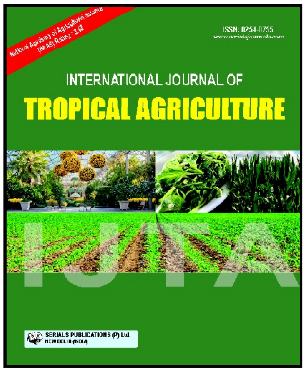

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Induction of Resistance in Chickpea (*Cicer arietinum* L.) Against Wilt Pathogen (*Fusarium oxysporum* f. sp. *Ciceri*) by Applying Non-convectional Chemicals

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Abstract: Non-conventional chemicals, *viz.*, Salicylic Acid (SA), Acetyl Salicylic Acid (ASA), DL-β-amino-*n*-butyric acid (BABA), gamma-amino-*n*-butyric acid (GABA), Amino-*iso*-butyric acid (AIBA), Indole-3–pyruvic acid (IPA), Indole-3–acetic acid (IAA), Nicotinic acid (NA), Isonicotinic Acid (INA), DL-Norvaline, Benzoic Acid and Cycloheximide were applied as foliar sprays to chickpea (Cicer arietinum) and the plants were subsequently challenged against *Fusarium* wilt, the causal agent of root rot in chickpea. All the chemicals reduced mortality of chickpea from *Fusarium oxysporum* f. sp. *ciceri* infection. Among them, Salicylic acid was significantly superior and reducing the wilt incidence followed by DL-β-amino-*n*-butyric acid (BABA) and Nicotinic acid (NA) these chemicals also increased germination of chickpea seed and reduced per cent mortality compared to control. Individual treatment of the chemicals showed better results than their combinations as plant mortality was reduced and accumulation of SA, BABA and NA increased in their individual treatments.

Key words: Chick pea; *Fusarium oxysporum* f. sp. *Ciceri*; Induced resistance; Non-conventional chemicals.

INTRODUCTION

Chickpea (Cicer arietinum) generally known as "Chana"/"Gram" or "Bengal Gram" in India is an important leguminous food grain. Chickpea is a highly nutritious grain legume crop it is widely appreciated as health food. It is a protein-rich supplement to cereal-based diets, especially to the poor in developing countries, where people are vegetarians or cannot afford animal protein. India is the largest producer of chickpea with about 63% of the total area under chickpea production lying in India with about 70% of the world total production, the total area 10.22 million hectare and production 9.88 million tones and productivity 967 kg/ha during the year, 2013-14 (http://eands.dacnet.nic.in). Highest production has been received from Madhya Pradesh by 39%, followed by Maharashtra (14%), Rajasthan (14%), Andhra Pradesh (10%), Uttar Pradesh (7%), Karnataka (6%) and other remaining states and UTs of India (10%) . The chickpea crop widely influenced by various pest, diseases and *Fusarium* wilt is regarded as one of the most important biotic stress which reduced its productivity at national level.

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *ciceri* is wide spread disease that occurs in most chickpea growing areas. It is soil borne pathogen that infects plants through roots at all the stages of the plant growth. Various strategies for controlling this disease have been introduced over the years including soil cultural practices, fungicide treatment etc. but serious losses still occur, largely because the effectiveness of these approaches is variable and often short lived (Jarvis,1988). Recent studies showed that the mechanism underlying the expression of plant defense gene upon microbial infection (Dixon and Lamb, 1990) have led to the conclusion that artificial manipulation of the natural plant defense system could provide a biologically/environmentally safe and commercially valuable alternative to the existing pathogen control methods (Sequeira, 1990; Dong and Cohen, 2002).

