Pak. J. Phytopathol., Vol. 25 (02). 155-159

Official publication of Pakistan Phytopathological Society
Pakistan Journal of Phytopathology
ISSN: 1019-763X (Print), 2305-0284 (Online)
http://www.pakps.com

CHEMICAL AND BIOLOGICAL MANAGEMENT OF *FUSARIUM OXYSPORUM* F.SP *MELONGENAE*

[a]Parsa Sahar, [a]Shahbaz T. Sahi, [b]Abdul Jabbar, [a]Abdul Rehman, [a]Kashif Riaz, [a]Abdul Hannan*
[a] *Department of Plant Pathology, University of Agriculture Faisalabad, Pakistan.*
[b] *Department of Agronomy,, University of Agriculture, Faisalabad, Pakistan.*

A B S T R A C T

Fusarium wilt is a serious threat to vegetable crops especially eggplant. Current study was designed to evaluate four systemic fungicides (Topsin-M, Difenoconazole, Alliete and Nativo), and *Bacillus subtilis* in laboratory and under greenhouse conditions. For this purpose poison food technique was applied in laboratory and soil was drenched by chemicals under greenhouse. The results of *in-vitro* study revealed that Topsin -M inhibited the growth of pathogen by 76.66% followed by Difenoconazole 67.50% and Alliete 53.50% at 800ppm after 9 days at 25°C. While the Nativo was the least effective fungicide with 42.40% efficiency as compare to control. Furthermore foliar applications of Topsin-M (0.05%, 0.10% and 0.20%) were evaluated under greenhouse conditions. The results of greenhouse investigations showed Topsin-M with 0.1% concentration was significantly effective (55.35%) in reducing the disease incidence. The study was further extended to evaluate *Bacillus subtilis*, which caused 52.50% reduction in mycelial growth of *F. oxysporum* f.sp *melongenae* under dual culture technique. The antagonistic effect was further evaluated to control the disease incidence under greenhouse conditions where it caused 72% reduction in disease incidence over control. Although efficacy of *Bacillus subtilis* was low under lab conditions but it was higher in green house studies when applied in soil. This work represented the first report of antagonistic potential of *Bacillus subtilis* against *F. oxysporum* f.sp *malongenae* and suggested to evaluate against other diseases also.

Keywords: Antagonism, Biocontrol, Dual Culture Technique, Eggplant wilt

INTRODUCTION

Eggplant is a widely grown vegetable crop in fields as well as under tunnels in Asia, Africa and America (Kalloo and Berg 1993; Sihachakr *et al.,* 1994). The eggplant is native to the Indian Subcontinent and now it is present all over the world (Yiu, 2006 and Doijode, 2001). Worldwide production area of eggplant is approximately 1.6 million ha and production is nearly 4.2 million tons (F.A.O., 2012). China is the leading with 58 percent of the world production. In the Pakistan total area of production under this vegetable crop is about 9 thousands hectares with the 89 thousand tons of production and Pakistan ranking stands at 18th position in the world (F.A.O., 2007).

Eggplant is susceptible to several diseases particularly verticillium wilt (*Verticillium dahliae*), fusarium wilt (*Fusarium oxysporum* f.sp. *melongenae*) and bacterial wilt (*Ralstonia solanacearum*) (Kalloo and Berg 1993; Sihachakr *et al.* 1994).

The recommended management methods against the wilts are the rotation of crops, use of resistant varieties, solarization, soil sterility and use of fungicides (Yucel *et al*., 2007).

Now a day numerous soil borne diseases have been successfully managed by the application of certain bacteria, which are beneficial to plant and colonize in rhizosphere (Thomshaw, 1996; Abdel-Monaim *et al*., 2012). These bacteria stimulate growth as well as control plant pathogens through their antagonistic potential (Bloemberg and Lugtenberg, 2001). Current study was done for comparison of fungicides and biological control against Fusarium wilt of eggplant. The major thrust of this research is to find a suitable management strategy by evaluation of chemicals and *B. subtilis* as bio control agent.

* Corresponding Author:
Email: hannan@uaf.edu.pk

MATERIALS AND METHODS

Collection and identification of Fusarium wilt pathogen: Diseased roots of eggplant with characteristic symptoms of wilt were collected. The infested portion of the roots were cut into 8-10 mm long pieces, washed with tap water and dipped into 70% ethanol for 60 seconds. The pieces were given three washings in sterilized water. These segments were than plated on autoclaved PDA in petri plates and were incubated at 25 °C for one week. The strain of pathogen was purified by transferring a minute piece of mycelium and identifying through colony as well as microscopic characters including shape of macroconidia (Booth, 1977).

