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In vitro STUDY OF IMPACT OF SELECTED FUNGICIDES AND PHYTOEXTRACTS ON MYCELIAL GROWTH OF *Alternaria solani* THE CAUSAL ORGANISM OF LEAF BLIGHT OF POTATO AND TOMATO

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ABSTRACT

Leaf blight of potato and tomato caused by *Alternaria solani* is an important fungal disease that causes heavy loss to the growers. In case of suitable conditions the pathogen causes maximum damage to these vegetable crops and even the fruits of tomato are also attacked by the pathogen. In the present study the pathogen was isolated and pure culture was maintained on Potato Dextrose Agar medium. *In vitro* experiments were done to evaluate the fungitoxic effects of three different concentrations of 7 selected fungicides as well as leaf extracts of 7 different plants extracts separately. Among the fungicides, 100 inhibition was found when 2000 ppm of Difenconazole, Propinconazole and Benomyl was used. Diphenconazole and Propinconazole even at 1000 ppm, completely inhibited the mycelial growth of the pathogen *in vitro*. Lowest percentage of inhibition of mycelial growth was found in case of copper hydroxide. Even at 2000 ppm the percentage of inhibition was 68.72 only. Similarly, Thiophenate at 2000 ppm could inhibit the mycelial growth that was 78.85 only. Among the phytoextracts, leaf extract of *Acasia nilotica* at 30% inhibited 84.74 of mycelial growth, which was followed by the extract taken from the cloves of *Allium sativa*. At 30 the percentage of inhibition was 81.65. This was followed by the percentage of inhibition by leaf extract of *Azadirachta indica*, which were 78.54 at 30%. Minimum inhibition at 30% of leaf extract of *Phyllanthus niruri* was 68.66. In the present work it was noted that mycelial growth was inhibited at all the concentrations of the seven fungicides and leaf extracts of all the *plants* with different percentages of inhibition.

KEYWORDS: Leaf Blight, Fungicides, Phytoextracts, Mycelial Growth, *Alternaria solani*

Potato (*Solanum tuberosum*) and tomato (*Lycopersicon esculentum* Mill) are most remunerative and widely grown vegetables in the world. In some western countries potato is the staple food. Ganie *et al*., (2016) reported that potato is considered "The King" in food staples and hardly any domestic kitchen is available which does not use potato in one or other form as it possesses all the attributes to be a potential food crop. Kaur *et al*., (2004) reported that potato bears antioxidant properties and due to this it inactivates reactive oxygen species, reduce oxidative damage, lead to improved immune functions and reduce risk of cardiovascular diseases, cancer, cataract, diabetes and aging. Early blight, caused by *Alternaria solani* is a serious disease of potatoes. Pathogen causes heavy loss if the infection is severe. This may reduce the yield up to 20-50. This disease also causes dry rot of tuber that reduces the quality and quantity of marketable tubers. The pathogen over winters as mycelium or conidia in plant debris, infected tubers or on the alternative hosts. Lower leaves in contact with soil are generally infected first. From there the upper leaves and nearby plants are infected. The presence of the pathogen on infected plants can be recognized by the concentric rings on the necrotic patches.

The pulp and juice of tomato is very digestible, promoter of gastric secretion and blood purifier additionally it contains folates, potassium, vitamins A and C. Tomato has rank second next to potato in world acreage and ranks first among processing crops. It is being cultivated in 4.73 million hectares all over the world with production of 163.96 million tones and an average yield of 34.66 tones per hact (Kumar and Singh, 2017). The early blight disease caused by *Alternaria solani* is most important disease and may cause heavy damage in the yield (Saha and Das, 2012). From the survey of literatures, it was noted that different workers have tried to control the disease and have recommended certain fungicides. Similarly, phytoextracts have been used for its control. Some of them may be mentioned here. Mohan and Raveesha (2007), Lee *et al*., (2007), Saha *et al*., (2008), Venkataswamy *et al*., (2010), Zaheer (2010), Srivastva and Singh (2011), Dellavalle *et al*., (2011), Saran (2011), Shinde and Dhale (2011), Tapwal *et al*., (2011), Bhardwaj (2012), Gujar and Talwar (2012), Jagpat *et al*., (2013), Reddy *et al*., (2013), Sasodi and Singh (2013), Kantwa *et al*., (2014), Jha *et al*., (2014), all have used different plants extract to control the mycelial growth and sporulation of the above pathogens.

There are different workers who have tried to control this pathogen either through bioagents or chemical fungicides. Some of them are, Hamza *et al*., (2015), Waghe *et al*., (2015), Ganie *et al*., (2016), Ghazanfar *et al*., (2016), Rani *et al*., (2016), Devi *et al*.,

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(2017), Karalia *et al*., (2017), Kumar and Singh (2017), Shin *et al*., (2017), Hussain *et al*., (2018), Prasad *et al*., (2018). Keeping these ideas in mind present work was carried out to evaluate the efficacy of certain plant extracts and fungicides at different concentrations, separately for the inhibition of mycelial growth of *Alternaria solani, in vitro*.

