Journal BiNET
Scientific Publication

Published with Open Access at Journal BiNET
Vol. 19, Issue 02: 1628-1638
Journal of Bioscience and Agriculture Research

Journal Home: www.journalbinet.com/jbar-journal.html

Impact of biochemical changes on the development of powdery mildew of pea

Amna Ikram[1], Hafiz Muhammad Usman Aslam[2,3], Owais Yasin[2], Akhtar Hameed[2], Qurban Ali[4], Nasir Ahmad Khan[2], Salman Ghuffar[5], Waqas Ahmed Khan[2,3] and Faheem Ahmed[2]

[1]Department of Pest Warning and Quality Control of Pesticides, Punjab, Pakistan
[2]Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan
[3]Department of Plant Pathology and Microbiology, Iowa State University, USA
[4]Entomological Research Institute, Ayub Agricultural Research Institute, Punjab Pakistan
[5]PMAS Arid Agriculture University Rawalpindi, Pakistan.

✉ For any information: ask.author@journalbinet.com
Article received: 09.10.18; Revised: 26.02.19; First published online: 14 March 2019.

ABSTRACT

Powdery mildew of pea is an air-borne disease of global dispersal. Erysiphe pisi, which is responsible for powdery mildew of pea is the major threat for pea production in Pakistan and in the world. Although other fungal pathogens such as Erysiphe baeumleri and Erysiphe trifolii have also been reported by initiating this disease on pea and involved in 25–50% yield drop. In the current study 6 varieties of pea from which 3 resistant (Peas 2009, No. 267, F-16) and 3 susceptible (PF-450, Climax and Mateor) were cultivated in research area of the Department of Plant Pathology, University of Agriculture Faisalabad to analyze the biochemical deviations in pea leaves after the induction of powdery mildew disease. The results indicated that among six chemical elements (P, K, Ca, Mg, Zn and Fe) potassium, calcium and zinc contents were higher (1.34 ppm, 2.14 ppm, 1.25 ppm respectively) in diseased leaves while phosphorus, iron and magnesium contents were decreased (0.72 ppm, 7.40 ppm and 0.35 ppm respectively) in diseased leaves as compared to the healthy leaves in both the reaction groups (resistant, susceptible).

Keywords: *Pea, Nutrients, Susceptible and Biochemical changes*

Cite Article: Ikram, A., Aslam, H. M. U., Yasin, O., Hameed, A., Ali, Q., Khan, N. A., Ghuffar, S., Khan, W. A. and Ahmed, F. (2019). Impact of biochemical changes on the development of powdery mildew of pea. Journal of Bioscience and Agriculture Research, 19(02), 1628-1638.
Crossref: https://doi.org/10.18801/jbar.190219.197

I. Introduction

Plant diseases are the most important restraint to improve the production efficacy and the quality of crops as they diminish the nutrient availability, uptake, supply, or consumption by the plant (Dordas 2008). Pea (*Pisum sativum*) is a commonly grown crop in Pakistan and also in the world. This crop is

Published with open access at journalbinet.com
EISSN: 2312-7945,

prone to numerous biotic and abiotic factors and among all of them powdery mildew of pea caused by *Erysiphi pisi* gains a considerable importance (Iqbal et al. 2017). This disease was firstly reported in New Zealand in 1855 (Berkeley 1855). Powdery mildew of pea is more prevalent in late planted or late maturing peas and cause losses from 75 to 87% in the world. In Pakistan losses due to this disease was 50 to 60% (Hussain et al. 2002). In plants, the nutrients produced various types of effects on disease progression. Type of diseases, the number of elements, forms of elements and weather factors are the important aspects, which may influence the effects of nutrients on the disease progress. Minerals are the crucial part of plant nutrition and their inconsistency leads to the certain disorders in the plants either through upsetting the metabolism or anomalous physiology of the plants by supporting the plant pathogens or depressing the growth of plants (Sahi et al. 2010).

The most appropriate method for the improvement of plant health is to examine the elemental analysis of both the healthy and diseased plants. *Erysiphe pisi*, is a drastic pathogen of pea crop which reduces the up-taking ability of the plants. The infected plants fail to optimize the nutrients like Ca, N, P, Zn, Mg, Cu and Fe in their system and become more susceptible (Fondevilla et al. 2007). Nutrients are necessary for the development of plants and play a vital role in microbial growth, often unrealized and always have been an important means in disease control (Walters and Bingham, 2007). Nutrients are important for the development of plants because both the macro and micro nutrients are mandatory for producing the resistance against the pathogens (Oliveira et al. 2010). Every nutrient in the plants has different functions; some of them strengthen the plant cell wall and prevents the entry of pathogens in the plant cells (Fageria 2001). Unfortunately, Pakistan lacks the usability of nutrients to manage the certain plant disorders. In this research plan, the impact of biochemical changes was assessed on six pea varieties (resistant or susceptible) with the induction of powdery mildew disease.

II. Materials and Methods

Determination of biochemical changes in leaves of diseased and healthy pea plants

The plant populations were based on the two group's i.e. healthy and diseased plants. Each group was consisted of two reaction types namely resistant and susceptible. The resistant reaction type had Peas 2009, No. 267 and F-16 varieties while susceptible reaction type contained three varieties PF-450, Climax, Mateor. The same varieties were included in the resistant and susceptible types of diseased group. The samples comprising of healthy and diseased groups both from the resistant and susceptible reaction types were taken from the fields of University of Agriculture, Faisalabad, Pakistan and stored in the refrigerator. The standard analytical methods were employed for the determination of different biochemical compounds by using the 'Nested Design' (Gomez and Gomez, 1984; Figure 01).

