Edition. Elsevier Academic Press USA. ISBN 0-12-044565-4.
3. Ashoka, S. 2005. Studies on fungal pathogens of vanilla with Special references to *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. Thesis submitted to the University of Agricultural Sciences, Dharwad.
4. Babalola, D.A., Y.O. Makinde, B.T. Omonona, M.O. Oyekanmi. 2010. Determinants of post harvest losses in tomato production: a case study of Imeko – Afon local government area of Ogun state. Journal of Life and Physical Science. Acta Satech. 3 (2): 14 - 18.
5. Barker, G.R. and Y. Fauchs. 1980. Research on postharvest diseases of tomato. A survey of the organisms causing rot of stored tomato fruits. Preliminary report, volcanicentre, Bet-Dagan. 782: 1-12.
6. Braithwaite, M., K.W.L. Knight, D.J. Saville and B. J. R. Alexander. 1996. Evaluation of fungicides for control of acremonium sp. and alternaria sp. Flower spotting on calla lilies. Centre, MAF Quality Management, P O Box 24, Lincoln.
7. Bose, T.K. and M.G. SOM. 1986. *Vegetable Crops in India*. Nayaprakash Publishing, Calcutta. 773p.
8. Chandravanshi, S.S., B.P. Singh and M.P. Thakur, 1994. Persistence of different fungicides used against *Alternaria alternata* in tomato. Indian phytopathol., 47: 241-244.
9. Cooper, J. 2007. Fungal plant pathogens and symptomology. Mastergardeners. Pp 1-10.

10. David, J.C. 1991. *Alternariabataticola*. International Mycological Institute-(IMI). Descriptions of Fungi and Bacteria. *Mycopathologia*. 116: 45-46.
11. Ellis, M.B. 1971. *Dematiaceous hyphomycetes*. Commonwealth Mycological Institute, Kew, Surrey, England. p. 607-608.
12. Gonzalez-Fernandez, R.E. Parts and J.V. Jorrin-Novo. 2010. Proteomics of Plant Pathogenic Fungi. *Journal of Biomedicine and Biotechnology*. 1-36.
13. Grogan, R.G., K.A. Kimble and I. Misaghi. 1975. A stem canker disease caused by *Alternaria Alternata* f. sp. *Lycopersici*. *Phytopathol.* 65:880-886.
14. Haggag, K.H.E. and S.F. Farghaly. 2007. Effect of Metalaxyl and Chlorpyrifos-Methyl Against Early Blight (*Alternariasolani*, Sor.) and Whitefly (*Bemisia tabaci*, Genn.) In Tomato and Egg plant. *Journal of Applied Sciences Research*. 3: 723-732.
15. Hassan, H.A.H. 1996. *Alternariamycotoxins* in Black rot lesion of tomato fruits: Conditions and regulations of their productions. *Acta Immunologica Hungarica*. 43: 125-133.
16. Hawamdeh, A.S. and S. Ahmad. 2001. *In vitro* control of *Alternariasolani*, the cause of early blight of tomato. *Online journal of biological sciences*. 1: 949-950.
17. Issiakhem, F. and Z. Bouznad, *In vitro* evaluation of difenoconazole and Chlorothalonil on conidial germination and mycelial growth of *Alternaria alternata* and *A. solanica* causal agent of early blight in Algeria. Twelfth Euro Blight workshop Arras (France), 2010, 14, 297–30.
18. Kemmitt, G. 2002. Early blight of potato and tomato. The plant health instructor. DOI: 10.1094/PHI-I-2002-0809-01.
19. Khan, M.M., R.U. Khan and F.A. Mohiddin. 2007. Studies on the cost-effective management of *Alternaria* blight of rapeseed-mustard (*Brassica* spp.). *Phytopathol. Mediterr. Pp* 201–206.
20. Leslie, J.F. and B. A. Summerell. 2006. The Fusarium Laboratory Manual. 1st Ed. Blackwell Publishing Professional, 2121 State Avenue, Ames 50014, USA.
21. Mathur, K. and K.S. Shekhawat. 1986. Chemical control of early blight in kharif sown Tomato. *Indian J. Mycol. Plant pathol.*, 16: 235-236.
22. Mate, G.D., V.V. Deshmukh, D.J. Jiotode, N.S. Chore and M. Dikkar, 2005. Efficacy of plant products and fungicides on tomato early blight caused by *Alternariasolani*. *Research on crops*, 6: 349-351.
23. Morris, P.F., M.S. Connolly and D.A. Stclair. 2008. Genetic diversity of *Alternaria alternata* isolated from tomato in California assessed using RAPDs. Elsevier publisher.
24. Neely, D. 1969. The value of *in vitro* fungicide tests. Illinois Natural History Survey Biological Notes No. 64 Urbana, Illinois.
25. Nene Y.L. and P.N. Thapliyal. 1993. *Fungicides in Plant Disease Control*. Oxford and IBH Publishing Co., New Delhi, India. 579p.
26. Ong, K. 2011. Basic plant pathology training Pathogenic agents. Agri life extension texas. 1p.
27. Parvez, E., S. Hussain, A. Rashid and M.Z. Ahmed, 2003. Evaluation of different protectant and eradicant fungicides against early and late blight of potato caused by *Alternariasolani* (Ellis and Mart) Jones and Grount and *Phytophthora infestans* (Mont) De Bary under field conditions. *Pakistan J. Biological Science*. 6: 1942-1944.
28. Rehman, A., M. Saleem., S. Mehboob., A.A. Bokhari. 2011. Fungi Associated With Rhizosphere Soil In Mango Decline Orchards And Their *In vitro* Control. *Pak. J. Phytopathol.* 23: 112-117.
29. Reinprecht, L. 2010. Fungicides for Wood Protection – World Viewpoint and Evaluation/Testing in Slovakia. Book. Ch; 5. Pp 95-122.
30. Sherf, A.T. and A. Macnab. 1986. *Vegetable diseases and their control*. John Wiley and sons, New York. 728p.
31. Singh, R.S., 1985. *Diseases of vegetable crops*. Oxford and IBH publishing co., New delhi. 436p.
32. Singh, S.N., B.P. Yadav, S.K. Sinha and K. L. Ojha. 1997. Efficacy of plant extract in inhibition of radial growth of *Colletotrichum capsici*. *Journal of Applied biology*. 51: 180-183.
33. Strange, R.N. and P.R. Scott. 2005. Plant Diseases: A threat to global food security. *Annu. Rev. Phytopathol.* 43: 83-116.