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EFFECT OF DIFFERENT PLANT ACTIVATORS AGAINST *RHIZOCTONIA SOLANI* CAUSING ROOT ROT OF CHILLI

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ABSTRACT

Chilli is an important horticultural crop all over the world. Root rot of chilli is the most important disease caused by *Rhizoctonia solani* responsible for immense losses. Systemic acquired resistance (SAR) acting as a significant part in the proficiency of plants to protect themselves by means of hyper-sensitive reaction (HR) of plants toward the destructive pathogens. Additionally, SAR reveals as long time defense approach that may be weeks to months or sometimes throughout the entire season. The aim of this study was to induce systemic acquired resistance (SAR) against *R. solani* in chilli crop. Five plant activators salicylic acid, potassium dihydrogen phosphate (KH₂PO₄), di-potassium hydrogen phosphate (K₂HPO₄), benzoic acid and citric acid were evaluated under Lab. and field conditions with three different concentrations (1, 2 and 3%). Salicylic acid gave better result at 3% concentration. After 3rd application, salicylic acid at 21 days interval expressed minimum disease incidence as compared to others treatments. At concentration of 3 % after twenty-one days of spraying salicylic acid showed minimum disease incidence (9.667%) followed by KH₂PO₄ (16.433%), K₂HPO₄ (20.241%), benzoic acid (23.367%) and citric acid (28.667%) while control expressed maximum disease incidence (86.467%). So, it is concluded that salicylic acid gave better result than others treatments.

Keywords: Salicylic acid, Potassium dihydrogen phosphate (KH₂PO₄), di-potassium hydrogen phosphate (K₂HPO₄), benzoic acid and citric acid.

INTRODUCTION

Chilli (*Capsicum annum* L.) is an economically important and most cultivated crop worldwide. It covers the area of 177, 6000 hectares in all over the world with total production of 718, 2000 tons (Singh, 2007). In Pakistan it is considered as a very valuable cash crop and cultivated over an area of 38.4 thousand hectares with annual production 53.7 thousand tones which contributes 1.5% share in the total GDP. Sindh is a major chilli producer province followed by Punjab and Baluchistan respectively (Hussain and Abid, 2011). It is constituent of different essential elements including

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proteins, fibers, minerals, vitamins, fatty acids, carotenoids, capsaicinoids and some steam-volatile oils (Bosland and Votava, 2003). Fresh green and red chilli has an abundant source of vitamin C and A (Chandra *et al.*, 2010; Chang *et al.*, 2011).

Rhizoctonia root rot of chilli caused by *Rhizoctonia solani* is a major constraint to chilli production all over the world. *R. solani* is a soil borne plant pathogenic fungi with a wide host range (chilli, corn, alfalfa, soybean etc.) and distributed all over the world (Agrios, 2005). First time this fungus was reported on potato plants in 1858, by Julius Kuhn. It belong to family basidiomycete often produces sexual spores (basidiospores) which are mostly four in number. In nature, *R. solani* reproduce asexually by means of somatic mycelium. It occurs mostly in the start and mid of summer. Diseased plants characteristically seem in patches in the pot or field conditions. Different conditions are responsible for this

disease including, soil moisture, type of soil and delayed emergence of plants. The disease is more common at seedling stage but also affects mature plants consequently sever crop losses. Defoliation, wilting, vascular discoloration and stunting growth are the most prominent symptoms of root rot of chilli (Sanogo, 2003). Currently, there is no commercially reliable hybrid variety of chilli having resistance against *R. solani* (Muhyi and Bosland, 1992).

Systemic acquired resistance (SAR) means of specific signals transferring pathway which play a significant role in the entire life of plants to defend themselves against destructive pathogens. When SAR path is activated, it's just an indication of disease and consequently plant develop necrotic lesion due to hypersensitive response (HR). SAR stimulates a broad range induction of systemic resistance in the plants (Hunt, 1996). SAR is a very useful approach and an inquisitive model for transducing signals. Understanding the biochemical variations consequences of cautious conditions which could permit the development of improved disease resistant transgene or else inventive sort of defensive plant chemicals act as stimuli in plant breeding and genetics for disease resistance tools (Ryals *et al.*, 1996). The objective of this study was to manage the Rhizoctonia root rot by using different plant activators.