Pathogenicity test: Pathogenicity of *F. oxysporum* f.sp *melongenae* was done in pot experiment (13x15 cm) on healthy plants. The seven days old culture prepared on PDA was cut into small cubes and mixed in the soil of pots and to grow for seven days before transplanting the seedlings. The rate of inoculum per pot was ¼ petri plate (9 cm) three pots were kept under each treatment. Then 30 days old seedlings were transplanted in pots. The transplantation under uninfected soil served as control. To confirm the pathogenicity, the re-isolation and identification was done from the test plants by taking the diseased portion and isolated the pathogen when showed disease symptoms.

Evaluation of fungicides against *Fusarium oxysporum* f.sp *melongenae*: *In-vitro* experiments were carried out to find fungicidal effect against pathogen. Four fungicides namely Topsin-M, Difenoconazol, Alliete and Nativo were evaluated at three concentrations (400, 600 and 800 ppm) using poisoned food technique (Table: I). The desired concentrations were obtained by adding appropriate amount of stock solution of individual fungicide in PDA medium in conical flask. Amended PDA medium was poured separately into petridishes and replicated thrice for each treatment. PDA medium without fungicide served as control. Each petri plate was inoculated with mycelial disc of *F. oxysporum* (5 mm) taken from 7 day old colony on PDA. The inoculated plates were incubated at 25°C till the fungus growth covered the plate in control. The colony diameter was recorded after 3, 6 and 9 days of inoculation.

The most effective fungicide for inhibiting the mycelial growth of *F. oxysporum* in lab was further evaluated under green house by foliar application. The nursery was sown in trays under green house. The soil used in pots infested with *F. oxysporum* by mixing soil with mass culture of fungus @ ¼ petri dish of 9 cm diameter prepared on potato dextrose agar. 30 days old healthy seedlings were transplanted in each pot. Aqueous suspension of fungicides Topsin-M was sprayed into the pot soil with 0.05, 0.1 and 0.5% concentrations with three replications and repeated thrice after interval of one week.

The pots were irrigated when needed. The disease incidence was recorded on 7th days after application of treatment. The data was analyzed statistically to determine the difference between fungicide treatments.

Evaluation of *B. subtilis* against *F. oxysporum* f.sp *melongenae*: *Taegro (*A talcum based fungicide carrying *Bacillus subtilis* var. *amyloliquefaciens* Strain FZB24) was obtained from Novozymes Biologicals Inc USA. The product is recommended for control of soil borne plant pathogens. The product was standardized at 10^{10} cfu/g and was further diluted by sterilized water for desired applications. Both pathogen and antagonist were simultaneously inoculated at the opposite ends of petri plates. Three petri dishes were used for each concentration while water was poured in control. Inoculated plates were incubated at 25 °C for 7 days.

Further *B. subtilis* was also evaluated under greenhouse condition. The experiment was designed in pots containing sterilized soil. Same procedure was adopted to inoculate pots. When pathogen was established in the soil then 30 days old seedlings were transplanted in these pots. After 20 days of transplantation B*. subtilis* at $10^{6,}10^{8}$ and10^{10}cfu/g concentrations was added to the pots and application was done three times with one week interval. The pots without antagonist were served as control. Data was recorded before each application and subjected to statistical analysis for comparison of means.

RESULTS AND DISCUSSION

***In vitro* Evaluation of fungicides:** The four fungicides (Topsin-M, Difenoconazole, Alliete and Nativo @ 400,600 and 800 ppm) were evaluated against *F. oxysporum* f.sp *melongenae* by poison food technique. Among all, Topsin-M was superlative to reduce mycelial growth, followed by Difenoconazole and Alliete whereas Nativo was least effective with reference to control. The data revealed that the effect of fungicides in reducing mycelial growth varied but increased after increasing dose rate (Table II). The Topsin-M showed relatively

better results at 800 ppm with 76.66% reduction in mycelial growth over control followed by Difenoconazole, Alliete and Nativo (67.50, 53.50 and 42.40% respectively) as compare to control at 800ppm after 9 days of inoculation. Effectiveness of Topsin-M to controlling the Fusarium wilt has been reported by other workers as well (Penchala *et al.,* 2008). They tested five fungicides against *F. oxysporum* and found Topsin-M better than others.

Table 1. Fungicides and their active ingredients.