MATERIALS AND METHODS

Alternaria solani was isolated from the infected leaves of tomato and potato. Pure culture of the fungus was maintained on the Potato Dextrose Agar medium. Seven plants, having medicinal values were located and their leaves were collected for the preparation of extracts. Leaves were washed thoroughly in the running tap water and dried properly. 100 g of above leaves was grinded in mortar and Pestle in 100 ml sterilized distilled water.

The extract was filtered through four layered of pre-moistened muslin cloth. The final volume was adjusted to 100 ml with distilled water. The extract was centrifuged at 5000 rpm for 5 min, and clean supernatant was used as stock solution. From this stock solution required amount was added in 100 ml Potato Dextrose Agar medium before, it gelled to make the concentration 10, 20 and 30. So here, also poison food technique (Nene and Thapliyal, 1993) was used. Above sterilized and phytoextracts containing medium was dispensed in Petri plates in the aseptic condition in the cabin of Laminar air flow chamber. These plates were allowed to cool and were used for inoculation.

Seven different fungicides such as:

i. Difenconazole 25 EC - Trade Name : Score
ii. Propinconazole 25 EC - Trade Name : Tilt
iii. Carbindazin 50 WP - Trade Name : Fungi Gamel.
iv. Mancozeb 75 WP - Trade Name : Dithane M-45
v. Benomyl 50 WP - Trade Name : Benlate
vi. Thiophenate Methyl 70 WP - Trade Name : Roko
vii. Copper Hydroxide - Trade Name : Kocide

Were purchased from the shop of Agrochemicals of Chapra town. Required amount of the chemical fungicides was dissolved to prepare concentration of 500 ppm, 1000 ppm, 2000 ppm. This was also added in pre-gelled Potato Dextrose Agar medium and then dispensed in the Petri plates on cooling these plates containing medium were used for inoculation.

Inoculation

Pure cultures of *Alternaria solani* maintained in the laboratory were used for inoculation. With the help of pre-sterilized cork borer, from the actively growing periphery region of the plate, 7 mm discs were cut with precautions. One such disc was transferred aseptically to the centre of each Petri plates, containing the poisoned solid medium.

These plates were incubated at 26 ± 2^0C in dark. Petri plates containing non-poisoned medium was also inoculated which served as control each treatment was replicated three times and in each culture 15 plates were inoculated. These cultures were watched on an alternate day and plates showing infection were discarded after autoclaving. The radial growth of the fungus in poisoned medium was recorded at the time when the radial growth of mycelium in control reached 91.60 mm. The plates were turned upside down and a lien was drawn with the help of a marker from one end of the boundary of the mycelium to other end. This was measured to denote the radial growth.

Percent inhibition of radial growth in the poisoned medium was calculated by applying the formula:

PI = C-T/CX100

Where,

PI= Percent inhibition

C = Radial growth in control

T= Radial growth in poisoned or treated medium with pytoextract/fungicides.

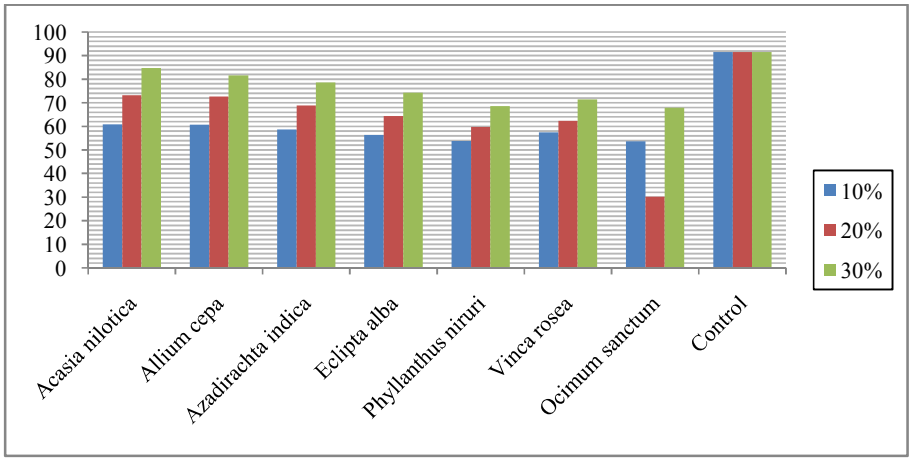
The mean of the data obtained has been represented by the graph 1 and 2.

RESULTS AND DISCUSSION

Evaluation of Phytoextracts

Data obtained in the present work on *in vitro* efficacy of phytoextracts of different concentrations against *Alternaria solani* have been represented by the graph 1. From the graph it may be noted that there were significant difference with respect to fungitoxic effect of different phytoextracts at all the three concentrations, and was reflected by the percentage of inhibition of radial growth of the fungal pathogen. Among the phytoextracts taken separately from seven *plant* species, extract taken from *Acasia nilotica* was most effective at all its concentrations with respect to the inhibition of radial growth of the fungus in culture. Here the percentage of inhibition was 84.74 at 30% concentration, followed by 73.26 at 20% and 60.82 at 10% concentrations of the