MATERIALS AND METHODS

This tentative work was conducted at Department of Plant Pathology, University of Agriculture, Faisalabad to evaluate systemic acquired resistant (SAR) through exogenous application of plant activators against Rhizoctonia root rot of chilli.

Collection of plant disease samples: Samples were collected on the basis of typical disease symptoms from the different localities of Faisalabad as well as Chiniot district and preserved in freezer at 4 °C for 5-7 days.

Isolation and pure culture of pathogen: Samples were surface sterilized with 5% bleach for 1 minute then washed three times with distilled water and plated on PDA medium. Pathogens were isolated from newly growing tip by using sterilized inoculating needle and

$$\text{Root infection (\%)} = \frac{\text{Total number of root pieces colonized by the fungus}}{\text{The total number of root pieces studies}} \times 100$$

Field experiment: All plant activators were also evaluated under in field conditions against *R. solani*. The plot size was 5×3 m², there were 10 rows per plot with row to row distance of 30 cm. The chilli seedlings were

plated on sterilized PDA media. Plates were incubated at 25°C± 2°C and observed daily for checking growth of colonies in petri plates (9cm dia). Sub-culturing was done from single spore technique to obtain pure culture.

Preparation of plant activators concentrations: Salicylic acid, citric acid, benzoic acid, KH₂PO₄ and K₂HPO₄ were purchased from Lyallpur chemical company, Faisalabad. Three concentrations 1, 2 and 3 % from each activator were prepared by dissolving in equal volumes of ddH₂O and C₂H₆O by adjusting pH 7 with 1N NaOH.

In-vitro evaluation of different plant activators: 1 ml concentration from each chemical was added and well distributed in Petri plates having diameter 5 cm with three replications, and then poured sterilized PDA even cover the bottom of the plate and gently shake to mix homogenously. Three days old culture of *R. solani* was selected for inoculation and 8 mm disks were seeded centrally in the Petri dishes then incubated at 27±1°C. Three petri dishes without plant activators inoculation were kept as control. Each treatment was repeated thrice to reduce experimental error. The percentage reduction of growth (RG) ratio was calculated according to the following formula (Amer, 1995):

$$\text{Reduction growth (\%)} = \frac{\text{RNT} - \text{RT}}{\text{RNT}} \times 100$$

Where: RNT = Radius for non-treated media (control)

RT = Radius for treated media.

Effect of different plant activators on plant growth and disease development

Pot experiment: Seedlings of commonly grown variety of chilli (Sanam) were transplanted into earthen pots of 20 cm diameter; having 2 kg steam sterilized sandy clay loam soil. The soil was artificially infested with pathogen inoculum at 10⁴ conidia/ g of soil before sowing (Zote *et al.*, 1996). The soil was also amended with the plant activators and with three concentrations, i.e. 1, 2 and 3 %. After transplanting, the sterilized water was applied to the seedlings. After 45 days, the plants were uprooted carefully and roots were washed with tap water. Data was recorded and root infection was calculated by following Formula.

transplanted in the field and irrigated accordingly to maintain 50% water holding capacity. After 3 weeks' different plant activators *viz.* Salicylic acid, Citric acid, Benzoic acid, KH₂PO₄ and K₂HPO₄ were sprayed at 1%,

2% and 3% concentrations to induce Systemic Acquired Resistance (SAR) against *R. solani* and 2nd spray was done after 14 days of first application. The treatments were repeated three times and control (without any treatment) was used for comparison. The application of plant activators was done early in the morning with the help of knapsack sprayer.

Collection of data: Data was recorded at 7 and 14 days of 1st spray application and again 21 and 28 days after 2nd spray application interval. This disease usually appears at seedling and flowering stages. It

was recorded at both stages as reported (Farooq et al., 2005). Disease was recorded visually and rated by using the following scale given by (Iqbal et al., 2005).

Sr.No.	Response	Scale
1	Highly Resistant	Less than 1% of plant wilted.
2	Resistant	1-10% of plants wilted.
3	Moderately Resistant	11-20% of plants wilted
4	Susceptible	21-50% of plants wilted
5	Highly Susceptible	51% or more of plants wilted

Disease incidence was calculated by the following formula:

$$\text{Disease incidence (\%)} = \frac{\text{Number of diseased plants}}{\text{Total number of plants inspected}} \times 100$$

STATISTICAL ANALYSIS

The statistical test was performed by using SAS statistical software. Means were sorted out by using Fisher’s least significant difference (LSD) procedure (Steel et al., 1997).

