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RELATIONSHIP BETWEEN INDUCED RESISTANCE AND MANGANESE, ZINC AND COPPER CONTENTS OF SUSCEPTIBLE CHICKPEA CULTIVARS AFTER THEIR INOCULATION WITH *ASCOCHYTA RABIEI*

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A B S T R A C T

The necrotrophic fungus *Ascochyta rabiei* causes Ascochyta blight (AB) disease in chickpea which infects all aerial parts of the plant, which results in severe yield loss. In order to evaluate the effects of resistance inducers Bion® (acibenzolar-S-methyl), salicylic acid (SA), potassium hydroxide (KOH) and plant extracts of neem (*Azadirachta indica* A. Juss.), datura (*Datura metel* L.) and garlic (*Allium sativum* L.), on the manganese zinc and copper contents of three chickpea cultivars 'C-44', 'Pb-91' and 'Bittle-98' to ascochyta blight disease an experiment was conducted in Pakistan during the year 2007-08. The data of minerals were taken by processing the tissue collected after 7th and 14th days and inoculation with spore's suspension of *Ascochyta rabiei*. Results revealed that the mineral contents increased significantly after the induction of resistance and upon inoculation with the pathogen after 14th day's time interval. This increase was more evident by the application of chemicals as compared to the plant extracts. Higher contents of manganese and zinc were recorded 14th days after treatment with (Bion®@ 1.2mM+ inoculation with *A. rabiei*) in all the cultivars. However, the copper contents significantly decreased in cultivar C-44 after treatment with salicylic acid, Bion® and neem while, it increased in all other treatments and cultivars. The over all results illustrated that resistance induction altered the mineral contents in all the three chickpea cultivars with more pronounced in C-44. In future study, molecular level research is required to get an insight into the resistance mechanisms.

Keywords: chickpea blight, *Ascochyta rabiei* (Pass.) Labr., *Cicer arietinum* L., resistance inducers, mineral contents.

INTRODUCTION

The chickpea (*Cicer arietinum* L.) is known for its high nutritional value as it contains carbohydrates, minerals and other trace elements (Zia-Ul-Haq *et al.,* 2007). Chickpea blight caused by *Ascochyta rabiei* (Pass) Labr., upsets the production statistics of the crop under favorable environmental conditions resulting in 50-70% crop loss (Malik and Bashir, 1984) or complete failure of the crop (Nene, 1984). The disease can be managed by some cultural practices (Ilyas *et al.,* 2007) and use of chemotherapeutants (Singh and Singh, 1990; Tripathi *et al.,* 1987) but the most economical practice is cultivation of cultivars resistant to blight disease (Nene and Reddy, 1987). These control strategies did not last long due to appearance of more virulent strains of the pathogen (Ali *et al.,*2009; Sarwar *et al.,*2000) and scarcity of resistance in the available germplam (Ghazanfar *et al.,*2010a). This necessitates exploring new methods to over come this disease. Plants have developed a wide variety of mechanisms that can be local or systemic and constitutive or inducible (Keen, 1990; Ryals *et al.,* 1994) to defend themselves. The understanding of these mechanisms against pathogens may lead to develop novel strategies to enhance disease resistance in crop plants (Pozo *et al.,* 2005). Plant defense responses against the pathogen are regulated by a complex network of signal molecules and transcriptional regulators. Biological control and chemical inducers are two of the most promising approaches for the

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control of plant diseases (Yu and Zheng, 2006). Induced Systemic Resistance (ISR) is a phenomenon whereby resistance to infectious disease is systemically induced. It is broad spectrum, long lasting, less likely to develop resistance in pathogens and may control soil born pathogens and plant viruses (Kuć, 2001). Various chemicals and plant extracts have the ability to induce resistance against a variety of pathogens. In this context (Ghazanfar *et al.,*2011) reported that among the inducing agents, Bion® was the most effective with 79% reduction in *A. rabiei* disease in cultivar C-44. The activity of the inducing agents is not due to antimicrobial activity or their ability to be transformed into antimicrobial agents but there is an increase (Sticher *et al.,* 1997) in phytoalexins, reactive oxygen species/free radicals, calcium, silicon/silicates, polyphenol oxidases, peroxidases and phenolic compounds etc.

Chickpea contains sufficient amount of all the important minerals (Zia-ul-Haq, 2007). The severity of the disease is affected by all the essential nutrients (Huber and Graham, 1999) and the balanced nutrition is necessary for any host plant to be resistant or susceptible to a pathogen (Filippi and Prabhu, 1998). However, no general rule exists how a particular nutrient can decrease or increase the severity of a disease or incidence of other diseases and it may have a completely opposite effect under different environments (Graham and Webb 1991; Huber, 1980; Marschner, 1995). The importance of nutrients in disease control has been recognized for many years but for some of the most severe diseases, the correct use of nutrients for the control of diseases in sustainable agriculture and successful disease management program has received little attention (Huber and Graham, 1999).

