RESEARCH ARTICLE

Response of heavy metal salts against *Alternaria* leaf spot infection on *Vigna mungo* (L.) Hepper seedlings by three techniques.

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ABSTRACT

The phytoalexins are both synthesized and accumulated in plants at infection site during compatible plant-pathogen interaction upon exposure to micro-organism and chemicals of diverse groups. The salts of heavy metals, well-known phytoalexin inducers, were used at 10^{-4} M concentration in different treatments to control *Alternaria* leaf spot infection in black gram seedlings caused by *Alternaria alternata* (Fr.) Keissler. Application of foliage spray, root-dip for two hours prior to transplanting and seed soaking for 24 h induced moderate to significant reduction in disease symptoms at different stages of growth after treatment against artificial inoculation of spore suspension of *Alternaria alternata*, an isolate of virulent pathogen. Seed soaking in aqueous dilute solutions of test chemicals comparatively seemed to provide the most effective and durable protection. The chlorides of barium, copper and mercury were proved most effective in reducing the infection and lesion expression significantly in most treatments after inoculation with virulent race of pathogen. Fungitoxicity in leaf diffusate was recorded decline with age in treated artificially inoculated seedlings. Little fungitoxicity appeared in leaf diffusates from 3- 4week old seedlings that had been treated uninoculated and moderate toxicity in that from untreated, inoculated seedlings. The treated seedlings inoculated at 3-and 4-week stage produced leaf diffusates with significantly greater toxicities which were in proportion to their abilities to resist attack.

Keywords: *Vigna mungo*(L.) Hepper, *heavy metal salts, leaf diffusates, phytoalexin, Fungitoxicity, pathogen, Alternaria alternata (Fr.)* Keissler.

INTRODUCTION

Black gram [*Vigna mungo* (L) Hepper], a highly prized legume pulse of Central Asia and Indian origin in recorded history, belongs to family Fabaceae, is extensively cultivated as staple food crop in the southern parts of Asia, Africa and Madagascar for its energizer nutritious beans as the seeds are excellent source of easily digestible plant protein (26%)

carbohydrate (48%); minerals (3.2%); vitamin-A, B_1, B_2, Niacin and low content of fat (1.4%); zero cholesterol and glycosides with calorific value 314 calories per 100 gram seeds. It is a store house of dietary fibers; calcium; potassium; iron; magnesium and copper (Savithsuri, 2013). Black gram contributes for over 40% of total world's legume seed production. India is leading producer and consumer of black gram seeds on the globe, contributing about 1.5 million tons of global annual output followed by major exporters, Myanmar and Thailand (Sharma *et al*, 2011). Lion's share of India's production, accounting for over 60% of the nation's total output is contributed by South India (Wikipedia, 2014).

In southern parts of Asia, seeds, sprouts and green pods of black gram are consumed as staple food for their high digestibility, low fat, zero cholesterol and lack of flatulence induction as well as employed as herbal medicine whereas it is used as fodder in *USA* and Australia (Wikipedia, 2014). The beans are boiled and eaten whole or after splitting make into *urid dal* which has an unpleasant mucilaginous texture hence it is often ground into flour or paste and extensively used in South Indian culinary preparations like *dosas, idlis*, *vada* that serve excellent for light breakfast. It is an important gradient of *dal makhani* in Punjabi cuisine and in tempering for South Indian dishes (Savithsuri, 2013). Regular consumption of germinated seed serves as energizer providing sufficient energy to body; reduces cholesterol; replenishes the iron content; enhances sperm count, motility; milk secretion in lactating mothers and improves cardiovascular health. The insoluble fiber content in seeds is proved to prevent constipation; soluble fiber enhances digestion; magnesium & foliate content aids in blood circulation and prevention of damages to the walls of arteries whereas the plenty of potassium in seeds helps to reduce hypertension and erectile dysfunction (Savithsuri, 2013).

In herbal medicine, black gram is proved to be effective liver stimulant, demulcent, cooling, dysmenorrhea, primary amenorrhea, aphrodisiac and nervine tonic. A hot poultice preparation from seeds and massaging with its herbal oil is reported effective in reducing inflammation of joints and muscle pain. Herbal preparation from seeds is also recommended in treatment of rheumatism, sexual problems including impotency, premature ejaculation and thickness of semen; nervous disorders such weakness of memory, nervous debility, partial & facial paralysis, schizophrenia and hysteria. Application of paste of cooked seeds for washing hairs cures dandruff and promotes hair growth. Seed decoction serve as promoter of digestive system, being useful for patient suffering from dyspepsia, gastric catarrh, dysentery and diarrhea while its external application is proved effective remedy in contracted knee and stiff shoulder. It is reported to have moistness increasing property coupled with bulk increasing quality aids in easy movement of bowel hence recommended in conditions like constipation, piles and colic. Apart from medicinal properties, black gram seeds help in increasing body bulk, energy level, strengthens the body and increases lifespan (Savithsuri, 2013).

A necrotrophic plant pathogen, *Alternaria* species remains as an increasing threat to legume crops around the globe including India, causing leaf spot and other diseases on over 380 plant species (Laemmlen, 2001). The susceptible cultivars of *Vigna mungo* (L) Hepper are affected with several seed borne diseases and some are seed transmitted (Rathod, 2012). Among them, *Alternaria* leaf spot, is a serious, incited by *Alternaria alternata* (Fr.) Keissler causing collor rot, stem lesions, damping off of seedlings, producing brown to black leaf spots, premature defoliation lead to a reduction of leaf count, adversely affect anabolism and reported reduction in annual productivity to the extent of 20-30% (Trivedi *et al*, 2013). The pathogen has been reported to attack cauliflower, cabbage, chili, papaya, wheat, rice sorghum etc. causing leaf spot, rots and blight diseases (Rathod, 2012). The pathogen remain in association with infected seeds with spores on the seed coat or mycelium under the seed coat, grows profusely in favourable environment and causes physiological damage to the seeds during refrigerated transport and storage (Meena *et al*., 2011). It can also cause upper respiratory tract infection and asthma in people with sensitivity (Wikipedia, 2014).