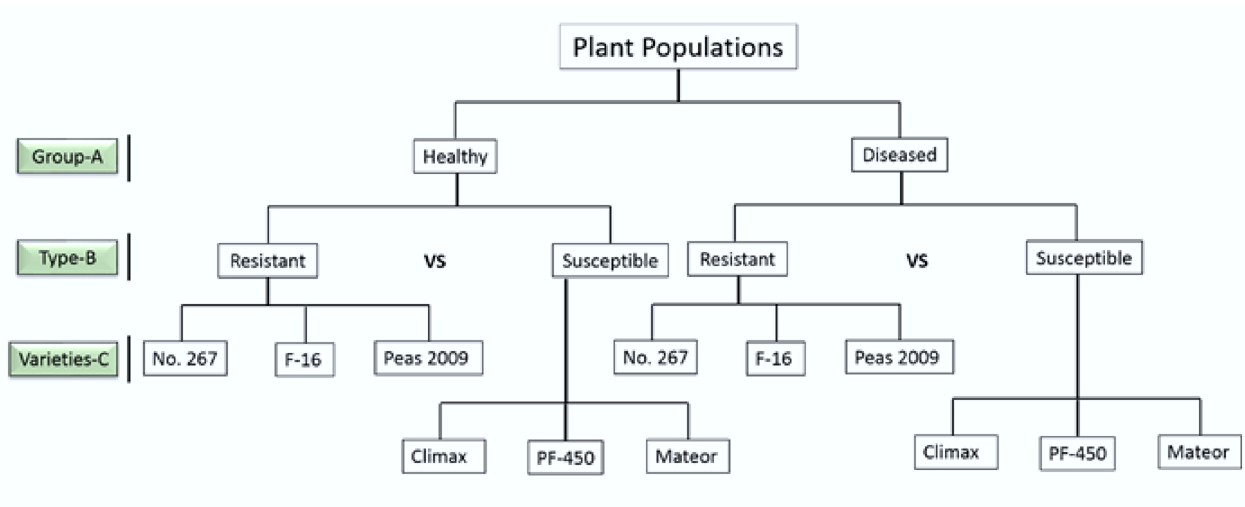

Figure 01. Nested structure of biochemical analysis.

Determination of ionic status (P, K, Ca, Mg, Zn and Fe) from leaves of diseased and healthy pea plants

Healthy and diseased plant samples (comprising leaves from both moderately resistant and susceptible cultivars) were harvested and leaves were oven-dried for 48 hours at 70°C. The samples were grounded in pestle and mortar. The dried samples (100 g) were boiled in 10 ml of 1.4 N HNO_3 (TH-550; Advantec, Tokyo, Japan) and digest on hot plate at 100°C for 30 min. After cooling, the suspension was diluted to 250 times with distilled water and analyzed for the determination of P, K, Ca, Mg, Zn and Fe (Bhargava and Raghupathi, 1993).

Determination of phosphorus from leaves of diseased and healthy pea plants

0.1 ml of sample solution already prepared by wet digestion method was taken in a volumetric flask. Then 8.6 ml of distilled water was added along with 1 ml of ammonium molybdate reagent. After swirling the flask to mix solution, 0.4 ml of aminonephthol sulphonic acid was added. The absorbency was measured by using the distilled water as reagent blank in place of sample solution at 720 nm on a spectrophotometer. Phosphorus concentration was determined by comparing the absorbency to a previously prepared standard curve (Fiske and Subbarow, 1925).

Determination of potassium from leaves of diseased and healthy pea plants

Potassium contents were measured by using the flame photometer following (AOAC 1990) method (KCl: standard). Standard curves for K was prepared by using the 10, 20, 30 and 40 ppm. Fresh working standards were prepared immediately before use.

Determination of calcium, magnesium, iron and zinc from leaves of diseased and healthy pea plants

Calcium (Ca), Magnesium (Mg), Iron (Fe) and Zinc (Zn) were determined by using the spectrophotometer. For the determination of these ions, calcium chloride, magnesium sulphate, iron sulphate and zinc oxide were used as standards respectively. The standard curves were obtained by using the 10, 20, 40, 80, 100 ppm for Ca; 5, 10, 15 and 20 ppm for Mg; 1, 2 and 3 ppm for Fe and 0.2, 0.3, 0.5 and 2 ppm for Zn, respectively.

Statistical analysis

The data were analyzed by using the Statistical Software (SAS 9.3) (SAS 2011). The data were subjected to analysis of variance (ANOVA) at 5 percent level of significance. Fisher's Least Significant Difference (LSD) test was used for statistical comparison among treatments.

III. Results

Determination of mineral contents from leaves of diseased and healthy pea plants

The samples of healthy (asymptomatic) and diseased plants from both the reaction groups were collected, oven dried, ground to fine powder and subjected to standard analytical method for the determination of P, K, Ca, Mg, Zn and Fe contents.

Determination of phosphorus (% dry weight) from leaves of diseased and healthy pea plants

Significant variation was observed between the healthy and diseased plants (avg. 0.82 and 0.72% respectively) during disease stress, with 0.013% percent of the total variance. No variation was expressed between the resistant (2.57%) and susceptible plants (0.69%) at $p \leq 0.05\%$ (Figure 02). With respect to phosphorus (P) concentration 10.90% of the total variance was shown by varieties in their

Published with open access at journalbinet.com
EISSN: 2312-7945,

natural tendencies (Table 01). The varieties No. 267 and PF-450 displayed maximum (0.91%) and minimum (0.67%) concentration of P to the extent of and percent respectively (Figure 02).