Manganese is another nutrient that influences the susceptibility and resistance of the host towards the diseases. Since Mn is a cofactor for both phenylalanine ammonia-lyase that mediates production of cinnamic acid and various other phenolic compounds and peroxidase involved in polymerization of cinnamyl alcohols into lignin (Burnell, 1988). Deficiency of Mn may prevent plants from effectively building up phenolics and lignin content which is considered the primary defense against fungal infection (Burnell, 1988; Barber and Ride, 1988; Matern and Kneusel, 1988). Graham and Rovira (1984) suggested an interaction between Mn and biosynthesis of defense-related phenolics and lignitubers as a mechanism of Mn-influenced increase in resistance to take-all fungus. Zn nutrition has role in disease suppression due to its protective role in the structural and functional integrity of cell plasma membranes (Welch *et al.,*1982; Welch and Norvell, 1993). Zinc-deficient plants exhibit low activity of the enzyme superoxide dismutase and have high levels of superoxide radical (O_2^-) (Cakmak and Marschner, 1988), leading to peroxidation of membrane lipids, loss of membrane integrity, increased membrane permeability (Thompson *et al.,*1987) with increased susceptibility to fungal infection. Plant diseases have been reported to be greatly influenced by copper, which mainly decreases the disease but in a few cases increases the symptoms. Copper may also play a role in disease resistance because of its involvement in many physiological functions. Copper is a micronutrient that participates in photosynthesis, respiration, antioxidant activity, cell wall metabolism and hormone perception (Pilon *et al.,* 2006). This essential role of copper in plant physiological processes, that influence plant resistance and susceptibility is sometimes overlooked (Evans *et al.,* 2007). However, (Molina *et al.,* 1998) demonstrated that in Arabidopsis, copper induces resistance against *Peronospora parasitica* in a partially SAR dependent way.

Since minerals play significant role in biochemical reactions yielding such metabolites that are involved in resistance/susceptibility of a crop against its pathogens, the objective of this study was to evaluate the effectiveness of resistance inducers (chemicals and plant extracts) in altering the manganese zinc and copper contents after inoculation with *A. rabiei* in susceptible chickpea cultivars.

MATERIALS AND METHODS

Plant Material: Seeds of three chickpea cultivars viz C-44, Pb-91 and Bittle-98 with susceptible response (Ghazanfar *et al.,*2010a) but good yield character (Ghazanfar *et al.,*2010b) were taken and cultivated in small plots in the experimental area of the Department of Plant Pathology, University of Agriculture, Faisalabad, during the year 2007-08. The experiment was laid out under split-split design with varieties in main plot, doses in subplots and inducers in sub-subplots with three replications. Each plot had six rows of 15 chickpea plants per row

Preparation of plant extracts: Extracts of neem (*Azadirachta indica* Juss.), datura (*Datura metel* L.) and garlic (*Allium sativum* L.) were prepared from leaves of neem and datura collected from research areas at the University of Agriculture, Faisalabad while cloves of garlic were purchased from a local market. Leaves of neem and datura were washed under tap water, surface sterilized with 1% sodium hypochlorite solution and rinsed with sterilized water before homogenizing (Yellow line DI-25 Basic, GmbH and CO, Germany) in sterile distilled water (1:1 w/v), and filtering

through a muslin cloth. Fresh garlic extracts were prepared by removing the outer, dry peel, surface-sterilizing for 2 min in 70% ethanol and washing in three changes of sterile distilled water. Cloves were crushed to a pulp in a sterile porcelain mortar with a pestle, the pulp was suspended in 100 ml water in a 250 ml Erlenmeyer flask, and then filtered through a muslin cloth. All plant extracts were heated to 40°C for 10 min to avoid contamination (Jaganathan and Narasimhan, 1988) and diluted to 5%, 10% or 15% concentration with sterile distilled water (v/v).

Chemicals: Aqueous solutions (0.5, 1.0 and 1.5mM) of salicylic acid (Sigma Aldrich, Germany), Bion® (0.4, 0.8 and 1.2 mM, Syngenta Crop Protection, Germany) and 25, 50 or 75mM KOH (Sigma Aldrich, Germany) were used for the induction of resistance.

Induction treatment: At early flowering stage, all the resistance inducing agents (chemicals and plant extracts) were sprayed on the plants until run off while the control plants were sprayed with distilled water only. The mass preparation of already isolated and preserved inoculum of *A. rabiei* on CSMA (Chickpea seed meal agar): chickpea seed meal 20g; glucose 20g; agar agar 20g; sterilized water to make volume one liter and pH 5.5) was carried by the method of (Ilyas and Khan, 1986). The conidial counts were adjusted with the help of haemocytometer. Four days after treatment, the plants were sprayed to run off with a spore suspension of *A. rabiei* ($1x10^5$ spores L^{-1}) that contained three drops/liter of Tween 80 as wetting agent in the evening since temperatures are lower at night to give better germination of conidia. Spray inoculation continued for three days to ensure maximum infection of plants. The plants were periodically sprinkled with water to maintain the humidity and favor germination of conidia.

Processing of plant samples for the estimation of manganese zinc and copper: Shoot samples from treated un-inoculated and treated inoculated chickpea plants of the three cultivars were collected 7 and 14 days after inoculation at the time when symptoms were fully developed on the inoculated control plants. Shoots were washed in a 0.2 % detergent solution to remove dirt, washed in 0.8 % HCl (to remove metallic contaminants from them), and then washed in deionized water to remove the previous two solutions. The samples were air-dried in the shade on paper towels and placed in paper bags prior to drying in an oven at 70°C for 72 hours to get a constant weight. These samples were ground with a Buhler grinder and then analyzed N, P and K (Bhargava and Raghupathi, 1995; Karla and Maynard, 1991). The percentage (content) of dry weight of these three nutrients were recorded as ppm (parts per million).