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 micro-organism (Atkinson and Urwin, 2012). Moreover, chemicals of diverse nature can also induced phytoalexin accumulation and thus providing protection in different plants (Eckadt, 2011; Zhuang *et al*., 2012; Bhajbhuje, 2013). This suggests the possibility that such chemicals of diverse nature may be useful in phytoalexin accumulation and inducing host resistance to cure plant disease. This has been successfully demonstrated in rice (Wilderman *et al*., 2004); peanut (Sobolev *et al*., 2007); *Cicer arietinum* (Raju *et al*., 2008); lima bean (Ballhom *et al*., 2009); strawberry (Amil-Ruiz *et al*., 2011); *Solanum melongena* L (Bhajbhuje, 2013) using diverse group of chemicals. The treatment by various methods involving foliage spray, root dip, and wet seed treatment with phytoalexin inducer chemicals induced most effective protection to plants against pathogens. Such induced protective effect had good correlation with the enhanced post-infection production of phytoalexin-like substance in the treated plants. After treatment, the susceptible plants become activated and these produced, when inoculated, much larger amount of fungitoxic substances that inhibited symptoms expression (Bhajbhuje, 2013; Horger *et al*., 2013). Battu *et al*., (2011) reported some antifungals, glycolalkaloid, phenol, ononitol, omethyl-scyllo-inositol in leaves of black gram. Bhajbhuje (2013) studied the role of heavy metals salts against *Alternaria* blight to *Solanum melongena* seedlings. Presently specific phytoalexin type substance has so far not been reported from black gram plants in response to infection of virulent strain of *Alternaria alternata*; hence it seemed to be worthwhile to study this phenomenon using salts of heavy metals with *Vigna mungo* (L.) Hepper -*Alternaria alternata* (Fr.)Keissler model following foliage spray, root dip and seed treatment techniques.

MATERIALS AND METHODS

Plant material: The seedlings were raised from healthy seeds of black gram [*Vigna mungo* (L.) Hepper] in 16 cm pots containing sand mixed sterile soil supplemented with farmyard manure and kept exposed to natural daylight.

Chemicals: Initially fungitoxicity of these aqueous solutions of the phytoalexin inducer salts of heavy metal of diverse nature including chlorides of mercury, copper, ferrous, barium, cadmium and lithium sulphate in dilute concentrations (10^{-2} to 10^{-5}) M were assayed for their toxic response on spore germination of *Alternaria alternata* following slide germination technique (CMI, 2010). The concentration (10^{-4} M) seemed to be most effective in inhibition of spore germination and germ tube growth, was selected for further treatment. This conc. effectively induced resistance in plants (Bhajbhuje, 2013; Horger *et al*., 2013).

Treatment: The aqueous solutions of selected conc. (10^{-4} M) of test chemicals were used to (a) spray seedlings, (b) soak seeds before sowing and (c) provide root-dip treatment. Prior to transplanting, the young seedlings of 2-wk old were dipped in test solutions for 2 hours. About 10 ml of solution was sprayed on foliage 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 FYM in 16 cm earthen pots. These pots were maintained in laboratory at 16-28^{0}C, where sufficient light was available and watered daily.

Inoculation: The seedlings in different treatments raised from treated or untreated seeds were inoculated at the age of 3 or 4-weeks by spraying foliage with the conidial suspension of 6 days old culture of *Alternaria alternata* in water till run-off. Control plants were sprayed with water. The pots with these plants were kept for 24 hours in a humid chamber with polythene cover to facilitate infection.

Disease assessment: Symptoms on the leaves were assayed a week after inoculation, and disease index was computed taking into consideration both number and size of lesions.

Collection of leaf diffusates and bioassay: Diffusates from 2 to 3 young leaves were obtained from both uninoculated and inoculated seedlings in different treatments at various growth stages, both before and after inoculation. After having been made cell-free, leaf diffusates were assayed for their effect on the germination of spores of *Alternaria alternata* following side germination method (CMI, 2010).

RESULTS AND DISCUSSION

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

In-vitro fungitoxicity of test chemicals:

Each test chemical was initially assayed for two concentrations (10^{-4} &10^{-5} M) for its fungitoxic effect on spore germination of *Alternaria alternata* following slide germination technique reported earlier (CMI, 2010) and the results on inhibition of spore germination and germ tube growth with all test chemicals are presented in Table 1. An aqueous solution of chlorides of mercury and copper and cadmium caused injury to spore of *Alternaria alternata* at low concentration (10^{-4}M) and induced total inhibition of spore germination and germ tube growth. Lithium sulphate, chlorides of ferrous and barium caused considerable inhibition at 10^{-4}M, reducing spore germination varied between 55 to 78% over control while moderate inhibitory effect to the extent of 40 to 51% was recorded with all test chemicals at 10^{-5}M concentration. Similar trend was recorded for germ tube growth with all concentrations of the test chemicals.

Table 1: Effects of aqueous dilute solution of heavy metal salts on spore germination & germ tube growth of *Alternaria alternata* (Fr.)Keissler[1]

Chemicals	Conc. (M)	Germination percentage[2]	Mean germ tube length[3]
Water (Control)		96	94
Mercuric chloride	10^{-4}	0 (-100.0)[4]	0 (-100.0)
	10^{-5}	48 (-51.0)	49 (-47.9)
Cupric chloride	10^{-4}	0 (-100.0)	0 (-100.0)
	10^{-5}	53 (-44.8)	58 (-38.3)
Barium chloride	10^{-4}	43 (-55.2)	45 (-52.1)
	10^{-5}	58 (-39.6)	64 (-31.9)
Ferric chloride	10^{-4}	21 (-78.1)	25 (-73.4)
	10^{-5}	51 (-46.9)	57 (-39.4)
Cadmium chloride	10^{-4}	0 (-100.0)	0 (-100.0)
	10^{-5}	49 (-48.9)	55 (-41.5)
Lithium sulphate	10^{-4}	35 (-63.5)	35 (-62.8)
	10^{-5}	56 (-41.7)	61 (-35.1)

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

Table 2: Effects of pre- & post-inoculation foliage *spray treatment* with salts of heavy metal on symptoms expression in black gram [*Vigna mungo* (L.)Hepper] seedlings at different intervals[1].