Table 01. Nested random effects of diseased and healthy leaves of pea plants

SOV	Variance component	Percentage of total
Group (A)	0.013	85.30
Type (B)	-0.002	0.00
Variety (C)	0.002	10.90
Error	0.001	5.80
Total	0.016	100.0

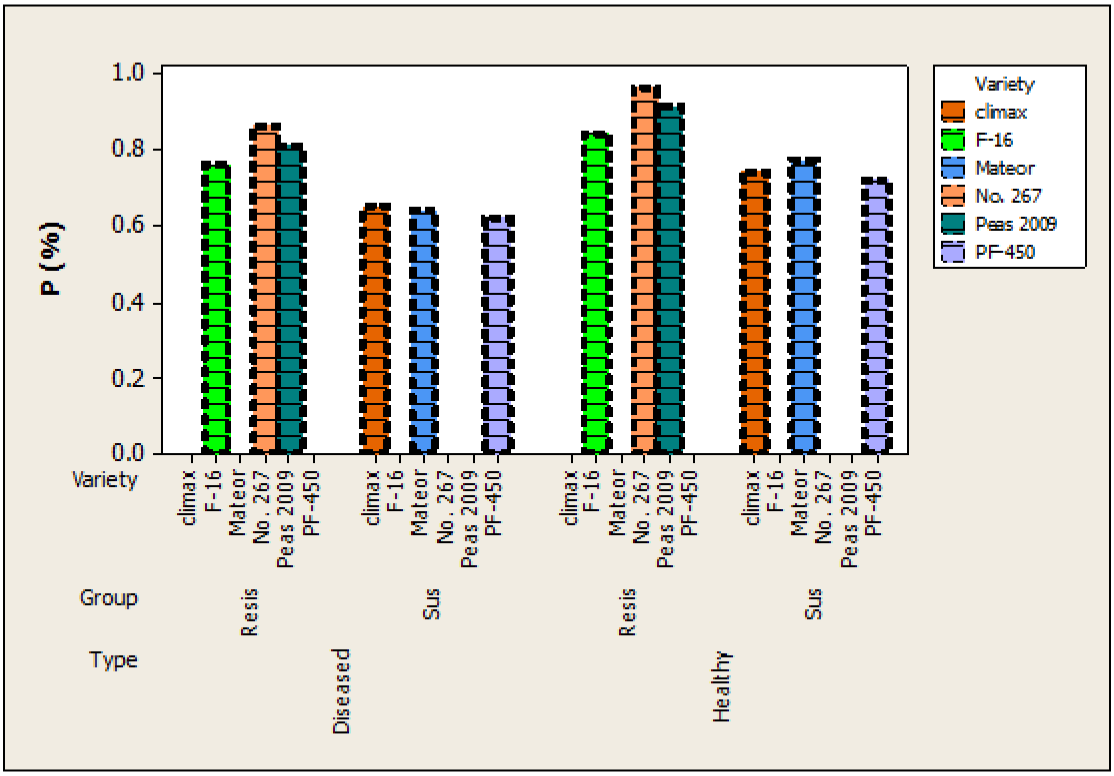

Figure 02: Concentration of phosphorus (%) in moderately resistant and susceptible Pea varieties of healthy and diseased plants.

Determination of calcium (dry weight in ppm) from leaves of diseased and healthy pea plants

Non-significant variation was observed of calcium contents between diseased and healthy plants which is 2.14 ppm and 1.80 ppm respectively. This variation was accounted for 22.61% of the total variance. Resistant and susceptible group (1.89 ppm and 2.05 ppm) expressed significant variation which is 45.87% (Figure 03). The total variance expressed by the varieties was 30.88%. No. 267 with 1.75 ppm and Climax with 2.09 ppm were the varieties with maximum and minimum calcium concentration respectively (Table 02 and Figure 03).

Table 02. Nested random effect's analysis of variance for calcium of diseased and healthy leaves of pea plants

SOV	Variance Component	Percentage of total
Group (A)	0.021	22.61
Type (B)	0.043	45.87
Variety (C)	0.029	30.88
Error	0.001	0.64
Total	0.094	100.0