STATISTICAL ANALYSIS

Experimental data were analyzed by ANOVA and the means were seperated by using Tukey's HSD (Honestly Significant Difference) test at 5% ($P_{0.05}$ at 5%) level of significance (Steel *et al.,*1997) using the software R. 2.12.1 (2008).

RESULTS

Manganese: The data regarding the Mn content of both the groups, induced un-inoculated and induced inoculated, are presented in (Table. 1). Application of disease resistance inducer chemical significantly ($P \leq 0.05$) increased the Mn contents in both the groups but this increase was more pronounced by the application of Bion (1197.28 ppm) as compared to all other chemicals. Application of salicylic acid also increased the manganese content in the cultivar C-44 but it was less (1189.99 ppm) as compared with Bion application at the highest dose rate on 14th day after induction and inoculation with *A. rabiei*, however, there was less increase (594.49 ppm). Application of neem leaf extracts also increased the Mn content (387.47 ppm) as compared to extracts of datura (251.94 ppm) and by garlic (202.88 ppm).

The induced un-inoculated plant of cultivar C-44 also showed the increasing trend by the application of Bion (1042.44 ppm) after 7th day of application followed by 1137.37 ppm in the salicylic acid and 518.35 ppm in case of KOH at the lowest dose rate. The plant extracts also caused increase but that was not significant with 340.11 ppm in neem after 14th day of application at 10% dose rate while in case of datura (218.37 to 218.81 ppm) and garlic (168.85 to 169.85 ppm) after 7th and 14th day (Table. 1). The induced and un-inoculated plants showed an increase in case of salicylic acid and Bion treated plants while KOH, neem, datura and garlic increased the small amount of Mn content without inoculation after 7th and 14th day of induction in the cultivar Pb-91. Mn content of 152.07 ppm was exhibited by plants of cultivar Bittle-98 after induction and inoculation with pathogen while salicylic acid showed 903.13 ppm followed by 903.13 ppm in Bion treated plants.

Zinc: The amount of zinc content of both groups, induced un-inoculated and induced inoculated, showed the increased trend (Table. 2) which was significant ($P \leq 0.05$), in case of Bion application (104.23ppm) followed by 99.41 ppm in salicylic acid treated plants with least amount of Zn contents (49.70 ppm) in C-44 on 14th day after induction and inoculation. In case of plant extracts, neem leaf extract exhibited significant increase of 33.97 ppm in Zn content as compared to datura (24.45 ppm) and garlic (25.45 ppm). Although the later two showed an increase but it was at par

with each other.

The Zn content in the cultivar Pb-91 was also increased by the application of resistance inducing agents and it was more pronounced after inoculation with the *A . rabiei* with maximum (87.07 ppm) recorded in Bion treated plants as compared to KOH (41.10 ppm) while salicylic acid treatment induced 82.2 ppm Mn content 14th days after inoculation and induction (Table. 2). The un-inoculated and induced plants also showed an increase but that was significant by the application of salicylic acid and Bion but non significant in KOH treated plants.

Application of plant extracts viz. garlic (19.78 ppm) and datura (19.91 ppm) exhibited the increase in Zn content but that was not statistically different from each other. On the other hand the neem leaf extract enhanced the Zn contents (26.62 ppm) which was different from all the other extracts in case of Bittle-98. Application of KOH resulted in 33.77 ppm Zn content which was significantly lower than that of Bion (72.92 ppm) and salicylic acid (67.54) at the highest dose rates (Table. 2) after 14th day of induction and inoculation.

Copper: The amount of copper content varies in all the three chickpea cultivars which was significantly different (Table. 3) in induced un-inoculated and induced inoculated plants. The highest increase was found in the Bion treated plants applied @ 1.2 mM which was 126.2 ppm in induced un-inoculated while 90.99 ppm in inoculated ones. KOH treated plants showed 61.73 ppm, whereas application of salicylic acid at highest dose rate decreased the Cu contents by 86.03 which were high without inoculation (122.13 ppm) at 1.5mM dose rate after 14th day of inoculation and induction of resistance in the cultivar C-44. The neem leaf extract decreased the Cu content from 42.02 to 39.99 ppm after 7th and 14th day; on the other hand, application of datura and garlic extract showed values of 29.53 ppm and 28.74 ppm, respectively, in the cultivar Pb-91. The trend of increased Cu content was continued in this cultivar by the application of salicylic acid (71.66 ppm) and Bion (77.19 ppm) while the lowest was recorded in KOH (35.83 ppm). The plant extracts showed the change in the Cu content with maximum produced by the neem (35.63 ppm) and lowest by the garlic (16.25 ppm). In case of un-inoculated plants Bion after 7th and 14th days produced Cu contents ranging from 34.47 to 37.47 ppm at 0.8mM dose rate (Table. 3). In cultivar Bittle-98, an increase was exhibited by the application of Bion (53.29 ppm) after 14th day of induction and inoculation with *A.rabiei* which was different with treatment where KOH was applied. Plant extract of datura showed maximum (18.41 ppm) copper content followed by neem (17.78 ppm) and garlic (15.02 ppm).

