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Research Article

Role of heavy metal salts on susceptibility of *Solanum melongena* L. seedlings to *Alternaria* early blight disease.

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ABSTRACT

The phytoalexins are low molecular weight, anti-microbial phenol compounds that are both synthesized by, and accumulated in plants at infection site during compatible plant-pathogen interaction upon exposure to heavy metal salts, were used at dilute concentration in different treatments to control Alternaria early leaf blight infection in Solanum melongena L. seedlings caused by Alternaria solani. Foliage sprays, root dips for two hours prior to transplanting or seed soaking for 24 hrs provided considerable to moderate protection to seedlings, at different stages after treatment, against artificial inoculation with the pathogen and mostly caused significant reduction in symptoms. Of the three methods, wet seed treatment appeared to provide the most effective and durable protection. Barium, mercury and Ferric chloride were found the most effective in reducing the infection and lesion expression significantly in most treatment after inoculation with virulent race of pathogen. The fungitoxicity in leaf diffusate was found decline with age in treated artificially inoculated seedling. Little fungi toxicity in leaf diffusates from 3 to 5-week old seedlings that had been treated uninoculated and moderate toxicity in that from untreated, inoculated seedlings. However, treated seedlings inoculated at the 3 and 5-week stage produced leaf diffusates with significantly greater toxicities which were in proportion to their abilities to resist attack.

KEYWORDS

Solanum melongena L., susceptible, heavy metals, leaf diffusate, phytoalexin.

INTRODUCTION:

Solanum melongena L. is a warm-season, non-tuberous, vegetable, of Solanaceae family, native to southern India and widely cultivated in Americas, Europe & Asia for its fleshy fruit as it is a rich source of dietary fiber, vitamins, potassium, and calcium; it also has low fat, zero cholesterol, and very low calorie content hence preferred food among weight-conscious consumers. India ranks second leading producer accounting around 27.55% of world total annual output after china (55.75%) followed by Egypt, Iran, Turkey, Indonesia, Iraq, Japan and others (IHD, 2012). In Ayurveda, the roots are used as anti-asthmatic & stimulant to cure syphilis and skin diseases; juice is employed for otitis, applied to ulcers of nose. Leaves infusion is used as an anodynes and remedy for throat and stomach troubles; given in the treatment of bronchitis, asthma and dysuria, and they stimulate the inter-hepatic metabolism of cholesterol. The burnt fruit as purgative, slightly bilious, beneficial in phlegm, obesity and also excellent remedy for liver complaints. The fruit bruised with vinegar, used as a poultice for abscesses and cracked nipples. The peduncle is used in intestinal hemorrhages, piles, and toothache. The seeds are used as a stimulant but are apt to lead to dyspepsia and constipation (FTRNR, 2010).

The susceptible cv. *Pusa Purple Long* (PPL) of *Solanum melongena* L. is seriously affected with *Alternaria* early leaf blight caused by *Alternaria solani* (E & M) (Jones & Grout) Mill. resulted in premature defoliation, reduction in size & quality of fruits, and reported to reduce the productivity to the extent of 20-30%. Studies have demonstrated a causal link between the post inflectional protection of phytoalexin-type antifungal substance in brinjal leaf tissue and host resistance to *Alternaria solani*. Phytoalexin accumulation at infection site makes a significant contribution to resistance, cultivars that are normally susceptible to a virulent race of pathogen (Bhajbhuje and Thakre, 2013).

The phytoalexins are low molecular weight, anti-microbial phenol compounds, known to produce and accumulate in leaves in response to wounding, to interaction with micro-organism or to treatment with certain chemicals of diverse nature including heavy metal salts and thus providing protection in different plants (Mazid et al., 2011; Bhajbhuje & Thakre, 2013). The susceptible plants after treatment become activated and these produced, when inoculated, much larger amount of fungitoxic substances that inhibited symptoms expression. This has been successfully demonstrated in *Lycopersicon esculentum* Mill (Bhajbhuje and Thakre, 2013). Whittaker and Stommel (2003) reported 3,5-dicaffeolquinic acid, hydroxycinnamic acid conjugation as antibacterial and antifungal compounds from *Solanum melongena* L. his suggests the possibility that heavy metal salts may be useful in phytoalexin accumulation and inducing host resistance to control plant disease. Presently specific phytoalexin type substance has so far not been reported from plant under study in response to infection of virulent strain of *Alternaria solani,* it seems considered that phytoalexin inducer chemicals might be of some use in reducing the disease symptoms expression and control of early leaf blight infection of *Solanum melongena* L. at the seedling stage when the crop often suffers heavy damage. This paper reports the results of studies on control of *Alternaria* early leaf blight disease using phytoalexin inducing heavy metal salts.

MATERIALS AND METHODS

Plants:

The plants of a moderately susceptible cv. '*PPL*' of *Solanum melongena* L. were raised from healthy seeds in 16 cm pots containing sterile soil supplemented with farmyard manure and kept exposed to natural daylight conditions.

Chemicals:

Solutions of six heavy metals salt viz., lithium sulphate and chlorides of mercury, cuprous, ferric, barium and cadmium were used for treatment. Initially fungitoxicity of the solution of test chemicals at dilute concentrations (10^{-3} to 10^{-5} M) as aqueous solution were assayed for their effect on the germination of spores of *Alternaria solani* following the slide germination technique reported earlier (Ainsworth, 1983). On the basis of the effectiveness of chemicals in inducing greatest inhibition of spore germination and germ tube growth, the concentration (10^{-4}M) was selected for further treatment.

