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IN-VITRO EVALUATION OF FUNGICIDES AND BIOAGENTS AGAINST TOMATO EARLY BLIGHT PATHOGEN *ALTERNARIA SOLANI* (L.)

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ABSTRACT
Early blight disease caused by *Alternaria solani* (L.) is one of the most destructive diseases of tomato in the tropical and subtropical regions. In the present study, efficacy of different fungicides at different concentration and bioagents against *A. solani* was assessed under *in vitro* conditions. A total of eight fungicides and seven bioagents were tested. It was found that, all the three concentrations of propineb (0.1%, 0.2% and 0.3%), hexaconazole (0.05%, 0.1% and 0.15%) and iprodione 25% + carbendazim (0.1%, 0.2% and 0.3%) and difenoconazole at 0.075% concentration showed cent per cent inhibition of *A.solani*. It was followed by lower concentrations of difenoconazole (0.05% and 0.025%) with 97.21 and 94.99 per cent inhibition of pathogen respectively. Among remaining fungicides, all the three concentrations of pyraclostrobin was significantly superior over copper hydroxide, azoxystrobin and trifloxystrobin 25% + tebuconazole in inhibiting mycelial growth and it ranged from 79.44 to 85.55 per cent. Among the bioagents tested, *Trichoderma viride* (KAU) and plant growth promoting microbial consortium (PGPM mix of KAU) were recorded cent per cent inhibition of pathogen. Among different bacterial antagonists *Bacillus subtilis* 1; an endophyte isolated from cocoa recorded maximum growth inhibition of 51.66 per cent.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a vital solanaceous vegetable crop of global importance grown in an area of 4.8 million hectares (FAO, 2017). In India tomato occupies an area of 0.797 million hectare, with a production of 20708 metric tons and productivity of 20.7 metric tons/ha in the year 2017 (FAO, 2017). Varied climatic adaptability and high nutritive value made the tomato cultivation more popular in the recent years. However, disease and pests pose serious threat to tomato cultivation among which, early blight is one of the most destructive diseases, causing considerable loss to quality and quantity of fruits. Under favourable conditions more than 80 per cent disease severity of early blight has been recorded in tomato crops (Kumar and Srivastava, 2013).

Primary methods of controlling the disease include preventing long periods of wetness on the leaf surface, cultural scouting, sanitation, and development of disease resistance varieties (Kirk *et al*., 2005 and Kumar and Srivastava, 2013). Moreover, the ultimate control of this disease is achieve through cultivation of resistant varieties, but it remains limited by the evolution of new strains of the pathogens. Hence, farmers mainly rely on fungicides for the control of pathogens *viz. Alternaria solani*, the casual organism of early blight. Several research workers have reported that timely application of fungicides is the best method to control early blight (Singh and Singh, 2006; Patel and Choudhary; 2010; Horsfield *et al*., 2010). According to Tofoli *et al*. (2010) fungicides *viz*. difenoconazole, tebuconazole, chlorothalonil, mancozeb, copper hydroxide and iprodione were effective against *A. solan*i. Sahu *et al*. (2013) reported that, the fungicides like pristine, maccani, boscalid, pyraclostrobin and mancozeb can be used not only to manage early blight disease but to increase the yield of tomato as well.

Although the use of chemicals to reduce or prevent losses caused by this agent seems simple and successful, the damage inflicted by the residual effects of chemicals on humans and environment should certainly be taken into account. So, biological control is an alternative method which provides environment-friendly, sustainable and promising strategies against plant diseases. Biocontrol methods such as seedling dip and foliar applications of *T. harzianum*, *T. viride* and *P. fluorescens* were found to decrease the early blight incidence up to 62 per cent and increased tomato yield up to 37 per cent (Ramanujam *et al*., 2015). Koley *et al*. (2015) studied efficacy of *B. subtilis* and *P. fluorescens* against *A. solani* and showed 52.77% and 47.22% of growth inhibition respectively. Basamma and Kulkarni (2016) also reported a significant reduction in early blight incidence of tomato under greenhouse conditions by application of *Bacillus subtilis*.

Unplanned, overdosed and wide use of fungicides often leads to serious environmental problems besides affecting the health of users and consumers. Hence, application of fungicides at proper dose and time interval is mandatory. Moreover, innovative and safe methods like use of biocontrol agents need to be identified and evaluated for continuous search to develop ecofriendly strategies to reduce the dependence on

harmful chemicals. Therefore, the present study was aimed to assess the efficacy of different doses of fungicides and bioagents against *Alternaria* leaf blight of tomato under *in vitro* conditions.

