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In vitro EVALUATION OF SELECTED FUNGICIDES AND PHYTOEXTRACTS ON POTATO LEAF SPOT DISEASE CAUSING ORGANISM, *Alternaria solani*

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ABSTRACT

Leaf blight of Potato is an important disease. It is caused by the fungal pathogen *Alternaria solani*. If the conditions such as moisture and temperature are favorable then the damage is to the greater extent. From the infected leaf of potato, the pathogen was isolated, identified and maintained as pure culture. Five commonly used fungicides at three different concentrations and three different concentrations of five different medicinal plants were evaluated for their fungitoxic effect *in vitro*. Three fungicides, Propinconazole, Difenconazole and Carbindazim at 2000 and 1000 ppm, inhibited the radial growth which was 100%. Similarly, Mancozeb and Benomyl at 2000 ppm completely inhibited the radial growth of the fungal mycelium. Benomyl at 1000 ppm could inhibit 96% of radial growth while Mancozeb at the same concentration inhibited 86%. Even at 500 ppm Propinconazole, Difenconazole and Carbindazim had higher percentage of radial growth inhibition than that of Benomyl and Mancozeb. Among the phytoextracts the inhibitory effect of *Acasia* was the highest at all the three concentrations than others that was 88.24% at 30%, 78.84 at 20% and 66.72 at 10%. This was followed by *Allium* extract that was 84.56, 76.75 and 62.54 at the similar concentrations. Extracts taken from *Azadirachta indica* inhibition the mycelial growth 78.54%, 68.89% and 58.64% respectively at three different concentrations as mentioned above. It was noted that extracts taken from *Phyllanthus niruri* could inhibit the radial growth of the mycelium which was the lowest at all the three concentrations used here.

KEYWORDS: Phytoextracts, Fungicides, Radial Growth, Fungal Pathogen, Potato Leaf Spot

Fungal pathogens are a big challenge before the plant pathologists as they damage different crops either in storage or in standing conditions. Potato is being cultivated for different purposes. In some western countries potato is staple food (Ganie *et al*; 2016) reported that potato is considered as "the king" among the staple food. Kaur *et al*; (2004) reported that potato bears antioxidant properties; therefore, it improves the immune systems. It also reduces the danger of cardiovascular diseases, cancer, cataract, diabetes and aging. The blight disease of potato is caused by *Alternaria solani* is called early blight. If conditions are favorable the pathogen can reduce 25-50% of the yield. The dry rot of tuber is also caused by this pathogen. Due to this there is qualitative reduction in the tuber. Due to change in the potato tuber the market value of the crop is also reduced.

The control this diseases, different workers have used certain fungicides as well as phytoextracts. Some of them may be mentioned here such as, Lee *et al*; (2007); Saha *et al*; (2008); Venkataswamy *et al*; (2010); Srivastva and Singh (2011); Dellavale *et al*; (2011); Bhardwaj (2012); Gujar and Talwar (2012); Reddy *et al*; (2013); Waghe *et al*; (2015); Ganie *et al*; (2016); Rani *et al*; (2016); Devi *et al*; (2017); Kumar and Singh (2017); Shin *et al*; (2017); Hussain *et al*; (2018) and Prasad *et al*; (2018). Keeping all these ideas in mind, experiments were done to evaluate the fungi toxic impact of selected fungicides and some phytoextracts on radial growth of mycelium of *Alternaria solani in vitro*, the causal agent of leaf spot disease of potato.

MATERIALS AND METHODS

Potato fields were located and plants showing symptoms were identified. Infected leaves were collected and the fungal pathogen was identified on the basis of the symptoms and microscopic studies. Here conidia were made the basis of identification. After this pure cultures were maintained on Potato Dextrose medium. For the extraction, five plants were collected Leaves were washed properly while *Allium* cloves were prepared after removing the scales. Air dried leaves were chopped off and 100 g of them was ground in mortar with the help of pestle in the presence of 100 ml pre-sterile distilled water. The extract was filtered through the three layered muslin cloth. The residue was extracted again and the final volume was made 100 ml by adding distilled water. The extract was centrifuged at 500 rpm for 5 min. Clean supernatant was taken for stock solution. From this stock solution desired amount was added in 100 ml PDA medium before it gelled so that the concentrations of 10, 20, and 30% were obtained. In this way poison food technique (Nene and Thapliyal, 1993) was adopted. Above medium along phytoextracts was dispensed in Petri Plates in the aseptic conditions of the laminar airflow cabinet. After cooling the plates were used for inoculation with fungal mycelial mat.

