



Research Article

Influence of Resistance Inducers on Nitrogen, Phosphorus and Potassium Contents of Susceptible Chickpea Cultivars after Inoculation with *Ascochyta rabiei*

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Abstract | Adequate nutrition plays a very important role in mitigating disease damage. Nevertheless, no specific rule exists that how a specific nutrient can alter the severity of the disease. Normally, plants are stressed by different environmental confines may be weakened and susceptible to disease and these include nutrient scarce plants. Tissue contents of potassium, nitrogen, and phosphorus of above parts of the ground of plant in three cultivars of chickpea ('C-44', Bittle-98' and 'Pb-91') after treatment with Bion® (acibenzolar-S-methyl), salicylic acid (SA), potassium hydroxide (KOH) or plant extracts of neem (*A. indica* A. Juss.), datura (*D. metel* L.) and garlic (*A. sativum* L.) were investigated 7 and 14 days after inoculation with *Ascochyta rabiei*. Elevated nitrogen (2.95%), potassium (1.05%) and potassium (533.00 ppm) contents in the tissues decreased disease severity (79.3%) in cv. C-44. These increases were significant ($P \leq 0.05$) after 14 days of inoculation with plant pathogen in plants pretreated with chemicals. There was no significant change in plants treated with the plant extracts except with extracts of *A. indica*. Chemically increased N, P, and K were highest in cultivar C-44 followed by Pb-91 and Bittle-98. A similar trend was observed with plant extract treated plants, with the greatest effect observed with *A. indica* extracts. The results indicate that chemicals reported to induce resistance to pathogens may increase mineral contents which prevent the spread of the pathogen.

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Introduction

Chickpea (*Cicer arietinum* L.) is susceptible to different factors (biotic and a-biotic) that influence the production of the crop. Chickpea blight disease caused by the pathogen, *Ascochyta rabiei* (Pass) Labr, may result in up to 70% crop losses (Nene, 1984; Malik and Bashir, 1984) when environmental conditions are favorable for the disease. Although plant disease can be controlled by a range of cultural

practices (Sattar, 1933; Luthra and Bedi, 1935) and chemo-therapentants (Tripathi *et al.*, 1987; Singh and Singh, 1990), cultivation of resistant cultivars is mainly valuable and cost-effective practices (Nene and Reddy, 1987). Plant resistance to an infectious organism is governed by a lot of biochemical metabolites through which enzyme-catalyzed biochemical reactions (Tenhaken and Barz, 1991) depend on the accessibility of definite anion; cation, and enzymes (Chen and Strange, 1991). Nutrients

play a very vital task in the development of plants and micro-organisms, so considered a significant factor for disease control strategies (Agrios, 2005). The severity of the disease is affected by all of the essential nutrients (Huber and Graham, 1999), and balanced nutrition necessary for any host plant to be resistant to pathogens (Filippi and Prabhu, 1998). The significance of plant nutrients in disease management strategy has been documented for many years but the accurate management of nutrients to manage some of the most severe diseases for sustainable agriculture have expected very slight attention (Huber and Graham, 1999).

Nitrogen is an important macro-nutrient obligatory for the normal development of plant parts (Chandra and Mishra, 1991). Nitrogen is an indispensable constituent of many metabolites like proteins, amino acids, phytochrome, nucleic acid, and chromosomes (Marschner, 1995). A deficiency of N results in impaired growth, reduction in protein synthesis, and inhibition of photosynthesis (Stitt and Krapp, 1998). Phosphorus is an integral part of many organic molecules such as cellular DNA, RNA, and ATP. It is also concerned with various metabolic processes in plants and pathogens but its task in resistance is unpredictable and not consistent (Kiraly, 1976). Potassium in plants has involved in various functions such as stomatal movement, enzyme activation, and turgor regulations (Peoples and Koch, 1979). Plants have deficient in potassium accumulate a considerable quantity of organic compounds because they function as an osmoticum in lack of adequate nutrients (potassium). High potassium status generally decreases the incidence of pests and disease% of the studies reported to decrease fungal and bacterial diseases due to a balanced K supply (Fuchs and Grossmann, 1972). The beneficial effect of potassium was instrumental in 69-70 (Amtmanns *et al.*, 2009).

Plant leaf extracts of *Azadirachta indica*, *Datura metal*, and *Allium sativum* have shown to be effective against *Alternaria* leaf spot, *Rhizoctonia solani* and *Alternaria solani* (Kagale *et al.*, 2004; Guleria and Kumar, 2006; Slusarenko *et al.*, 2008) by inducing resistance.

Minerals play a key role in different biochemical reactions concerned in resistance or susceptibility against various plant pathogens, study intended to conclude the influence of different treatments and inoculation with pathogen, *A. rabiei*, on the mineral

content of chickpea cultivars differing in resistance to this pathogen.

Materials and Methods

Climatic conditions

The temperature range between 8 to 12°C during the night and 14 to 35°C through the day with 10-h light period. The humidity ranges from 60-70%.