MATERIALS AND METHODS

Isolation, purification and maintance of the pathogen

A. solani was isolated from infected tomato leaves and purified by following single spore isolation method (Ho and Ko, 1997). Pure cultures of different isolates of *A. solani* were tested for the pathogencity on tomato leaves, reisolated and maintained on potato dextrose agar slants for further investigations.

In vitro evaluation of fungicides

In vitro evaluation of eight fungicides in three different dosses *viz*. propineb 70% WP (0.1%, 0.2% and 0.3%), copper hydroxide 77 WP (0.15%, 0.2% and 0.25%), pyraclostrobin 20% EC (0.025%, 0.05% and 0.075%), azoxystrobin 23% SC (0.1%, 0.15 and 0.2%), hexaconazole 5% EC (0.05%, 0.1% and 0.15%), difenconazole 25% EC (0.025%, 0.05% and 0.075%), iprodione 25% + carbendazim 25% WP (0.1%, 0.2% and 0.3%) and trifloxystrobin 25% + tebuconazole 55% (0.025%, 0.05% and 0.075%) was carried out by poisoned food technique (Zentmyer, 1955). The fungicides were mixed separately with sterilized potato dextrose medium in suitable proportion to get the desired concentrations and poured to sterilized Petri dishes @ 20 ml/plate. Eight mm sized disc from five day old culture of the pathogen was placed at the center of each Petri dish containing poisoned medium. Experiment was in Completely Randomized Design (CRD) with three replications for each fungicide. Observations were recorded, till pathogen attained full growth in control. Per cent inhibition of the pathogen was calculated using the formula suggested by Vincent (1927).

$$\% \text{ inhibition of pathogen} = \frac{C - T}{C} \times 100$$

C = Growth of the pathogen in control

T = Growth of the pathogen in treatment

In vitro evaluation of antagonists

Three endophytic *Bacillus* sp (*B. subtilis* 1, *B. subtilis* 2 and *B. subtilis* 3) isolated from cocoa leaves, three reference cultures *viz. Trichoderma viride* (KAU), *Pseudomonas fluorescens* (KAU) and *B. subtilis* (KAU) and one plant growth promoting microbial consortium (PGPM mix of KAU) were screened for their antagonistic activity against the pathogen, *A. solani* by adopting dual culture technique (Johnson and Curl, 1972). Antagonistic activity of *T. viride* was tested by employing deferred antagonism and bacterial antagonists by simultaneous antagonism methods. Monoculture of the pathogen served as control. Experiment was laid out in Completely Randomized Design (CRD) and three replications were kept for each antagonist. Observations were recorded daily till the pathogen attained full growth in control and per cent inhibition was calculated.

RESULTS AND DISCUSSION

In vitro evaluation of fungicides

The efficacy of eight fungicides against *A. solani* at different concentrations is given in Fig. I. All the fungicides tested were found to be effective against the pathogen; however, the efficiency varied with the chemical. There was a positive correlation between the concentration and per cent inhibition of growth of mycelium except propineb, hexaconazole iprodione 25% + carbendazim. All the three concentrations of propineb (0.1%, 0.2% and 0.3%), hexaconazole (0.05%, 0.1% and 0.15%) and %), iprodione 25% + carbendazim (0.1%, 0.2% and 0.3%) recorded cent per cent inhibition of the pathogen. Hence, it revealed that, even the lower concentration of these fungicides were effective against the pathogen. Difenconazole was significantly superior over copper hydroxide, pyraclostrobin, azoxystrobin and trifloxystrobin 25% + tebuconazole in inhibiting mycelial growth and recorded 94.99-100 per cent inhibition. The combination fungicide, trifloxystrobin 25% + tebuconazole, was the least effective and recorded only 73.33 per cent inhibition even at the concentration of 0.075 per cent.