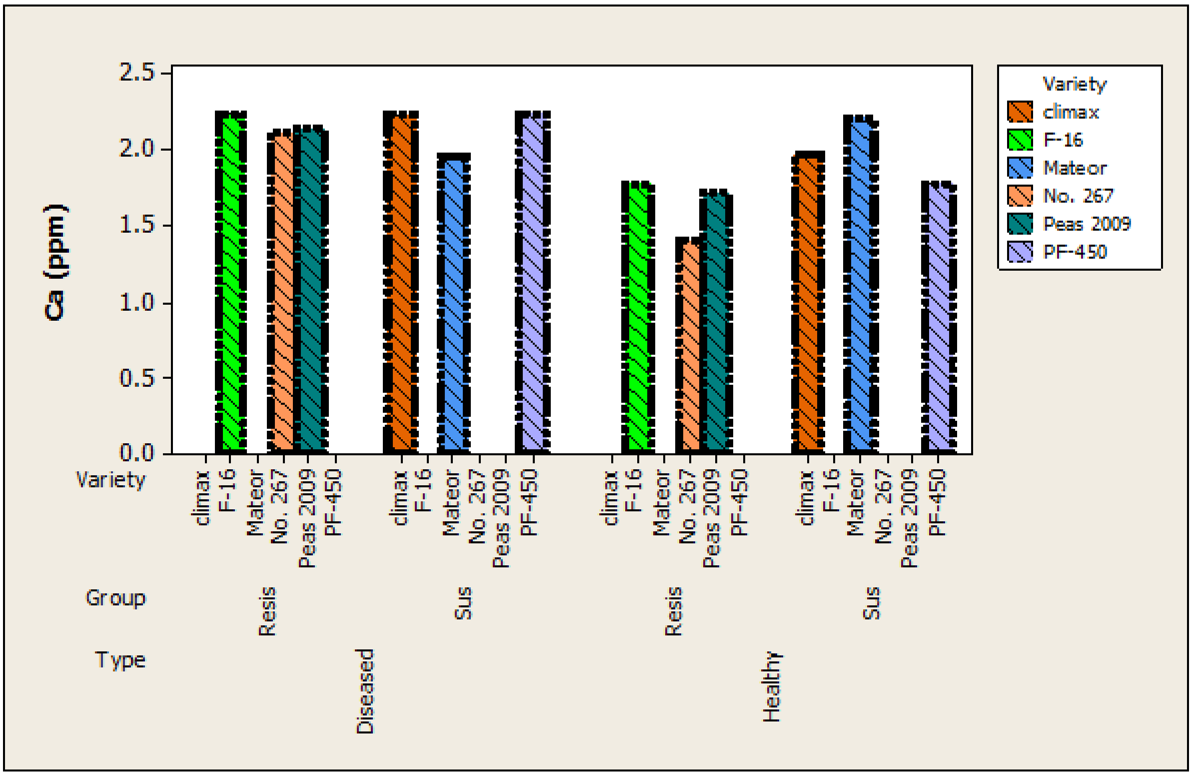

Figure 03: Concentration of calcium (ppm) in moderately resistant and susceptible pea varieties of healthy and diseased plants.

Determination of magnesium (dry weight in ppm) from leaves of diseased and healthy Pea plants

Non-significant variation was observed in magnesium contents when both the healthy (0.39 ppm) and diseased plants (0.35 ppm) was compared under the disease stress conditions with 14.74% of the total variance. Resistant and susceptible plants expressed significant variation (35.67%) in magnesium contents of pea leaves which is found to be 0.39 ppm and 0.35 ppm respectively (Figure 04). Varieties with 21.46% of the total variance exhibited their natural tendencies. The maximum and minimum concentrations of Mg contents were seen in the verities of No. 267 (0.42 ppm), PF-450 (0.35 ppm) and Mateor (0.35) respectively (Table 03 and Figure 04).

Table 03. Nested random effect's analysis of variance for magnesium of diseased and healthy leaves of pea plants

SOV	Variance component	Percentage of total
Group (A)	0.000	14.74
Type (B)	0.001	35.67
Variety (C)	0.000	21.46
Error	0.001	28.13
Total	0.002	100.0

Published with open access at journalbinet.com

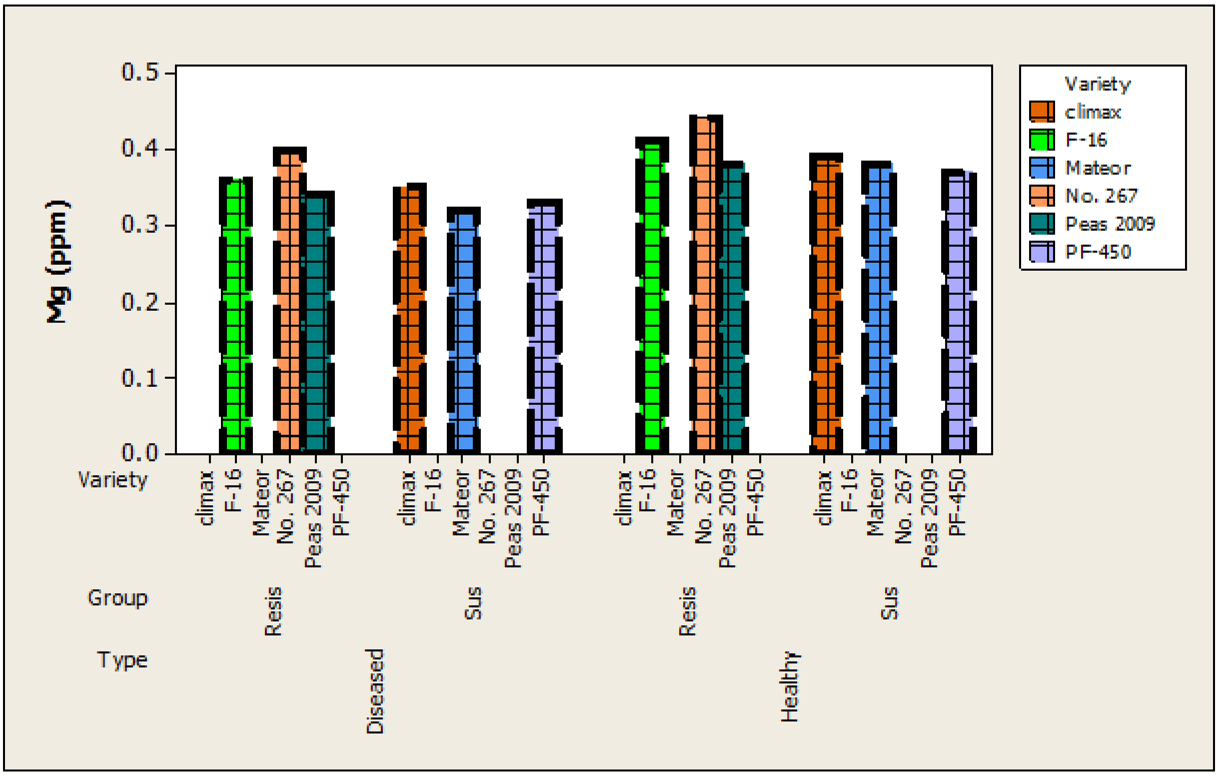

Figure 04: Concentration of magnesium (ppm) in moderately resistant and susceptible pea varieties of healthy and diseased plants.