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Five fungicides used were:

Propinconazole 25 EC

Difenconazole – 25 EC

Carbindazim- 50 WP

Mancozeb- 75 WP

Benomyl – WP

Above fungicides were procured form the traders of Agrochemicals in Chapra town. Here also stock solutions were prepared and added in 100 ml semi melted PDA medium to get 500, 1000, 2000 ppm per liter. Above medium was dispensed in the culture plate and on cooling used for the inoculation of fungal mat.

INOCULATION

With the help of pre-sterilized cork borer, 6 mm diameter, mycelial mat was taken from the periphery of pre-cultured *Alternaria solani*. Above disc was placed in the middle of the plate under aseptic condition. Inoculated plates were incubated in the culture room at 26 ± 1^0C temperature, in dark. Petri plates having culture medium without phytoextracts or fungicides were used as control. When the radial growth in control plate reached 91.25 mm, then growth in poisoned medium was measured. For this plates were kept upside down and a line was drawn from one end to other. From this length to the length of disc was deducted that gave the actual growth of the fungus in the presence of phytoextracts or the fungicide.

Percent Inhibition = PI

PI = C-T/CX100

Where,

C= Radial growth in control

T = Radial growth in treated plate.

The means of the data were presented in graph 1 and 2.

RESULTS AND DISCUSSION

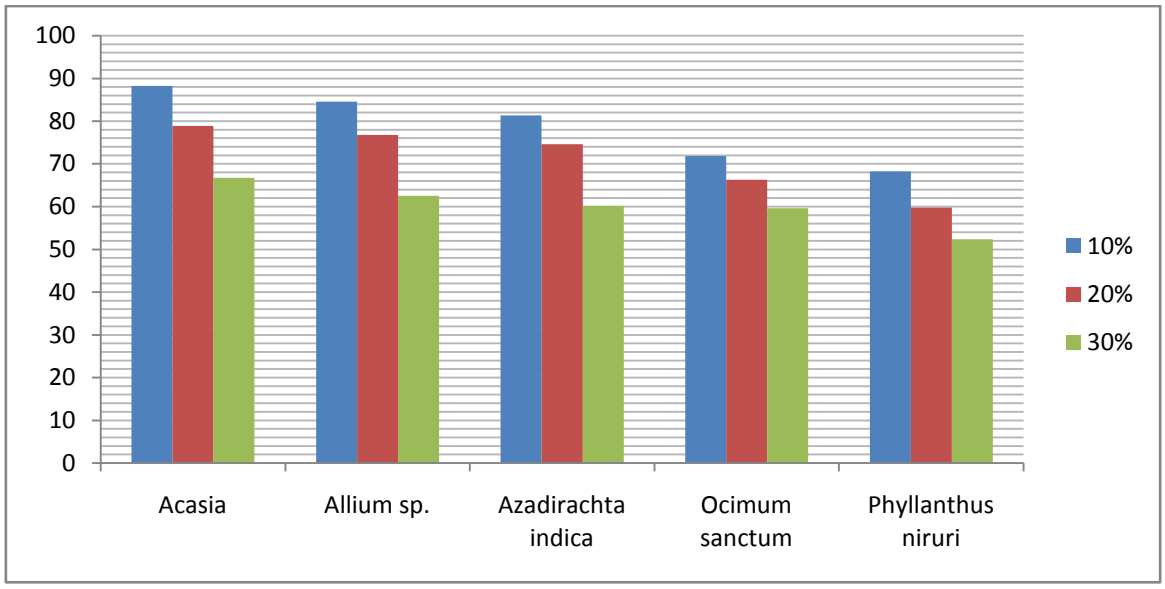
From the graph-1, it was noted that at 30% concentrations of phytoextracts taken from *Acasia* could inhibit the radial growth which was 88.24 in 30% followed by 78.84 in 20% and 66.72 in 10%. This was followed by the extract taken from *Allium* at 30% which was 84.56, at 20% 76.75% and at 10% 62.54. Phytoextract taken from *Azadirachta* at 30% was 81.38, at 20% 74.62 and at 10% 60.18 respectively. Next to it was phytoextracts at 30% 71.81, 66.32 at 20% and 59.65 at 10%. Here minimum inhibition of mycelial growth was 68.24 at 30%, 59.78 at 20% and 52.36 at 10% respectively.

