

“TOXICITY OF BACTERIAL PATHOGENS ISOLATED FROM ONION CROP GROWN IN ALWAR DISTRICT OF RAJASTHAN, INDIA”

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ABSTRACT

Toxins are chemical substances secreted by microorganisms which may affect host plants by causing a disease. Toxins have been identified in a wide range of phytopathogens. These have been classified as pathotoxins, vivotoxins and phytotoxins based on their mechanism of action and interaction with the host plants. Three replicates of twenty onion healthy seeds were treated with toxins of studied bacterial pathogens. These treated seeds were incubated to study the effects on seed germination, seedling health and seedling growth, and seedling vigour index (SVI). Toxin treated seeds showed less germination and seedling vigour index as compared to untreated seeds. Raised seedlings also showed symptoms of corresponding bacterial diseases. Seedling vigour index was recorded for toxin treated seeds 431.42 (*Pseudomonas aeruginosa*) and 587.61 (*Pseudomonas syringae* pv. *syringae*) as compared to 910 (healthy untreated).

Key words: Bacteria, disease, onion, symptoms, toxins.

INTRODUCTION

Toxins are chemical substances secreted by microorganisms which are injurious to plants and involved in disease development. In his experiment, Antony de Bary (1886) succeeded to show soft rot symptoms by applying a sterile extracts from rotten carrots to healthy tissues (Huang, 2001, Usman, 2018). Many metabolic reactions take place in the living plant cells. The disruption in these metabolic reactions leads to plant diseases. Toxins directly affect on host protoplast and leads to death of the cells. Fungal and bacterial pathogens may produce toxins in infected plants as well in culture media. Toxins are effective in very low concentrations and are very poisonous. Several toxins have been shown to be involved in disease development (Graniti, 1991). Toxins are extremely poisonous and effective in very low concentrations. Toxins injure host cells either by affecting osmotic relations or by affecting metabolic reaction disrupting physiological process in the plant. Toxins have been classified on the basis of their chemical characteristics and may be classified on their biological activity, antimetabolites, membrane affecting compounds and enzyme inhibitor efficacy. However, toxins broadly classified on the basis of their host selectivity and on general roles in disease developments (Durbin, 2012). In their disease development toxins are divided in three classes namely pathotoxins, vivotoxins and phytotoxins. Bacterial toxins show extreme diversity in their structure, mode of action, intracellular receptor recognition, enzymatic activity and mode of secretion. In addition to their role in disease development, bacterial toxins may be used as tools for diagnosis,

prevention and therapy of diseases (Popoff, 2018). In the present study, toxic effects of culture filtrates of two bacterial pathogens *Pseudomonas aeruginosa* and *Pseudomonas syringae* pv. *syringae* were studied.

Materials and methods

Culture filtrate of bacterial pathogens namely *Pseudomonas aeruginosa* (CF-pa) and *Pseudomonas syringae* pv. *syringae* (CF-ps) isolated from diseased onion plants were selected to study the phytotoxic effects on onion seeds. Crude toxins were prepared by incubating pure cultures on Nutrient broth for the production of toxins. Bacterial suspensions (4ml) were inoculated in sterile broth medium (50 ml) and incubated at $25\pm 2^{\circ}\text{C}$ for up to 30 days. The obtained bacterial suspensions were filtered through whatman filter paper no. 1 and centrifuged for 25 min. at 1000 rpm. The extracted clear supernatant solution was used as a crude toxin sample. Twenty seeds of healthy onion seed samples rinsed with sterilized distilled water and soaked in culture filtrate of the studied samples for 24 hrs under sterilized conditions. Treated onion seed samples were again rinsed with sterilized distilled water and placed in petri plates containing three layer moistened blotter papers. Seeds were also treated with sterile distilled water served as check. These treated seeds containing petri plates incubated up to 15 days to observe seed germination, symptoms, healthy and symptomatic seedlings and root and shoot length. Seedling vigor index was also calculated using formula Seedling vigor index (SVI) = (Seedling length) X Percent seed germination (Maiti et al. 2012).

