RESEARCH ARTICLE

In vitro fungitoxic effect of some plant growth regulators on spore germination and germ tube emergence of *Alternaria solani*

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ABSTRACT

The inhibitory effect of five plant growth regulators including indol-3-acetic acid; indol-3-butyric acid; naphthalene acetic acid; 2,4-dichlorophenoxy acetic acid; and phenyl acetic acid was evaluated *in vitro* against the leaf blight pathogen, *Alternaria solani*. All test chemical inducers at 10^{-2} to 10^{-4}M conc. significantly reduced spore germination and germ tube growth of the test pathogen. Pathogenic fungus, *Alternaria solani* had sensitive response against 10^{-2} conc. of naphthalene acetic acid, reducing spore germination by 84% and germ tube growth by 14% over untreated control. Also, complete inhibition spore germination of test fungal pathogen was confined when all chemical inducers were evaluated at 10^{-1}M conc. Fungal spore germination and germ tube growth increased significantly as the conc. of chemical inducers was decreased. Among the test growth regulators, indol-3-acetic acid had least inhibitory effect against test pathogen of these parameters undertaken. The results of the present study revealed the possibility of usage plant growth regulators in inducing phytoalexin compound in susceptible plant cultivars.

Key words: Fungitoxicity, susceptible, abiotic elicitors, chemical plant resistance inducers.

INTRODUCTION

The Deuteromycetes ubiquitous fungal genus *Alternaria* comprises diverse saprophytic as well as Endophytic species and is known for its notoriously destructive plant pathogen members (Mamgain *et al*., 2013). Out of the total 299 known species representing genus *Alternaria*, majority of them lack sexuality, although few species have been found to have sexual stage in

their life cycle.The genus is characterized by the formation of polymorphous, multicellular and pigmented conidia either singly or in short or longer chains by asexual method. The conidia are broadest near the base; taper gradually to an elongated bead and provided with cross, longitudinal as well as oblique septa. It has been found to have a drastic effect on the members belonging in the plant families such as Cucurbitaceae, Brassicaceae, Solanaceae, Poaceae etc. which are having nutritional as well as economical food value. It is associated with a wide variety of substrates including seeds, plants, agricultural products, animals, soil and the atmosphere. It is also common allergen in humans, growing indoors and causing hay fever or hypersensitivity reactions that sometimes lead to asthma. Some species readily cause opportunistic infections in immuno-compromised people such as AIDS patients (Mamgain *et al*., 2013). Several taxa are also important postharvest pathogens, causative agents of phaeohyphomycosis in immuno-compromised patients or airborne allergens.

Some saprophytic species representing genus *Alternaria*, are agents of decay and decomposition and growing profusely on dead and decaying debries of plant and animal origin producing a variety of primary and secondary metabolites. Several pathogenic species mostly causing leaf blight infection considered the major problems in agricultural production throughout the world, reducing yield and quality of crops and produce more than 70 phytotoxins of host selective (host specific) and nonspecific types (Trivedi *et al*., 2013). Host-selective toxins (HSTs) are toxic only to host plants while nonspecific toxins can affect variety of non-host plants. The *Alternaria* HSTs involve a diverse group of low-molecular-weight substances such as alterotoxins, alternariol, tenuazonic acid; alternaric acid and were found in culture filtrates as families of closely related compound and were reported to play a crucial role in determining host specificity and contributing to disease development. The *Alternaria* HSTs cause necrosis on leaves of susceptible cultivars at concentrations as low as 10^{-8} to 10^{-9} M and no necrosis on leaves of resistant cultivars even at higher concentrations (Otani *et al.*, 1995) The toxin from secondary metabolites penetrate host tissues, directly act on living host cell protoplasm and damage the metabolically active cells to influence the course of disease development (Mamgain *et al*., 2013).

Majority species of *Alternaria* caused leaf spot and early blight resulting in defoliation, reduction in size and quality of fruits, ultimately adversely affects productivity (Mamgain *et al*., 2013).). The leaf spots may be control by foliar application of effective organic fungicides, but these are reported hazardous and their residual toxicity in plant parts, fruits poses carcinogenic disorders to consumers (Trivedi, *et al*., 2013) and also helps to increase level of air pollutants. To overcome these, the concept of screening for disease resistance has been developed (Eckadt, 2011). Phytoalexin accumulation at infection site in leaves, stem, cotyledons and hypocotyl (Ingham, 1982), in response to wounding (Rahe and Arnold, 1975), to interaction with micro-organism (Iriti & Franco, 2009) or to treatment with certain chemicals (Ismile *et al*., 1987; Bhajbhuje, 2013) makes a significant contribution to resistance, cultivars that are normally susceptible to a virulent race of pathogen thus providing protection in different plants. A little is known about induction of resistance by application of plant growth regulators in plants and control of leaf blight pathogen, it seemed to be worthwhile to report the fungitoxicity of plant growth regulators at variable concentrations against leaf blight pathogen, *Alternaria solani* (Ellis & Martin) Jones & Grout.