Among the fungicides it was noted that Propinconazole 25 SE, Difenconazole 25 SE, Carbindazim 50% at 2000 and 1000 ppm, completely inhibited the mycelial growth of the fungus *in vitro*. Similarly, Mancozeb and Benomyl at 2000 ppm also inhibited 100% mycelial growth of the fungus. Maximum inhibition of mycelial growth was noted at 500 ppm of Difenconazole which was 96% followed by Propinconazole at the same concentration that was 91.66%. At this concentration Carbindazim inhibited mycelial growth which was 88%. Minimum inhibition of mycelial growth was noted at 500 ppm in the case of Benomyl which was 72% where as maximum in the case of Difenconazole that was 96.00% followed by Propinconazole 91.66 and Carbindazim 88%.

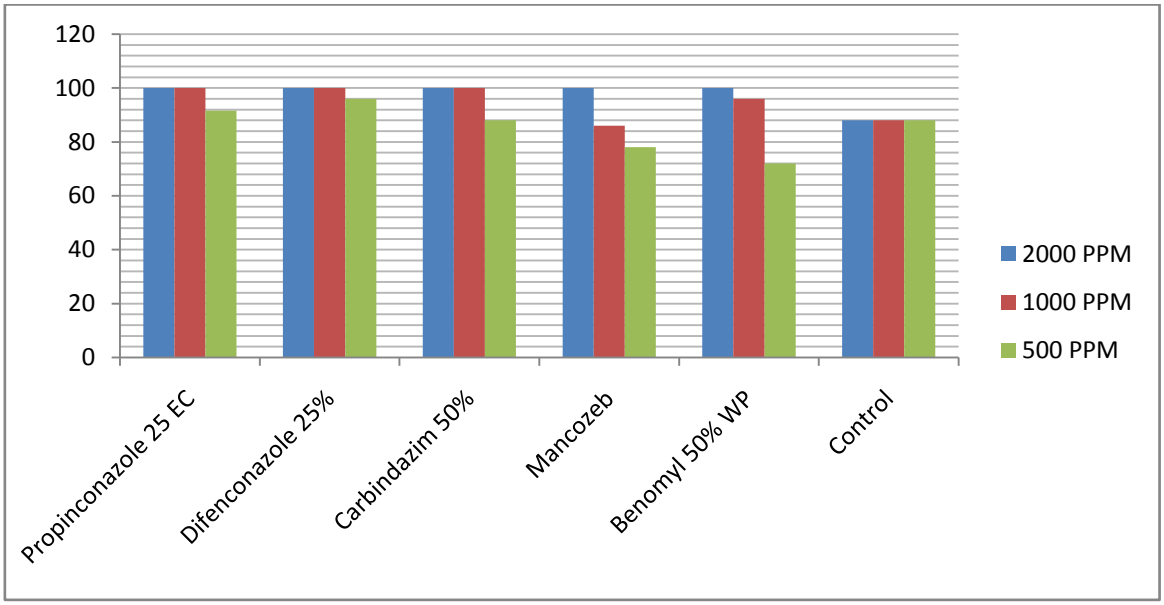
DISCUSSION

To control the fungal diseases and for the higher yields, chemical fungicides are being used all over the world. The residues of these chemicals are becoming a serious threat to our water bodies and the soil itself. Because phytoextracts have no such toxic effects, so there are search to find out suitable plant extracts that may be used to control the fungal diseases without any side effects. Different workers have performed experiments to confirm the antifungal activity of certain phytoextracts such as 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); Jha *et al*; (2014); Singh *et al*; (2014); Hussain *et al*; (2015); Rathod *et al*; (2015); Nagegba *et al*; (2018); Chaudhary *et al*; (2019). According to the above workers, extracts taken from the medicinal plants have antifungal activity at different concentrations. Although there are differences of such activities among different plants which may be due to presence of the secondary metabolites. Therefore, findings of the present works are in agreement with the findings of the above workers. The concentrations of different fungicides which may be effective in controlling the growth of fungal pathogens are one of the important factors in the use of these chemical fungicides. For this different workers have suggested different concentrations. On the basis of *in vitro* experiments, Rani *et al*; (2016); Theja and Devappa (2016); Kumar *et al*; (2017); Waghe *et al*; (2017); Prasad *et al*; (2018) have expressed their views regarding the concentrations which may be

optimum to control the pathogen. In the present work also attempts were made to determine the concentrations of the fungicides so that it may not be used at higher concentrations.



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



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

CONCLUSION

Use of chemical fungicides to control the fungal pathogens of different crops are posing a threat on the aquatic animals. They are entering our food chain and we are becoming prey of these fungicides. There is a need for alternative agents. Phytoextracts are the best candidate. However, most of the results are on the basis of *in vitro* experiments. There should be *in vivo* trial to determine specific concentrations of specific phytoextracts against specific fungal pathogen. It should be applicable to chemical fungicides also.

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