DISCUSSION

Numerous studies have been published on the subject of nutritional predisposition of plants to attack by pests and diseases, yet the role of nutrition to improve resistance and tolerance of plants lags behind (Graham, 1980; Marschner, 1995). Although the available literature did not provide any information about the effect of induced resistance on the mineral contents of plant tissue but the present study showed that application of resistance inducers inflicts variation of minerals contents. There is increase in the Mn contents in all the three chickpea cultivars and this boost was more obvious by the application of Bion and least with garlic fourteen-day post application of treatments. These increased levels provide 79% disease reduction in case of *A. rabiai* infection in the cultivar C-44 (Ghazanfar *et al.,*2011). Severity of root rot pathogens (*Rhizoctonia solani* and *Rhizoctonia bataticola*) of cowpea was also reduced by the application of Mn (Kalim *et al.,* 2003). Similar results were also reported by (Randhawa, 1994) that there was increase in the Mn contents of chickpea cultivars upon inoculation with the *A. rabiei*. (Sahi *et al*., 2010) reported significant increase in manganese contents of resistant lines of lentil but on the contrary there was highly significant decrease in the manganese content of susceptible lines after inoculation with *Ascochyta lentis*. Three times foliar spray with either jasmonic acid or salicylic acid significantly increased Mn contents in the tomato plant against *Fusarium oxysporum* but it was more with AM fungi plus JA in both leaves and roots (El-Khallal, 2007). The susceptibility of bent grass was increased to *Gaeumannomyces graminis* (Sacc.) Arx. & D. Olivier var. *avenae* (E.M. Turner) Dennis, disease under Mn deficiency (Heckman *et al.,* 2003). Manganese is considered to be the most premeditated micronutrient in the development of resistance in plants in case of both root and foliar diseases (Graham and Webb, 1991; Heckman *et al.,* 2003). Mn also has direct role in the synthesis of lignin, biosynthesis of phenol, photosynthesis and various other functions (Marschner, 1995; Graham and Webb, 1991). Mn also inhibits the initiation of an enzyme amino peptidase, which provides essential amino acids for fungal growth and also pectin methyl-esterase that degrades host cell walls. Furthermore, manganese controls lignin and suberin biosynthesis (Römheld and Marschner, 1991) which are biochemical barriers to fungal pathogen invasion (Hammerschmidt and Nicholson, 2000; Vidhyasekaran, 2004) by the activation of various enzymes in the shikimic acid and phenyl propanoid pathways (Marschner, 1995) as they are phenolic polymers resistant to enzymatic degradation (Agrios, 2005).

Table 1. Mean comparison (± S.E) of manganese content (ppm) in cultivars C-44, Pb-91 and Bittle-98 after 7 and 14 days of the treatments with resistance inducers (chemicals and plant extracts)