MATERIALS AND METHODS

The plant growth regulators of diverse chemical nature induce phytoalexin in plants, when applied in dilute concentration, were screened for fungitoxic effect against leaf blight causing pathogen *Alternaria solani*. The stock solution of

0.1M concentration for five plant growth regulators was prepared separately in volumetric flask and each was diluted to the concentration between 10^{-2} to 10^{-4}M. These solutions of different concentrations of test chemicals were screened for fungitoxic assay employing the slide germination technique (CMI, 2010). *Alternaria solani* was isolated from infested leaves and stored seeds of tomato as internal seed borne pathogen and maintained in laboratory on PDA nutrient medium at 25±1^{0}C. One drop of different conc. of test chemicals was placed on cover slip and added one drop of spore suspension of test pathogen in the drop. The spores were allowed to grow in drop of water serve as control. The coverslip with spores in drop was inversely placed on cavity glass slide in triplicate. Slides of different treatments were randomly distributed into large Petri dishes made into moist chamber and kept these for 24 hrs. in darkness. One drop of lecto-phenol was put on each spot to fix the germinated spores. Germination of spores was counted in terms of percentage on the basis of 300 spores and germ tube growth was measured on the basis of 90 germlings from each spot observed randomly. These concentrations were selected on the basis of their effectiveness in inducing resistance in plants (Bhajbhuje, 2014).

RESULTS AND DISCUSSIOINS

Altogether five plant growth regulators in aqueous dilute solution (10^{-2} to 10^{-4}M) are screened to study *in vitro* fungitoxic effect on spore germination & germ tube growth of pathogen following slide germination method (CMI, 2010). A drop of spore suspension in Czapek's Dox broth was placed 3 cm apart on each of three slides per treatment. The slides were randomly distributed into large Petri plates made into moist chamber and kept at room temperature in darkness. After 24 h of incubation, the percent spore germination was recorded from each spot on the basis of 50 spores and germ tube growth on the basis of 15 germlings.

The results presented in Table 1 revealed that an aqueous solution of all five plant growth regulators at different concentrations caused injury to spore of *Alternaria solani* inhibiting spore germination and germ tube growth. An absolute inhibitory effect was induced with naphthalene acetic acid (NAA) and phenyl acetic acid (PAA), when treated with 10^{-1}M aqueous solution of test chemical inducers. Naphthalene acetic acid (NAA) caused greater inhibitory effect at 10^{-2}M conc. reducing the spore germination by 84% over the untreated control, followed by phenyl acetic acid (PAA) causing reduction in this parameters by 71 %. Moderate inhibitory effect to the extent of 61% and 65% for spore germination was recorded with 2, 4-dichloro-phenoxy acetic acid (2, 4-D) and Indol-3-butyric acid (IBA) at 10^{-2}M conc. respectively. Indol-3-acetic acid (IAA) had least inhibitory effect at this conc. on spore germination (Table 1).

The inhibitory effect for all the plant growth regulators was declined with dilution of aqueous stock solution. The greatest declining of inhibitory effect was recorded with naphthalene acetic acid (NAA), reducing the spore germination by 35% and 6%, when treated with conc. 10^{-3} to 10^{-4}M. Least inhibition of spore germination was confined at 10^{-4}M with Indol-3-acetic acid (IAA) while remaining test chemical inducers indol-3-butyric acid (IBA); 2,4-dichlorophenoxy acetic acid (2,4- D); phenyl acetic acid (PAA) had considerable to moderate inhibitory effect at conc. 10^{-3} M while it was declined to 2-9% when treated with 10^{-3} M conc. over untreated control (Table 1).

