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EVALUATION OF NATURAL PLANT EXTRACTS, ANTAGONISTS AND FUNGICIDES AGAINST EARLY BLIGHT CAUSED BY *A. SOLANI IN VITRO*

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ABSTRACT
Early blight (*Alternaria solani*) of tomato is most destructive disease in tropical and subtropical countries. In natural epidemics, early blight is strongly influenced by environmental conditions causing the Leaf blight, stem blight and apical fruit rot. The bio assay of fungicides, botanicals and bio agents forms the prerequisite for the field evaluation. *In vitro* evaluation of twelve fungicides, six bioagents and ten botanicals against *Alternaria solani* casing early blight of tomato revealed that contact fungicidemancozeb @ 0.2%, systemic fungicide, hexaconazole @ 0.1 % and the combi fungicide Hexazconazole 4% + Zineb 68% @ 0.2%recorded the maximum inhibition of 87.21% 88.88%, 88.88% mycelial growth respectively. Among the bioagents tested *Trichoderma harzianum* found effective in inhibiting the mycelial growth (77.50%) followed by *T. viride* (75.14%). Among the ten plant extracts evaluated, Jatropa leaf extract @ 10per cent was found to be effective in inhibiting the mycelial growth of *A. solani* (62.78%). The efficacy of fungicides, bioagents and botanicals can be further evaluated in combinations of spray options under field conditions.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill) is one of the most popular and widely grown vegetable crop. The crop is grown for its edible fruits, which can be consumed either fresh or it can be processed to several products like puree, paste, soup, juices, ketchup, whole canned fruits *etc.* Early blight also known as target spot disease incited by *Alternaria solani* (Ellis and Martin) Jones and Grout is one of the world's most catastrophic disease incurring loss both at pre and post harvest stages in tomato growing tracks of India. *Alternaria* rot has been considered as the most common disease of tomato and other plants and causes heavy losses in quality of the fruits, thus rendering large quantity of tomato fruits unfit for consumption (Hassan, 1996; Singh *et al.*, 1997). All aboveground parts of the plant can have symptoms of this disease. Leaf spots are circular, up to 1/2" in diameter, and dark to light brown Spots may occur singly or in large numbers on the leaf. The leaf may turn yellow, then brown and fall off. Older leaves are usually affected before the disease works up the plant. This disease, which in severe cases can lead to complete defoliation, is most damaging on tomato (Peralta *et al.*, 2005). In natural epidemics of early blight are strongly influenced by environmental conditions, even though severe disease appears every year in northern India (Kumar and Srivasthava, 2013).The use of fungicides is an age old practice in absence of resistant cultivars. Certain protective fungicides although hazardous to environment are still used for the control of fungal diseases (Nwankiti *et al.*, 1990; Vaish and Sinha, 2003). Mancozeb (0.2%) was found most effective for inhibiting the mycelial growth of *A. solani* (Choulwar *et al.*, 1989). The Fungicidal spectrum of Mancozeb in controlling early blight of tomato was confirmed by Singh *et al.* (2001). However, use of bio agents and plant extracts have been suggested by some workers as alternative to synthetic chemicals in order to counter the potential hazardous effect on the environment associated with the use of synthetic chemicals (Ejechi and Ilondi,1999; Singh, *et al.*, 1997; Amadioha, 2000). Natural plant products and bio agents are important sources of new agrochemicals for the control of plant diseases (Kagale *et al.*, 2004). Furthermore, biocides of plant origin are non-phytotoxic, systemic and easily biodegradable (Qasem and Abu-Blan, 1966). Many studies have shown that plant extracts effectively controlled various plant pathogens *in vitro* (Talibi *et al.*, 2012). The leaf extracts of *D. stramonium, A. indica*, and *A. sativum* at 5% concentration caused the highest reduction of mycelial growth of *A. solani* (44.4%, 43.3% and 42.2%, respectively) as reported by Nashwa and Abo-el Elyousr, 2011).These plant extracts are potential as environmentally safe alternatives and as components in integrated management programs (Bowers and Locke, 2004). Hence the attempt has been made to evaluate some new agro chemicals, plant exctracts 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. The combination of Mancozeb with *T. viride* was found most effective in reducing the disease (Balal and Singh, 2011). Keeping the above points in view the *in vitro* evaluation

of fungicides, bio agents and botanicals to know their bio efficacy.