Constitutive barriers (physical and chemical) present in plant system prior to injection are collectively responsible for natural resistance in plant. Induced resistance in plants can be activated be preinfection with necrotizing pathogen or pretreatment of plants with either natural or synthetic chemicals (Kessmann *et al*, 1994; Sticher *et al*, 1997; Agrios,1997). Chowdhary (2000) tested the effectiveness of four different non-convectional chemicals of diverse nature at concentration of 10-3 to 10-6M in controlling *Fusarium* wilt of chickpea in pot experiment. Among these chemicals, Indole acetic acid, Cycloheximide and Cycocel showed very strong protective effect at the lower than higher concentration in susceptible cultivar ICC-4951. The speed and extent of the plant response to microbial attack appears to be the key determinants in the outcome of a given interaction, and it is reasonable to assume that a faster response to a pathogen may enhance the resistance in a previously susceptible plant. Enhanced disease resistance in plants was found to occur in response to biotic (pathogens or non pathogens) and a biotic inducing agents such as salicylic acid, K_2HPO_4, oxalic acid, chitosan etc. (Gaffney *et al.*, 1993; Benhamou *et al*, 1994; Bashir *et al.*, 1997). The present paper reports that seed treatment with non-convectional chemicals induces systemic resistance in chickpea seedlings against *Fusarium* wilt.

MATERAL AND METHODS

Isolation Maintenance of Fungus

Highly virulent strain of *Fusarium oxysporum f. sp. ciceri* was isolated from infected chickpea plant by using potato dextrose agar media. The infected parts were cut aseptically into small pieces and surface sterilized with mercuric chloride (0.1%) for one minute followed by two or three washing with sterilized distilled water and then placed aseptically on solidified PDA in Petri plates. Inoculated Petri plates were incubated at 25±1k ºC for 8 days. Growth was further subculture to maintain pure culture.

Studies on Induce Resistance

Chemicals

The following chemical compounds were used as resistance inducer against vascular wilt pathogen and obtained from "Sigma Chemie" Gmbh (Deisenhofer, Germany) through Centre for Biochemical Technology (CBT) Govt. of India.

1. Salicylic Acid derivatives:
   (a) Salicylic Acid (SA)
   (b) Acetyl Salicylic Acid (ASA),
2. Butyric acid derivatives:
   (a) DL-β-amino-*n*-butyric acid (BABA)
   (b) γ-amino-*n*-butyric acid (GABA)
   (c) Amino-*iso*-butyric acid (AIBA)
3. Indole derivatives:
   (a) Indole-3-pyruvic acid (IPA)
   (b) Indole-3-acetic acid (IAA)
4. Nicotinic acid derivatives:
   (a) Nicotinic acid (NA)
   (b) Isonicotinic Acid (INA)
5. Miscellaneous
   (a) DL-Norvaline
   (b) Benzoic Acid,
   (c) Cycloheximide.

Test for Fungitoxicity

All the chemicals were tested for their fungitoxicity by "Poison food technique" (Grover and Moore, 1962). A test tube containing 5 ml distilled water was sterilized in autoclave at 1.5 kg/cm^2 for 15 minutes. Solution was prepared by adding aseptically a required amount quantity of chemicals in 100 ml medium .Quantity of ingredient for 100 ml PDA was dissolved in 95 ml distilled water and autoclaved. Solutions of respective chemicals were thoroughly mixed in melted cooled medium 25 ml/ 250 ml and 20 ml medium was poured in each sterilized Petri plates. Medium without chemical served as control. After solidification mycelia disc of 5 mm diameter from actively growing culture was ascetically transferred to the centre of each Petri plates. Petri plates were incubated at 25 ± 1°C for 72 hours. The radial diameter of the fungus was recorded in each treatment (Bhattacharya and Roy, 1998).

Phyto toxic Test

To investigate the phytotoxicity of the chemical used, rolled towel paper test was followed. Seeds of susceptible variety H-208 were dipped in solution of chemical for 24 hrs. About 50 seeds from each treatment were placed on water saturated papers, separately. These were incubated at 28 ± 2°C for a week. After one week, seeds were observed for percent germination, phytotoxicity if any.

In *vitro* Test

Application of different chemicals tested for their induced resistances were screened in glass house through seed treatment. Different concentrations mentioned below were used after assessing the fungitoxicity against *Fusarium Oxysporum* f. sp. *ciceri.*

(a) Indole-3-pyruvic acid: 2.0 u Mol,

(b) Indole-3-acetic acid: 0.2 m Mol

(c) Others: 2.0n Mol

The solution of chemical compounds were prepared in 10 ml sterilized distilled water by dissolving a requisite quantity of each chemical separately.