Determination of zinc (dry weight in ppm) from leaves of diseased and healthy pea plants

Healthy and diseased plants expressed non-significant variation of zinc with 19.67 percent of the total variance (Table 04). Similar results were obtained between resistant (1.14 ppm) and susceptible plants (1.21 ppm). 1.13 ppm and 1.23 ppm concentrations were retained by Peas 2009 and Mateor which accounted for 7.66 percent of the total variance (Figure 05).

Table 04. Nested random effect's analysis of variance for zinc of diseased and healthy leaves of pea plants

SOV	Variance component	Percentage of total
Group (A)	0.002	19.67
Type (B)	0.008	67.83
Variety (C)	0.001	7.66
Error	0.001	4.84
Total	0.012	100.0

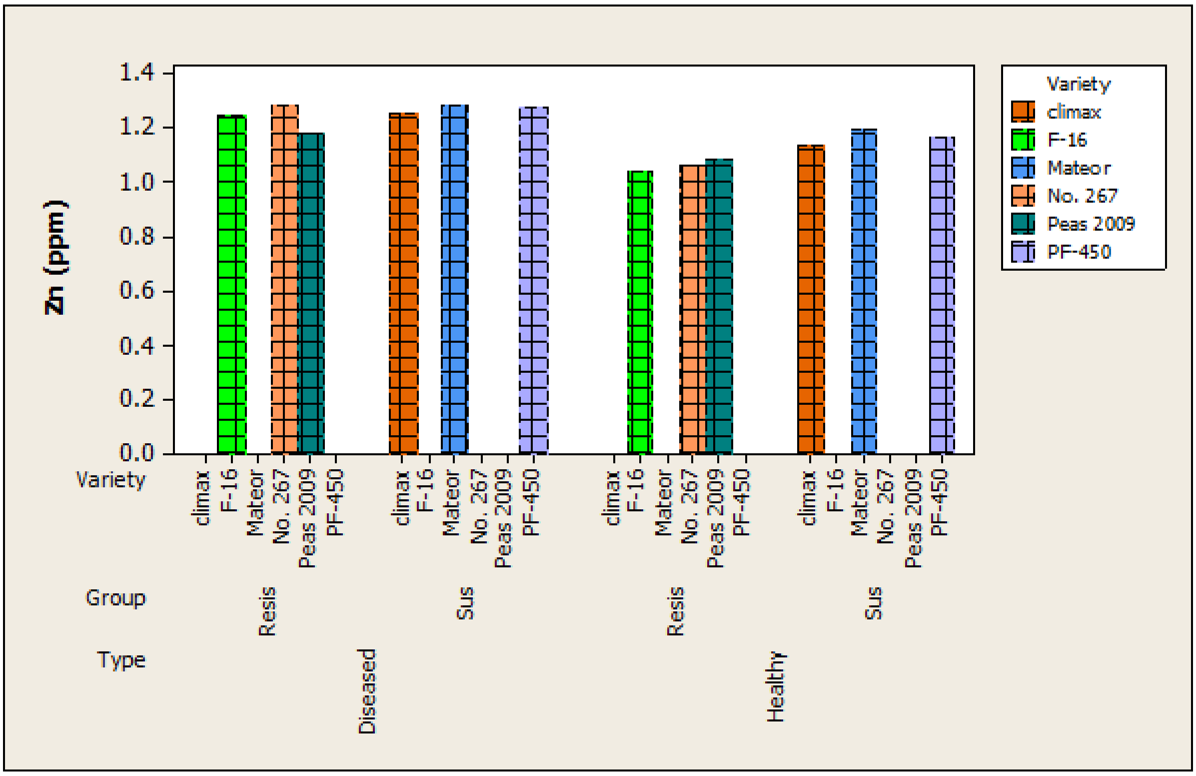

Figure 05: Concentration of zinc (ppm) in moderately resistant and susceptible pea varieties of healthy and diseased plants.

Determination of iron (dry weight in ppm) from leaves of diseased and healthy pea plants

Non-significant variation was observed between the healthy and diseased plants (averaging to 8.68 ppm and 7.40 ppm respectively) during disease stress (Figure 06) with 14.80 percent of the total variance at $p \leq 0.05\%$ (Table 05). No variation was expressed between resistant (8.55 ppm) and susceptible plants (7.53 ppm). 50.45 percent of the total variance was shown by varieties in their natural tendencies. Maximum (9.3 ppm) concentration was displayed by variety named "Peas-2009" and minimum (6.8 ppm) by PF-450 (Figure 06).

Table 05. Nested random effect's analysis of variance for iron of diseased and healthy leaves of pea plants

SOV	Variance component	Percentage of total
Group (A)	0.241	14.80
Type (B)	0.566	34.72
Variety (C)	0.823	50.45
Error	0.001	0.04
Total	1.631	100.0

Published with open access at journalbinet.com
EISSN: 2312-7945, © 2019 The Authors, Research paper.