Plant material and experimental design

Seeds of three chickpea cultivars (C-44, Bittle-98 and Pb-91) that are susceptible to *A. rabiei* were cultivated in small plots of 6.0 m² in the field conditions. The experiment was a planned split plot design with varieties in the central plot, doses in subplots, and inducers in subplots with three replications. Each plot had six (6) rows and fifteen (15) chickpea plants per row.

Preparation of plant extracts

Plant extracts e.g, datura (*Datura metel* L.), garlic (*Allium sativum* L.), and neem (*Azadirachta indica* Juss.) were prepared from leaves collected from research areas from UAF, Pakistan while cloves of garlic were purchased from the local market. The leaves of selected plants were then washed in tap water, and rinsed with sterilized water before homogenizing 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 and surface-sterilizing for 1 min in ethanol solution (70%) and washed in sterile double distilled water. The cloves were firm to a pulp and suspended in 100 ml distilled water and then filtered via muslin cloth. All extracts were heated at 40°C for almost ten (10) mint. to avoid contamination and diluted to different concentrations, 5, 10 or 15% with double distilled water (v/v) (Jaganathan and Narasimhan, 1988).

Resistance inducers 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 applied.

Application of inducers and challenge inoculation with A. rabiei

At early flowering, the chemicals and extract of plants were sprayed on plants until runoff while the check (control) plants were sprayed only with double

distilled water. Pathogen inoculum was prepared and the conidial concentrations were adjusted with a hemocytometer (Ilyas and Khan, 1986). Four days after treatment, the plants were sprayed to run off with spore suspension of the plant pathogen, *A. rabiei* (1×10^5 spores L^{-1}) that contained Tween 80 (3 drops/liter) as a wetting agent in the late evening because the temperature was lesser at night to give better germination of spores (conidia). Spray inoculation continued for three days to guarantee maximum infection of plants. The plants were periodically sprayed with water (H_2O) to maintain the moisture and favor germination of conidia.

Processing of plant samples

Shoot samples from treated inoculated and un-inoculated chickpea plants of the three (3) cultivars were collected 7 and 14 days after inoculation at which time symptoms were fully developed on the inoculated control plants. The shoots were washed with a detergent solution (0.2%) to remove dirt, washed in HCl (0.8%) to get rid of metallic contaminants, and then washed in deionized water to eliminate the prior two solutions. The samples were dried up on paper towels in the shade and placed in paper bags before drying in the oven for 72h at $70^\circ C$ to get constant weight. These samples were ground with the grinder and then analyzed Nitrogen, Phosphorus, and potassium (Bhargava and Raghupathi, 1995; Karla and Maynard, 1991). Nitrogen and Phosphorus were recorded as a percentage of dry weight and potassium as ppm.

Statistical analysis

Experimental data were evaluated by ANOVA and the means were separated by Tukey's HSD test at 5% level of significance (Steel *et al.*, 1997) using the software R. 2.12.1 (2008). The experiment was repeated three times independently to overcome the pseudo replications.

Results and Discussion

In a preliminary experiment, resistance was induced in three chickpea cultivars by the application of Bion® (acibenzolar-S-methyl), salicylic acid (SA), potassium hydroxide (KOH), and plant extracts of garlic, neem, and datura. The data recorded considerable disease fall (79.3%) was observed by Bion® in the cv. C-44 (@1.2 mM (Figure 1B) compared to SA (salicylic acid), however, the least was observed by potassium hydroxide. Among different plant extracts tested,

the utmost disease reduction (43.5%) against the disease was seen after application of *A. indica* (Figure 1D) and other extracts, *D. metel* and *A. sativum* did not attest effective to reduce the disease.

Nitrogen

Salicylic acid significantly increased ($P \leq 0.05$) nitrogen in inoculated plants (Table 1). This increase was highest in cultivar C-44 (2.95 %) intermediate in Pb-91 (2.65 %) and the least in Bittle-98 (2.51 %). In all the three cultivars, the increase in nitrogen was highest with the highest rate of salicylic acid and inoculation with the pathogen. Bion (with 1.2mM) increased N to 2.86 % in C-44, 2.76 % in Pb-91 and 2.48 % in Bittle-98 14 days after treatment in inoculated plants. KOH increased nitrogen in the three cultivars the least (1.48, 1.3, and 1.26 % in C-44, Pb-91, and Bittle-98, respectively) (Table 1). Plant extracts did not significantly increase nitrogen. Overall, the application of chemicals increased nitrogen more than the plant extracts with the increase more pronounced by salicylic acid at the highest rate tested and the least with by KOH in cultivar C-44.

Phosphorus

The phosphorus contents (Table 2) of chemically treated inoculated and un-inoculated plants of three chickpea cultivars were notably different ($P \leq 0.05$). Generally, the phosphorus in all three inoculated cultivars was increased 14 days after treatment by the higher rate of chemicals and plant extracts except with KOH where P in the treated, un-inoculated plants was higher (0.51, 0.63, and 0.78 %) compared to treated inoculated plants (0.35, 0.45 and 0.49 percent) of cultivar C-44 but opposite in Pb-91 and Bittle-98. The plant extracts did not appreciably change the amount of phosphorus in the three chickpea cultivars.