Soil Inoculation

Twelve gram of inoculums was mixed to the top of 2.0 kg soil filled in plastic pots and thoroughly mixed by hand and left for 3 days to allow complete establishment of the pathogen in the soil. Ten seeds of susceptible chickpea cultivar H-208 surface disinfested with $HgCl_2$ solution (0.1%) for one minutes was used for sowing after air drying. The seeds were dipped in test tube containing salt solutions for 24 hrs. Then these treated seeds were sown in separate pots containing challenge inoculums. All treatments were replicated thrice, all pots were kept in glasshouse and adequate moisture was maintained. The data were recorded on the number of seed germinated, mortality percentage and disease control percentage.

RESULT AND DISCUSSION

All the chemicals showed non-fungi toxic response against *Fusarium oxysporum* f.sp. *ciceri* when tested in *in vitro* by poison food technique. Effect of various non-chemicals was assessed on germination of chickpea seed for percent increase in germination and percent mortality. Maximum germination (95%) was observed in pots wherein the seeds were treated with salicylic acid followed by NA (90.6%) and BABA (90.3%) (Figure 1). There was non-significant difference between these chemicals. All treatments were significantly superior to check. All the chemicals tried in present investigation reduced the wilt incidence as compared to check. In general Salicylic acid was significantly superior in reducing the wilt incidence (74.7%) followed by DL-β-amino-*n*-butyric acid (BABA) (70.7%) and Nicotinic acid (NA) (70.5%), respectively. Maximum wilt incidence was recorded in isonicotinic acid (INA), which results 47.8% disease control, over check. The germination percentage of chickpea seeds were increases 49.9% through salicylic acid followed by NA (42.3%), whereas in cycloheximide treated seeds are also exhibited 20.6% increased in germination.

Result revealed that exogenous application of salicylic acid significantly reduced the wilt incidence (74.7%) over control followed by Benzoic acid, DL-β-amino-*n*-butyric acid (BABA) and nicotinic acid tried as seed treatment. These chemicals also increased germination of chickpea seed and reduced per cent mortality. The findings of the present investigations corroborated with the earlier reports that the resistance can be induced by non-convectional chemicals against various pathogens of different crops *viz.*, chickpea, tomato, cotton and rice

Table 1
Effect of some non conventional chemicals on chickpea wilt under green house condition

Treatment	*Germination (%)*	*Increase in germination (%)*	*Mortality (%)*	*Disease control (%)*
DL-b-amino-n-butyric acid (BABA)	90.3[a]	42.0	28.3[a]	70.7
Salicyclic acid (SA)	95.4[a]	49.9	24.4	74.7
Acetyl salicyclic acid (ASA)	87.5[ab]	37.5	36.3[b]	62.3
DL-norvalin (NOR)	86.4[ab]	35.8	35.06	63.7
Amino iso butyric acid (AIBA)	77.5[c]	21.7	37.7[bc]	60.9
Indole-3-acetic acid (IAA)	86.0[ab]	35.1	34.5[b]	64.2
Cyclo heximide (Cydo)	70.4[d]	10.6	33.5[b]	65.2
Indole-3-pyruvic acid (IPA)	80.3[cd]	26.3	33.6[b]	65.2
Nicotinic acid (NA)	90.6[a]	42.3	28.5[a]	70.5
g–amino-n-butyric acid (GABA)	85.6[af]	34.6	40.1[c]	58.4
Benzoic acid (BA)	82.5[bcf]	29.6	27.7[a]	71.3
Isonicotinic acid (INA)	72.6[cd]	14.1	50.5	47.7
Check	63.6	–	96.66	–
CD (0.05)	5.46	–	2.93	–
CV	3.95	–	4.48	–

*Values in each vertical column followed by same letter do not differ significantly.