RESULTS

The data analysis revealed that salicylic acid expressed statistically better results as compared to all others treatments. At *in-vitro* experiment 1, 2 and 3%

concentrations of salicylic acid showed minimum mycelial growth (3.63, 2.46 and 1.14 cm) followed by KH₂PO₄ (3.70, 2.63 and 1.8 cm), K₂HPO₄ (3.76, 2.50 and 1.94 cm), benzoic acid (4.56, 3.47 and 2.2 cm) and citric acid (3.66, 3.49 and 2.3 cm) while control expressed maximum mycelial growth (5 cm). Salicylic acid exhibited significant reduction in fungal growth colony at concentration of 3% followed by all other concentrations (Figure 1).

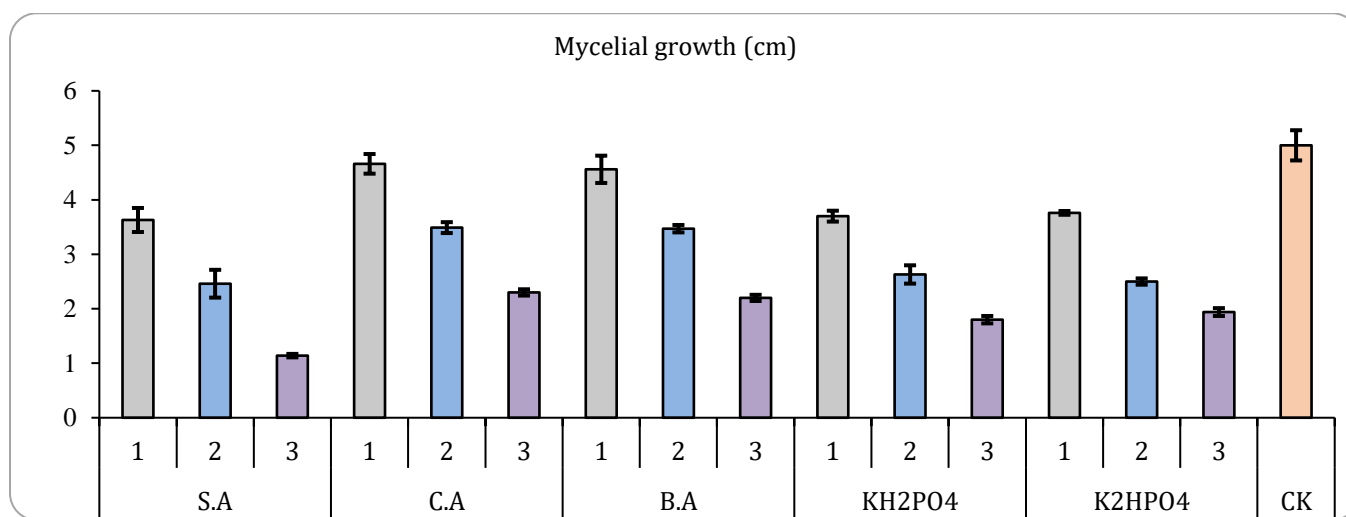


Figure. 1. Mycelial growth for *R. solani* culture incorporated with salicylic acid, citric acid, benzoic acid, KH₂PO₄ and K₂HPO₄ concentrations. S.A= Salicylic acid, C.A= Citric acid, B.A= Benzoic acid and CK= Control with 1, 2 and 3% different concentrations.

Similarly, the effect of different plant activators on the growth of chilli plants showed that minimum root infection (4.34 %) was observed in salicylic acid and maximum infection (11.30 %) was observed in citric acid treated plants at concentration of 3 % as compared to other concentrations (Figure 2). Maximum root and shoot length (6.78 and 17.88 cm)

was observed in case of salicylic acid at higher concentration while minimum (4.97 and 13.06 cm) lengths of root and shoot were recorded in citric acid at same concentrations. Likewise, root and shoot weight (6.49 and 8.50 gm) also expressed maximum growth in case of salicylic acid treated plants followed by all other plant activators (Figure 3).