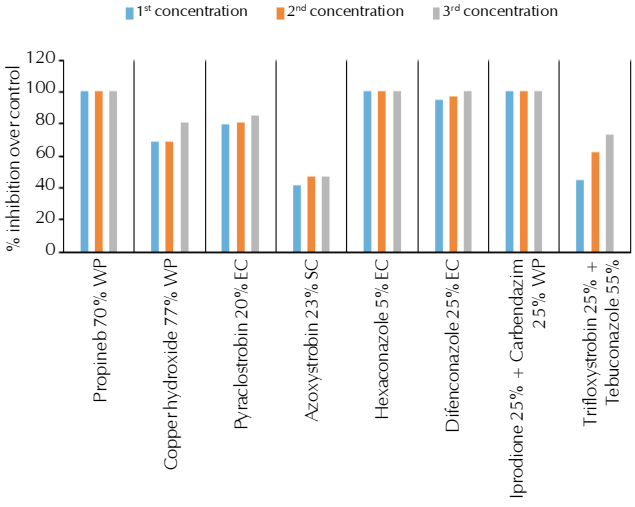

Figure1: *In vitro* evaluation of fungicides against *Alternaria solani*

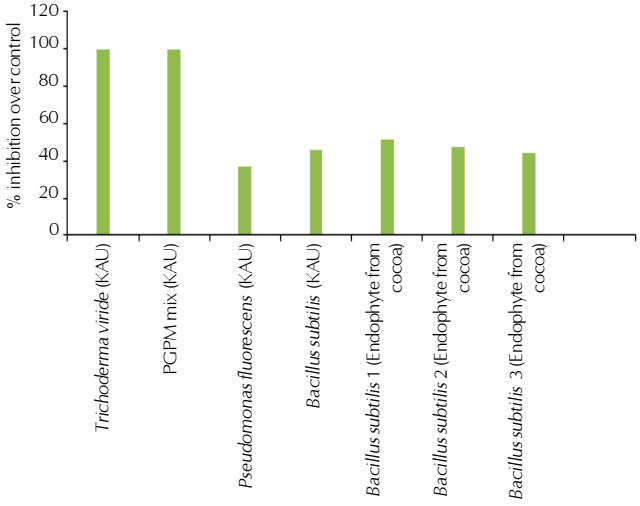

Figure 2: *In vitro* evaluation of bioagents against *Alternaria solani*

Azole fungicides like hexaconazole and difenconazole can inhibit the pathogen either by destroying their cell membrane or its permeability or by inhibiting metabolic processes of the pathogen and hence are extremely effective (Fisher *et al.*, 2004). Similarly, propineb, a contact fungicide which interferes at different locations in the metabolism of the pathogen; on several points of the respiration chain, in the metabolism of carbohydrates and proteins, in the cell membranes and this multi-site mode of action of propineb also prevents development of resistance in the pathogen. Strobilurin group fungicides like azoxystrobin, pyraclostrobin and trifloxystrobin are broad spectrum in action and excellent inhibitors of spore germination and comparatively less effective for direct mycelial inhibition than above chemicals. Moreover, strobilurins inhibits mitochondrial respiration by blocking electron transfer at the cytochrome bc_1 complex (Jiang *et al.*, 2009). The induction of the alternative oxidase respiratory pathway at the cytochrome bc_1 target site has been proposed as the likely reason for the low mycelial sensitivity to strobilurins displayed by the pathogen. Complete inhibition of mycelial growth of *A. solani* by hexaconazole at 0.025 per cent has been reported earlier by Singh and Singh (2006). Efficacy of fungicides like mancozeb, copper oxychloride, copper hydroxide, chlorothalonil, difenoconazole, azoxystrobin and propineb against *A.solani* has also been proved by many workers (Patel *et al.*, 2007 and Genie *et al.*, 2013). The present results were also in agreement with Chohan *et al.*, (2015) and Gazanafar *et al.*, (2016) who studied the effect of different fungicides *viz.* difenconazole, tebuconazole, chlorothalonil, mancozeb, propineb, copper hydroxide and iprodione against Alternaria under *in vitro* conditions.