Potassium

Potassium in the chemically treated, inoculated C-44 cultivar ranged from 480.8 to 527.86 ppm and was significantly higher than salicylic acid treated un-inoculated plants (Table 3). Cultivars Pb-91 and Bittle-98 also showed the same trend with a maximum of 310.1 ppm and 158.3 ppm potassium 14 days after treatment and inoculation. The highest K (533.0 ppm) was with the highest rate of Bion, The potassium in Pb-91 and Bittle-98 was also increased by Bion and inoculation. The lowest potassium (79.16 ppm) was in Bittle-98 which was significantly lower than Pb-91 and C-44 after application of KOH and

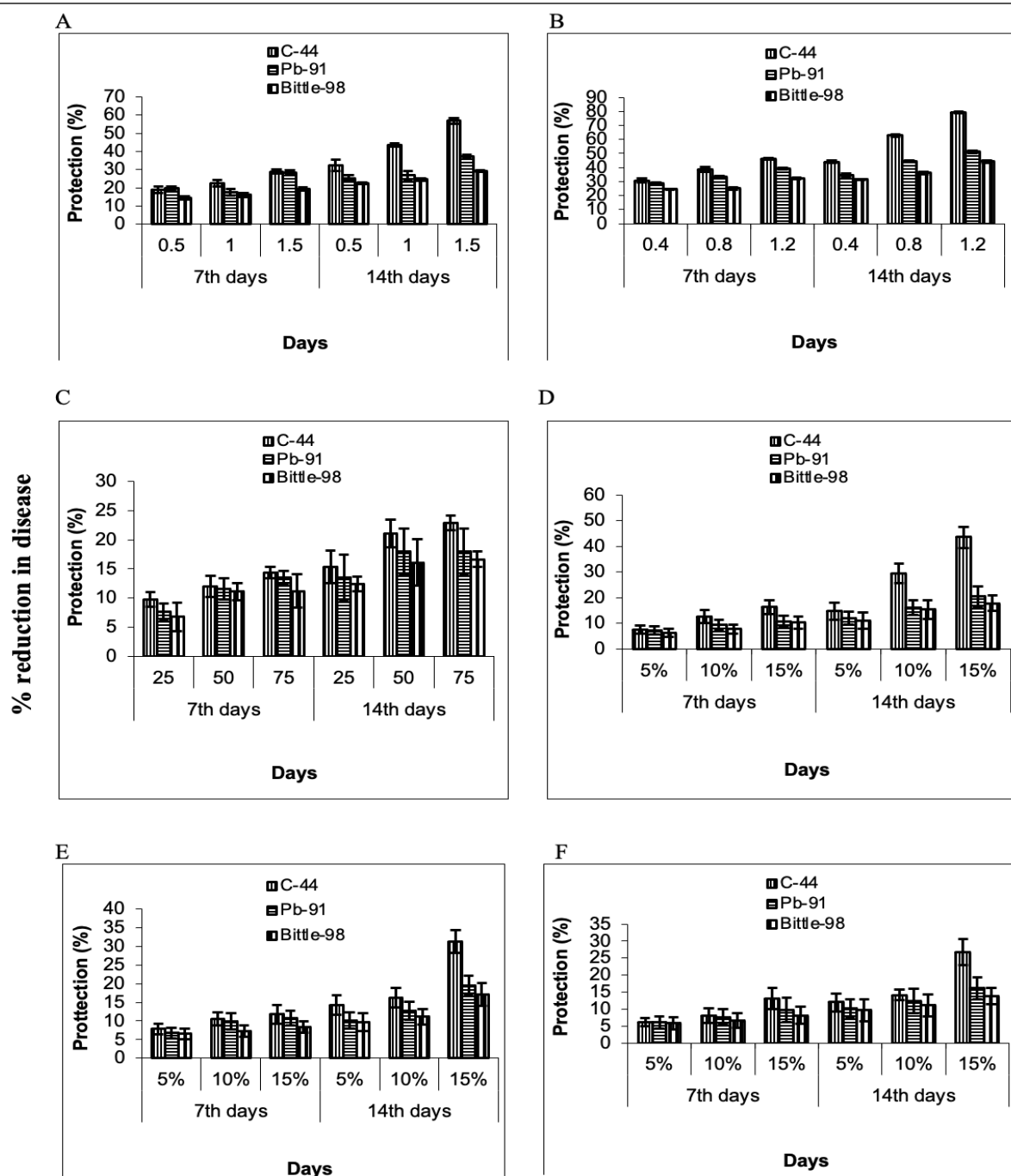


Figure 1: Mean comparison of % reduction in *Ascochyta* blight disease by the application of (A) salicylic acid (B) Bion (C) KOH (D) Neem (E) Datura (F) Garlic in three chickpea cultivars. Data are means of three repeated experiments. The error bar represents the standard error of the mean according to the least significant difference test at $P \leq 0.05$.

inoculation with *A. rabiei*. Increased Potassium also was observed in chemically treated un-inoculated plants. Plant extracts also increased potassium in plant tissues but too much lower levels compared to the chemicals. The potassium in C-44 (174.89 ppm) was highest 14 days after treatment with neem extract compared with the treated un-inoculated C-44 plant (69.4 ppm) (Table 3). The lowest potassium (29.2 ppm) was found in Bittle-98 treated with the highest rate of garlic extract.