`	Active ingredient	Company name
Topsin-M	Thiophenate methyl 70% WP	Arysta Life Sciences, Pakistan
Difenoconazole	Difenoconazole 25% W/V	Saver Enterprises, Pakistan
Aliette	PhositileAlomenium 80% W/W	Bayer Crop Sciences, Germany
Nativo	Tibuconazole 50% W/W Trifloxisroben 25% W/W	Bayer Crop Sciences, Germany

Table 2. %age reduction in colony growth of *Fusarium oxysporum* over control by poisoned food technique.

	After 3 days			After 6 days			After 9 days		
	400ppm	600ppm	800ppm	400ppm	600ppm	800ppm	400ppm	600ppm	800ppm
Topsin-M	70.17 b	64.69 d	72.5 ab	67.68 c	70.57 b	76.81 a	67.56 c	69.73 b	76.66 a
Difenconazole	54.40 g	48.97 h	57.11 f	50.72	57.14 f	64.43 e	52.17 f	57.50 f	67.50 c
Aliette	35.50 k	25.30	40.38 ij	37.68 j	42.42 i	51.20 g	40.64 ij	43.81 i	53.50 g
Nativo	15.60 o	07.55 p	21.92 n	22.31 n	25.28 m	36.55 k	25.64 m	27.23 l	42.44 i

The means sharing similar letters are not significantly different at (P>0.05) by DMR test. LSD= 0.1941.

Evaluation of Topsin-M against *Fusarium* wilt of eggplant under greenhouse: The pots were filled with infested soil and placed undisturbed for one week in greenhouse. The Topsin-M was effective under laboratory hence further evaluated by drenching in pots under greenhouse. Three doses (0.05, 0.10 and 0.20%) of Topsin-M were formulated and drenched in soil before transplantation. The results indicated that, Topsin-M @ 0.20% exhibited minimum disease incidence 28.50, 24.40 and 25.00% after 1st, 2nd and 3rd application respectively (Fig 1).

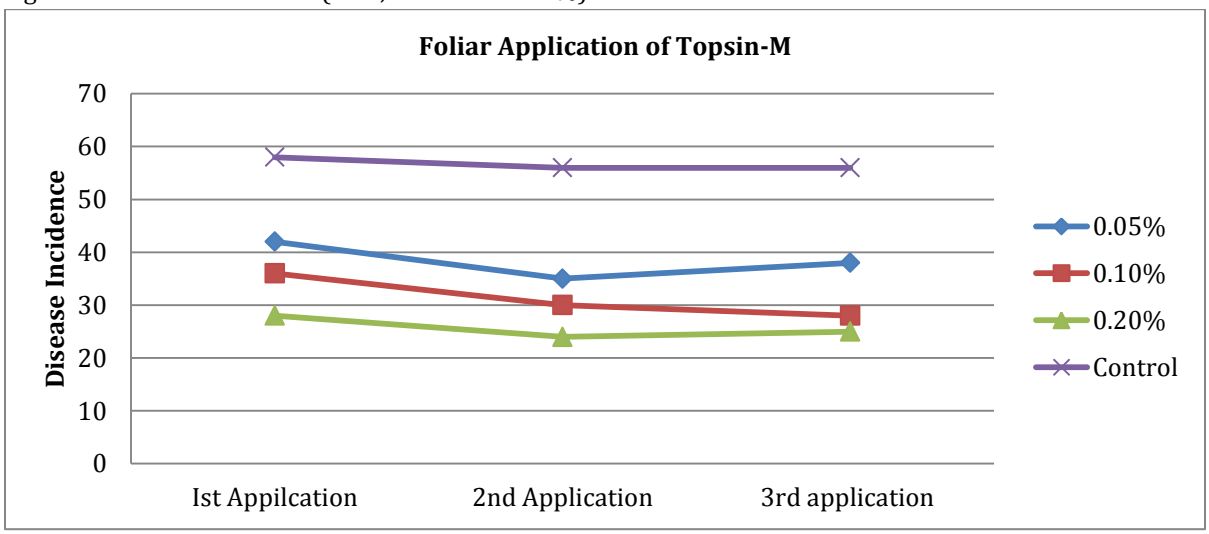

Figure 1. Evaluation of Topsin-M against *Fusarium* wilt of eggplant under greenhouse conditions.

***In vitro* Evaluation of *Bacillus subtilus* against Fusarium wilt of eggplant:** In dual culture test *B. subtilis* strain showed significant growth inhibition of pathogen on the PDA medium. *B. subtilis* showed 50 and 48% reduction in colony growth over control at 10^{10} colony forming unite (CFU) after 6th and 9th day respectively. (Fig. 2). Khan *et al*., 2011 tested *B. subtilis* against *F. oxysporum* and found that the bacterial isolate inhibited the growth of pathogenic fungi and recommended to purify antifungal compounds.