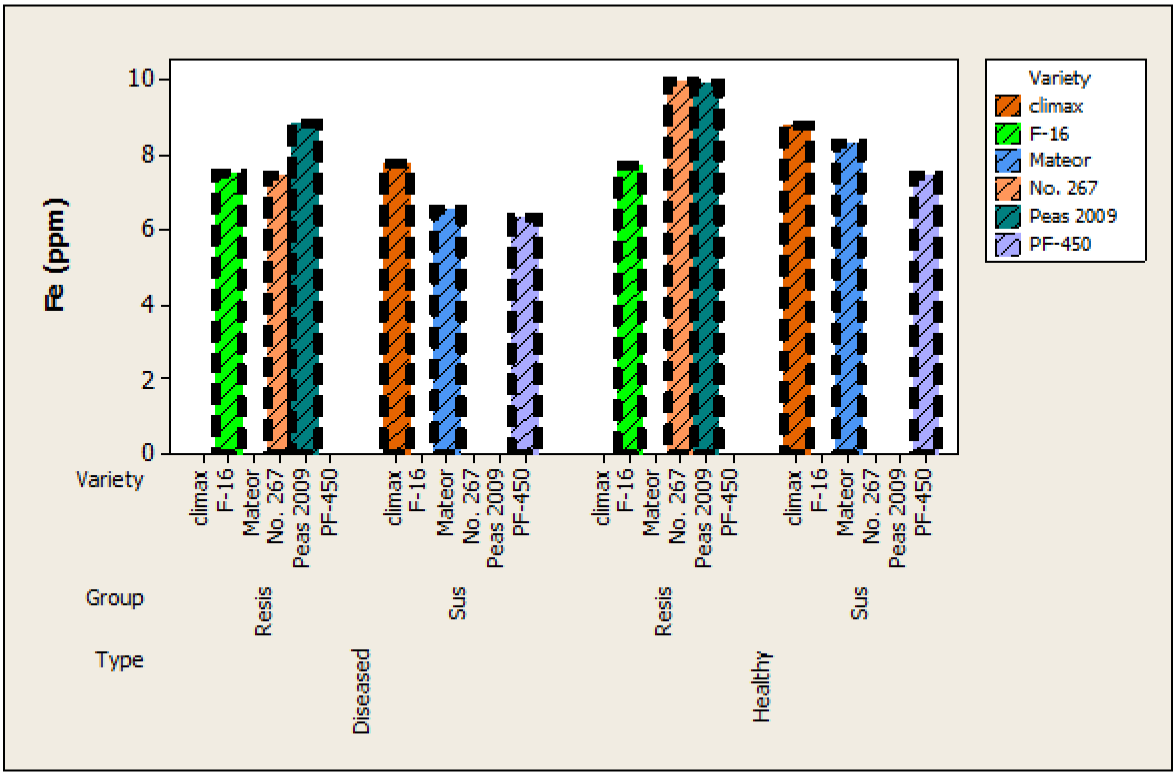

Figure 06: Concentration of iron (ppm) in moderately resistant and susceptible pea varieties of healthy and diseased plants.

Determination of potassium (dry weight in ppm) from leaves of diseased and healthy pea plants

Healthy and diseased plants showed significant variation (1.22 ppm and 1.34 ppm) respectively under disease stress (Figure 07) and accounted for 0.00 percent of the total variance but non-significant variation was revealed by resistant and susceptible plants. Varieties exhibited their natural tendencies with respect to K concentration explaining 46.94 % of the total variance (Table 06). Peas-2009 and PF-450 accumulated the maximum and minimum concentration of potassium (1.17 and 1.38 ppm) respectively (Figure 07).

Table 06. Nested random effect's analyses of variance for potassium of diseased and healthy leaves of pea plants

SOV	Variance component	Percentage of total
Group (A)	-0.001	0.00
Type (B)	0.006	48.40
Variety (C)	0.006	46.94
Error	0.001	6.66
Total	0.013	100.0

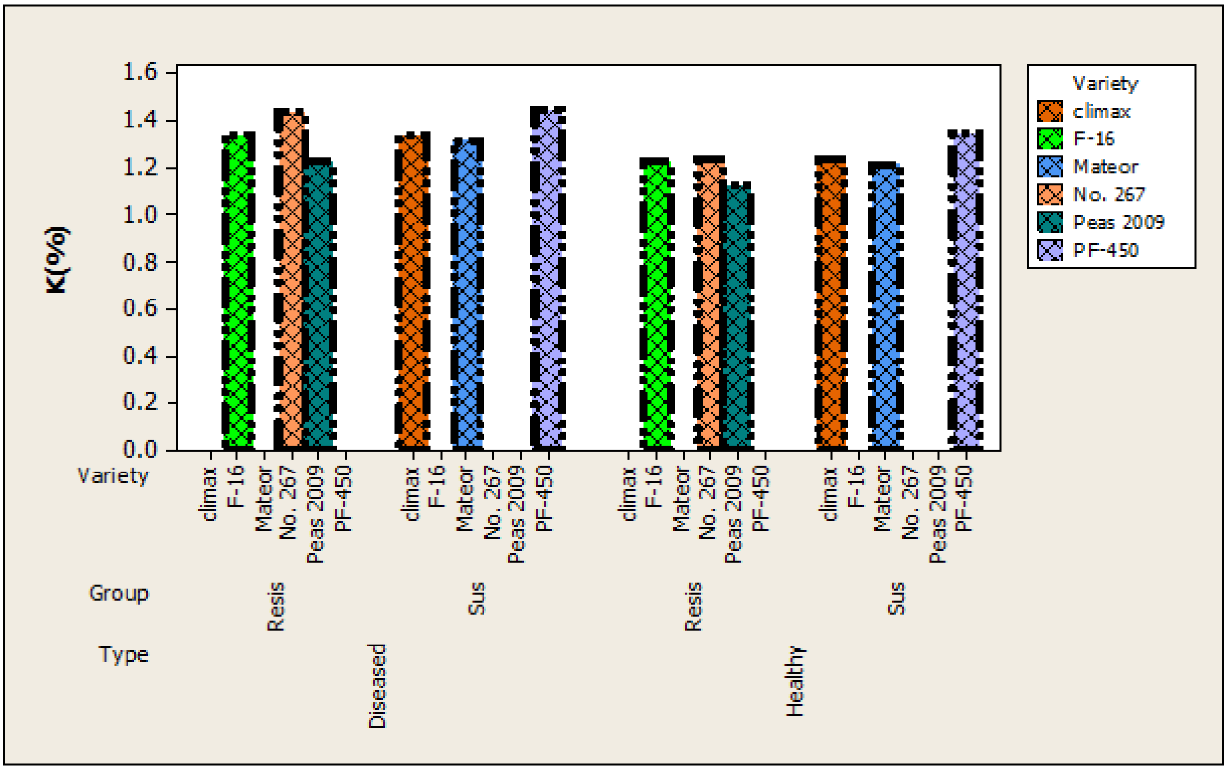

Figure 07: Concentration of potassium (percentage) in moderately resistant and susceptible pea varieties of healthy and diseased plants.