Treatments		Cultivars								
		C-44			Pb-91			Bittle-98		
		Doses (Mm)								
	Salicylic acid	0.5	1.0	1.5	0.5	1.0	1.5	0.5	1.0	1.5
7d	Ind. and Un-Inoc.	1032.52±11.88	1042.82±6.71	1053.48±10.98	916.53±0.85	923.97±0.88	932.09±6.03	766.25±5.44	773.81±5.37	783.20±6.10
	Ind. and Inoc	1157.83±9.27	1168.35±5.12	1185.69±4.93	979.26±5.51	988.77±5.51	997.18±6.78	880.40±5.23	885.49±5.88	896.02±3.10
14d	Ind. and Un-Inoc.	1037.37±10.85	1046.48±10.08	1055.89±11.98	922.04±3.27	928.22±4.80	933.26±4.71	771.57±0.72	779.32±0.73	789.30±5.38
	Ind. and Inoc	1164.31±17.64	1171.81±6.59	1188.99±5.55	981.92±1.04	992.16±0.69	999.00±0.54	881.46±0.48	890.32±5.52	903.13±2.08
	Bion	0.4	0.8	1.2	0.4	0.8	1.2	0.4	0.8	1.2
7d	Ind. and Un-Inoc.	1040.67±4.92	1048.29±5.94	1059.59±5.60	921.37±5.22	929.43±5.38	941.78±5.55	771.59±6.22	781.75±5.14	789.65±0.42
	Ind. and Inoc	1164.57±10.13	1175.38±12.11	1190.51±5.39	983.47±1.69	989.94±5.51	1011.23±10.49	884.67±6.23	891.66±0.75	898.44±5.81
14d	Ind. and Un-Inoc.	1042.44±11.33	1052.93±9.15	1063.00±10.96	926.41±6.44	935.62±2.49	949.71±5.06	779.04±5.73	791.66±5.39	798.34±0.91
	Ind. and Inoc	1170.22±9.50	1184.16±11.26	1197.28±5.16	988.04±6.19	997.28±12.11	1023.81±11.26	885.44±2.76	894.43±2.61	901.52±5.68
	KOH	25	50	75	25	50	75	25	50	75
7d	Ind. and Un-Inoc.	516.26±2.79	521.41±2.23	526.74±3.10	458.27±2.74	461.99±1.35	466.05±2.72	383.13±1.84	386.91±3.33	391.60±1.60
	Ind. and Inoc	578.92±1.66	584.18±2.66	592.85±3.01	489.63±4.93	494.39±2.55	498.59±2.55	385.79±2.79	389.66±2.63	394.65±2.12
14d	Ind. and Un-Inoc.	518.35±1.76	523.24±2.77	527.95±1.91	459.35±4.49	464.11±2.16	466.63±2.04	385.79±2.70	389.66±1.79	394.65±2.52
	Ind. and Inoc	582.16±6.82	592.58±4.64	594.49±1.88	490.96±5.31	496.08±3.01	499.50±4.56	440.73±1.35	444.99±2.41	451.57±0.87
	Azadirachta indica	5%	10%	15%	5%	10%	15%	5%	10%	15%
7d	Ind. and Un-Inoc.	335.90±2.12	338.91±0.40	342.38±1.40	297.87±0.28	300.29±0.28	302.93±1.68	249.03±0.75	251.82±1.11	255.21±1.29
	Ind. and Inoc	376.63±0.97	379.38±2.10	386.02±2.74	318.26±1.28	321.35±0.35	324.08±1.86	252.43±1.48	255.95±2.80	260.52±1.93
14d	Ind. and Un-Inoc.	337.15±0.85	340.11±1.34	343.83±2.19	298.58±0.87	301.67±0.75	303.64±2.21	250.76±1.38	253.95±1.77	256.86±1.25
	Ind. and Inoc	378.40±1.34	382.18±1.50	387.42±1.67	318.46±3.89	322.45±4.58	326.68±2.14	286.81±1.90	289.91±0.39	294.52±2.70
	Datura metel	5%	10%	15%	5%	10%	15%	5%	10%	15%
7d	Ind. and Un-Inoc.	211.89±1.50	218.37±1.02	220.60±0.99	191.30±0.16	193.19±1.55	194.89±1.03	158.21±0.65	161.8±0.14	163.76±1.22
	Ind. and Inoc	243.09±1.72	245.63±2.50	248.91±0.77	204.42±2.29	207.41±1.63	209.50±2.24	165.66±1.03	168.61±0.28	172.03±0.70
14d	Ind. and Un-Inoc.	216.90±1.22	218.81±0.11	220.78±0.21	192.09±1.28	194.08±0.20	195.13±0.22	161.66±1.38	162.95±1.17	165.03±1.85
	Ind. and Inoc	245.11±0.93	248.35±0.88	251.94±1.45	205.97±1.90	208.45±1.38	211.55±1.09	184.64±1.29	188.42±1.13	192.83±1.96
	Allium sativum	5%	10%	15%	5%	10%	15%	5%	10%	15%
7d	Ind. and Un-Inoc.	166.92±0.69	168.92±0.64	170.31±1.35	148.17±1.10	149.71±1.51	151.02±1.14	123.87±0.08	125.10±0.11	126.95±0.96
	Ind. and Inoc	187.51±1.59	190.88±1.88	197.02±1.03	158.98±1.82	162.85±1.68	167.54±1.05	129.74±0.69	132.32±1.34	136.94±1.30
14d	Ind. and Un-Inoc.	168.37±1.20	169.85±1.11	171.37±0.54	149.52±0.68	153.39±1.70	157.54±1.26	125.74±1.03	127.65±0.99	132.60±1.25
	Ind. and Inoc	189.23±1.97	194.44±2.23	202.88±2.16	161.08±1.83	165.40±2.42	173.51±1.77	142.83±1.27	146.55±1.02	152.00±1.60

HSD value for treatments = 7.290397
HSD value for doses = 0.462741
HSD value for varieties = 3.701927

Table 2. Mean comparison (± S.E) of zinc content (ppm) in cultivars C-44, Pb-91 and Bittle-98 after 7 and 14 days of the treatments with resistance inducers (chemicals and plant extracts)