The similar trend was confined for germ tube emergence with all concentration of the plant growth regulators tested. The spores of leaf blight pathogen; *Alternaria solani* remained dormant and did not produce germ tube, when treated with 10^{-1}M aqueous solution of all five plant growth regulators excepting indol-3-acetic acid (IAA). All the test chemical inducers excluding indol-3-acetic acid (IAA) at 10^{-2}M concentration had 10 to 18% inhibitory effect on

germ tube growth compared to untreated control. Indol-3-acetic acid (IAA) at 10^{-2}M induced 3% inhibitory effect. Phenyl acetic acid (PAA) and 2,4-dichlorophenoxy acetic acid (2,4-D) caused greater inhibition at 10^{-2}M conc., reducing the mean germ tube growth to the extent of 18 and 17% respectively while remaining test chemical inducers had 10-14% inhibitory effect on the same parameter over untreated control (Table 1). The inhibitory effect was declined with decrease in concentration of all the plant growth regulators tested. The greatest declining of inhibitory effect was recorded with Phenyl acetic acid (PAA) reducing the germ tube growth by 16% and 13% at conc. 10^{-3} to 10^{-4}M respectively. Indol-3-acetic acid (IAA) had little inhibitory effect; Naphthalene acetic acid (NAA) induced 9% inhibition while remaining test chemical inducers

Table 1: Effect of plant growth regulators at dilute concentration on spore germination and germ tube growth of *Alternata solani*

S. No.	Plant growth regulators	Percent spore germination			Mean germ tube growth		
		10^{-2} M	10^{-3} M	10^{-4} M	10^{-2} M	10^{-3} M	10^{-4} M
1.	Indol-3-acetic acid (IAA)	44 (-55.1)	71 (-27.6)	96 (-2.0)	92 (-3.1)	94 (-2.1)	95 (-1.0)
2.	Indol-3-butyric acid (IBA)	34 (-65.3)	64 (-34.7)	92 (-6.1)	86 (-10.4)	89 (-7.3)	91 (-5.2)
3.	Naphthalene acetic acid (NAA)	16 (-83.7)	48 (-51.0)	84 (-14.3)	83 (-13.5)	86 (-10..4)	87 (-9.4)
4.	2,4-dichlorophenoxy acetic acid (2,4-D)	38 (-61.2)	68 (-30.6)	94 (-4.1)	88 (-16.7)	92 (-4.2)	94 (-2.1)
5.	Phenyl acetic acid (PAA)	28 (-71.4)	56 (-42.9)	89 (-9.2)	79 (-17.7)	81 (-15.6)	84 (-12.5)
	Water (Control)	98	98	98	96	96	96

1. Results have been expressed as percentage in terms of control; 2.Average of 300 spores; 3.Average of 90 germlings; 4.Values in parentheses indicate percentage reduction or increase in terms of control

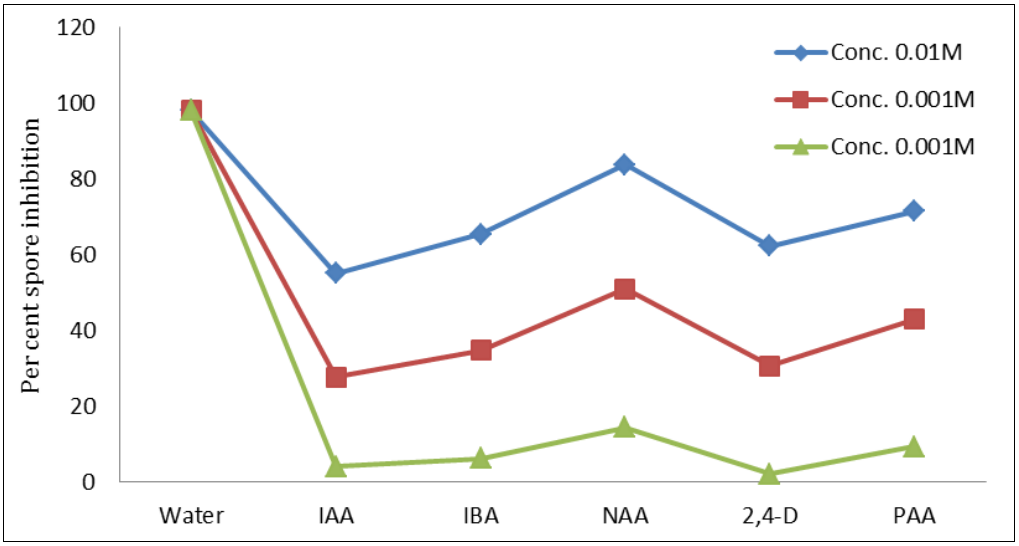

Fig. 1: Fungitoxicity of plant growth regulators on spore germination *Alternata solani*
(IAA= indol -3-acetic acid; IBA=Indol-3-butaric acid; NAA= Maphthalene acetic acid; 2,4-D+ 2,4-dichlorophenoxy acetic acid; PAA-phenyl acetic acid)