Treatment:

The solutions of selected concentration (10^{-4} M) of test chemicals were used to (a) spray seedlings, (b) soak seeds before sowing and (c) provide root-dip treatment. The young seedlings of 2-week old were dipped in the test solutions for 2 h prior to transplanting into pots. About 10 ml of solution was sprayed on leaves of 25 plants growing in pots till dropping. Seeds were soaked for 24 hour in test solution and being sown in sterile soil supplemented with farmyard manure in 16 cm earthen pots. None of the treatment had any adverse effect on seed germination or any phytotoxic effect on *Solanum melongena* L. seedlings. The pots were kept on racks at room temperature of 16-28^{0}C, where sufficient light was available and watered daily.

Inoculation:

The seedlings were inoculated at the age of 3 or 4 weeks by spraying foliage with the conidial suspension (C.5 x 10^5/ ml) of virulent isolate of *Alternaria solani*. Inoculum was obtained from 6 days old cultures grown on PDA medium incubated at 28^{0}C. Control plants were sprayed with water. After inoculation, pots with these plants were kept in a humid chamber having polythene cover for 24 hour to facilitate infection (Ainsworth, 1983).

Disease assessment:

Symptoms on the leaves were assessed a week after inoculation. A disease index was computed taking into consideration of both the number and size of lesion as mean of observations on ten plants, single from each of these pots per treatment as the method described earlier (Bhajbhuje and Thakre, 2013)

Collection of leaf diffusates and bioassay:

Diffusates were obtained from leaves collected 3 days after inoculation, washed in distilled water, dried between blotting papers and cut into 2.5 cm long pieces. Two gm. of leaf pieces were taken in sterile glass tubes with 5 ml of distilled water and incubate at 28^{0}C for 24 hrs. After having been made cell-free, leaf diffusates were assayed for their effect on the germination of spores of *Alternaria solani* following the side germination method (Ainsworth, 1983).

RESULTS AND DISCUSSION:

Six heavy metal salts were screened to study the parameters (i) *In vitro* fungitoxic effect on spore germination & germ tube growth of pathogen; (ii) Effect of foliage spray, root-dip & wet seed treatment on symptoms expression; (iii) Persistence of protective effects and (iv) Fungitoxicity of leaf diffusate of treated healthy & inoculated plants against pathogen. Initially, *in vitro* fungitoxicity of test chemicals was assayed

In-vitro fungitoxicity of test chemicals

Each test chemical was initially assayed at two concentrations (10^{-4} &10^{-5} M) for its fungitoxic effect on spore germination of *Alternaria solani* following slide germination technique. Two drops of spore suspension in different media were placed 3 cm apart on each of three slides per treatment. After 16 hrs. of incubation at room temperature in darkness, 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 data presented in Table I records inhibition of spore germination and germ tube growth with all test chemicals. The greatest inhibitory effect was recorded with mercury, cadmium and cupric chloride, causing 96 to 98% reduction in spore germination. Barium chloride, Ferric chloride and lithium sulphate caused considerable (58 to 81%) inhibition at 10^{-4}M while. moderate inhibitory effect (37 to 47%) was recorded with all test chemicals at 10^{-5}M. Similar trend was recorded for germ tube growth with all concs of the test chemicals. None of these chemicals at other concs and barium chloride at any of its two conc. induced any inhibitory effect on spore germination. These results are confirmed with the earlier findings with *Helminthosporium sativum* from *Capsicum annum* (Bhajbhuje, 1989); *Alternaria solani* f. sp. *lysopersici* from *Lycopersicon esculentum* (Bhajbhuje & Thakre, 2013). The chlorides of mercury, copper & cadmium are highly toxic compared to others tested at very low conc. to fungal spores of *A. solani*.

Direct toxicity of heavy metal salts of varying origin to the fungus does not seem to explain the reduction of symptoms. Non-toxic chemicals such as ferric chloride and barium chloride generally provided stronger protection than mercuric, cadmium and cupric chloride, a highly toxic one. These 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 workers from time to time. The inhibition of spore germination may be attributed to variable toxic effect of test chemicals. Similar results were reported with conidia of *Alternaria solani* (Singh and Khanna, 1969), *A. alternata* (Bhajbhuje, 1989), *A. porae*, (Feofilova et al., 2012), *A. solani* (Bhajbhuje and Thakre, 2013). The hydrolytic products of the chemicals even at low conc. caused injuries to fungal spores and disturb the 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.

Effect of different treatments on symptoms expression:

Foliage spray: Heavy metal salts were used at 10^{-4} conc. to spay pot grown *Solanum melongena* L. seedlings 3 days before their inoculation at the age of 3-week. The concentrations selected for chemicals were mostly those which were expected to induce the production of fungitoxic substance, inhibit spore germination germ tube growth of the pathogen in significant manner. The foliage spray with all the chlorides excepting ferric chloride provided greatest protection to the seedling, reducing the disease symptoms by 73 to 86%.

Moderate inhibition was recorded with ferric chloride and lithium sulphate, causing 40 % & 56% reduction respectively. The number of successful infections was appreciably reduced in most of the treatments, the maximum reduction in lesions number was recorded with mercuric and ferric chloride the least with lithium sulphate.

The considerable inhibition was noticed with barium, cadmium and cupric chlorides, reducing the lesion number varies between 69 to 73%.