The nutritional status and availability influence the

development and metabolisms of the plant as well as their level of susceptibility or resistance to pathogens (Krauss, 1999; Ruan and Wu, 2000). Successful invasion of pathogens not only depends upon their ability to evade defense mechanisms but also to utilize the nutrients available in plants (Solomon *et al.*, 2003). In the current study, we investigated the impact of several chemicals reported to induce disease resistance on nitrogen, phosphorus, and potassium content after inoculation with *A. rabiei* in three chickpea cultivars. We found that nitrogen increased and that was more pronounced after inoculation with

A. rabiei. Although the varieties under study were susceptible to *Ascochyta* blight yet by the application of resistance inducers they showed a reduction in the disease with increase nitrogen contents (Ghazanfar *et al.*, 2010). The reasons for this increase might be due to fungal autolysis, increased nitrogen assimilation, increased flux from intercellular sources, and increased apoplast protease activity (Soloman and Oliver, 2001). In earlier studies, the effect of nitrogen on disease progress was variable but the causes of this irregularity are inadequately understood (Hoffland *et al.*, 2000). The results presented here are consistent with studies of Randhawa (1994) who reported an increase of

nitrogen in chickpea cultivars after inoculation with *A. rabiei*. Comparable results also have been reported by Soloman and Oliver (2001) in describing higher total nitrogen in tomato leaves after inoculation with *Cladosporium flavum*. Nitrogen increased in lentil plants after inoculation with *A. lentis* and this increase was higher in the susceptible clusters compare with the resistant ones as described by Sahi *et al.* (2007). The supply of nitrogen greatly influences the activity of three resistance-related enzymes: Chitinases, chitosanases, and preoxidases, and their level were much lower in plants cultivated under limited Nitrogen than high nitrogen conditions (Dietrich *et al.*, 2004).