Chemicals	Conc (M)	Disease symptoms[2] (Age of the plant)					
		2-week (Pre-inoculated)[2]		3-week (Post-inoculated)		4-week (Post-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
Water (Control)		32	19.3	38	23.8	44	26.3
Mercuric chloride	10^{-4}	07 (-78.1)[3]	3.2 (-83.4)	08 (-78.9)	5.1 (-78.6)	13 (-70.5)	6.8 (-74.1)
Cupric chloride	10^{-4}	10 (-68.8)	3.9. (-79.8)	11 (-71.1)	7.2 (-69.7)	15 (-65.9)	8.1 (-69.2)
Barium chloride	10^{-4}	12 (-62.5)	4.6 (-76.2)	09 (-76.3)	5.8 (-75.6)	12 (-72.7)	7.1 (-73.0)
Ferric chloride	10^{-4}	20 (-37.5)	11.8 (-38.9)	21 (-44.7)	8.4 (-56.3)	26 (-40.9)	12.6 (-43.7)
Cadmium chloride	10^{-4}	09 (-71.9)	4.9 (-74.6)	10 (-73.7)	6.9 (-71.0)	16 (-63.6)	9.1 (-65.4)
Lithium sulphate	10^{-4}	15 (-53.1)	8.1 (-58.0)	16 (-57.9)	10.2 (-57.1)	29 (-34.1)	14.9 (-43.3)
C.D. (P = 0.05)			1.7		1.2		1.4
C.D. (P = 0.01)			1.9		1.8		1.6

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.

Barium chloride at any of its two concentrations did not induce significant inhibitory effect on spore germination. This report is in confirmation with earlier finding of Ashraf and Ali (2007) who reported inhibitory response of heavy metals on microbial community. Ezzouhri *et al*., (2009) reported the heavy metal tolerance level of some filamentous fungal organisms including *Alternaria alternata, Aspergillus niger, Geotrichum candidus, Penicillium sp*., and *Fusarium sp*. The effectiveness of variable concentration of heavy metals and other diverse group of chemicals was confirmed on spores of *Alternaria brassicicola* (Meena *et al*., 2011), *Alternaria porae* (Feofilova *et al*., 2012) and *Alternaria solani* (Bhajbhuje, 2013). The chlorides of mercury, copper and cadmium are highly toxic over other test chemicals at very low conc. to spores of *Alternaria alternata* (Fr.) Keissler.

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* (Ashraf and Ali, 2007; Meena *et al*., 2011), *A. porae* (Feofilova *et al*., 2012), *A. solani* (Abdel-Kader *et al*., 2012; Bhajbhuje, 2013). 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.

Effect of different treatments on symptoms expression:

(a) *Foliage spray*: The salts of heavy metal were used at 10^{-4}M concentration to spay pot grown foliage of black gram seedlings 3 days before their inoculation at the age of 3-week. The concentration 10^{-4}M was selected for the test chemicals which expected to induce synthesis of fungitoxic substance that inhibited spore germination and germ tube growth of isolate of virulent pathogen in significant manner. The foliage sprayed with all the chlorides excepting ferric chloride provided greatest protection to the seedling, reducing the disease symptoms by 75 to 83% over control. Moderate inhibitory effect was recorded with ferric chloride and lithium sulphate, causing 39% and 58% reduction in disease symptoms respectively. The number of successful infection was appreciably reduced in most of the treatments. Pronounced reduction in count of lesions was recorded with mercuric and least with ferric chloride. Lithium sulphate, chlorides of copper and cadmium induced considerable inhibition; reducing a count of lesions varies between 53 to 72% over control. Significant inhibition of lesion expression was recorded in all treatments, excepting ferric chloride and lithium sulphate which induced plants with some small lesions and fewer large lesions over control. Ferric chloride had 38% inhibition on symptom expression (Table 2).

The symptoms were assayed after 3- & 4-week of inoculated plants and recorded in table 2. The most pronounced protective effect,71-79% was achieved at the age of 3-week with chlorides of mercury, barium and cadmium when seedlings were artificially inoculated by virulent isolate of pathogen, while other treatments induced 56–69% inhibitory effect over control. The induced inhibitory effect was gradually declined in all the treatments 4-week growth stage, causing reduction in disease index which varies between 43-74%. The chlorides of mercury and barium had greatest inhibitory effect at 4-week stage in treated inoculated seedlings. The declining in inhibitory effect was pronounced with ferric chloride and lithium sulphate (Table 2).

Moreover, a count of lesions was reduced in all the treatments at 3- to 4-week growth stage. The most pronounced effects being recorded at 3-wk stage with chlorides of barium and mercury , reducing lesion count varies between 76% and 79% over control respectively, others treatment had moderate effect, reducing the lesion count to 45 – 71%. After inoculation, greater reduction in count of lesions was recorded with barium chloride. In all the treatments, considerable inhibition in lesion count was recorded to the extent of 34-71% at 4-week growth stage. The lesion expansion was inhibited in all cases; stronger inhibition was recorded with the chlorides of mercuric and barium. In such treatments, there were proportionately more small lesions and a few large lesions compared to control (Table 2).

(b)*Root-dip treatment*: Since transplanting of black gram seedlings, the potential of treatment with salts of heavy metal at their stage was explored. Seedlings at the age of 2-week were removed from pots, given a root-dip in the aqueous solution of test chemicals, at 10^{-4}M concentration for 2 h and then transplanted to other large sized pots containing sterile soil amended with FYM. Plants were left exposed to natural leaf spot infection. The observations were recorded up to 4-week (Table 3). Excluding ferric chloride, the inhibition in symptoms expression to the level of 58-83% over control was recorded in different treatments at the 2-week growth stage. The most protective effect was achieved with all chlorides excluding ferric chloride used for the root-dip treatment causing 75 to 83% inhibition in mean disease index. The lithium sulphate had moderate inhibitory effect, reducing the disease symptoms expression by 58% while only 40% inhibitory effect over control was confined to seedling receiving ferric chloride treatment. Such induced protective effects gradually declined with the age of seedlings in most of the treatments (Table 3).

At 3-week growth stage, the inhibition in symptoms expression was recorded to the extent of 52-77% in different treatment. The chlorides of mercury, copper and barium induced pronounced protective effect on symptoms expression, reducing the disease index varies between 71-77% while other test chemicals had moderate inhibitory effect on symptoms expression, causing reduction in mean disease index per plant to the extent of 52-66% over control (Table 3).