Table 1 : Effects of heavy metal salts on spore germination & germ tube growth of *Alternaria solani*[1]

Chemical	Conc. (M)	Germination percentage[2]	Mean germ tube length[3]
Water (Control)		98	96
Mercuric chloride	10^{-4} 10^{-5}	2 (-97.96)[4] 51 (-47.96)	4 (-95.83) 56 (-41.67)
Cupric chloride	10^{-4} 10^{-5}	2 (-97.96) 55 (-43.88)	3 (-96.88) 57 (-40.63)
Barium chloride	10^{-4} 10^{-5}	41 (-58.16) 61 (-37.76)	44 (-54.17) 67 (-30.21)
Ferric chloride	10^{-4} 10^{-5}	18 (-81.63) 52 (-46.94)	23 (-76.04) 58 (-39.58)
Cadmium chloride	10^{-4} 10^{-5}	3 (-96.94) 52 (-46.94)	3 (-96.88) 55 (-42.71)
Lithium sulphate	10^{-4} 10^{-5}	32 (-67.34) 57 (-41.84)	34 (-64.58) 62 (-36.46)

1. Results have been expressed as percentage in terms of control. 2. Average of 3000 spores
3. Average of 90 germlings 4. Values in parentheses indicate percentage reduction or increase in terms of control

Lithium sulphate had 54% inhibitory effect on symptom expression. Significant inhibition of lesion expression was recorded in all treatments, excepting ferric chloride and lithium sulphate which gave plants with some small lesions & fewer large lesions than controls (Table 2).

The symptoms were assayed after 3- & 5-week of inoculated plants.The most pronounced protective effect was achieved at the age of 3-week with mercury, barium and ferric chloride when seedlings were artificially inoculated by virulent isolate of pathogen, while other treatments induced 54–67% inhibitory effect. The induced inhibitory effect was gradually declined in all the treatments at 4- & 5-week growth stage, causing reduction in disease index varies between 46-59% and 23-39% respectively. The Mercury, barium and ferric chloride had greatest inhibitory effect at 5-wk stage in treated inoculated seedlings (Table 2).

Moreover, lesion number was reduced in all the treatments at 3- to 5-wk growth stage. The most pronounced effects being recorded at 3-wk stage with chlorides of ferric and barium, reducing lesion number by 70 & 73% respectively, others treatment had moderate effect, inhibiting the lesion number to the extent of 54 – 68% (Table 2). In all the treatments, the lesion number was found reduced to 56-63% and 22-41% at 4- & 5-week stages respectively. The lesion expansion was inhibited in all cases; stronger inhibition was recorded with the barium and ferric chloride. In such treatments, there were proportionately more small lesions and fewer large lesions compared with those in the controls (Table 2).

Root-dip treatment: Since transplanting of *Solanum melongena* L. seedlings, the potential of treatment with heavy metal salts at their stage was explored. Seedlings were removed from pots when 2-week old, given a root-dip in the solution of test chemicals, at their more effective conc. for 2 hrs. and then transplanted to other large sized pots containing sterile soil supplemented with FYM. Plants were left exposed to natural leaf spot infection.

Since such infection was of a mild nature, observations were continued up to 5 wk (Table 3). Excluding the ferric chloride, the best protective effect was achieved over control with all chlorides used for the root-dip treatment. At the 2-week growth stage, reduction in disease index varied between 40-86% in different treatments. The most pronounced protective effect was recorded with chlorides of mercury, cuprous, cadmium and barium, causing 73 to 86% inhibition in mean Such disease index. The ferric chloride and lithium sulphate had moderate inhibitory effect, reducing the disease index by 40% and 56% respectively. induced protective effects gradually declined with time to lower levels in most of the treatments. Excluding the barium and mercury chloride, the marginal symptom inhibition was noticed in all root-dip treatments at 5-week growth stage, causing 17 to 37% reduction in disease index (Table 3).

The declining trend was significant at 3- & 4-week growth stage in all the root-dip treatments excepting barium chloride, reducing the disease index varies between 33 – 75% and 28 to 67% respectively. At later stages of plant development, 5- to7-week stages, declining in symptoms inhibition was moderate compared to control. Similar induced gradual declining trend was noticed in mean number of lesions in all the root-dip treatments with test chemicals. The inhibitory effect was mostly on the number of infection rather than on lesion expansion. All the treatments had considerable inhibitory effect at 2 –wk in this respect. Such induced inhibitory effects in lesion number gradually declined with further growth of seedlings. Excluding barium & mercuric chloride, only 17 - 34% inhibition in mean lesion number per plant was recorded in all the treatments at the 5-wk stage (Table 3).

Wet seed treatment: All the test chemicals were used at effective (10^{-4}) conc for soaking seeds in their solutions for 24 h. The seedlings were raised in pots from soaked seeds and transplanted, each into single large-sized earthen pot containing sterile soil supplemented with FYM and were left exposed to natural infection. The plants of 4-week old receiving different treatments were inoculated with spore suspension of a virulent pathogen, *Alternaria solani.* The symptoms assayed prior to inoculation were recorded in table 4.

The symptoms recorded at various developmental growth stages from the seedling receiving seed treatment of heavy metals salts prior to inoculation revealed considerable to moderate inhibition in fungal infection on the leaves in most of the treatments (Table 4).

The most pronounced inhibitory effect was noticed at 2-week growth stage with chlorides of barium, mercury cuprous & cadmium, causing reduction in the disease index varies between 74 to 87%. The ferric chloride and lithium sulphate induced moderate inhibitory effect, reducing disease index by 54% and 58% respectively. The inhibitory effects gradually declined to lower levels in all the treatments, induced reduction in disease index to the extent of 27 - 67% at 5-week stage.