Treatments		Cultivars								
		C-44			Pb-91			Bittle-98		
		Doses (Mm)								
	Salicylic acid	0.5	1.0	1.5	0.5	1.0	1.5	0.5	1.0	1.5
7d	Ind. and Un-Inoc.	58.97±1.53	66.31±1.11	72.10±0.91	44.67±1.10	49.47±0.45	54.82±1.37	32.32±0.57	38.29±0.95	42.51±0.62
	Ind. and Inoc	86.59±1.13	92.50±1.20	94.14±1.28	64.66±1.12	70.78±1.04	79.06±1.16	50.32±0.45	55.54±0.73	61.25±0.54
14d	Ind. and Un-Inoc.	61.53±1.23	67.18±1.09	76.58±1.29	45.88±1.32	51.78±0.91	57.14±0.97	33.61±1.23	40.63±0.87	43.93±0.83
	Ind. and Inoc	90.50±1.25	95.89±2.04	99.41±0.31	69.62±1.24	76.63±1.19	82.20±1.19	52.07±1.00	59.28±0.87	67.54±1.26
	Bion	0.4	0.8	1.2	0.4	0.8	1.2	0.4	0.8	1.2
7d	Ind. and Un-Inoc.	61.53±1.86	67.18±1.09	76.58±1.29	47.73±0.70	52.45±0.44	58.33±0.60	33.93±2.65	40.63±2.64	44.22±2.33
	Ind. and Inoc	91.50±0.72	98.22±0.48	99.79±2.37	70.29±0.61	76.96±0.91	83.20±0.58	53.40±0.55	62.25±0.54	69.64±0.45
14d	Ind. and Un-Inoc.	68.09±1.88	71.13±0.58	82.05±1.89	49.33±2.01	56.82±0.77	61.25±0.64	38.26±0.58	44.62±0.41	48.33±0.38
	Ind. and Inoc	95.47±0.66	100.20±0.61	104.23±0.57	73.50±0.69	80.48±0.43	87.07±0.73	58.18±0.60	68.07±0.71	72.97±0.91
	KOH	25	50	75	25	50	75	25	50	75
7d	Ind. and Un-Inoc.	29.49±0.76	33.16±0.99	36.05±1.03	22.34±0.54	24.73±0.23	27.41±0.68	16.16±0.29	19.15±0.47	21.25±0.30
	Ind. and Inoc	43.30±1.41	46.25±1.17	47.07±1.20	32.33±1.45	35.39±0.52	39.53±1.92	25.16±0.80	27.77±0.36	30.63±0.27
14d	Ind. and Un-Inoc.	30.77±1.11	33.59±0.99	38.29±1.05	22.94±1.36	25.89±1.03	28.57±0.48	16.81±0.61	20.32±0.43	21.97±1.11
	Ind. and Inoc	44.92±1.49	47.95±1.02	49.70±1.25	34.81±1.11	38.32±0.59	41.10±0.59	26.04±1.07	29.64±0.84	33.77±0.62
	Azadirachta indica	5%	10%	15%	5%	10%	15%	5%	10%	15%
7d	Ind. and Un-Inoc.	19.16±0.49	21.55±0.36	23.43±0.29	14.52±0.35	16.08±0.14	17.82±0.44	10.50±0.18	12.44±0.31	13.82±0.20
	Ind. and Inoc	28.14±0.36	29.40±0.70	30.26±0.68	21.02±0.36	23.34±0.62	25.69±0.37	15.35±0.66	18.05±0.23	19.91±0.75
14d	Ind. and Un-Inoc.	20.00±0.40	21.84±0.35	24.89±0.42	14.91±0.43	16.83±0.30	18.57±0.31	10.92±0.40	13.20±0.28	14.27±0.26
	Ind. and Inoc	29.41±0.83	31.16±0.66	33.97±1.38	22.63±0.40	27.57±0.69	30.05±1.07	17.26±0.62	21.60±0.54	26.62±0.68
	Datura metel	5%	10%	15%	5%	10%	15%	5%	10%	15%
7d	Ind. and Un-Inoc.	12.33±0.32	13.86±0.23	15.07±0.19	9.34±0.23	10.68±0.32	11.80±0.41	7.09±0.33	8.67±0.65	9.55±0.70
	Ind. and Inoc	18.44±0.56	20.67±0.45	24.02±0.96	13.85±0.21	15.80±0.52	18.53±0.81	11.52±0.63	13.61±0.67	16.81±0.11
14d	Ind. and Un-Inoc.	12.86±0.26	14.05±0.22	16.01±0.26	9.59±0.27	11.83±0.83	13.61±0.67	7.69±0.87	9.49±0.75	10.51±0.55
	Ind. and Inoc	19.26±0.40	21.05±1.00	24.45±0.27	15.56±0.82	19.02±0.24	21.52±0.56	13.22±0.86	16.06±0.51	19.78±0.64
	Allium sativum	5%	10%	15%	5%	10%	15%	5%	10%	15%
7d	Ind. and Un-Inoc.	9.54±0.24	11.38±0.69	13.32±0.24	7.55±0.47	8.66±0.65	9.86±0.22	5.22±0.09	6.52±0.48	7.53±0.57
	Ind. and Inoc	14.66±0.65	17.62±0.82	21.55±0.70	12.12±0.86	17.11±1.05	28.44±1.06	8.47±0.34	9.98±0.52	14.23±0.59
14d	Ind. and Un-Inoc.	10.61±0.81	14.19±0.39	14.77±0.68	8.08±0.83	10.04±1.02	13.24±0.69	7.10±1.05	10.23±0.43	11.76±0.32
	Ind. and Inoc	15.96±0.84	19.83±1.03	25.40±0.62	15.92±0.24	22.39±1.26	32.29±0.77	11.75±0.81	16.25±0.75	19.91±0.80

HSD value for treatments = 2.087515
HSD value for doses = 0.238958
HSD value for varieties = 0.348339

Table 3. Mean comparison (± S.E) of copper content (ppm) in cultivars C-44, Pb-91 and Bittle-98 after 7 and 14 days of the treatments with resistance inducers (chemicals and plant extracts)