Table 1: Mean comparison (\pm S.E) of nitrogen content (% dry weight) 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.	1.64±0.022	1.88±0.008	1.97±0.008	1.42±0.017	1.46±0.013	1.56±0.021	1.14±0.025	1.21±0.017	1.47±0.008
	Ind. and Inoc	2.33±0.007	2.55±0.030	2.77±0.021	2.30±0.053	2.40±0.056	2.61±0.008	2.18±0.005	2.30±0.054	2.46±0.006
14d	Ind. and Un-Inoc.	1.78±0.013	1.93±0.025	1.99±0.064	1.49±0.005	1.51±0.007	1.62±0.051	1.40±0.004	1.45±0.011	1.51±0.003
	Ind. and Inoc	2.37±0.053	2.59±0.055	2.95±0.027	2.33±0.038	2.46±0.050	2.65±0.027	2.31±0.016	2.36±0.012	2.51±0.006
Bion		0.4	0.8	1.2	0.4	0.8	1.2	0.4	0.8	1.2
7d	Ind. and Un-Inoc.	2.22±0.013	2.22±0.008	2.32±0.015	1.51±0.011	1.63±0.02	1.71±0.007	1.40±0.023	1.49±0.024	1.53±0.016
	Ind. and Inoc	2.67±0.011	2.70±0.023	2.75±0.026	2.46±0.016	2.65±0.03	2.71±0.009	2.13±0.031	2.25±0.020	2.45±0.019
14d	Ind. and Un-Inoc.	2.22±0.014	2.35±0.017	2.37±0.018	1.57±0.013	1.70±0.02	1.76±0.011	1.49±0.019	1.51±0.017	1.60±0.027
	Ind. and Inoc	2.68±0.010	2.81±0.012	2.86±0.013	2.54±0.027	2.68±0.01	2.76±0.013	2.31±0.041	2.36±0.013	2.48±0.061
KOH		25	50	75	25	50	75	25	50	75
7d	Ind. and Un-Inoc.	0.82±0.006	0.94±0.016	0.99±0.009	0.71±0.012	0.73±0.014	0.78±0.006	0.57±0.010	0.61±0.024	0.74±0.015
	Ind. and Inoc	1.17±0.004	1.28±0.012	1.39±0.014	1.15±0.031	1.20±0.047	1.31±0.013	1.09±0.011	1.15±0.008	1.23±0.019
14d	Ind. and Un-Inoc.	0.89±0.011	0.97±0.010	1.00±0.021	0.75±0.023	0.76±0.007	0.81±0.008	0.70±0.021	0.73±0.011	0.76±0.013
	Ind. and Inoc	1.19±0.013	1.30±0.027	1.48±0.017	1.17±0.014	1.23±0.016	1.33±0.011	1.16±0.013	1.18±0.013	1.26±0.017
A. indica		5%	10%	15%	5%	10%	15%	5%	10%	15%
7d	Ind. and Un-Inoc.	0.53±0.01	0.61±0.005	0.64±0.013	0.46±0.011	0.47±0.006	0.51±0.009	0.37±0.010	0.40±0.004	0.48±0.011
	Ind. and Inoc	0.76±0.01	0.82±0.016	0.91±0.021	0.74±0.017	0.78±0.008	0.86±0.021	0.71±0.014	0.75±0.017	0.80±0.018
14d	Ind. and Un-Inoc.	0.57±0.02	0.63±0.014	0.66±0.014	0.49±0.019	0.50±0.011	0.53±0.013	0.46±0.011	0.47±0.008	0.49±0.009
	Ind. and Inoc	0.78±0.01	0.84±0.017	0.96±0.011	0.76±0.012	0.81±0.016	0.88±0.017	0.76±0.013	0.78±0.013	0.82±0.014
D. metel		5%	10%	15%	5%	10%	15%	5%	10%	15%
7d	Ind. and Un-Inoc.	0.34±0.002	0.39±0.011	0.41±0.005	0.30±0.004	0.30±0.011	0.33±0.016	0.23±0.007	0.26±0.004	0.32±0.012
	Ind. and Inoc	0.48±0.008	0.54±0.010	0.59±0.011	0.48±0.009	0.51±0.017	0.55±0.006	0.45±0.011	0.48±0.011	0.52±0.017
14d	Ind. and Un-Inoc.	0.37±0.011	0.40±0.008	0.42±0.010	0.30±0.012	0.32±0.014	0.34±0.005	0.29±0.005	0.31±0.008	0.33±0.009
	Ind. and Inoc	0.50±0.013	0.57±0.006	0.64±0.002	0.50±0.005	0.53±0.008	0.56±0.015	0.49±0.003	0.50±0.017	0.53±0.021
A. sativum		5%	10%	15%	5%	10%	15%	5%	10%	15%
7d	Ind. and Un-Inoc.	0.26±0.006	0.30±0.011	0.32±0.007	0.21±0.010	0.24±0.013	0.26±0.011	0.17±0.004	0.20±0.010	0.24±0.012
	Ind. and Inoc	0.39±0.004	0.43±0.008	0.47±0.015	0.38±0.012	0.39±0.008	0.43±0.013	0.34±0.011	0.38±0.013	0.40±0.013
14d	Ind. and Un-Inoc.	0.28±0.010	0.33±0.005	0.34±0.019	0.23±0.006	0.26±0.021	0.27±0.009	0.24±0.013	0.25±0.011	0.25±0.010
	Ind. and Inoc	0.40±0.012	0.44±0.010	0.50±0.016	0.38±0.014	0.45±0.018	0.48±0.014	0.38±0.014	0.39±0.012	0.43±0.021

HSD value for treatments = 0.02912; HSD value for doses= 0.00090; HSD value for varieties= 0.01494.