Table 3: Effects of *root-dip treatment* at the time of transplanting with heavy metal salts on symptom expression in black gram [*Vigna mungo* (L.) Hepper] seedlings at different intervals[1].

Chemicals	Conc (M)	Disease symptoms[2] (Age of the plant)					
		2-week		3-week		4-week	
		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)		32	19.3	36	24.2	41	27.5
Mercuric chloride	10^{-4}	07 (-78.1)[3]	3.2 (-83.4)	07 (-80.5)	5.5 (-77.3)	15 (-63.4)	10.1 (-63.3)
Cupric chloride	10^{-4}	10 (-68.8)	3.9. (-79.8)	12 (-66.7)	6.3 (-73.9)	18 (-56.1)	12.4 (-54.9)
Barium chloride	10^{-4}	12 (-62.5)	4.6 (-76.2)	10 (-72.2)	7.1 (-70.6)	17 (-58.5)	11.9 (-56.7)
Ferric chloride	10^{-4}	20 (-37.5)	11.8 (-38.9)	20 (-47.2)	10.2 (-57.8)	24 (-41.5)	14.8 (-46.2)
Cadmium chloride	10^{-4}	09 (-71.9)	4.9 (-74.6)	14 (-61.1)	8.3 (-65.7)	20 (-51.2)	13.6 (-50.5)
Lithium sulphate	10^{-4}	15 (-53.1)	8.1 (-58.0)	15 (-58.3)	11.6 (-52.1)	26 (-36.5)	16.4 (-40.4)
C.D. (P = 0.05)			1.7		1.8		1.6
C.D. (P = 0.01)			1.9		2.1		2.2

1. Results have been expressed as percentage in terms of control.
2. Disease symptoms recorded an interval of a week of uninoculated plant.
3. Values in parentheses indicate percentage reduction or increase in terms of control.

The declining trend was significant at 4-wk growth stage in all the root-dip treatments, inhibited symptoms expression by 40-63% over control. The seedlings receiving root-dip treatment with chlorides of mercury, copper and barium had greatest inhibitory effect. The moderate protective effect to the extent of 40 – 51 % was confined with lithium sulphate and chlorides of ferric and cadmium. At later stages of plant development, the declining in symptoms inhibition was moderate with chlorides of mercury, copper and barium while others had little inhibitory effect (Table 3).

Similar induced gradual declining trend was noticed in mean count of lesions in all the root-dip treatments with test chemicals. The inhibitory effect was mostly on the count of lesions rather than on lesion expansion. All the treatments had pronounced inhibitory effect at 2 – week growth stage in this respect. Such induced inhibitory effects in lesion count gradually declined with further growth of the seedlings. Excluding chlorides of barium, copper and mercury, only 47 - 61% and 36-51% inhibition in mean lesion count per plant was recorded in all the treatments at the 3- & 4-week growth stage respectively (Table 3).

(c)*Wet seed treatment*: An aqueous solutions of the test chemicals at effective (10^{-4}M) concentration were used for soaking healthy seeds for 24 hours. The seedlings were raised from treated seeds with aqueous solution of test chemicals in large sized pots containing a sterile soil supplemented with FYM. These pots containing seedlings were left exposed to natural infection. The plants of 3-week old receiving different wet seed treatments of test chemicals at 10^{-4}M conc. were inoculated with spore suspension of *Alternaria alternata* Keissler. The results on symptoms expression before and after inoculation are presented in Table 4. The symptoms recorded at various developmental growth stages from the seedling receiving wet seed treatment prior to inoculation revealed considerable to moderate inhibition in fungal infection on the leaves in most of the treatments. The most pronounced inhibitory effect was confined at 2-week growth stage with chlorides of barium, mercury, copper and cadmium, causing reduction in the disease index varies between 78 to 89%. The ferric chloride and lithium sulphate induced moderate inhibitory effect, inhibiting the symptoms expression by 50% and 63% over control respectively. The declining effect at 3-week growth stage was marginal in all treatments excluding barium chloride where the inhibition in disease index was enhanced to 86% against 68% in 2-week stage. The inhibitory effects continued declining in seedlings receiving wet seed treatment with all test chemicals to lower level, reducing in disease index by 50-78% at 4-week stage (Table 4).

Table 4: Effects of *wet seed treatment* with heavy metal salts on symptom expression in uninoculated black gram [*Vigna mungo* (L.) Hepper] seedlings at different intervals[1].

Chemicals	Conc (M)	Disease symptoms of uninoculated seedlings[2] (Age of the plant)					
		2-week		3-week		4-week	
		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)		32	19.3	38	23.8	44	26.3
Mercuric chloride	10^{-4}	05 (-84.4)[3]	2.1 (-89.1)	06 (-84.2)	4.2 (-82.4)	12 (-72.7)	6.9 (-73.8)
Cupric chloride	10^{-4}	08 (-75.0)	2.8 (-85.5)	09 (-76.1)	4.7 (-80.3)	13 (-70.5)	8.1 (-69.2)
Barium chloride	10^{-4}	11 (-65.6)	6.1 (-68.4)	06 (-84.2)	3.4 (-85.7)	11 (-75.3)	5.8 (-77.9)
Ferric chloride	10^{-4}	17 (-46.9)	9.7 (-49.7)	21 (-44.7)	10.7 (-55.0)	21 (-52.3)	11.7 (-50.4)
Cadmium chloride	10^{-4}	09 (-71.9)	4.2 (-78.2)	10 (-73.7)	3.9 (-83.6)	15 (-65.9)	8.7 (-63.1)
Lithium sulphate	10^{-4}	12 (-62.5)	7.1 (-63.2)	14 (-53.2)	9.3 (-60.9)	24 (-45.5)	13.2 (-49.8)
C.D. (P = 0.05)			2.17		2.4		1.7
C.D. (P = 0.01)			3.2		4.1		3.8

1. Results have been expressed as percentage in terms of control
2. Disease symptoms recorded an interval of a week of uninoculated plant.
3. Values in parentheses indicate percentage reduction or increase in terms of control.