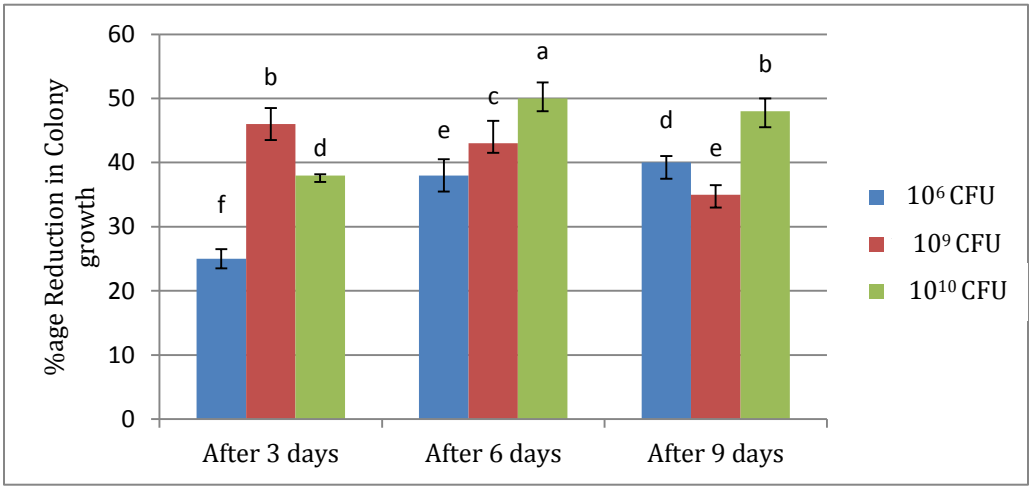

Figure 2. In vitro Evaluation of *Bacillus subtilis.*
The means sharing similar letters are not significantly different at (P>0.05) by DMR test. LSD = 0.28.

Evaluation of *Bacillus subtilis* against fusarium wilt of eggplant under greenhouse conditions: The efficacy of *Bacillus subtilis* as biocontrol agent against Fusarium wilt of eggplant was evaluated under the greenhouse conditions. The bioagent was applied directly into soil after three weeks of transplantation while *F. oxysporum* was incorporated seven days before transplantation. Data showed that *B. subtilis* significantly reduced disease incidence where 71 and 72% disease reduction was observed at 10^{9} and 10^{10} colony forming units (CFU) over control (Fig. 3). In this regard, Saleh (1997) added antagonist and find their effect on growth of the pathogenic fungi, his studies proved role of *B. subtilis* has good potential to inhibit rhizospheric microbes. Control of plant pathogens through biological means is no doubt a safe technique, hence more work is required to establish new trend in agriculture. Application of bioagents reduce density of inoculum of the pathogen which minimize incidence of diseases for longer period of time (Berger *et al.*, 1996 and Harris *et al.*,1994).

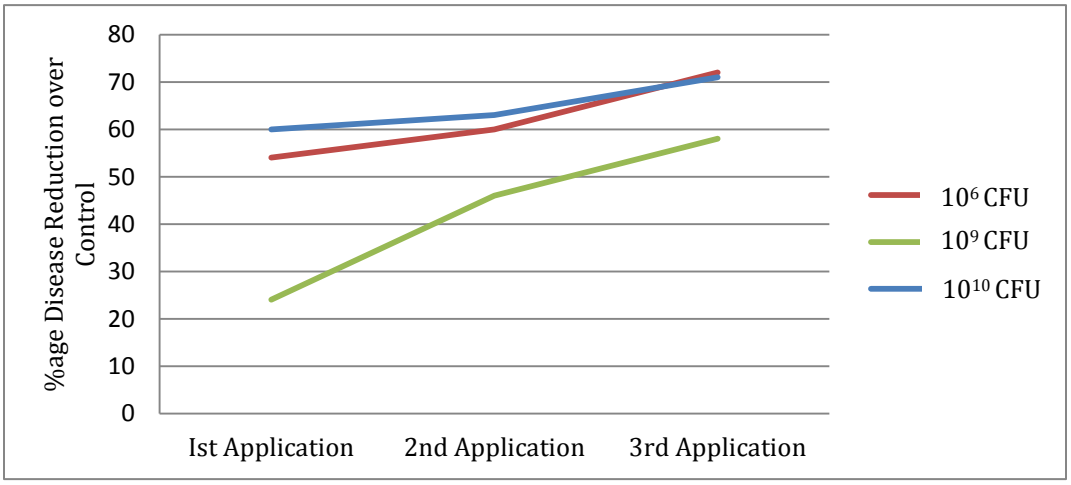

Figure 3: Evaluation of *Bacillus subtilis* under greenhouse condition.