Table 2: Mean comparison (\pm S.E) of phosphorus content (% dry weight) 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.	0.40±0.008	0.58±0.011	0.73±0.019	0.33±0.006	0.44±0.012	0.54±0.006	0.16±0.002	0.25±0.004	0.34±0.010
	Ind. and Inoc	0.68±0.023	0.88±0.012	0.95±0.014	0.43±0.011	0.54±0.014	0.68±0.012	0.26±0.001	0.33±0.007	0.45±0.01
14d	Ind. and Un-Inoc.	0.51±0.013	0.63±0.014	0.78±0.011	0.38±0.012	0.47±0.004	0.62±0.019	0.18±0.011	0.26±0.011	0.40±0.009
	Ind. and Inoc	0.70±0.020	0.91±0.019	0.98±0.016	0.47±0.014	0.56±0.010	0.72±0.020	0.30±0.016	0.42±0.013	0.52±0.016
Bion		0.4	0.8	1.2	0.4	0.8	1.2	0.4	0.8	1.2
7d	Ind. and Un-Inoc.	0.54±0.011	0.65±0.013	0.73±0.018	0.38±0.003	0.49±0.002	0.57±0.010	0.18±0.002	0.28±0.006	0.40±0.010
	Ind. and Inoc	0.83±0.013	0.91±0.027	0.98±0.006	0.48±0.010	0.58±0.005	0.72±0.011	0.30±0.006	0.40±0.003	0.52±0.013
14d	Ind. and Un-Inoc.	0.62±0.005	0.69±0.023	0.76±0.011	0.41±0.004	0.52±0.003	0.60±0.020	0.22±0.003	0.32±0.010	0.46±0.005
	Ind. and Inoc	0.83±0.024	0.96±0.020	1.05±0.023	0.51±0.011	0.61±0.011	0.79±0.014	0.39±0.010	0.46±0.004	0.59±0.011
KOH		25	50	75	25	50	75	25	50	75
7d	Ind. and Un-Inoc.	0.20±0.003	0.29±0.004	0.36±0.009	0.17±0.004	0.22±0.006	0.27±0.020	0.08±0.001	0.13±0.002	0.17±0.006
	Ind. and Inoc	0.34±0.016	0.44±0.019	0.48±0.010	0.22±0.003	0.27±0.001	0.34±0.011	0.13±0.001	0.17±0.010	0.22±0.005
14d	Ind. and Un-Inoc.	0.51±0.021	0.63±0.023	0.78±0.013	0.19±0.001	0.24±0.002	0.31±0.017	0.09±0.002	0.13±0.003	0.20±0.011
	Ind. and Inoc	0.35±0.006	0.45±0.009	0.49±0.017	0.23±0.011	0.28±0.003	0.36±0.013	0.15±0.004	0.21±0.001	0.26±0.017
A. indica		5%	10%	15%	5%	10%	15%	5%	10%	15%
7d	Ind. and Un-Inoc.	0.12±0.002	0.19±0.001	0.24±0.010	0.11±0.003	0.14±0.006	0.18±0.010	0.05±0.001	0.08±0.001	0.11±0.002
	Ind. and Inoc	0.23±0.011	0.29±0.006	0.32±0.009	0.13±0.006	0.18±0.003	0.22±0.003	0.09±0.010	0.11±0.003	0.15±0.003
14d	Ind. and Un-Inoc.	0.33±0.003	0.41±0.020	0.51±0.013	0.12±0.010	0.15±0.014	0.20±0.006	0.06±0.001	0.09±0.001	0.13±0.010
	Ind. and Inoc	0.25±0.012	0.31±0.016	0.35±0.011	0.15±0.011	0.19±0.010	0.24±0.007	0.11±0.003	0.14±0.004	0.17±0.006
D. metel		5%	10%	15%	5%	10%	15%	5%	10%	15%
7d	Ind. and Un-Inoc.	0.08±0.001	0.12±0.010	0.16±0.003	0.07±0.001	0.09±0.002	0.11±0.010	0.04±0.001	0.06±0.001	0.08±0.001
	Ind. and Inoc	0.16±0.010	0.24±0.011	0.28±0.013	0.09±0.002	0.12±0.001	0.16±0.012	0.07±0.002	0.14±0.010	0.16±0.010
14d	Ind. and Un-Inoc.	0.14±0.001	0.18±0.006	0.21±0.010	0.09±0.001	0.10±0.006	0.14±0.006	0.07±0.001	0.11±0.004	0.15±0.006
	Ind. and Inoc	0.22±0.003	0.30±0.005	0.34±0.014	0.11±0.001	0.14±0.001	0.19±0.013	0.07±0.002	0.18±0.011	0.18±0.011
A. sativum		5%	10%	15%	5%	10%	15%	5%	10%	15%
7d	Ind. and Un-Inoc.	0.06±0.003	0.09±0.002	0.12±0.004	0.05±0.001	0.07±0.004	0.09±0.006	0.02±0.001	0.04±0.002	0.06±0.002
	Ind. and Inoc	0.14±0.009	0.19±0.013	0.25±0.014	0.08±0.010	0.10±0.009	0.15±0.013	0.05±0.002	0.08±0.003	0.16±0.011
14d	Ind. and Un-Inoc.	0.17±0.012	0.20±0.007	0.25±0.011	0.07±0.003	0.08±0.011	0.10±0.006	0.03±0.006	0.05±0.001	0.07±0.003
	Ind. and Inoc	0.17±0.011	0.22±0.013	0.28±0.016	0.08±0.002	0.13±0.014	0.17±0.011	0.06±0.003	0.10±0.010	0.19±0.009

HSD value for treatments = 0.0346; HSD value for doses= 0.00108; HSD value for varieties= 0.01454.

Contrary to these results, increased nitrogen made plants more susceptible to pathogens in different host-pathogen interactions (Nam *et al.*, 2006). Increasing nitrogen also increases lesion density with leaf rust (Robert *et al.*, 2005). There are many other reports of increased severity of diseases with an increase in the concentration of leaf nitrogen (Olesen *et al.*, 2003; Neumann *et al.*, 2004).

The orthophosphate ion (PO_4^{3-}) has been documented by Walters and Bingham (2007) very significant for all cells, as it is included in various important molecules such as ATP, nucleic acids, and phosphoproteins.