Table 5: Fungitoxic effect of leaf diffusates from seedlings of black gram receiving wet seed treatment (both uninoculated and inoculated) on spore germination and germ tube growth of (both uninoculated and inoculated) on spore germination and germ tube growth of *Alternaria alternata* (Fr.) Keissler

Chemicals	Germination of spores(%) and germ tube growth (µm) in leaf diffusates[3] Age of the plant (weeks)									
	2-weeks		3-weeks				4-weeks			
	Uninoculated		Uninoculated		Inoculated		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
Water (Control)	96	102	98	126	86 (-12.3)	109 (-13.5)	98	134	91 (-7.1)	121 (-9.7)
Mercuric chloride	41 (57.3)[1]	39 (-61.8)	52 (-45.9)[1]	58 (-53.9)	16 (-83.6)	22 (-82.5)	96 (-2.1)	131 (-2.2)	24 (-75.5)	34 (-74.6)
Cupric chloride	43 (-55.5)	41 (-59.8)	58 (-40.8)	64 (-49.2)	19 (-80.6)	26 (-79.4)	95 (-3.1)	129 (-3.7)	28 (-71.4)	39 (-70.9)
Barium chloride	51 (-46.9)	44 (-59.3)	54 (-44.8)	68 (-46.0)	17 (-82.7)	24 (-81.0)	96 (-2.1)	132 (-1.5)	26 (-73.5)	36 (-73.1)
Ferric chloride	49 (-48.9)	48 (-52.9)	60 (-38.8)	71 (-36.5)	22 (-77.6)	31 (-75.4)	95 (-3.1)	128 (-4.5)	31 (-68.4)	41 (-69.4)
Cadmium chloride	45 (-53.1)	42 (-58.8)	53 (-45.9)	66 (-47.8)	31 (-68.4)	36 (-71.4)	94 (-4.1)	131 (-2.2)	39 (-60.2)	51 (-61.9)
Lithium sulphate	51 (-42.9)	61 (-40.2)	63 (-35.7)	71 (-36.5)	38 (-61.2)	46 (-63.5)	95 (-3.1)	132 (-1.5)	61 (-37.8)	72 (-46.3)
C.D.(P = 0.05)		**2.6**		**3.3**		**2.5**		**2.2**		**1.9**
C.D.(P =0.01)		**3.4**		**4.1**		**2.9**		**2.8**		**2.7**

1. Values in parenthesis indicate percentage reduction or increase in terms of uninoculated control.
2. Aqueous solutions of all the heavy metal salts 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.

The seed soaking in aqueous solution of test chemicals seemed to be the most effective for most of the treatment, induced 61-86% protective effect at 3-week stage. The inhibitory effect gradually declines in most of the treatment with age of plant except barium chloride. A greatest inhibition was recorded at 2-week growth stage to the extent of 61 to 82% in the seedling receiving seed treatment (Table 4).

Lesion count was reduced significantly in young seedlings in most of the treatments, the greatest effects being recorded with chlorides of copper, mercury and cadmium, reducing the lesion count at 2-week stage to the extent of 71 to 84%. Such induced inhibitory effects gradually declined to 49 to 63% in 4-wk stage except chlorides of mercury and barium, while lesion expansion was inhibited in all cases, there were proportionately more small lesions and fewer large lesions. The stronger inhibition was recorded with chlorides of mercury, copper and barium (Table 4). The seed treatment provides substantial protection and long persistence of such effect at significant level. These results are confirmed with earlier finding in *Solanum melongena* L (Bhajbhuje, 2013). The phytoalexin accumulation in response to seed treatment was confined in rice (Wilderman *et al*., 2004); peanut (Sobolev *et al*., 2007); *Cicer arietinum* (Raju *et al*., 2008); mustard (Meena *et al*., 2011); and strawberry (Amil-Ruiz *et al*., 2011).

***Fungitoxicity of leaf diffusates*:**

Leaf diffusates were obtained initially from 2-wk old uninoculated plants and later from both uninoculated and inoculated plants receiving wet seed treatments 3 days later each inoculation and bio-assayed for fungitoxicity (Table 3). Leaf diffusates from uninoculated plants in different treatments elicited supportive response to spore germination with very mild toxic effect. However, diffusates from comparable inoculated plants in all treatment induced significant levels of fungitoxicity, causing 61 to 84% spore inhibition compared to only 12% in control at 3-week stage. Some relation was evident between each level of toxicity and degree of resistance. Most pronounced toxicity was recorded with chlorides of mercury, copper & barium while a moderate toxicity was confined with lithium sulphate over control. The leaf diffusates from 4-week-old inoculated plants was seemed to be comparatively less fungitoxic, causing 38-75% reduction in spore germination compared to only 7% reduction in control indicated fungitoxicity of leaf diffusates gradually declines with plant's age (Table 5).

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 inoculated plants induced significant levels of fungitoxicity, inhibiting germ tube growth by 64 to 83%, compared to only 14% inhibitory effect in control at 3-wk stage. The greatest toxicity was reported at this stage with chlorides of mercury and barium; considerable with cadmium & ferric chloride while others had moderate toxic effect on germ tube growth. A little toxicity (2-4%) was noted with leaf diffusates from 4-wk-old uninoculated plants receiving treatments with chlorides of copper, ferric and cadmium while mild toxicity, 1-2% was recorded for others compared to 46-75% inhibition in inoculated treated plants (Table 5).

Fungitoxic effect of leaf diffusates on both spore germination and germ tube growth has been reduced with all treated plants, when inoculated 3 days after 3-wk growth stage with spore suspension of *Alternaria alternata.* The leaf diffusates from 3-wk old inoculated plants receiving seed treatment with mercury and barium chlorides were reported comparatively effective, causing highest inhibition in spore germination followed by cupric chloride while others had moderate inhibitory effect. The leaf diffusates from plants receiving these treatments were highly toxic to pathogen, reducing 63–82% germ tube growth. A fungitoxicity was gradually declined with age of plants. Diffusates from 4-wk old inoculated plants with all chlorides except cadmium chloride and lithium sulphate was reported considerably toxic to pathogen, inhibiting 68–75% spore germination and 69 -74% germ tube growth while other treatments induced moderate toxicity for these parameters(Table 5).