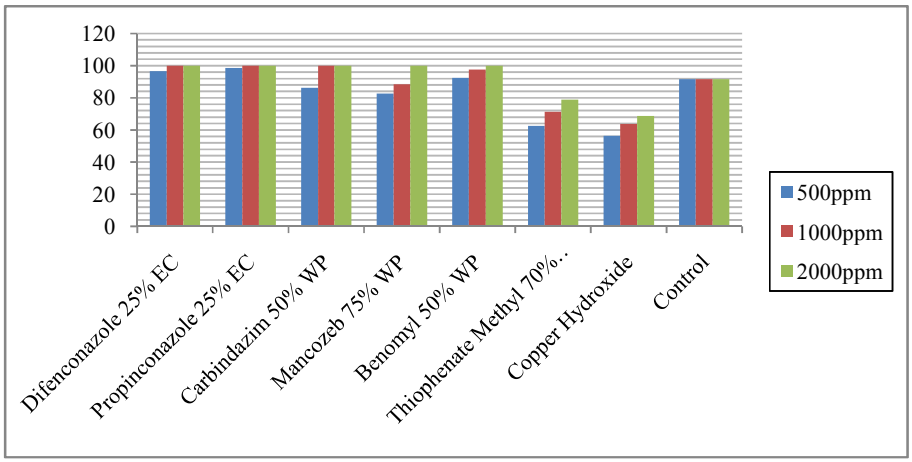
extract. This was followed by the extracts taken from the cloves of *Allium cepa*, which was 81.65 at 30, 72.75 at 20 and 60.18 at 10 concentration respectively. It may be noted form the graph that at similar concentrations of leaf extract of *Azadirachta indica* the percentage of inhibition was 78.54, 68.88 and 58.64 respectively. Minimum inhibition of radial growth of mycelium was inhibited by the leaf extract taken from the *plants* of *Phyllanthus niruri* at all the three concentrations followed by the leaf extracts of *Ocimum sanctum* that was 68.84, 61.18 and 55.76 respectively.



Graph 1: Showing percentage of inhibition of radial growth of mycelium of *Alternaria alternata* cultured in the medium poisoned with different concentrations of phytoextracts

Evaluation of Fungicides

Seven different fungicides at three different concentrations were assessed *in vitro* to determine the most effective fungicide against *Alternaria solani*. Here also poison food technique was applied and 500, 1000, 2000 ppm of the all the seven fungicides were used separately. The data obtained are represented by the graph 2. It was found that Difenconazole 25 EC, Propinconazole 25 EC, Carbindazim 50 WP, at 1000 and 2000 ppm concentrations inhibited the radial growth of fungal mycelium 100% and similarly, Benomyl 50 WP at 2000 ppm, inhibited the radial growth that was 100%. Therefore, among the seven fungicides, most effective fungicide was Difenconazole 25 EC which exhibited 100% inhibition of mycelial growth at 1000 and 2000 ppm. Even at 500 ppm the percentage of inhibition was higher than rest of the fungicides at this concentration. Form the graph it may be noted that copper hydroxide at all the concentrations used revealed lowest percentage of inhibition in the radial growth of mycelium of the fungus *in vitro*.



Graph 2: Showing percentage of inhibition of radial growth of mycelium of *Alternaria alternata* cultured in the medium poisoned with different concentrations of fungicides

Efficacy of phytoextracts taken from different plants as fungitoxicant has been assessed by different workers. Okigbo and Ojbonuaya (2006), Satish *et al*., (2007), Chang et al., (2008), Saran (2011) Talibi *et al*., (2012), Zare *et al*., (2012), Sherwani *et al*., (2013), Singh and Srivastva (2013), Srinivas *et al*., (2013).

Singh *et al*., (2014), Hussain *et al*., (2015), Rathod *et al*., (2015), Mathivanan and V.R. Prabhavathy (2007), Nagegba *et al*., (2018), Chaudhary et al., (2019), Singh et al (2019). All these workers concluded that phytoextracts taken from different plants in general and medicinal plants in particular revealed fungitoxic activities at different concentrations. However, the quantum of activities varied which may be due to presence of different secondary metabolites. All these findings therefore, corroborate with the findings of the present work as here also the variations in fungitoxic effect was noted.

Different workers have also evaluated the efficacy of different fungicides at different concentrations to assay the efficacy *in vitro*. Rani *et al*., (2016), Theja and Devappa (2016), Kumar *et al*., (2017), Wagh *et al*., (2017), Prasad *et al*., (2018), have evaluated different concentration of selected fungicides against different fungal pathogen. Findings of present work are in agreement with the findings of these workers as the fungitoxic effect increases along with the increased concentrations. Similarly, all the fungicides do not inhibit mycelial growth at the same concentration.

It may be concluded that keeping the idea of impact of chemical fungicides on our environment, its cost and impacts on non-target organisms, it is essential to search an alternative agent to control the diseases of our crops. Phytoextracts are more potent candidate for it. Here among the fungicides Difenconazole 25 EC, Propinconazole 25 EC, Carbindazim 50 WP and Benomyl may be more suitable fungicides. Likewise the phytoextracts from *Acasia nilotica*, *Allium cepa*, *Azadirachta indica* extracts may be preferred.

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