Table : 2: Effects of pre- & post-inoculation **spray treatment** with heavy metal salts on symptom expression in pot-grown *Solanum melongena* L. seedlings at different intervals[1]

Chemicals	**Conc. (M)**	**Disease symptoms[2] (Age of the plant)**							
		2-week (Pre-inoculated)		**3-week (Inoculated)**		**4-week (Inoculated)**		**5-week (Inoculated)**	
		Mean no. of Lesions/ plant	Mean disease index/ plant	Mean no. of Lesions/ plant	Mean disease index/ plant	Mean no. of Lesions/ plant	Mean disease index/ plant	Mean no. of Lesions /plant	Mean disease index/ plant
Water (Control)		28	15.4	37	19.4	41	23.6	48	25.2
Mercuric chloride	10^{-4}	06 (-78.6)[3]	2.1 (-86.4)	12 (-67.6)	5.9 (-69.6)	17 (-58.5)	9.6 (-59.3)	31 (-35.4)	16.2 (-35.7)
Cupric chloride	10^{-4}	08 (-71.4)	2.6 (-83.1)	13 (-64.9)	6.5 (-66.5)	19 (-53.7)	10.1 (-57.2)	33 (-31.3)	17.1 (-32.1)
Barium chloride	10^{-4}	07 (-73.1)	2.4 (-84.4)	10 (-72.9)	5.7 (-70.6)	15 (-63.4)	9.8 (-58.5)	32 (-33.3)	16.2 (-35.7)
Ferric chloride	10^{-4}	17 (-39.3)	9.2 (-40.3)	11 (-70.3)	5.8 (-70.1)	16 (-61.0)	9.6 (-59.3)	28 (-41.7)	15.2 (-39.7)
Cadmium chloride	10^{-4}	08 (-69.2)	4.1 (-73.4)	14 (-62.2)	7.1 (-63.4)	20 (-51.2)	11.2 (-52.5)	34 (-29.2)	18.7 (-25.8)
Lithium sulphate	10^{-4}	12 (-53.9)	6.7 (-56.5)	17 (-54.1)	8.9 (-54.1)	22 (-46.3)	12.6 (-46.6)	37 (-22.9)	19.2 (-23.8)
C.D. (P = 0.05)			1.9		1.4		1.7		1.8
C.D. (P = 0.01)			2.8		2.1		2.4		2.7

1. Results have been expressed as percentage in terms of control
2. Disease symptoms recorded 3 days after spraying
3. Values in parentheses indicate percentage reduction or increase in terms of control.

Table : 3 : Effects of root-dip treatment at the time of transplanting with heavy metal salts on symptom expression in pot-grown *Solanum melongena* L seedlings at different intervals[1]

Chemicals	Conc (M)	Disease symptoms[2] (Age of the plant)							
		2 -weeks		3 -weeks		4 -weeks		5 -weeks	
		Mean no. of Lesions /plant	Mean disease index/plant	Mean no. of Lesions/plant	Mean disease index/plant	Mean no. of Lesions /plant	Mean disease index/plant	Mean no. of Lesions/plant	Mean disease index/plant
Water (Control)		28	15.4	37	18.3	39	22.8	46	23.8
Mercuric chloride	10^{-4}	06 (-78.6)[3]	2.1 (-86.4)	09 (-75.7)	4.5 (-75.4)	13 (-66.7)	7.2 (-68.4)	26 (-43.5)	13.2 (-44.5)
Cupric chloride	10^{-4}	08 (-71.4)	2.6 (-83.1)	10 (-72.9)	4.9 (-73.2)	15 (-61.5)	8.4 (-63.2)	30 (-34.8)	14.9 (-37.4)
Barium chloride	10^{-4}	07 (-73.1)	2.4 (-84.4)	07 (-81.1)	3.4 (-81.4)	12 (-69.2)	7.4 (-67.5)	24 (-47.8)	12.9 (-45.8)
Ferric chloride	10^{-4}	17 (-39.3)	9.2 (-40.3)	24 (-35.1)	12.3 (-32.8)	28 (-28.2)	16.1 (-29.4)	36 (-21.7)	18.2 (-23.6)
Cadmium chloride	10^{-4}	08 (-69.2)	4.1 (-73.4)	11 (-70.3)	5.2 (-71.6)	15 (-61.5)	8.9 (-60.9)	29 (-36.9)	14.8 (-37.8)
Lithium sulphate	10^{-4}	12 (-53.9)	7.3 (-52.6)	19 (-48.7)	9.7 (-47.0)	24 (-38.5)	14.3 (-37.3)	38 (-17.4)	19.4 (-18.5)
C.D. (P = 0.05)			2.4		2.1		1.9		2.8
C.D. (P = 0.01)			3.9		4.2		2.8		3.2

1. Results have been expressed as percentage in terms of control
2. Disease symptoms recorded 3 days after spraying
3. Values in parentheses indicate percentage reduction or increase in terms of control

Table :4: Effects of wet seed treatment with heavy metal salts on symptom expression in pot- grown uninoculated *Solanum melongena* L seedlings at different intervals[1].