***In vitro* evaluation of antagonists**

All the seven antagonists showed some antagonistic activity against the pathogen. However, *Trichoderma viride* (KAU) and plant growth promoting microbial consortium (PGPM mix of KAU) showed cent per cent inhibition of pathogen by the overgrowth mechanism of antagonism, causing complete disintegration of the pathogen (Fig.2). However, less than 50 per cent inhibition was observed for all the bacterial antagonists, except *Bacillus subtilis* 1 (Endophyte from cocoa) which showed 51.66 per cent. Among different bacterial antagonists *P. fluorescens,* showed the lowest growth inhibition (36.66%) of the fungus *A. solani* over the control. Efficacy of *Trichoderma* against *Alternaria* sp. have been reported by many workers (Verma *et al.,* 2008; Pandey, 2010; Begum *et al.*, 2010). *Trichoderma* inhibit the growth of the pathogen through its rapid growth potential and competition for food and space (Devi *et al.*, 2012). *Trichoderma* can also inhibit the pathogen through the production of volatile and non-volatile compounds (Sumana and Devaki, 2012). *Bacillus* species as a group offer several advantages over other bacteria for protection against pathogens because of their ability to form endospores, and broad-spectrum activity of their antibiotics (Abdalla *et al.*, 2014). Antagonistic activity of endophytic strains of *B. subtilis* from coconut and cotton against *A. solani* of tomato has been earlier observed by Sundaramoorthy and Balabaskar (2012). Similarly, Koley *et al.*, 2015 and Ramakrishna *et al.* (2018) also recorded 40-50 per cent growth inhibition of *A. solani* by *B. subtilis*.

In the present attempt we tried to find out suitable fungicides with its effective dose and bio-control agents against *A. solani*, the casual organism of early blight of tomato. Though, all the fungicides and bioagents evaluated *in vitro* were found effective against the pathogen, among different fungicides tested all the three concentrations of propineb (0.1%, 0.2% and 0.3%), hexaconazole (0.05%, 0.1% and 0.15%) and %), iprodione 25% + carbendazim (0.1%, 0.2% and 0.3%) were found to be most effective and among the bioagents, *T.viride* (KAU) was most efficient. Hence, the present study revealed that, even the lower concentration of these fungicides were effective against the pathogen. However, further studies are needed to evaluate their potential under field conditions and that will help the farmers to dissuading the use of over doses of chemicals to avoid residual toxicity in the tomato fruits, in addition of reducing the cost of chemical.

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REFERENCES

Abdalla S. A., Algam S. A. A., Ibrahim E. A. and Naim A. M. E. 2014. In vitro screening of Bacillus isolates for biological control of early blight disease of tomato in Shambat soil. *World J. Agric. Res*. **2(1):** 47-50.

Basamma, R. H. and Kulkarni, S. 2016. Bioefficacy of b diseases of tomato *Bacillus subtilis* against foliar fungal diseases of tomato. *Int. J. Appl. Pure Sci. Agric*. **2:** 220-227.

Begum, M. F., Rahman, M. A. and Alam, M. F. 2010. Biological control of Alternaria fruit rot of chili by *Trichoderma* species under field conditions. *Mycobiology*. **38(2):** 113-117.

Chohan, S. R., Perveen, M. A., Mehmood, S., Naz, C. and Akram, N. 2015. Morpho-physiological studies, management and Screening of Tomato Germplasm against *Alternaria solani,* the causal agent of tomato early blight. *Int. J. Agri. Biol*. **17(1):** 111-118.

Devi, S. S., Sreenivasulu, Y., Saritha, S., Kumar, M. R., Kumar K. P. and Sudhakar, P. 2012. Molecular diversity of native *Trichoderma* isolates against *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.). A causal agent of Fusarium wilt in tomato (*Lycopersicon esculentum* Mill.). *Arch. Phytopathol. Pl. Prot*. **45(6):** 686-698.

Fisher, N., Brown, A. C., Sexton, G., Cook, A., Windass, J. and Meunier, B. 2004. Modeling the Qo site of crop pathogens in Saccharomyces cerevisiae cytochrome b. *Eur. J. Biochem*. **271 (11):** 2264-2271.

Food and Agriculture Organization Corporate Statistical Database 2017. Available from: http://en.wikipedia.org/wiki/Food_and_Agriculture_Organization_Corporate_Statistical _Database.

Genie, S. A, Qaisar, A, Qazi N., Shabi, U. R. and Dar, W. 2013. Integrated management of early blight of potato under Kashmir valley conditions. *Afr. J. Agric. Res*. **8(32):** 4318-4325.

Ghazanfar, M. U., W. Raza, K. S. Ahmed, M. H. Rasheed, J. Qamar and Haider, N. 2016. Evaluation of different fungicides against *Alternaria solani* (Ellis and Martin) Sorauer cause of early blight of tomato under laboratory conditions. *Int. J. Zool. Studies*. **1(5):** 8-12.