Indol-3-butyric acid (IBA); 2,4-dichlorophenoxy acetic acid (2, 4-D); phenyl acetic had mild inhibitory effect at conc. 10^{-4} M compared to untreated control (Table 1). Of the five plant growth regulators screened against *Alternaria solani*, naphthalene acetic acid (NAA) caused greater inhibition of spore germination, but had moderate inhibitory effect on germ tube emergence, while Phenyl acetic acid (PAA) induced considerable inhibitory effect on spore germination but had greater reduction in germ tube growth. Moderate to considerable inhibitory effect was recorded with Indol-3-butyric acid (IBA) and 2,4-dichlorophenoxy acetic acid (2,4-D) while Indole-3-acetic acid had least inhibitory effect on both parameters studied (Fig. 1). It is in agreement with earlier finding of Ashraf and Ali (2007) who reported inhibitory response of chemical inducers on microbial community.

Ezzouhri *et al*., (2009) reported the chemical tolerance level of some filamentous fungal organisms including *Alternaria alternata, Aspergillus niger, Geotrichum candidus, Penicillium sp*., and *Fusarium sp*. Swami and Alane (2013) screened crude extract of various parts of some botanicals containing variable concentration of chemicals against some dominant seed borne fungal pathogens of green gram and reported the inhibitory level of the test crude extracts against these pathogens including *Alternaria alternata, Phytophthora sp., Fusarium oxysporum, Aspergillus niger, Rhizoctonia solani, Curvularia lunata* and *Cladosporium* and reported the inhibitory level of the test crude extracts against these pathogens. The effectiveness of variable concentration of diverse group of chemicals was confirmed on spores of *Alternaria brassicicola* (Meena *et al*., 2011), *Alternaria porae* (Feofilova *et al*., 2012) and *Alternaria alternata* (Bhajbhuje, 2014).

Direct toxicity of heavy metal salts of varying origin to the fungal pathogen does not seem to explain the reduction of symptoms. Chlorides of copper and barium are non-toxic, provided stronger protection than mercuric and cadmium chloride, a highly toxic one. These test chemicals may exert inhibitory influence upon fungal spores germination and impose upon them exogenous dormancy. This is clearly shown by sensitivity of fungal spores to chemicals by several researchers. The inhibition of spore germination may be attributed to variable toxic effect of test chemicals. Similar findings were reported with conidia of *Alternaria tenuis* (Bhajbhuje, 1989); *A. tenuissima* (Singh *et al*., 2000); *A. alternata* (Meena *et al*., 2011; Bhajbhuje, 2014), *A. porae* (Feofilova *et al*., 2012), *A. solani* (Abdel-Kader *et al*., 2012). The hydrolytic products of the chemicals possibly at low conc. induced dormancy or may cause injuries to fungal spores by dissolving the protective thick wall layers and plasma membrane or ruptured them making porous. Aqueous solution of test chemicals diffused through ruptured cell wall and porous plasma membrane to cytoplasm, react with functional cytoplasmic components of spore and seems to disturb a series of physiological processes of spore germination leading to any of the change (i) an inhibitors of trehalose degrading enzymes is destroyed; (ii) the trehalose degrading enzyme is synthesized from its precursor, the conversion being analogous to the trypinogen-trypsin transformation; (iii) the enzyme is thought to be spatially separated from its substrate inside a dormant spores and activation may bring the two together and (iv) a series of interlocking enzyme reactions are shifted from one steady state level (Feofilova *et al*., 2012). In the present investigations, the variable inhibition of fungal spore germination and germ tube growth may be attributed to the differential toxic effect of the test chemicals.

CONCLUSION

The experimental findings reveal that an aqueous solution at 10^{-2}M concentration of plant growth regulators seemed to provide more vigorous defence response to virulent pathogen, *Alternaria solani*. These phytoalexin inducer test chemicals stimulated production of large amount of

fungitoxic substances in susceptible tissue on post-infection of virulent pathogen which make plant resistant to some extent and readily respond to infection. Of the test chemical inducers, naphthalene acetic acid at 10^{-2}M may serve as very promising compounds for use in plant disease control.

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