IV. Discussion

The greatest total of substances is required from outside all the plants for the culmination of their life which are called nutrients. Plants also required simple nutrients like Ca, Mg, N, P, K, Na and S (macro nutrients), Cu, Fe, Mn, Zn, Mo, Cl and B (micro nutrients) for metabolic progressions. The mineral nutrition is a significant aspect of plant growth that directs the production of all plants. Here in the current research an effort has been made to know the consequence of powdery mildew fungal pathogen on mineral nutrition of *Pisum sativum* L. The prime factor directing the mineral elements of plant material is specific and genetically fixed for the various mineral nutrients (Grusak et al. 2001).

Potassium is an important factor for the development of plants and various other reformist processes such as translocation of proteins and carbohydrates, photosynthesis, protein synthesis, stability of ribosomes, nitrogen uptake, glycolysis and phosphorylation in plants. Most of the plants require 1% K+ for their optimum growth (Cakmak 2005). However, the amounts of phosphorus in the plant cells were analyzed in the range of mM (Hnatowich et al. 2012). It is evidenced from the fact that infection of barley with powdery mildew or rust fungi has been shown to lead to increased P concentration in leaves, either through increased root uptake and subsequent transport to shoots or decreased re-translocation out of infected leaves (Douchkov et al. 2016).

K+ is generally reported to decrease disease severity in many host plants, including diseases caused by necrotrophic and biotrophic pathogens. In many cases, this seems to be restricted to the deficiency range, i.e. K+ undersupplied plants tend to be more prone to the infection than plants getting an adequate supply of K+. It was noticed that the powdery mildew fungus on grasses (*Erysiphe graminis*) escalating the potassium uptake in plants (Horsfall 2012).

Calcium is also considered as a non-toxic mineral nutrient and is very active in detoxifying the high concentrations of other mineral elements in plants. Calcium activities of many enzymes have been either stimulated or inhibited by the application of this nutrient element. It binds with the proteins, nucleic acids and lipids to affect the cell adhesion, membrane chromatin organization and enzymatic conformation (White and Broadley, 2003). This is commonly found in plants Cat-channel blocker and chlorpromazine; a calmodulin antagonist was seen on the development of *Erysiphe pisi* (Singh 2000).

Magnesium is a small, mobile and strongly electropositive divalent cation in the plants, found both in bound as well as free form. Mg plays an important part in making of chlorophyll in plants. It also acts enzymatic reactions involved in organic acid synthesis. Magnesium activates the enzymes such as ATPase, carboxylase, glutamate synthetase and Fructose 1,6-diphosphatase (Cakmak and Yazici, 2010). While zinc is an important element for different metabolic processes i.e. nitrogen metabolism, carbohydrate metabolism, protein synthesis, auxin synthesis; particularly IAA synthesis. It works as a catalyst for many enzymes, which is essential for chlorophyll biosynthesis. The critical toxicity levels of zinc in leaves of crop plants are more than 400–500 mg kg-1 on dry weight basis (Broadley et al. 2007).

V. Conclusion

Erysiphe pisi is a severe fungal pathogen which is responsible for powdery mildew disease of pea. The efficacy of six chemical elements (P, K, Ca, Mg, Zn and Fe) was assessed on six pea verities (resistant or susceptible). With the induction of disease, potassium, calcium and zinc contents were higher (1.34 ppm, 2.14 ppm, 1.25 ppm, respectively) in diseased leaves while phosphorus, iron and magnesium contents were decreased (0.72 ppm, 7.40 ppm and 0.35 ppm, respectively) in diseased leaves as compared to the healthy leaves in both the reaction groups (resistant, susceptible).

Author contributions

A.I. and H.M.U.A wrote the article and corrected the manuscript before final submission. A.I., W.A.K and F.A. conducted the experimental works. O.Y. and A.H. was involved in statistical analysis. N.A.K, Q.A. and S.G. was designed the study.

References

[1]. AOAC (1990). Official methods of analysis of the Association of Official Analytical Chemists. Vol. 1: The Association.

[2]. Berkeley, M. (1855). Vibrio forming cysts on the roots of cucumbers. Gardener's Chronicle and Agricultural Gazette, 14, 220.

[3]. Bhargava, B. and Raghupathi, H. (1993). Analysis of plant materials for macro and micronutrients. Methods of analysis of soil, plant, water and fertilizers. Fertilizers Development and Consultation Organization, New Delhi, India. pp. 49-82.

[4]. Broadley, M. R., White, P. J. Hammond, J. P. Zelko, I. and Lux, A. (2007). Zinc in plants. New phytologist. 173(4), 677-702. https://doi.org/10.1111/j.1469-8137.2007.01996.x

[5]. Cakmak, I. (2005). The role of potassium in alleviating detrimental effects of abiotic stresses in plants. Journal of Plant Nutrition and Soil Science, 168(4), 521-530. https://doi.org/10.1002/jpln.200420485

[6]. Cakmak, I. and Yazici, A. M. (2010). Magnesium: a forgotten element in crop production. Better Crops, 94(2), 23-25.