Perrenoud (1990) directed effect of phosphorus on pathogen survival, development and multiplication; it may alter plant metabolism to affect a pathogen's food supply, stomatal function and plant defense to influence a pathogen's establishment in the plant. Our results confirmed that phosphorus increased in both, treated inoculated chickpea plants and treated un-inoculated but it increased more after inoculation with *A. rabiei*. Increased phosphorus in treated inoculated plants indicates that there are an intact and consistent uptake and translocation of phosphorus since it is used to form different cell constituents like phospholipids, nucleic acids, the coenzymes NADP,

Table 3: Mean comparison (\pm S.E) of potassium 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.	167.85±1.19	184.11±2.11	200.96±0.98	131.91±1.23	145.44±2.34	164.44±3.25	68.91±1.46	85.00±2.46	94.93±2.52
	Ind. and Inoc	480.86±6.71	498.10±0.78	509.57±4.92	262.86±1.63	283.05±2.16	296.73±2.19	120.13±2.05	134.41±1.10	146.22±1.15
14d	Ind. and Un-Inoc.	182.67±2.38	195.96±2.93	213.58±2.44	141.01±3.38	154.34±2.54	164.79±2.96	79.56±0.98	92.26±1.49	102.00±2.82
	Ind. and Inoc	491.88±4.87	517.93±4.86	527.86±6.64	268.10±0.92	288.89±4.97	310.14±5.09	131.16±1.08	144.48±2.10	158.32±1.51
Bion		0.4	0.8	1.2	0.4	0.8	1.2	0.4	0.8	1.2
7d	Ind. and Un-Inoc.	182.63±1.89	190.96±3.20	204.37±2.63	141.59±0.71	154.47±2.81	168.31±0.54	71.59±0.89	92.37±0.67	103.62±0.71
	Ind. and Inoc	491.81±1.00	518.06±0.79	528.43±5.29	270.72±5.12	288.91±5.04	310.19±4.74	131.94±0.94	144.40±2.60	158.03±1.10
14d	Ind. and Un-Inoc.	189.24±0.90	195.84±0.80	207.05±0.76	144.66±0.53	159.14±0.67	172.97±0.80	76.97±0.71	98.24±0.51	107.19±0.85
	Ind. and Inoc	495.68±2.79	523.94±6.32	533.01±4.90	280.25±5.41	297.63±5.20	319.12±3.22	139.85±2.52	151.45±0.67	163.06±2.21
KOH		25	50	75	25	50	75	25	50	75
7d	Ind. and Un-Inoc.	83.93±1.60	92.06±1.05	100.49±1.52	65.96±1.57	72.73±1.62	82.22±1.46	34.46±0.50	42.50±1.62	47.47±0.64
	Ind. and Inoc	240.43±6.11	249.05±5.67	254.79±3.37	131.44±5.40	141.53±0.41	148.37±0.77	60.07±1.58	67.21±1.60	73.11±0.57
14d	Ind. and Un-Inoc.	91.34±0.59	97.98±1.01	106.79±3.19	70.51±0.48	77.18±0.36	82.40±1.48	39.78±1.53	46.13±1.49	51.01±0.50
	Ind. and Inoc	245.94±3.26	258.97±3.92	263.93±2.01	134.06±1.94	144.45±0.47	155.07±2.73	65.58±0.54	72.24±0.28	79.16±1.04
A. indica		5%	10%	15%	5%	10%	15%	5%	10%	15%
7d	Ind. and Un-Inoc.	54.55±0.73	59.83±0.80	65.31±0.97	42.87±1.32	47.27±0.77	49.82±0.30	22.40±0.33	27.63±0.27	30.85±0.41
	Ind. and Inoc	156.28±1.96	162.55±1.51	165.61±2.24	85.77±1.04	92.33±0.88	96.77±1.52	39.05±0.32	43.69±0.35	47.52±0.77
14d	Ind. and Un-Inoc.	59.37±0.89	63.69±1.57	69.41±0.36	45.83±0.31	50.17±0.24	53.56±1.56	25.86±0.32	29.99±0.71	33.16±0.32
	Ind. and Inoc	159.86±5.44	168.66±1.06	174.89±2.23	87.14±0.87	96.56±0.49	103.46±2.02	42.63±0.92	47.62±0.70	52.12±0.92
D. metel		5%	10%	15%	5%	10%	15%	5%	10%	15%
7d	Ind. and Un-Inoc.	35.10±0.19	38.83±0.50	42.35±0.50	26.58±0.76	29.75±0.86	34.38±0.28	14.08±0.50	17.44±0.35	19.85±0.27
	Ind. and Inoc	101.55±0.73	104.48±2.56	109.22±0.74	55.63±0.68	59.85±0.80	64.71±2.09	25.45±0.48	28.44±0.55	31.24±0.65
14d	Ind. and Un-Inoc.	38.53±0.52	42.97±1.33	45.99±0.69	30.15±0.85	33.94±0.78	35.46±0.63	16.63±0.20	19.63±0.22	21.66±0.36
	Ind. and Inoc	104.18±2.84	108.60±1.65	116.37±1.69	58.06±1.53	66.41±1.53	69.18±0.90	29.09±1.03	31.21±0.69	36.10±0.77
A. sativum		5%	10%	15%	5%	10%	15%	5%±	10%	15%
7d	Ind. and Un-Inoc.	27.14±0.14	30.10±0.46	32.82±0.46	20.99±0.98	23.85±0.79	26.92±0.71	11.14±0.16	13.74±0.13	15.35±0.20
	Ind. and Inoc	78.07±1.02	81.53±1.63	83.38±2.01	43.17±1.64	48.43±1.55	51.64±1.32	20.09±0.77	23.73±1.14	25.97±1.36
14d	Ind. and Un-Inoc.	29.86±1.05	33.35±1.02	35.53±1.16	22.80±0.65	25.29±0.81	28.31±0.95	12.86±0.16	15.92±0.90	17.49±1.15
	Ind. and Inoc	79.86±1.60	86.73±2.57	89.34±1.65	43.68±1.29	49.70±1.83	59.47±1.82	21.21±0.17	25.36±1.08	29.26±0.78

HSD value for treatments = 19.7488; HSD value for doses= 0.4058; HSD value for varieties= 3.0674.