Treatments		Cultivars								
		C-44			Pb-91			Bittle-98		
		Doses (Mm)								
	Salicylic acid	0.5	1.0	1.5	0.5	1.0	1.5	0.5	1.0	1.5
7d	Ind. and Un-Inoc.	103.95±2.20	110.94±3.17	113.82±2.84	26.76±0.52	30.29±0.43	32.55±0.62	18.30±0.63	23.00±0.85	28.38±1.14
	Ind. and Inoc	71.95±0.80	77.51±0.93	81.79±0.81	53.14±1.28	60.59±1.46	66.88±1.34	27.84±0.63	29.80±0.59	35.92±1.25
14d	Ind. and Un-Inoc.	103.70±2.54	114.9±2.67	122.13±2.44	28.66±0.51	34.47±0.89	38.22±0.67	24.29±0.96	27.89±0.70	31.74±0.87
	Ind. and Inoc	73.19±1.00	79.87±0.48	86.03±0.93	59.39±1.12	64.29±1.58	71.66±1.05	31.97±0.78	40.62±1.42	43.43±1.39
	Bion	0.4	0.8	1.2	0.4	0.8	1.2	0.4	0.8	1.2
7d	Ind. and Un-Inoc.	104.41±2.50	115.36±2.76	123.43±5.14	28.66±0.51	34.47±0.89	39.22±0.58	28.56±0.36	33.40±0.48	38.48±0.55
	Ind. and Inoc	72.85±1.53	80.09±3.18	87.34±1.68	60.14±0.96	65.57±0.58	73.40±0.50	40.62±0.68	43.43±0.39	48.17±0.54
14d	Ind. and Un-Inoc.	109.16±3.18	119.25±3.24	126.20±3.24	31.72±1.14	37.47±0.74	42.23±0.64	31.27±0.65	36.50±0.57	41.13±0.57
	Ind. and Inoc	75.38±0.62	84.24±2.16	90.99±2.63	64.36±0.52	70.19±1.75	77.19±1.69	47.35±0.61	50.25±0.58	53.29±0.52
	KOH	25	50	75	25	50	75	25	50	75
7d	Ind. and Un-Inoc.	35.98±0.40	38.75±0.46	40.90±0.40	13.38±0.26	15.15±0.21	16.28±0.31	9.15±0.31	11.50±0.42	14.19±0.57
	Ind. and Inoc	51.59±0.92	55.47±2.06	56.91±1.68	26.57±0.64	30.30±0.73	33.44±0.42	13.92±0.31	14.90±0.29	17.96±0.62
14d	Ind. and Un-Inoc.	36.59±0.50	39.94±0.53	43.01±0.86	14.33±0.25	17.23±0.44	19.11±0.33	12.15±0.48	13.94±0.35	15.87±0.43
	Ind. and Inoc	51.85±0.51	56.81±1.10	61.73±1.29	29.70±0.55	32.14±0.50	35.83±0.52	15.98±0.39	20.31±0.34	21.72±0.19
	Azadirachta indica	5%	10%	15%	5%	10%	15%	5%	10%	15%
7d	Ind. and Un-Inoc.	33.54±1.45	36.72±1.84	39.99±1.18	9.70±0.70	12.51±0.65	15.91±0.89	5.95±0.20	7.48±0.27	9.22±0.37
	Ind. and Inoc	23.39±0.54	25.19±0.30	26.58±1.48	17.27±0.42	25.03±1.30	29.07±0.92	9.05±0.20	9.69±0.19	11.67±0.41
14d	Ind. and Un-Inoc.	34.70±1.33	37.36±1.27	42.02±1.65	12.98±0.99	17.53±0.57	23.75±0.98	7.89±0.31	9.06±0.22	10.31±0.28
	Ind. and Inoc	24.45±0.92	26.29±0.46	29.62±0.57	19.63±0.55	29.89±1.12	35.63±0.76	10.72±0.44	13.87±0.88	17.78±0.77
	Datura metel	5%	10%	15%	5%	10%	15%	5%	10%	15%
7d	Ind. and Un-Inoc.	15.04±0.17	16.20±0.19	17.10±0.17	5.93±0.42	7.00±0.36	9.14±0.38	3.82±0.13	5.47±0.74	5.93±0.23
	Ind. and Inoc	21.57±0.30	23.86±0.91	26.13±1.08	11.78±0.53	14.33±0.59	18.32±0.79	7.82±0.51	8.90±0.43	11.51±0.82
14d	Ind. and Un-Inoc.	15.64±0.44	17.70±0.54	18.65±0.52	6.66±0.59	8.543±0.54	10.08±1.01	5.74±0.82	5.83±0.14	7.63±0.72
	Ind. and Inoc	22.35±1.11	25.37±1.16	29.53±1.12	13.08±0.23	15.44±0.67	21.65±0.65	10.35±1.00	13.83±1.02	18.41±0.74
	Allium sativum	5%	10%	15%	5%	10%	15%	5%±	10%	15%
7d	Ind. and Un-Inoc.	11.30±0.45	12.86±0.66	13.55±0.34	4.66±0.35	5.90±0.60	7.93±0.27	3.29±0.35	4.38±0.46	5.92±0.84
	Ind. and Inoc	17.35±1.07	19.60±0.75	20.40±1.22	8.92±0.25	10.46±0.45	13.47±0.43	7.83±0.42	9.48±0.37	12.47±0.53
14d	Ind. and Un-Inoc.	12.50±0.73	13.58±0.80	14.57±0.73	5.30±0.36	7.24±0.25	8.84±0.23	4.59±0.33	5.51±0.52	8.13±0.14
	Ind. and Inoc	18.43±0.73	24.92±1.06	28.74±1.09	10.27±0.24	12.06±1.29	16.25±1.46	9.17±0.51	12.57±0.68	15.02±0.51