[7]. Dordas, C. (2008). Role of nutrients in controlling plant diseases in sustainable agriculture. A review. Agronomy for sustainable development, 28(1), 33-46. https://doi.org/10.1051/agro:2007051

[8]. Douchkov, D., Lueck, S. Hensel, G. Kumlehn, J. Rajaraman, J. Johrde, A. Doblin, M.S. Beahan, C.T. Kopischke, M. and Fuchs, R. (2016). The barley (*Hordeum vulgare*) cellulose synthase-like D2 gene (HvCslD2) mediates penetration resistance to host-adapted and nonhost isolates of the powdery mildew fungus. New Phytologist, 212(2), 421-433. https://doi.org/10.1111/nph.14065

[9]. Fiske, C. H. and Subbarow, Y. (1925). The colorimetric determination of phosphorus. J. biol. Chem. 66(2), 375-400. https://doi.org/10.1111/j.1439-0523.2006.01312.x

[10]. Fondevilla, S., Carver, T., Moreno, M. and Rubiales, D. (2007). Identification and characterization of sources of resistance to *Erysiphe pisi* Syd. in Pisum spp. Plant breeding, 126(2), 113-119.

[11]. Fageria, V. D. (2001). Nutrient interactions in crop plants. Journal of plant nutrition, 24(8), 1269-1290. https://doi.org/10.1081/PLN-100106981

[12]. Gomez, K. A. and Gomez, A. A. (1984). Statistical procedures for agricultural research: John Wiley & Sons.

[13]. Grusak, M. A., Broadley, M. R. and White, P. J. (2001). Plant Macro and Micronutrient Minerals. eLS. 1-6. https://doi.org/10.1038/npg.els.0001306

[14]. Hnatowich, G. L., Steckler, S. J., Leggett, M. and Priest, K. (2012). Methods and compositions for increasing the amounts of phosphorus available for plant uptake from soils. Google Patents.
[15]. Horsfall, J. G. (2012). Plant disease: an advanced treatise: How Plants Suffer from Disease: Elsevier.
[16]. Hussain, S. I., Mahmood, T., Khokhar, K., Laghari, M. and Bhatti, M. (2002). Screening of pea germplasm for yield and resistance towards powdery mildew. Asian Journal of Plant Sciences, 1(3), 230-231. https://doi.org/10.3923/ajps.2002.230.231
[17]. Iqbal, A., Shah, S., Nisar, M. and Ghafoor, A. (2017). Morphological characterization and selection for high yielding and powdery mildew resistant pea (*Pisum sativum*) Lines. Sains Malaysiana, 46(10), 1727-1734. https://doi.org/10.17576/jsm-2017-4610-08
[18]. Oliveira, S. R., Neto, J. A. G., Nobrega, J. A. and Jones, B. T. (2010). Determination of macro and micronutrients in plant leaves by high-resolution continuum source flame atomic absorption spectrometry combining instrumental and sample preparation strategies. Spectrochimica Acta Part B: Atomic Spectroscopy, 65(4), 316-320. https://doi.org/10.1016/j.sab.2010.02.003
[19]. Sahi, S. T., Ghazanfar, M. U., Afzal, M., Wakil, W. and Habib, A. (2010). Influence of inoculation with Ascochyta lentis on mineral contents (Na, Ca, Mg, Zn, Cu and Fe) of susceptible and resistant lines of lentil (*Lens culinaris* Medik.). Pak. J. Bot. 42(1), 375-382.
[20]. SAS I. (2011). SAS/STAT 9.3 user's guide: SAS Institute.
[21]. Singh, U. (2000). Pea-powdery mildew-an ideal pathosystem. Indian Phytopathology. 53(1), 1-9.
[22]. Walters, D. and Bingham, I. (2007). Influence of nutrition on disease development caused by fungal pathogens: implications for plant disease control. Annals of Applied Biology, 151(3), 307-324. https://doi.org/10.1111/j.1744-7348.2007.00176.x
[23]. White, P. J. and Broadley, M. R. (2003). Calcium in plants. Annals of botany, 92(4), 487-511. https://doi.org/10.1093/aob/mcg164

HOW TO CITE THIS ARTICLE?

Crossref: https://doi.org/10.18801/jbar.190219.197

MLA
Ikram, et al. "Impact of biochemical changes on the development of powdery mildew of pea." Journal of Bioscience and Agriculture Research 19(02) (2019): 1628-1638.

APA
Ikram, A., Aslam, H. M. U., Yasin, O., Hameed, A., Ali, Q., Khan, N. A., Ghuffar, S., Khan, W. A. and Ahmed, F. (2019). Impact of biochemical changes on the development of powdery mildew of pea. Journal of Bioscience and Agriculture Research, 19(02), 1628-1638.

Chicago
Ikram, A., Aslam, H. M. U., Yasin, O., Hameed, A., Ali, Q., Khan, N. A., Ghuffar, S., Khan, W. A. and Ahmed, F. "Impact of biochemical changes on the development of powdery mildew of pea." Journal of Bioscience and Agriculture Research 19(02) (2019): 1628-1638.

Harvard
Ikram, A., Aslam, H. M. U., Yasin, O., Hameed, A., Ali, Q., Khan, N. A., Ghuffar, S., Khan, W. A. and Ahmed, F. 2019. Impact of biochemical changes on the development of powdery mildew of pea. Journal of Bioscience and Agriculture Research, 19(02), pp. 1628-1638.

Vancouver
Ikram, A, Aslam, HMU, Yasin, O, Hameed, A, Ali, Q, Khan, NA, Ghuffar, S, Khan, WA and Ahmed, F. Impact of biochemical changes on the development of powdery mildew of pea. Journal of Bioscience and Agriculture Research. 2019 March 19(02): 1628-1638.