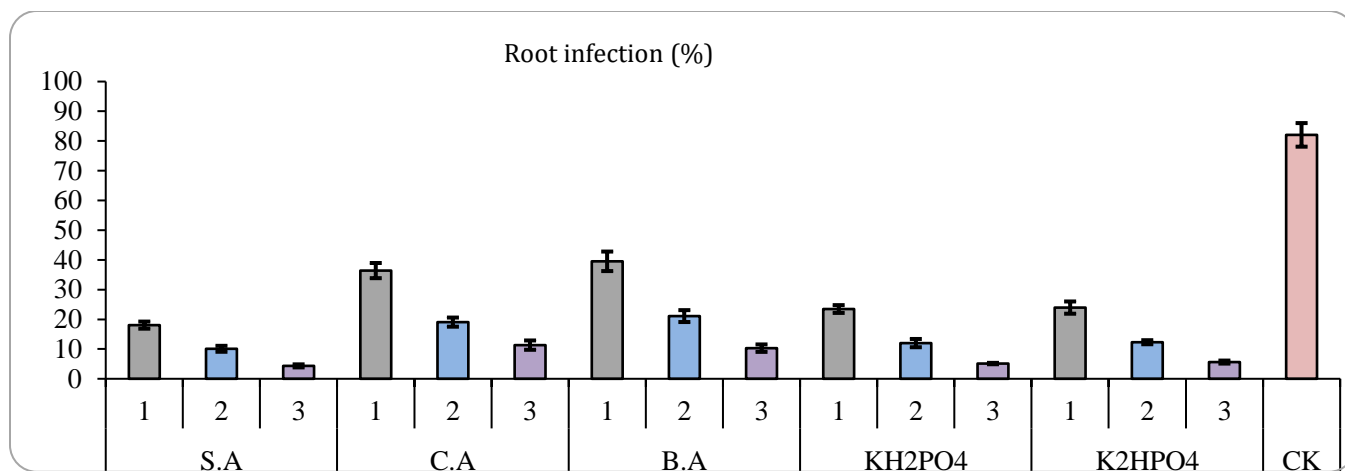


Figure 2. Efficacy of plant activators on root infection (%) caused by *R. solani*. S.A= Salicylic acid, C.A= Citric acid, B.A= Benzoic acid and CK= Control with 1, 2 and 3% different concentrations.