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 production of antifungal compounds in the leaves themselves by plant during successive growth period. Battu *et al*., (2011) reported some antifungals, glycolalkaloid, phenol, ononitol, omethyl-scyllo-inositol from leaves of *Vigna mungo* L. Pathogenesis in leaves exhibited antimicrobial activity (Karthikeyan *et al*., 2009). The volatile extract of blackgram leaves reported antifungal, induced total inhibition of mycelial growth and sporulation of indicating a defense response against these pathogenic fungi (Battu *et al*., 2011).

Significant to moderate levels of fungitoxicity was recorded in the leaf diffusates from 3-week-old uninoculated seedlings in different treatment while considerable to mild toxicity was observed from 4-wk-old seedlings receiving treatment with chlorides of mercury, barium and copper while mild toxicity was recorded with others treatments. The fungitoxicity rapidly declined with plant's age and disappear between 5-6-week in most of the treatments. It is possible that the substance was metabolized and also diluted by plant growth so as to leave moderate toxic effect at the 4-week stage. The untreated plants themselves developed considerable, 7-13% fungitoxicity when inoculated after 3- or 4-week. The greater post-infection development of fungitoxicity in the seed treated plants, even 4-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. These results are confirmed with earlier reports as in Peanut (Sobolev *et al*., 2007); *Cicer arietinum* (Raju *et al*., 2008); *Fragaria vesca* (Amil-Ruiz, *et al*., 2011). Bhajbhuje (2013) reported similar finding while studying the role of diverse group of phytoalexin inducer chemicals on *Solanum melongena*.

Persistence of protective effect:

All the test chemicals, when applied through foliage spray, root-dip and wet seed treatment, effectively protected 2-week-old black gram [*Vigna mungo* (L) Hepper] seedlings against *Alternaria* leaf spot infection incited by *Alternaria alternata*. The seedling receiving treatment through foliar spray induced significant protective effect in young stage against the pathogen. The persistence of protective effect was recorded with foliage spray to the extent of 14-51% at the age of 5-

week growth and was persisted to 17-54%, when seedlings given a root-dip in the aqueous solution of test chemicals in same age of plant. The most pronounced protective effect was recorded in inoculated seedlings at 3-week growth stage receiving wet seed treatment and it was persisted to the extent of 38 to 76% at the age of 5-week growth. In most of the treatments, the pronounced protective effect was achieved to a significant level after artificial inoculation of the seedlings by spore suspension of isolate of virulent pathogen. At the age of 5-week, the greatest protective effect was recorded with barium chloride, causing 51%, 58% and 76% reduction in symptoms in foliage spray, root-dip and wet seed treatment respectively. The mercury chloride induced 71% protection to inoculated seedlings receiving wet seed treatment while 51% and 54 % protective effect was recorded with foliage spray and root dip treatment respectively. The considerable protective effect was noticed with cupric chloride with all the three techniques. Lithium sulphate and ferric chloride were observed comparatively least protective in inoculated seedlings (Table 6).

The inhibitory effect was observed persisted in all the inoculated seedlings receiving wet seed treatment of test chemicals at 6-week growth stage. The most pronounced persistence was recorded with chlorides of barium and mercury followed by cupric chloride. Excluding ferric chloride, lithium sulphate and cadmium chloride, the considerable inhibitory effect was persisted in inoculated plants up to 6-week stage, reducing the symptoms varies between 47 to 57%, indicated protective effect declined with age of seedlings. Little to mild inhibitory effect was persisted in the seedlings receiving foliage spray and root-dip treatment, causing reduction to the extent of 7-23% and 7-30% respectively compared to 32-57% with wet seed treatment at 6-week old stage (Table 6).

Table 6: Comparison between foliage spray, root-dip and wet seed treatment with chemicals on symptom expression in pot-grown black gram [*Vigna mungo* (L.) Hepper] seedlings inoculated with *Alternaria alternata* (Fr.) Keissler after 5 & 6-weeks.

Chemicals	Disease symptoms (Age of the plant : 5-week)						Disease symptoms[2] (Age of the plant : 6-week)					
	Foliage spray		Root-dip treatment		Wet seed treatment		Foliage spray		Root-dip treatment		Wet seed treatment	
	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	Mean no. of lesions /plant	Mean disease index /plant	Mean no. of lesions /plant	Mean disease index /plant
Water (Control)	48	28.6	45	28.1	42	22.3	52	32.2	49	30.4	51	26.2
Mercuric chloride	25 (-47.9)[1]	14.1 (-50.7)	21 (-53.3)	12.8 (-54.4)	12 (-71.1)	6.8 (-69.5)	41 (-21.2)	24.7 (-23.3)	36 (-26.8)	21.9 (-27.9)	23 (-54.9)	11.6 (-55.7)
Cupric chloride	28 (-41.7)	16.5 (-42.3)	25 (-44.4)	14.9 (-46.9)	15 (-64.3)	8.1 (-63.7)	45 (-13.5)	28.3 (-12.1)	39 (-20.4)	23.8 (-21.7)	25 (-51.0)	12.3 (-53.1)
Barium chloride	24 (-50.0)	14.1 (-50.7)	20 (-55.6)	11.9 (-57.7)	10 (-76.2)	5.4 (-75.8)	41 (-21.2)	24.9 (-22.7)	35 (-28.6)	21.3 (-29.9)	22 (-56.9)	11.2 (-57.3)
Ferric chloride	39 (-18.6)	22.7 (-20.6)	35 (-22.2)	21.6 (-23.1)	18 (-57.1)	9.6 (-56.9)	48 (-7.7)	29.4 (-08.7)	43 (-12.2)	26.2 (-13.8)	28 (-45.1)	13.7 (-47.7)
Cadmium chloride	30 (-37.5)	18.7 (-34.6)	26 (-42.2)	15.7 (-44.1)	20 (-52.4)	10.7 (-52.0)	44 (-15.4)	26.8 (-16.8)	39 (-20.4)	23.2 (-23.7)	28 (-45.1)	14.5 (-44.7)
Lithium sulphate	42 (-12.5)	24.4 (-14.7)	38 (-15.5)	23.2 (-17.4)	25 (-40.5)	13.8 (-38.1)	50 (-03.8)	29.7 (-07.8)	45 (-06.3)	28.2 (-07.2)	36 (-29.4)	17.8 (-32.6)
C.D.(P= 0.05)		1.2		1.1		1.6		1.3		1.5		1.9
C.D. (P = 0.01)		1.3		1.4		2.1		1.6		1.8		2.8

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.