HSD value for treatments = 8.242425
HSD value for doses = 0.355438
HSD value for varieties = 1.557818

Copper greatly influenced by many plant diseases by increasing or decreasing their symptoms but the role of copper in resistance and susceptibility is overlooked in some cases (Chmielowska *et al.,* 2010). Copper cations were found to be involved in the synthesis of solanapyrone A, B and C along with other mineral elements. After having the information about the involvement of Cu cations in the formation of phytotoxins and ultimate disease condition, it would be easy to deduce that the induced plants upon inoculation had higher content of Cu in Pb-91 and Bittle-98 but less in C-44, so would easily be in a position to spare the required amount of cations for the formation of phytotoxins. This was most likely due to the involvement of Cu cations in the formation of phytotoxins as already reported (Chen and Strange, 1991). The present results are in line with (Randhawa, 1994) who reported decreased copper in chickpea cultivars resistant to *A. rabiei* and increased copper content in susceptible cultivars as the cultivars under study were susceptible (Ghazanfar *et al.,*2010). However, in the cultivar C-44 the copper contents decreased after inoculation with *A. rabiei* in salicylic acid, Bion and neen treated plants. The reason of this response is not known although the stress copper plants are less symptomatic to disease (Chmielowska *et al.,*2010). Thus more research is needed at molecular level to explore this phenomenon. The results reported by (Yardımcı *et al.,* 2007) showed that healthy alfalfa plant leaves contained more Cu contents (48.50 ppm) as compared to infected one. Wheat variety Kenya was less susceptible to mildew, *E. graminis* grown on copper deficient sand culture as compared to more susceptibility when grown on boron deficient and fairly resistant to infection when grown upon a balanced nutrient solution (Schutte, 1967).

The results of our study have showed that zinc contents increased in both the induced inoculated and non inoculated plants of chickpea and reduced the chickpea blight disease severity (Ghazanfar *et al.,* 2011). The reason behind this reduction in disease was that Zn plays an important role in maintaining the integrity or stability of the host plant's membranes (Thongbai *et al.,* 1993). On the other hand, Zinc-deficient plants contain low levels of superoxide dismutase (Cakmak and Marschner, 1988a) and, therefore, high levels of superoxide radicals, leading to peroxidation of membrane lipids, loss of membrane integrity and increased membrane permeability (Cakmak and Marschner, 1988b; Welch and Norvell, 1993; Welch *et al.,*1982). Zinc as an activator of Cu/Zn-SOD, is involved in protection against oxidative damage by the detoxification of superoxide radicals (Cakmak, 2000). There is invariable response to the level of Zn in decreasing or increasing or no effect towards plant susceptibility to disease (Grewal *et al.,* 1996). The application of Zn reduced disease severity due to its toxic effect directly to pathogen and not through the plant's metabolism (Graham and Webb, 1991). Zinc also plays a role in the synthesis of protein and starch (Römheld and Marschner, 1991). Application of Zn to the soil reduced infections by *Fusarium graminearum* (Schwabe) and root rot diseases, e.g. caused by *Gaeumanomyces graminis* (Sacc.) in wheat (Grewal *et al.,* 1996). The results of the present findings are in line of (Sahi, *et al.,* 2010) who found that zinc content of susceptible lentil lines was higher than that of the resistant ones prior to inoculation with the pathogen and it even increased in both the groups with the increase being more pronounced in case of susceptible group. These results are similar to those recorded by (Randhawa, 1994) in case of chickpea blight caused by *A. rabiei* interaction but opposite to those obtained by (Reddy and Khare, 1984) in lentil rust (*Uromyces fabae)* interaction. Zinc has also been reported to completely inhibit the mycelial growth of *Aspergillus carneus* and *A. ellipticus* at 500 mg L^{-1} (Moslem and Parvez, 1992). The results of (Streeter *et al.,* 2001) are also in line with the present finding that Zn-sufficient plants are more tolerant to the effects of root pruning by the fungus than Zn-deficient plants.

CONCLUSION

As far as the estimation of findings towards mineral contents of induced un-inoculated and induced inoculated of three chickpea cultivars are concerned; only one paper (El-Khallal, 2007) was available with more relevant study. Other studies (Sahi *et al.,* 2007, 2010) although related with the present experiments but effect of induced resistance by the use of chemicals and plant extracts on Mn, Cu, and Zn was not properly explored. This is the maiden attempt toward the role of these micronutrients in chickpea blight. In future, molecular level studies are needed to get an insight into the mechanisms.

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Muhammad U. Ghazanfar	:	Conceive idea of research write original manuscript.
Waqas Raza	:	Conduct research and collection of data
Waqas Wakil	:	Edited manuscript
Muhammad K. Bashir	:	Proof read the manuscript.