Chemicals	Conc. (M)	Disease symptoms of uninoculated seedlings[2] (Age of the plant)							
		2 -weeks		3 -weeks		4 -weeks		5 -weeks	
		Mean no. ofLesions/ plant	Mean disease index/plant	Mean no. of Lesions/ plant	Mean disease index/plant	Mean no. of Lesions /plant	Mean disease index/plant	Mean no. of Lesions/ plant	Mean disease index/plant
Water (Control)		28	15.4	34	18.3	38	20.6	44	23.8
Mercuric chloride	10^{-4}	04 (-85.7)[2]	1.9 (-87.7)	07 (-79.4)	3.5 (-80.9)	11 (-71.1)	5.5 (-73.3)	17 (-61.4)	8.6 (-63.9)
Cupric chloride	10^{-4}	06 (-78.6)	2.4 (-84.4)	08 (-76.5)	4.1 (-77.6)	13 (-65.8)	6.5 (-68.4)	18 (-59.1)	9.4 (-60.5)
Barium chloride	10^{-4}	05 (-82.1)	2.2 (-85.7)	08 (-76.5)	3.9 (-78.7)	10 (-73.7)	5.3 (-74.3)	14 (-68.2)	7.8 (-67.2)
Ferric chloride	10^{-4}	15 (-46.4)	8.3 (-53.9)	19 (-44.1)	8.9 (-51.4)	23 (-39.5)	12.6 (-38.8)	31 (-29.6)	17.3 (-27.3)
Cadmium chloride	10^{-4}	08 (-71.4)	3.9 (-74.7)	07 (-79.4)	3.6 (-80.3)	13 (-65.8)	6.8 (-67.0)	19 (-56.8)	9.6 (-58.8)
Lithium sulphate	10^{-4}	12 (-57.1)	6.5 (-57.8)	15 (-55.9)	7.9 (-56.8)	22 (-42.1)	12.1 (-41.2)	28 (-36.4)	15.4 (-35.3)
C.D. (P = 0.05)			2.1		2.4		1.7		2.6
C.D. (P = 0.01)			3.2		4.1		3.8		3.7

1. Results have been expressed as percentage in terms of control 2. Disease symptoms recorded 3 days after spraying 3. Values in parentheses indicate percentage reduction or increase in terms of control

The declining effect at 3- & 4-week growth stages was marginal in all the treatments, excluding the chlorides of mercury & barium, the inhibition in disease symptoms varies between 51–78% and 39-68% respectively (Table 4).

Lesion number was reduced significantly in young seedlings in most of the treatments, the greatest effects being recorded with barium & mercury chloride, reducing the lesion number at 2-wk stage to the extent of 82 and 85% respectively. Such induced inhibitory effects gradually declined to 29 to 59% in 5-wk stage except mercury chloride and barium chloride, while lesion expansion was inhibited in all cases, there were proportionately more small lesions and fewer large lesions. The stronger inhibition was recorded with mercuric chloride, cadmium chloride and barium chloride. Similar declining trend was reported at 3- & 4-week growth stages in this respect (Table 4).

The Seed soaking in chemical solution seemed to be the most effective form for most of the of treatment induced 51-81% inhibitory effect followed by 32-75% inhibition in root-dip while 54-70% reduction was recorded for foliage spray treatment in this respect at 3-week stage. The inhibitory effect gradually declines in most of the treatment with age of plant. At 5-week stage, a greatest inhibition was recorded to the extent of 35 to 67% in the seedling receiving seed treatment, compared to root-dip and foliar spray treatment, reducing disease incidence to the extent of 18-44% and 23-35% respectively. The seed treatment provide substantial protection and long persistent of such effect at significant level. The foliar spray induced instant protection, but persisted for short duration. These results are confirmed with earlier findings in peanut (Sobolev et al., 2007); in tomato (Bhajbhuje and Thakre, 2013).

Persistence of protective effect:

All the test chemicals, when applied through foliar spray, root dip and wet seed treatment, effectively protected 2-wk-old *Solanum melongena* L. seedlings against *Alternaria solani* infection. The most pronounced protective effect was

Table 5: Effect of wet seed treatment with chemicals on symptom expression in pot-grown *Solanum melongena* L seedlings inoculated with *Alternaria solani* after 4 & 5-weeks

Chemicals	Disease symptoms (Age of the plant : 5-week)						Disease symptoms² (Age of the plant : 6-week)					
	Proportion of different size group				Mean no. of lesions /plant	Mean disease index /plant	Proportion of different size group				Mean no. of lesions /plant	Mean disease index /plant
	Very small	Small	Medium	Large			Very small	Small	Medium	Large		
Water (Control)	44.2	26.1	21.8	7.9	44	23.8	44.2	26.1	21.8	8.7	48	25.6
Mercuric chloride	63.4	18.3	16.5	1.8	16 (-63.6)¹	8.4 (-64.7)	58.3	21.7	17.4	2.6	21 (-56.3)	11.4 (-55.5)
Cupric chloride	61.5	24.3	12.3	1.9	17 (-61.4)	9.2 (-61.3)	57.2	22.3	16.7	3.8	23 (-52.1)	12.6 (-50.8)
Barium chloride	69.1	18.5	11.2	1.2	10 (-77.3)	6.1 (-74.4)	65.8	17.7	14.7	1.8	16 (-66.7)	9.2 (-64.1)
Ferric chloride	66.3	22.9	9.4	1.4	12 (-72.7)	7.2 (-69.8)	62.6	18.7	16.4	2.3	19 (-60.4)	10.6 (-58.6)
Cadmium chloride	59.7	23.8	14.8	1.7	18 (-59.1)	9.3 (-60.9)	51.2	25.8	18.8	4.2	23 (-52.1)	13.8 (-46.1)
Lithium sulphate	53.8	26.3	16.3	3.6	21 (-52.2)	11.8 (-50.4)	48.1	29.7	17.3	4.9	31 (-35.4)	15.7 (-38.7)
C.D. (P = 0.05)						1.2						2.5
C.D. (P = 0.01)						3.1						3.9

1. Values in parenthesis indicate percentage reduction or increase in terms of inoculated control
2. Symptoms were assessed a week after each inoculation at the age of 5 and 6- week

recorded in seedlings receiving wet seed treatment compared to foliar spray and root-dip treatment were artificially inoculated at 4-wk growth stage and the symptoms were assayed at the age of 5-& 6-week growth stage.