Moreover, significant to moderate inhibition in lesion count was recorded for all the treatments at 5-week stage, the most pronounced effects being recorded with barium chloride reducing lesion count followed by chloride of mercury and copper while, other treatments had moderate protective effect, reducing the lesion count to the extent of 12-38%; 15-42% and 40-57% with seedlings receiving foliage spray, root-dip and seed treatment respectively. The lesion expansion was inhibited in all cases; stronger inhibition was recorded in seedlings receiving wet seed treatment with chlorides of barium, mercuric and cadmium. Substantial protective effect was also recorded for 6-week-old inoculated plants with most of the wet seed treatments, causing 29-57% inhibition in this respect. Greatest effect was significant with chlorides of barium and mercury followed by cadmium and cupric chloride. The lesion expansion was significantly inhibited and lesion count was appreciably reduced in inoculated seedlings receiving seed treatment. In such treatments, there were proportionately more small lesions and fewer large lesions compared with those in control. The reduction in count of infections in most of the wet seed treatments and the inhibition of lesion expansion 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. The most persistent effect for longer duration was confined with wet seed treatment over foliar spray and root-dip treatment.

The experimental results revealed that *in vitro*, chlorides of mercury, copper and cadmium inhibited absolute spore germination and germ tube growth but allow mild lesion formation in 5-6-week old plants. At this stage, moderate to little toxicity was evident in leaf diffusate from any treatment. This may suggest that large amount of fungitoxic substance was produced in test plant in response to seed treatment compared to foliage spray and root-dip treatment. Little symptoms were produced when the production of fungitoxic substances soon attains a level inhibitory to the invading organism. Pronounced inhibition of germ tube growth in some treatments in relation to spore germination seemed to be of particular significance when such toxicity is confined relating to disease resistance as a post-interaction phenomenon. Among the three techniques employed in induction resistance in *Vigna mungo* seedlings, mostly seed treatment interfered with infection process itself, since barium and mercuric chloride induced significant inhibition in the lesions count as well as mean lesion size implying appreciable inhibition of lesion count was less pronounced but that on lesion size appeared to be greater for most of the seed treatments where plants had proportionately more small lesions and a few large lesions over untreated plants. These results coincided with earlier findings with *Solanum melongena* L. (Bhajbhuje, 2013). Karthikeyan *et al*., (2009) induced systemic resistance in *Vigna mungo* against urdbean leaf crinckle virus by chemicals.

The symptom expression was significantly inhibited at 2-3 week and moderately at 4- 5-week stages in most of the wet seed treatments. The protective effect of seed treatment was persisted in 6-wk-old plants but it was declined by 5-8% over 5-week-old plants with all the treatment. This may be quite possible that production of phytoalexin-like substances was initially stimulated and detected in young seedlings but at later stages, there may be insufficient quantity of accumulation of phytoalexins-like substances by little aged seedlings (Eckadt, 2011) or applied chemicals may have been metabolized in host tissues and their concentrate probably were diluted by seedling growth so as to leave little toxic effect at later stages (Bhajbhuje, 2013). It seems unlikely that as the chlorides of mercury and barium had their direct toxic effect on the pathogen but these test chemicals have provided substantial protection to *Vigna mungo* seedlings; it was in all probability an induced effect. Pronounced production of fungitoxic substance in the treated plants in different treatments when inoculated may induced moderate to high level of fungitoxicity as compared to little or mild toxicity developed in untreated plants and relationship between such post infection levels of toxicity in treated plants and levels of resistance induced in such stimulated high post-infection productions of fungitoxic substances in susceptible tissue may be due to a conditioning effect of treatment chemicals which make plant resistant and readily respond to infection by producing large amount of fungitoxic substances (Eckadt, 2011; Amil-Ruiz, *et al*., 2011; Bhajbhuje, 2013). As the conditioning effect wakens with time, the production of extra fungitoxic substances diminishes and the induced protective effect also declines.

The salts of heavy metals, the known phytoalexin inducer chemicals, were used at dilute concentration

(10^{-4}) for wet seed treatment, most of the six heavy metal salts tested appeared to provide moderate to substantial protection to *Vigna mungo* against *Alternaria* leaf spot infection, resulting from both artificial inoculation and natural infection with virulent pathogen, *Alternaria alternata* Keissler. This indicated successful induction of resistance in susceptible plants by seed treatment with such chemicals which often persisted at appreciable levels even up to 5-6-week. growth stage. The wet seed treatment of chemicals seemed to be the most effective, provided substantial and long persistence protection to significant level. Although apparent enhanced post-infection production of fungitoxic substance(s) was strong in *Vigna mungo*. Some antifungals viz., glycolalkaloid, phenol, ononitol, omethyl-scyllo-inositol have been reported in leaves (Battu, *et al*., 2011); however synthesis and accumulation of efficient phytoalexin has not so far been reported against infection of a virulent strain of leaf spot causing pathogen, *Alternaria alternata* Keissler, any suggestion about the possible involvement of such compounds in development of resistance is conjectural. Nevertheless, an involvement of some kind of fungitoxic substance in this process is strongly indicated.

Since there is no correlation between *in-vitro* fungitoxicity of the phytoalexin inducer chemicals and the resistance induced 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 induce 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 experimental findings reveals that an aqueous solution at dilute conc. of salts of heavy metals applied by foliar spray, root-dip and wet seed treatment, induced significant reduction in infection and provided considerable to moderate protection to *Vigna mungo* (L).Hepper seedlings, at different growth stages against artificial inoculation with a leaf spot causing virulent pathogen, *Alternaria alternata* (Fr.)Keissler. Seeds soaked in aqueous dilute solutions of test chemicals comparatively seemed to provide the most effective and durable protection over other techniques. An aqueous solution at 10^{-4}M concentration of chlorides of barium, copper and mercury by wet seed treatment provided more vigorous defence response to virulent pathogen as well as induced long persistent substantial protection at significant level. 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. Barium, copper and mercury chlorides at dilute concentration may serve as very promising compounds for use in plant disease control. Wet seed treatment proved to be the most effective over others in seed producing plants.