Ho, W. C. and Ko, W. H. 1997. A simple method for obtaining single spore isolates of fungi. Botanical Bulletin of Academia Sinica. p. 38.

Horsfield, A. Trevor, W., Kent, D., Doug, W. and Scott, P. 2010. Effect of fungicide use strategies on the control of early blight (*Alternaria solani*) and potato yield. *Australas. Plant Pathol*. **39:** 368-375.

Jiang, J., Ding, L., Michailides, T. 2009. Molecular characterization of field azoxystrobin-resistant isolates of *Botrytis cinerea*. *Pestic. Bio.Che. Phys.* ***93:*** *72-76*.

Johnson, J. F. and Curl, A. E. 1972. *Methods for Research on the Ecology of Soil-Borne Plant Pathogens*. Burgess Publishing Co. New York. p. 142.

Koley, S., Smahapatraand, S. and Kole, P. C. 2015. *In vitro* efficacy of bio-control agents and botanicals on the growth inhibition of *Alternaria solani* causing early leaf blight of tomato. *Int. J. Bio-res. Env. Agril. Sci.* **1(3):** 114-118.

Kirk, W. W., Abu-El Salem, F. M., Muhinyuza, J. B., Hammerschmidt, R. and Douches, D. S. 2005. Evaluation of potato late blight management utilizing host plant resistance and reduced rates and frequencies of fungicide applications. *Crop Prot*. **24:** 961-970.

Kumar, S. and Srivastava, K. 2013. Screening of tomato genotypes against early blight (*Alternaria solani*) under field condition. *The Bioscan*. **8(1):** 189-193.

Pandey, A. 2010. Antagonism of two Trichoderma Species against *Alternaria alternata* on *Capsicum frutescens*. *J. Exp. Sci*. **1(5):** 18-19.

Patel, N. A., Dange, S. R. S. and Patel, S. I. 2007. Efficacy of chemicals in controlling fruit rot of tomato caused by *Alternaria* tomato. Indian *J. Agric. Res*. **39(1):** 72-75.

Patel, R. L. and Choudhary, R. F. 2010. Management of *Alternaria solani* causing early blight of tomato with fungicides. *J. Plant Dis. Sci*. **5(1):** 65-67.

Ramakrishnan, A., Desai, R., Uma, G., and Maheswari, U. 2018. Biocontrol activity and PGPR ability of different isolates of Pseudomonas and Bacillus on tomato *Int. J. Pure App. Biosci*. **6(6):** 728-735.

Ramanujam, B., Sriram, S., Rangeshwaran, R. and Basha, H. 2015. Biocontrol efficacy of fungal and bacterial antagonists against early blight of tomato caused by *Alternaria solani*. *Indian J. Hortic.* **72(1):** 147-148.

Sahu, D. K., Khare, C. P., Singh, P. and Thakur, M. P. 2013. Evaluation of newer fungicide for management of early blight of tomato in Chhattisgarh. *The Bioscan*. **8(4):** 1255-1259.

Singh, P. C. and Singh, D. 2006. *In vitro* evaluation of fungicides against *Alternaria alternata*. *Ann. Plant Protect. Sci.* **14(2):** 500-502.

Sumana, K. and Devaki, N. S. 2012. *In vitro* evaluations of some bioagents against tobacco wilt pathogen. *J.Biopest*. **5(1):** 18-22.

Sundaramoorthy, S. and Balabaskar, P. 2012. Consortial effect of endophytic and plant growth promoting Rhizobacteria for the management of early blight of tomato incited by *Alternaria solani*. *J .Plant Pathol. Microb*. **3:** 7.

Tofoli, J. G., Domingues, R. J., Garcia, J. O and Kurozawa, C. 2010. Tomato early blight control by fungicides and its effects on yield. *Summa Phytopathologia*. **29:** 225-233.

Verma, M., Brar, S., Tyagi, R. D., Surampalli, R. Y. and Valéro, J. R. 2008. Antagonistic fungi, *Trichoderma* spp.: Panoply of biological control. *Biochem. Eng. J.* **37:** 1-20.

Vincent, J. M. 1927. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*. **159:** 850.

Zentmyer, G. A. 1955. The world of Phytophthora. In: Erwin, D. C., Bartnicki-Garcia, S. and Tsao, P. H. (eds), Phytophthora: Its Biology, Taxonomy, Ecology and Pathology, American Phytopathological Society, St Paul, Minnesota, USA. pp. 1-8.