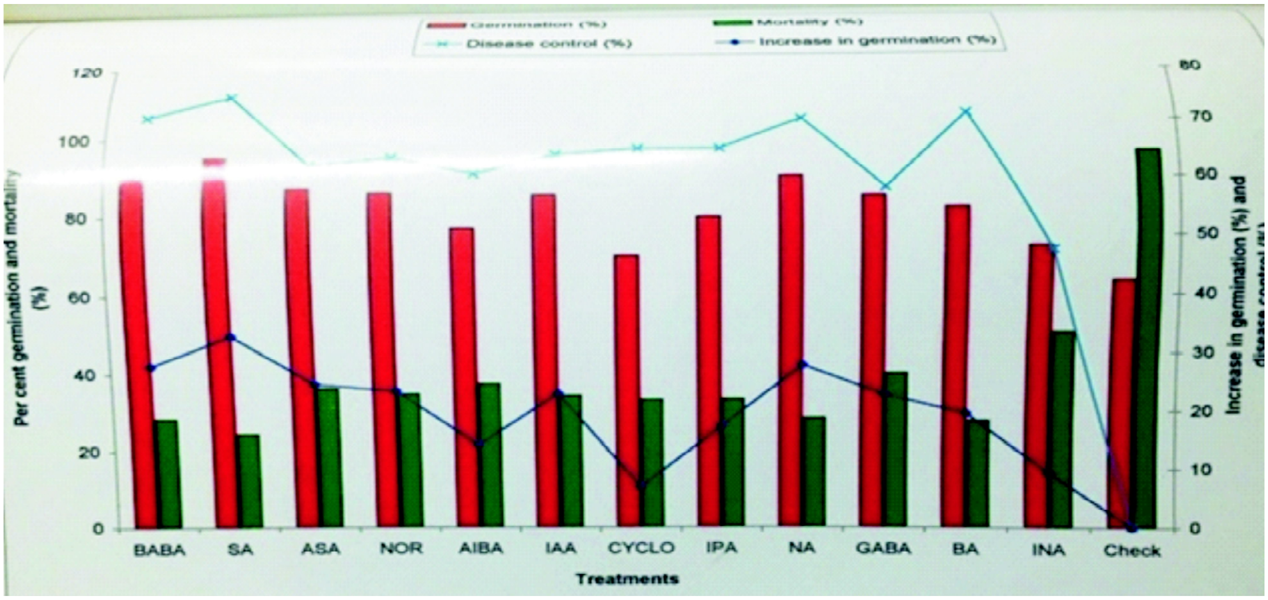

Figure 1: Effect of some non conventional chemicals on chickpea wilt under glasshouse conditions

(Bashir *et al*., 1997; Benhamou *et al*., 1994; Bhattacharya *et al*., 1998; Chowdhury, 2000; Dong and Cohen. 2002; Kessmann *et al*., 1994). Test chemicals act possibly by inducing metabolic changes leading to development of toxic factors, as a result the internal environment unfavorable for pathogen growth and activity induced the resistance and protection against *Fusarium* infection under natural conditions. The curative property of SA and its derivatives has been known since Hippocrates described the presence of salicylates to provide relief during childbirth (Weissman, 1991). Significant knowledge advances have been made in the last 20 yrs about the metabolism and signaling mechanism in plant defense activation when applying SA (Durner *et al*., 1997). The role of SA is well-known at the beginning of defense responses against different abiotic and biotic stress (Catinot *et al*., 2008).

The results presented here show that exogenously applied Salicylic acid provided protection to chickpea plant similar to that of Benlate, which is a standard fungicide. This protection may be due to induction of a set of plant defence reactions that culminate in the creation of a toxic environment adversely affecting the pathogen and causing fungal growth inhibition. These observations are in consistence with the proposed role of Salicylic acid as active signaling molecules and shed more light on the potential of induced resistance as a valuable alternative means of disease control. We concluded that resistance inducing chemicals have the potential to control chickpea wilt when applied at the proper time and dose rate. These chemicals can be integrated with suitable fungicides since they have a synergistic effect with fungicides and persist longer; it would help reduce the number of fungicide applications by enhancing the natural resistance of chickpea cultivars even if they were susceptible to *fusarium* wilt.

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