In most of the treatments, the pronounced protective effect was achieved to a significant level after artificial inoculation the seedlings by virulent isolate of pathogen. At the age of 5-wk, the greatest protective effect was recorded with ferric chloride and barium chloride, reducing the symptoms by 73% & 77% respectively. The considerable, 65% protective effect was noticed with mercury chloride while others inhibited the disease index varies from 59 to 61% in inoculated seedlings. Lithium sulphate had comparatively least inhibitory effect (Table 5). Excluding lithium sulphate and cadmium chloride, the considerable inhibitory effect was persisted in inoculated plants up to 6-week tage, reducing the symptoms varies between 50 to 64%, indicated protective effect declined with age of plant.

Moreover, substantial reduction in lesion number was recorded for all the treatments at 5-wk stage, the most pronounced effects being recorded with barium and ferric chloride followed by mercury chloride, reducing lesion number by 73%,77% and 64% respectively, others treatment had moderate protective effect, reducing the lesion number to the extent of 52 to 61%, while lesion expansion was inhibited in all cases, stronger inhibition was recorded with chlorides of barium, ferrous, mercuric and cadmium. Substantial protective effect was also recorded for 6-wk-old inoculated plants with most of the treatments, causing 52-66% inhibition in this respect. Greatest effect was particularly significant with barium (67%), ferric (60%) and mercury chloride (56%). At this stage, 52% inhibitory effect on lesion was recoded for inoculated plants receiving treatment with cadmium and cupric chloride. The size of the lesions was significant reduced in inoculated plants with pathogen (Table 5). In such treatments, there were proportionately more small lesions and fewer large lesions compared with those in the controls. The number of infections was reduced in most of the treatments and lesion expansion was also inhibited in some, suggesting that the induced resistance may have operated at two stages i.e., initially by limiting the number of successful infection and subsequently by restricting lesion size. This may imply some post inflectional changes in the leaf tissue in treated plants that may limit the *in vitro* activity of the pathogen.

Fungitoxicity of leaf diffusate:

In the final experiment, seedling were raised in pots from soaked seeds and inoculated at the age of 3-week. Leaf diffusates were obtained initially from 2-week-old uninoculated plants and later from both uninoculated and inoculated plants in different treatments 3 days later each inoculation and these were bio-assayed for fungitoxicity. Leaf diffusates collected from uninoculated plants in different treatments favored spore germination with inducing very mild toxic effect. However, diffusates from comparable inoculated plants in all treatment induced significant levels of fungitoxicity, reducing spore germination by 55 to 76% as compared to only 11% reduction in control at 3-week stage. Some relation was evident between each levels of toxicity and the degree of resistance. Most pronounced toxic effect was recorded with barium chloride, while a mild toxicity was found with leaf diffusate from lithium sulphate over to control in this respect (Table 6).

The leaf diffusate from 4-week old inoculated plants receiving seed treatment was found comparatively less fungitoxic, causing 41-71% reduction in spore germination compared to only 5-9% reduction in control indicated fungitoxicity of leaf diffusate gradually declines with age of the plant. Little toxicity to spore germination was reported in leaf diffusate from 5-week old uninoculated plants receiving seed treatment with mercury and ferric chloride. Remaining plants receiving treatment induced 4-6% fungitoxicity in this respect (Table 6).

Leaf diffusates from uninoculated plants in different treatments supported germ tube growth almost to the same extent inducing very little toxic effect. However, diffusates from comparable inoculated plants induced significant levels of fungitoxicity, reducing germ tube growth by 55 to 78%, compared to only 10% reduction in control at 3-week stage. A greatest toxicity was reported at this stage with barium chloride, considerable with mercuric & ferric chloride while other treatments had moderate toxic effect on germ tube growth. This fungitoxicity in plants raised from treated seeds declined with further growth of the plants.

Table 6 : Fungitoxic effect of leaf diffusate of wet seed treated *Solanum melongena* L. plants (both uninoculated and inoculated) on spore germination and germ tube g growth.