It was concluded that application of *Bacillus subtilis* showed relatively better results as compare to fungicides in field trials especially. In future this biocontrol agent should be commercialized for the management of other plant diseases as they have least ecological impact and hazard to consumers as well.

ACKNOWLEDGEMENT

The authors would like to acknowledge The Researcher Home Faisalabad (an agro chemical company) for providing Bioproduct (Teagro) after importing from USA. Moreover the authors also acknowledge the services of Dr. Javaid Saleem (Seed Manager, Allah Dine Group of Companies, Pvt. Ltd. Pakistan) for providing seed.

REFEREMCES

Abdel-Monaim, M., M. Abdel-Gaid and M. El-Morsy. 2012. Efficacy of rhizobacteria and humic acid for controlling *Fusarium* wilt disease and improvement of plant growth, quantitative and qualitative parameters in tomato. ESci J. Plant Pathol. 1(1): 39-48.

Berger, F., L.D. White, R. Frazer and C. Lieifert. 1996. Effect of pathogen inoculum, antagonist density, and plant species on biological control of Phytophthora and Pythium damping-off by *B. subtilis* in High humidity fogging glasshouses.Phytopathology.86:428-433.

Booth, C., 1977. Fusarium laboratory guide to the identification of major spacies, Commonwealth Mycological Institute, Kew Surrey, England.

Bloemberg, G.V. and B.J.J. Lugtenberg. 2001. Molecular basis of plant growth promotion and biocontrol by rhizobacteria, Current Opinion of Plant Biology. 4: 343-350.

Doijode, S.D., 2001. Seed storage of horticultural crops. Haworth Press: International Standard Book England. 157.

Food and Agriculture Organization. 2007. FAO stat Database Collection http://apps.fao.org/page/collection.

Food and Agriculture Organisation. 2012 FAO stat Database Collection http://apps.fao.org/page/collection.

Harris, A.R., D.A. Schisler, M.H.Ryder and P.G. Adkins. 1994. Bacteria suppress damping-off caused by *Pythium* var. *sporangiiferum* and promote growth. Soil biol. Biochem.26:1437.

Kalloo, G. and B.O.Berg. 1993.Genetic improvement of vegetable crops.Pergamon Press Ltd, Oxford UK.587–604.

Khan, A.A., Naseem, R. Lavarti and B. Prathibha, 2011.Screening and Potency Evaluation of Antifungal from Soil Isolates of *B. subtilis* on Selected Fungi Advanced Biotech, 10: 35-37.

Penchala, R.G., S.V.Ramakrishna Rao and K.Gopal. 2008. *In vitro* evaluation of antagonists and fungicides against the red gram wilt pathogen *Fusarium oxysporum* f.sp. *udam* (butler).Legume Res., 31: 133 – 135.

Saleh, O.I., 1997. Wilt, root rot and seed diseases of groundnut in El-Minia Governorate, Egypt.*Egypt.* Journal of Phytopathology.25: 1-18.

Sihachakr, D., M.C.Daunay, I. Serraf, M.H. Chaput, I. Mussio, R. Haicour, L. Rossignol and G. Ducreux. 1994. Somatic hybridization of eggplant with its close and wild relatives. In: Bajaj YPS (ed) Biotechnology in agriculture and forestry, somatic hybridization in crop improvement, Springer-Verlag, Berlin.255–278.

Thomshaw, L.S., 1996. Biological control of plant pathogens.Current Opinion. 77: 343-347.

Van der, H.R.S., C. Running, J.J. Giovannoni and S.D. Tanksley. 2002. Deduction about the number, organization, and evaluation of genes in the tomato based on analysis of a large expressed sequence tag collection and collective genomic sequencing. The plant cell. 14: 1441-1456.

Yiu, H.H., 2006. Handbook of Food Science, Technology, and Engineering by CRC Press. International Standard Book.United States.

Yucel, S., Y.H. Elekcioolu, C. Can, M.A. Soout and A. Ozarslandan, 2007. Alternative techniques to control wilts in vegetables. Turkish Journal of Agriculture. 31: 47-53.