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REFERENCES

Abdel-Kader MM, El-Mougy NS, EI-Gannal NG, Abd-EI-Kareem F, Abd-Alla (2012) Laboratory evaluation of some chemicals affecting pathogenic fungal growth. *Jour. Appl. Sci. Res*., 8(1) : 523-530.

Amil-Ruiz F, Blanco-Portales R, Munoz-Blanco J, Caballero JL (2011) The strawberry plant defense mechanism: A molecular review. *Plant Cell Physiol*., 52 (11):1873-1903.

Ashraf A, Ali TA (2007) Effect of heavy metals on soil microbial community and mung beans seed germination. *Pak. J. Bot*., 39(2): 629-636.

Atkinson NJ, Urwin PE (2012) The interaction of plant biotic and abiotic stresses: from gene to field. *Journal of Experimental Botany*, 63(3): 1-21.

Ballhom D., Kautz, S, Heil M, Hegeman AD (2009) Cyanogenesis of wild lima bean (*Phaseolus lunatus* L.) is an efficient direct defence in nature. *Plant Singhing and Behavior,* 4(8): 735-745.

Battu G, Ch KVL, Male SNA, Priya TH, Malleswar, Reeshma, SK (2011) Phytopharmacological review of *Vigna* species. *Pharmanest*, 2(1): 61-67.

Bhajbhuje MN (1989) Investigations on mycoflora associated with vegetable seeds from Vidarbha Region. Ph.D. Thesis, R.T.M. Nagpur University, Nagpur, M.S. India.

Bhajbhuje MN (2013) Role of heavy metal salts on susceptibility of *Solanum melongena* L. seedlings to *Alternaria* early blight disease. *Int. J. of Life Sciences,* 1 (1): 51-62.

CMI (2010) Commonwealth Mycological Institute. Description of Pathogenic fungi and bacteria. Kew Surrey, England. Pp 451-460.

Eckadt NA (2011) Induction of Phytoalexin Biosynthesis: WRKY33 - Is a Target of MAPK Singling. *Plant Cell*, 23(4): 1190.

Ezzouhri L, Castro E, Moya M, Espinola F, Lairini K (2009) Heavy metal tolerance of filamentous fungi isolated from polluted sites in Tangier, Morocco. *African J. Microbiol. Res*., 3(2):35-48.

Feofilova E, Ivashechkin A, Alekhin A, Sergemma Y (2012) Fungal spores: Dormancy, germination, chemical composition and role in biotechnology (review), *Appl Biochem, & Microbiol*., 48(1) : 1-21.

Horger A, Fones HN, Preston GM (2013) The current status of the elemental defense hypothesis in relation topathogens, PMCID: PMC3797420, *Front Plant Science*, 4 : 395.

Karthikeyan G, Doraisamy, Rabindran R (2009) Induction of systemic resistance in blackgram (*Vigna mungo*) against urdbean leaf crinckle virus by chemicals. *Archives of Phytopath & Pl. Protection*, 42(1): 1-8.

Laemmlen F (2001) *Alternaria* Diseases. Division of Agric. & National Resources, Univ. of California. Publication 8040. http://anrcatalog.ucdavis.edv. (Retrieved April, 17, 2014).

Meena PD, Chattopadhyay C, Kumar A, Awasthi RP, Singh R, Kaur S, Thomas L, Goyal P, Chand P (2011) Comparative study on effect of chemicals on *Alternaria* blight in Indian mustard – A multilocaion study in India. *J. Environ. Biol*., 32(3) : 375.

Raju S, Jayalaxmi SK, Sreeramulu K (2008). Comparative study on the induction of defence related enzymes in two cultivars of chickpea (*Cicer arietinum* L.) genotype by salicylic acid spermine and *Fusarium oxysporum* f. sp. *Cicero*. *Australian Jour. Crop Sci*., 2(3): 121-140.

Rathod S (2012) Seed borne *Alternaria* species: A review. *Currant Botany*, 3(2): 21-23.

Savithasuri (2013) Ayurveda, medicinal properties and health benefits of black gram- *Vigna mungo*. http://www/ayurhelp.com/plants/blackgram (Retrieved, April, 12, 2014).

Sharma OP, Bambawale OM, Gopali JB, Bhagat S, Yelshetty S, Singh SK, Anwar R, Singh OM (2011) Field guide Mung bean and Urd bean. Government of India, Department of Agriculture and co-operation, NCIMP, ICAR, New Delhi, India.

Singh SK, Singh UP, Tuli L, Prithviraj B , Sarma BK (2000)Effect of spore conc, of *Alternaria tenuissima* on germination & development of germ tubes on hosts & non-hosts. *Indian Phytopath*., 53(4) : 419-422.

Sobolev V, Guo B, Robert H (2007)Interrelationship of Phytoalexin Production and Disease Resistance in Selected Peanut Genotypes. *Jour. Agric. and Food Chem*., 55 : 2195-2200.

Trivedi A, Sharma S K, Hussain T, Sharma SK, Gupta PK (2013) Application of biodynamic preparation, bio-control agent and botanicals for organic management of virus and leaf spots of black gram (*Vigna mungo* L. Hepper). *Acad. J. Agric. Res*., 1(4): 60-64.

Wikipedia (2014) Black gram – *Vigna mungo* (L) Hepper. Wikimedia Foundation, Org. en.wikipedia,org/wiki. Inc. (Retrieved April 15, 2014).

Wilderman PR, Xu M, Jin Y, Coates RM, Peters R (2004) Identification of Syn-Pimara-7,15-Diene Synthase reveals functional clustering of Terpene synthases involved in rice Phytoalexin/Allelochemical biosynthesis, *Plant Physiology*, 135 : 2098-2105.

Zhuang X, McPhee KE, Coram TE, Peever TL, Chilvers MI (2012) Rapid transcriptase characterization and parsing of sequences in non-model host-pathogen interaction; pea – *Sclerotinia sclerotiorum*. BMC *Genomics,* 13 : 1-19.