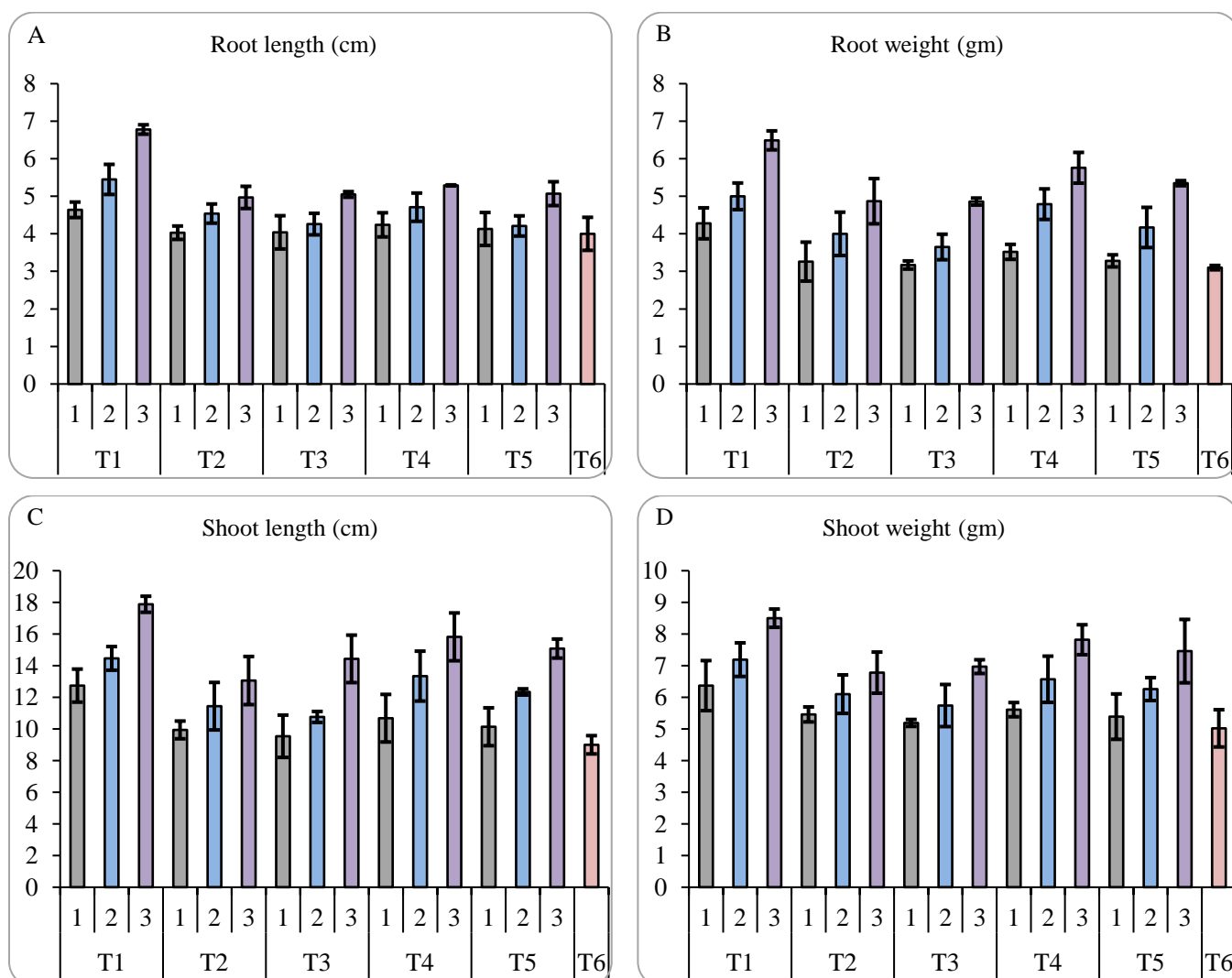


Figure 3. Effect of plant activators on plant growth and disease development. T1= Salicylic acid, T2= Citric acid, T3= Benzoic acid, T4= KH₂PO₄, T5= K₂HPO₄ and T6= control with 1, 2 and 3% different concentrations.

The statistical analysis showed that 3rd application of salicylic acid after 21 days of interval minimum disease incidence was recorded as compared to others applications after consecutive days. Concentration of 3 % after 21 days of spraying salicylic acid showed

minimum disease incidence (9.667%) followed by KH_2PO_4 (16.433%), K_2HPO_4 (20.241%), benzoic acid (23.367%) and citric acid (28.667%) while control expressed maximum disease incidence 86.467% (Figure 4).