NAD ATP, and other energy-yielding compounds. Almost similar findings were reported by [Sahi et al. \(2007\)](#) who reported that Phosphorus was higher in un-inoculated plants of susceptible lentil lines than the resistant ones, but upon inoculation Phosphorus also increased in resistant lentils. The present results are also consistent with [Randhawa \(1994\)](#) who reported an increase in phosphorus contents in chickpea cultivars of both resistant and susceptible groups after infection with *Ascochyta rabiei*. The application of foliar phosphorus induced systemic protection (PISP) against powdery mildew of roses, mango, and nectarines ([Reuveni and Reuveni, 1998](#)) by increasing

the activity of peroxidase, phenylalanine, lipoxygenase, and ammonia-lyase (PAL). A similar effect might occur in this case where increased phosphorus (P) induced systemic resistance in chickpea plants. Increased phosphorus reduces the intensity of disease in different host-pathogen interactions with downy mildew, rice bacterial blight, blue mold, barley yellow dwarf virus and rice blast ([Huber and Graham, 1999](#); [Reuveni et al., 2000](#)). Phosphate applied to plants might direct apoplastic calcium to amend membrane integrity and therefore influence the movement of apoplastic enzymes and discharge elicitor-active oligogalacturonides from the cell wall of different

plants (Gottstein and Kuc, 1989; Walters and Murray, 1992). On the other hand, phosphorus may enhance the severity of *sclerotinia* in garden plants and downy mildew of onion (Develash and Sugha, 1997). The results of our research is consistent with that of Shehu and Aliero (2010) who reported increased phosphorus in leaves inoculated with *Alternaria porri* compared to healthy ones.

Application of the proper rate of potassium, balanced with other nutrients, decreases the susceptibility of plants (Huber and Graham, 1999). Potassium is involved in different metabolic functions (respiration, photosynthesis) and the synthesis of high molecular weight compounds. Potassium deficiency increases parasitic diseases due to a disturbance of metabolic functions. Present studies indicated that there is increased potassium in chickpea cultivars especially cultivar C-44, after chemical treatment and inoculation with *A. rabiei*. This noticeable increase in potassium of chemically treated; inoculated susceptible chickpea may be attributed to a change in the physiological and biochemical plant defense system. Similarly, Sahi *et al.* (2007) found that potassium increased in both resistant and susceptible lentil lines after inoculation with *A. lentis*. These results are also consistent with those of Randhawa (1994) and Reddy and Khare (1984). Our results are consistent with El-Khallal (2007) who reported high potassium in salicylic acid-treated plants. A converse association between disease incidence and potassium nutrition has been revealed for sheath blight of rice (*Thanatephorus cucumeris*), rice blast (*Pyricularia oryzae*). A restorative effect of potassium was reported for bacterial diseases in rice such as bacterial leaf blight (Krauss, 1999). By increasing potassium availability and uptake the severity of stripe rust decreased. Almost 2450 references were reviewed by Perrenoud (1990) on the use of potassium. He reported that 70% of fungal diseases were decreased, 69% of bacterial diseases, 63 % of mites and insects and 41% of virus diseases by potassium. Higher applications of potassium increased the yield of fungal, bacterial, and viral pathogen-infected plants. Potassium deficiency results in decreased of protein synthesis and accumulation of simple nitrogen compounds such as amides that are used by plant pathogens (Dordas, 2008). There were no observed differences in crop response concerning different potassium sources. The balance between nitrogen and potassium affects the disease susceptibility of plants. Examples of disease reduction in obligate and

facultative parasites by potassium included severity of leaf blight (*Helminthosporium*) and increase wheat yield and seedling rot caused by *Rhizoctonia solani* (Sharma and Duveiller 2004; Sharma *et al.*, 2005). Excess potassium harmed the growth of tomato plants because they were more susceptible to *Verticillium* wilt caused by *V. dahliae* (Burge and Simmons, 1982).

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Novelty Statement

Adequate nutrition plays a key role in mitigating disease damage. Hence, current study indicates that chemicals reported to induce resistance to plant pathogens may increase mineral contents which prevent the spread of the pathogen.

Author's Contribution

Dr. Muhammad Usman Ghazanfar conceived and designed research and wrote the manuscript. Dr. Waqas Wakil analyzed the data and help in conduct of experiments. Dr. Shahbaz Talib Sahi supervised the experiment and improved the draft. Dr. Waqas Raza helped in analysis, submission and correspondence of paper.

Conflict of interest

The authors have declared no conflict of interest.

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