Chemicals	**Germination of spores(%) and germ tube growth (µm) in leaf diffusates[3] Age of the plant (days)**													
	2-weeks		**3-weeks**				**4-weeks**				**5-weeks**			
	Healthy (Uninoculated)		**Healthy (Uninoculated)**		**Inoculated**		**Healthy (Uninoculated)**		**Inoculated**		**Healthy (Uninoculated)**		**Inoculated**	
	Germination[4] (%)	Mean germ tube length[5]	Germination (%)	Mean germ tube length	Germination (%)	Mean germ tube length	Germination (%)	Mean germ tube length	Germination (%)	Mean germ tube length	Germination (%)	Mean germ tube length	Germination (%)	Mean germ tube length
Water (Control)	98	159	98	192	87 (-11.2)	172 (-10.4)	98	218	89 (-9.2)	197 (-9.6)	98	226	92 (-6.1)	213 (-5.8)
Mercuric chloride	32 (-67.4)[1]	56 (-64.8)	38 (-61.2)	72 (-62.5)	28 (-71.4)	53 (-72.4)	92 (-6.1)	203 (-6.8)	37 (-62.2)	74 (-66.1)	95 (-3.1)	220 (-2.7)	42 (-57.1)	94 (-58.4)
Cupric chloride	39 (-60.2)	61 (-61.6)	46 (-53.1)	91 (-52.6)	35 (-64.3)	71 (-63.0)	90 (-8.2)	209 (-4.1)	41 (-58.1)	86 (-60.6)	92 (-6.1)	211 (-6.6)	47 (-52.0)	106 (-53.1)
Barium chloride	36 (-63.3)	58 (-63.5)	40 (-59.2)	79 (-58.1)	23 (-76.5)	42 (-78.1)	93 (-5.1)	207 (-5.1)	28 (-71.4)	61 (-72.0)	93 (-5.1)	214 (-5.3)	32 (-67.3)	76 (-66.4)
Ferric chloride	56 (-42.9)	89 (-44.0)	63 (-35.7)	121 (-36.9)	26 (-73.5)	52 (-72.9)	91 (-7.1)	201 (-7.8)	30 (-69.4)	68 (-68.8)	95 (-3.1)	218 (-3.5)	36 (-63.3)	84 (-64.2)
Cadmium chloride	42 (-57.1)	65 (-59.1)	48 (-51.0)	94 (-51.0)	38 (-61.2)	73 (-62.0)	90 (-8.2)	199 (-8.8)	51 (-48.0)	114 (-47.7)	94 (-4.1)	216 (-4.4)	56 (-42.9)	128 (-43.4)
Lithium sulphate	47 (-52.0)	72 (-54.7)	54 (-44.9)	103 (-46.4)	43 (-56.1)	86 (-55.2)	89 (-9.2)	197 (-9.6)	58 (-40.8)	132 (-39.5)	92 (-6.1)	211 (-6.6)	64 (-34.7)	146 (-35.4)
C.D. (P = 0.05)		10.8		13.2		14.3		11.2		16.9		14.6		12.2
C.D. (P = 0.01)		13.2		19.6		16.8		14.3		18.9		18.4		17.7

1. Values in parenthesis indicate percentage reduction or increase in terms of uninoculated control, **2.** All the chemicals were tested at 10^{-4}M conc
3. Following inoculation, leaf diffusates were collected from both uninoculated (healthy) and inoculated plants after 3 days,
4. Average of 300 spores , **5.** Avg. of 90 germlings.

A little toxicity (4-7%) was noted with leaf diffusates from 4-week old uninoculated plants in treatments with barium, mercury & cupric chloride while mild toxicity, 8-10% was recorded for others compared to 41-71% reduction in germ tube growth in inoculated treated plants. A little to mild toxicity was reported from leaf diffusate of 5-week old uninoculated plants. A leaf diffusate from inoculated plants receiving treatments induced greatest fungitoxicity with barium and ferric chloride, causing 35-58% inhibition in germ tube growth while others had moderate effect.

Inoculation with compatible race of pathogen itself led to the development of considerable fungitoxicity in plants grown from untreated seeds. This seems to have resulted from secretion of antifungal compounds in the leaves themselves by plant during successive growth period. Sharma et al., (2011) reported mild antifungal property of leaves of *Solanum melongena* L. Reduced fungitoxicity effect of leaf diffusate on both spore germination and germ tube growth was recorded with all plants receiving treatment, when inoculated 3 days after 3-wk stage with spore suspension of *Alternaria solani*. The leaf diffusate from 3-week old inoculated plant with treatment of barium chlorides was reported comparatively more effective, causing 78 % reduction in spore germination followed by ferric chloride (73%) and mercury chloride (72%), while 56-64% inhibition was recorded with other treatments. The leaf diffusate from plants receiving these treatments were highly toxic to pathogen, reducing 55 – 78% the germ tube growth. A leaf diffusate of 4-week old inoculated plants receiving the seed treatment with all chlorides except cadmium chloride and lithium sulphate was reported considerably toxic to pathogen, inhibiting 58–71% spore germination as well as 60-72% germ tube growth while other treatments induced moderate toxicity for these parameters. A fungitoxicity was gradually decline with further growth of plants receiving seed treatment. A considerable to moderate fungitoxicity was reported for spore germination and germ tube growth from leaf diffusate of 5-wk old uninoculated plants (Table 6).

Markedly to greater levels of fungitoxicity was recorded in the leaf diffusates collected from 3-week old treated uninoculated seedlings in different treatment while mild to moderate toxicity was observed from 4-week old seedlings. The leaf diffusate from 5-wk-old plants had little toxicity with barium chloride, cupric chloride and lithium sulphate while negligible toxicity was recorded with others treatments. The fungitoxicity rapidly declined with plants age to disappear between 6-7-weeks in most of the treatments. It is possible that the substance was metabolized also diluted by plant growth so as to leave little toxic effect at the 5-week stage. The untreated (control) plants themselves developed considerable, 6-11% fungitoxicity when inoculated after 3-or 5-week, those in most of the seed treatments developed more toxicity under similar condition. The greater post-infection development of fungitoxicity in the seed treated plants, even 5-week, after seed treatment, appears to be more significant than the initial development of toxicity in them. This seems to have resulted from the interaction between seed treatment and infection, mediated possibly through some alteration in host metabolism. The results are confirmed as reported earlier by Hargreaves (1979) in *Phaseolus vulgaris* and *Pisum sativum*. Bhajbhuje and Thakre (2013) reported similar finding while studying the effect of phytoalexin inducer chemicals of diverse groups stimulated phytoalexin accumulation in cv. *Pusa Rubi* of *Lycospersicon esculentum* Mill.