Results and discussion

Onion seeds soaked in studied bacterial crude toxins and distilled water were observed for various parameters such as seed germination, symptomatic seedlings, seedling length and seedling vigour index on 8th day of incubation. Seeds treated with CF-pa showed 58.3% germination as compared to check 100% while CF-ps treated seeds showed 71.66% germination. The results revealed that toxins produced by *P. syringae* pv. *syringae* was less toxic to the onion seeds as compared to *P. aeruginosa*. The raised seedlings from CF-pa treated seeds showed 45% symptomatic while CF-ps treated seeds showed higher percentage (60%) of symptomatic seedlings. Seedling length was also reduced due to the toxicity of studied bacterial pathogens (Fig. 1). Seedling vigour index (SVI) was calculated for each experiment and it was found that overall SVI of healthy seeds was 910 and SVI of CF-pa and CF-ps treated seeds was reduced as low as 47.41% and 64.57% showing severe effects of toxins produced by these bacterial pathogens isolated from diseased onion plants. The toxins produced by *P. syringae* pv. *syringae* includes coronatine, tabtoxins, syringomycin, phaseolotoxins and syringopeptin. These phyto-bacterial toxin produces virulence and toxicity in host plants. Due to presence of wide variety toxins the bacterial pathogen has been divided into pathovars (pathogenic variants) (Bender et al. 1999). Syringomycin G, syringopeptin 25A and syringomycin E were found responsible for antimicrobial activity and toxic activity on *Rhodotorula pilimanae*. Syringomycin was found more active compare to syringopeptin 25A in antimicrobial assay (Iacobellis et al. 1992). These are frequently used biological and pharmacological tools (Aktories and Schmidt, 2015). The results of present study were also similar to the available literature confirming the severity of bacterial toxins.

The symptoms of *P. aeruginosa* affected seedlings were appeared as brown to dark brown lesions and blighting appearance. Withering of seedlings, yellow to irregular brown lesions were observed as symptoms of *P. syringae* pv. *syringae* toxins affected seedlings. Coronatine phytotoxine secreted by *Pseudomonas syringae* is a specific virulence factor for inducing disease symptoms. The bacterial pathogen invade through stomata and grows in apoplast (Geng *et al.* 2012). Different Pathovars of *P. syringae* are known to produce coronatine, phaseolotoxin, tabtoxin and syringomycin and syringopeptin (causes various symptoms) (Hu *et al.* 1998, Cazorla *et al.* 1998, Bender *et al.* 1999, Trigiano, Windham and Windham, 2004, Agrios, 2005, Geng *et al.* 2012, Gallarato *et al.* 2012).

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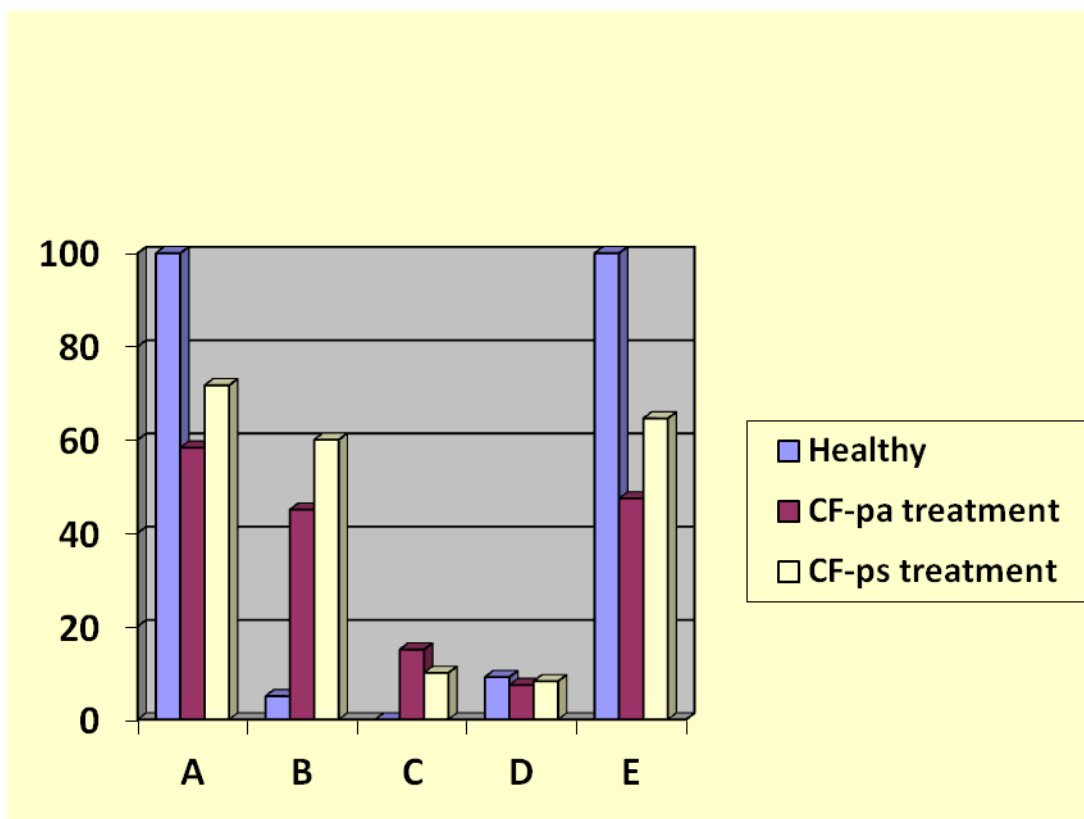


Fig.1. Effects of bacterial toxins on onion seeds and seedlings. (Where A=Seed germination, B=Symptomatic seedlings, C=Seedling mortality, D=Seedling length, E=Seedling vigour index SVI)