MATERIALS AND METHODS

In vitro evaluation of bio agents and botanicals against *Alternaria solani* (Ellis and Martin) Jones and Grout was carried out through dual culture and poisoned food technique, respectively (Ganie *et al.,* 2013).

In vitro evaluation of fungicides and plant extracts against *Alternaria solani*

The fungicides and plant extracts were evaluated through poisoned food technique (Carpenter, 1942; Nene and Thapliyal, 1993, Shravelle, 1961, Genie *et al.,* 2013). For the fungicide evaluation, the desired amount *i.e.* 0.05%, 0.1 % and 0.2% of non-systemic and combi fungicides and 0.025%, 0.05% and 0.1% of non-systemic fungicides was added to sterile PDA medium and poured in to petriplates.

For evaluation of plant extracts, the leaves of Durant, Eucalyptus, Garlic, Clerodendron, Neem, Communist weed, Congress weed, Tulasi, Pongamia, Jathropa were collected and washed with sterilized distilled water and dried at room temperature, crushed and suspended in 80% ethanol and filtered after one hour through Whatman No. 1 filter paper. These where evaporated to dryness on a water bath (40 ± 2ºC), on cooling, their aqueous suspensions were prepared in the ratio of 1:1 (W/v) by adding sterilized distilled water. The extract of 5 mL, 10mL of each was taken from each botanical and was poured in 95, 90 and 85mL luke warm PDA in 250mL conical flask.

For both fungicides and plant extracts 5 mm mycelial disc was placed at the center. Suitable check was maintained without addition of fungicide or plant extracts. Nine days old 5 mm mycelial disc of *Alternaria solani* was placed in the centre of petriplates and incubated at 27 ±1ºC for nine days and three replications were maintained for each treatment. The diameter of the colony was measured in two directions and average was recorded. Per cent inhibition of mycelial growth of the fungus was calculated by using the formula by Vincent (1947).

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

Where,

I = Per cent inhibition of mycelia growth

C = Growth of mycelium in control (mm)

T = Growth of mycelium in treatment (mm)

In vitro evaluation of pathogen by bioagents using dual culture technique

An antagonistic activity of various fungal antagonists viz., *Trichoderma harzianum, Trichoderma viride* Pers. and *Trichoderma virens* (Miller *et al.,*) von Arx were tested *in vitro* against *Alternaria solani* by dual culture technique (Utkhede and Rahe, 1983). The *T. viride* was evaluated against five phytopathogens by following dual culture technique (Morton and Srouble, 1955). *The* antagonistic potential of *Trichoderma harzianum, T. viride, T. koningii, T. virens, Pseudomonas fluorescens* and *Bacillus subtillis* was assessed against *Alternaria solani* by dual culture technique on PDA medium. For this 20 mL of sterilized and cooled medium (PDA) was poured in each petriplates (90 mm diameter) and was allowed to solidify. A 5 mm disc of *A. solani* was plated at one end of the medium with the help of sterilized cork borer. Just opposite to it 5 mm disc of the *Trichoderma* spp. was placed at another end 0.5 to 1.0 cm away from edge of petriplates. For this a week old culture of *A. solani* and *Trichoderma* spp. in petriplates on sterilized PDA medium were used.

RESULTS AND DISCUSSION

In vitro evaluation of fungicides against *Alternaria solani*

In the absence of resistant cultivars, use of fungicides to manage the disease is an old-age practice. When there is outbreak of epidemic for any reason perhaps use of fungicides is one of the best options available. These fungicides have to be used judiciously according to the need and kind of organism involved. Availability of new fungicides necessitates evaluation of fungicides under *in vitro* conditions to know their efficacy, and initiate spray schedule in field conditions.

The results indicated that (Table 1) there was a significant difference among contact fungicides in inhibiting the growth of *A. solani*. Among the three contact fungicides evaluated, mancozeb (63.20%) was significantly superior over other treatments followed by chlorothalonil (55.74%). Least inhibition was observed in propineb (51.18%). Among the different concentration of contact fungicides tested against *A. solani* mancozeb at 0.2 per cent (87.21%) was very effective. Least inhibition of mycelial growth of the pathogen was observed in propineb (29.63%) at 0.05 per cent concentration.