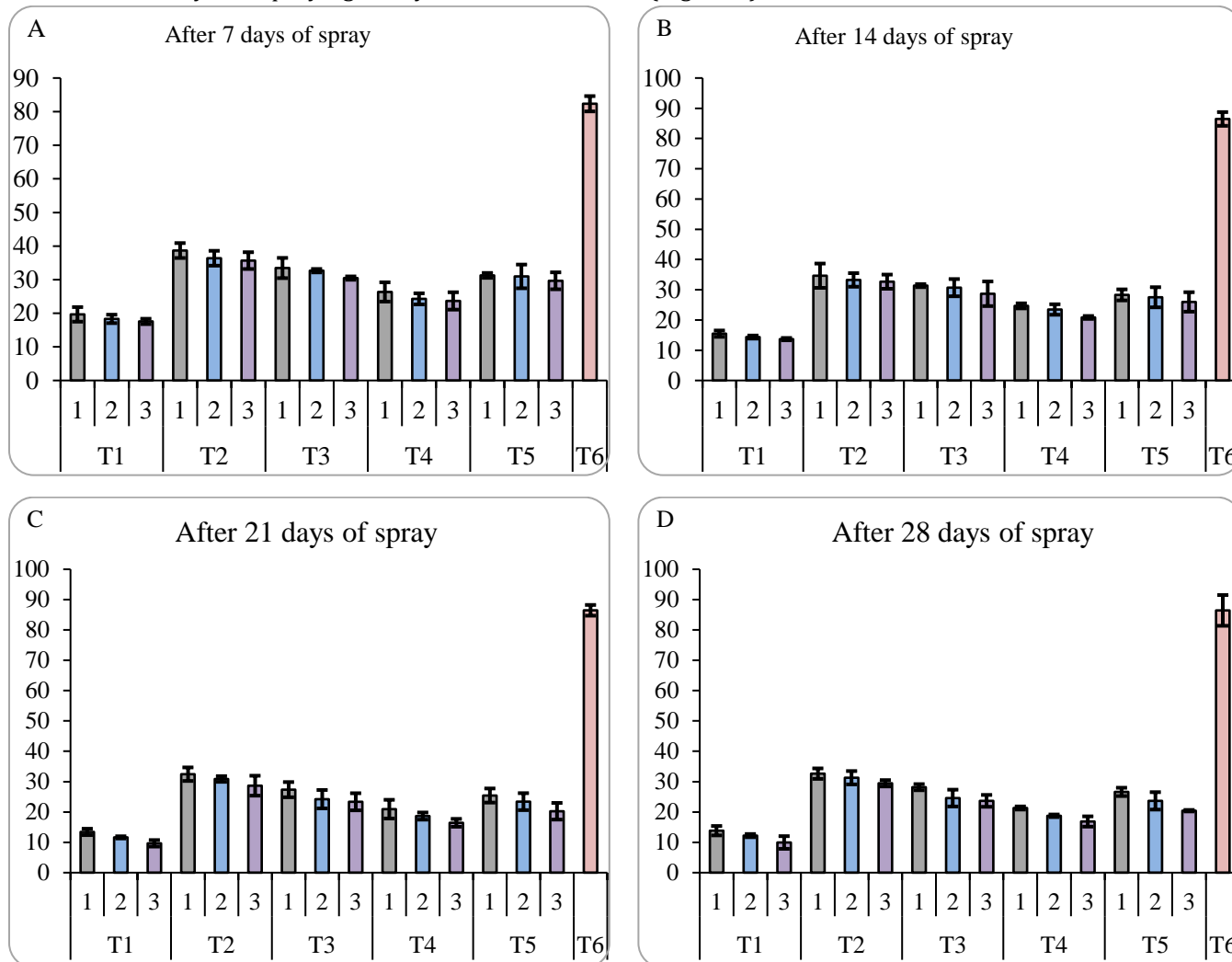


Figure 4. Effect of plant activators on disease incidence (%) of *R. solani*. T1= Salicylic acid, T2= Citric acid, T3= Benzoic acid, T4= KH_2PO_4 , T5= K_2HPO_4 and T6= control with 1, 2 and 3% different doses.

DISCUSSION

To understand the biochemical modifications result to defensive conditions which could permit the development of the transgene with improved disease resistance or else innovative sort of plant defensive chemicals acting as stimuli to plant genetics for disease resistance tools (Ryals *et al.*, 1996). In this study five plant activators salicylic acid, potassium dihydrogen phosphate (KH_2PO_4), di-potassium hydrogen phosphate (K_2HPO_4), benzoic acid and citric acid were evaluated with three different concentrations (1, 2 and 3%). It was

noted that, salicylic acid at all concentrations (1, 2 and 3%) significantly ($P \leq 0.05$) decrease the mycelial growth, root infection, shoot infection and disease incidence as compare to other plant activators but 3% concentration is significantly more effective than other concentrations. Plants treated with salicylic acid showed best growth followed by all other plant activators. Benzoic acid performance is better than citric acid; KH_2PO_4 , K_2HPO_4 while citric acid is better than KH_2PO_4 , K_2HPO_4 and KH_2PO_4 showed better results than K_2HPO_4 . In tobacco and cucumber plants salicylic acid play a vital

role to induce the conventional SAR (Yalpani *et al.*, 1991). Salicylic acid induces resistance through activating signal molecule and helps to trigger plant defense gene expression (Enyedi *et al.*, 1992). Salicylic acid enhances both localized acquired resistance (LAC) and systemic acquired resistance (SAR) in plants (Hammerschmidt *et al.*, 2001). In chickpea salicylic acid induced PR protein that increases resistance against black gram diseases (Ramanathan and Vidhyase, 2002). Salicylic acid reliant on signaling pathway that leads to systemic manifestation of wide range spectrum and long-term disease resistance that is effective against different fungi and other pathogens (Heil and Bostock, 2002).

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