The Seedlings were inoculated after 3 and 5-week and developed 22-50% and 14- 37% less symptoms as compared to that in the control treatment (Table 6). Maximum effect was recorded with barium and ferric chloride and least with lithium sulphate. Diffusates from treated, uninoculated plants induced no or negligible fungitoxic effect at either stage of sampling, but those from comparable plants inoculated after 3 or 5-week induced mild to fairly high levels of toxicity causing respectively 27 to 47% and 17 to 33% reductions in germ tube growth as against only mild toxicity in the diffusates from untreated, inoculated plants causing respectively 11 and 6% reductions. The fungitoxicity in leaf diffusates from inoculated plant declines between the first and second sampling for all treatments.

The results obtained in the present investigation reveals that *in vitro,* chlorides of mercury, copper and cadmium inhibits 96-98% spore germination and germ tube growth but allow mild lesion formation in 6-wk old plants. At this stage of growth, moderate toxicity was evident in leaf diffusate of plants from any treatment. This may suggests that some quantity fungitoxic substance was produced in *Solanum melongena* L. plants in response to seed treatment (Sharma et al., 2011). Little or no symptoms are produced where the production of fungitoxic substances soon attains a level inhibitory to the invading organism. Greater inhibition of germ tube growth in some treatments in relation to spore germination seems to be of particular significance when such toxicity is viewed in the background of disease resistance as a post-interaction phenomenon. Seed treatment interfered with the infection process itself, since the barium and ferric chloride caused significant reductions in the number of lesions, and as well as mean lesion size implying appreciable inhibition of lesion number was less pronounced but that on lesion size appeared to be greater for most of the treatments where plants showed proportionately more small lesions and fewer large lesions as compared with the untreated plants.

The symptom expression was significantly inhibited at 2-3 week and moderately at 5-7 week stages in most of the seed treatments. The protective effect of seed treatment was persisted in 6-week old plants but it was declined by 10-18% over 5-week old plants with all the treatment. This may be quite possible that production of phytoalexin-like substances was initially induced and detected in younger seedlings insufficient quantity of accumulation of phytoalexins-like substances by little aged seedlings (Dahiya and Rimmer, 1989) or applied chemicals may have been metabolized in the host tissues and their concentrate probably were diluted by seedling growth so as to leave little toxic effect at 5-7 week stage (Bhajbhuje and Thakre, 2013). It seems unlikely; therefore, that mercury, barium and ferric chloride may have provided any substantial protection to *Solanum melongena* L. seedlings through their direct toxic action on the pathogen; it was in all probability an induced effect. Enhanced production of fungitoxic substance in the treated plants in different treatments when inoculated may induce moderate to high level of fungitoxicity as compared only mild toxicity developed in untreated plants and relationship between such post infection levels of toxicity in treated plants and levels of resistance induced in the such stimulated high post-infection productions of fungitoxic substances in susceptible tissue may be due to a conditioning effect of treatment chemicals which make susceptible plant respond to infection by producing large amount of fungitoxic substances (Mert-Turk, 2002; Eckadt, 2011; Bhajbhuje and Thakre, 2013). As the conditioning effect wakens with time, the production of extra fungitoxic substances diminishes and the induced protective effect also declines.

Heavy metals salts, a known phytoalexin inducer chemicals, were used at dilute concentration (10^{-4}) for foliage spray, root dip or wet seed treatment, most of the six heavy salts tested appeared to provide moderate to substantial protection to *Solanum melongena* L. seedlings against *Alternaria* blight leaf symptoms, resulting from both artificial inoculation and natural infection with *Alternaria solani*. This indicated successful induction of resistance in susceptible plants by treatment with such chemicals which often persisted at appreciable levels even up to 5-7 week growth stage.

Seed soaking in chemical solution seemed to be the most effective form of treatment, provided substantial and long persistence protection to significant level. Although apparent enhanced post-infection production of fungitoxic substance(s) was strong in *Solanum melongena* L. Since some *antibacterial, antifungal, steroidal, glycosides*, *hydroxyl-cinnamic acid* conjugation and *solanine* are reported from other cultivars but no efficient phytoalexin has so far been reported from cv. *PPL* of Solanum melongena L. against infection of a virulent strain of leaf spot causing pathogen, *Alternaria solani*, any suggestion about the possible involvement of such compounds in the development of resistance in them is conjectural. Nevertheless, an involvement of some kind of fungitoxic substance in this process is strongly indicated. A mild antifungal properties of plant crude extract was reported from *Solanum melongena* L, (Sharma, 2011).

Since there is no correlation between *in-vitro* fungitoxicity of the phytoalexin inducer chemicals and the protection provided by them to seedlings, these chemicals may act as *sensitizers*, normally remain suppressed in compatible host pathogen interactions and activate the dynamic defense potential of the host and may induced series of changes in the process of metabolism of the susceptible host; appear to modify the host-parasite interactions in such a way as to result in inhibition of symptom development and an expression of host resistance.

CONCLUSION

The heavy metal salts, a known phytoalexin inducer chemicals, at dilute concs applied by foliage sprays, root dips and wet seed treatment, caused significant reduction in disease symptoms and provided considerable to moderate protection to *Solanum melongena* L. seedlings, at different stages after treatment, against artificial inoculation with the *Alternaria* early blight causing pathogen. Seed soaking seemed to be the most effective, provided long persistent substantial protection at significant level. The ability seed treatment with dilute solutions of barium, mercuric and ferric chloride, test to condition the susceptible host tissues towards a more vigorous defiance response to the pathogen, Alternaria solani, and serve as very promising compounds for use in plant disease control.

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