Among the systemic fungicides, hexaconazole at 0.1 per cent (88.88%) gave maximum inhibition of mycelial growth of pathogen followed by at 0.05 per cent (86.66%) concentration. The least effective fungicide was difenconazole at 0.025 per cent (77.03%) concentration. The systemic fungicide hexaconazole (86.90%) gave maximum inhibition followed by propiconazole (85.20%). Least inhibition of mycelial growth was observed in difenconazole (80.98%) (Table 2). The results are in conformity with Arun Kumar (2011) as he reported that Hexaconazole (0.1%) was found effective followed by Chlorothalonil (0.2%) and Mancozeb (0.2%).

Among the five combi-fungicides evaluated, Hexazconazole 4% + Zineb 68% 72WP (Avtar) at 0.2 per cent (88.88%) was superior in inhibiting the mycelial growth over other treatments, which is on par with Carbendazim 12% +Mancozeb 63%) 75WP (Saaf) and Carbendazim 25%+ Iprodione 25% 50WP (Quintal) at 0.2 per cent. Least inhibition was observed in Captan 70% + Hexaconazole 5%) 75WP (Taquat) (54.81%) at 0.5 % (Table 3). The results are in agreement with findings of several workers like Natarajan (1980); Kamble *et al.,* (2000). Arunkumar (2006), Mallikarjun (1996) and Patel and Choudhary (2010) reported the effectiveness of triazoles in inhibiting mycelial growth. At 150 ppm, Difenoconazole (91.95%) gave maximum inhibition of the mycelial growth followed by Cabriotop (64.36%) as reported by Abdussamee *et al.,* 2014. Maximum inhibition of *Alternaria alternata* in treatment with Carbendazim 12% +Mancozeb 63%) 75WP

0.2% (90.36%), followed by Mancozeb at 0.25% (88.88%) was observed by Waghe *et al.,* 2015.

In vitro evaluation of bioagents against *Alternaria solani*

Six bioagents were evaluated for their efficacy against *A. solani* through dual culture technique as explained in material and methods. The results of the study are presented in Table 4. *Trichoderma harzianum* recorded highest inhibition (77.50%) followed by *T. viride* (75.14%), *T. koningii* (73.19%) and *T. virens* (71.53%). The least inhibition of the fungus was observed in *Bacillus subtilis* (52.02%) and *P. fluorescens* (36.22%) antagonist. Among bioagents, significantly higher mycelial growth inhibition of *A. solani* was recorded in the case of *T. harzianum* (71.85%), which was followed by *T. viride* (65.93%) and *T. virens* (58.65%). 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%) was reported by Ganie *et al*. (2013). The effectiveness of *Trichoderma harzianum* ISO-1, *T.harzianum* ISO-2 and *T. piluliferum* against A. solani was reported by (Shikha Thakur, 2014).

In vitro evaluation of plant extracts against *Alternaria solani*

The ten plant extracts were evaluated at two concentrations in the laboratory for their efficacy against *A. solani* through poison food technique as detailed in Material and Methods. The data are presented in Table 5.

The results revealed that, the plant extracts were effective at 10 per cent than 5 per cent concentration. Among the ten plant extracts evaluated, Jatropha at 10 per cent concentration was found to be best in inhibiting the mycelial growth of *A. solani* (62.78%) and found significantly superior over all the other extracts, followed by Pongamia (38.70%), Garlic (37.04%), Durant (34.44) and Neem leaf extract (32.41%) at 10 per cent. The least inhibition of mycelial growth of *A. solani* was recorded in Tulasi (12.22%) followed by Eucalyptus (13.70) at 5 per cent concentration. The present investigation of various botanicals inhibiting the growth of *A. solani* is in line with the earlier findings (Anamika and Sobita, 2011; Arunkumar, 2008; Kota, 2003; Ogbebor and Adekunle, 2008; Patni*et al*., 2005). Arunkumar (2006) reported least inhibition by tulasi extract. In *in vitro* study the leaf extracts of *D. stramonium, A. indica*, and *A. sativum* at 5% concentration caused the highest reduction of mycelial growth of *A. solani* Nashwa (2012). Roshan (2014) reported the effectiveness of *J. curcas* followed by *D. strumarium* and *A. indica* extract in inhibition of mycelial growth of *A. solani*. Ethyl acetate extract of *Azadiractha indica* in retarding fungal growth of *A. solani* at 0.19 mg was reported by Shahnaz *et al*. (2013).Yanar *et al*. (2011) showed the effectiveness of *L. nobilis, S. officinalis, H. lupulus*in inhibiting *A. solani*. The effectiveness of *D. stramonium*, (61.12% mycelial growth inhibition) of *A. solani* was reported by Ganie *et al*. (2013).

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