Macrophomina phaseolina alters the biochemical pathway in Vigna radiata chastened by Zn$^{2+}$ and FYM to improve plant growth

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**ABSTRACT**

Mung bean [Vigna radiata (L.) Wilczek] is an important cash pulse crop extensively cultivated in the arid region of Pakistan, which encounters intimidating charcoal rot disease caused by *Macrophomina phaseolina* (Tassi) Goid. The current research was conducted to check the potential of Zn (1.25, 2.44 and 5 mg kg$^{-1}$) and FYM [farmyard manure (1% and 2%)] in mono-, bi- and trilateral interaction in managing disease and improving yield. Suppression of plant immunity by *M. phaseolina* was indicated by the change in activities of antioxidant enzymes (CAT and SOD) and cell wall strengthening enzymes (POX and PAL) that revealed inability of the protein receptor to identify the pathogen elicitor. FYM improved soil physicochemical properties and beneficial microbes activity, which released antimicrobial protein- and plant defense-stimulating protein and in response to ROS (reactive oxygen species) signaling molecules plant susceptibility was reduced. However, Zn as a co-factor chastened the ROS in stressed cells by upregulation of antioxidant enzymes in favor of the plant. The complex interaction of FYM and Zn potentially hijacked the further multiplication of pathogen. Finally, soil amendment improved biological attributes and grain yield to profitable farming in terms of harvest index percentage and benefit–cost ratio.

**Introduction**

Mung bean [Vigna radiata (L.) Wilczek] belongs to the family Fabaceae, and is a nutrient-rich, warm-season (30–35°C) and short-duration crop that required low fertile soil with less water to complete its life cycle. It was therefore cultivated under both irrigated and arid conditions (Islam et al. 2012). In Pakistan, mung bean is being cultivated on 146 thousand hectares with 98 thousand tons of production on an annual basis, with marginal yield per unit area (Hanif et al. 2013). Charcoal rot disease caused by *Macrophomina phaseolina* (Tassi) Goid is an economically important constraint in mung bean production especially in arid regions of the world and in arid to tropical regions of Pakistan.

*M. phaseolina* is a soil- and seed-borne fungus of family Ascomycetes with sclerotial and pycnidial forms. Its heat-tolerant sclerotia can survive for 2–15 years in soil or in root debris and when host tissues decompose, these are released in clusters in the upper few inches of the soil surface and can colonize living and dead tissues. Pycnidiospores are produced on infected aerial plant parts (stem and leaf tissues) to facilitate secondary dispersal. In addition to that, the fungus exhibits the potential to attack plants on almost every growth stage and may cause death of young seedlings due to the formation of dark irregular lesions on the epicotyls and hypocotyls that extend to the cotyledons. In adult plants, pathogen invasion blocks xylem and caused host death due to seedling blight; stem and pod rot (Beas-Fernández et al. 2006). Up till now, cultural, chemical or biological management options were found ineffective against *M. phaseolina* and no registered fungicide is available. However, Quintozene and Captan are some available fungicides that manage disease to some extent. However, the revolution to sustainable agriculture in an eco-friendly way cannot be achieved using such chemicals that are health hazards. Micro- and macronutrients could provide a solution to intractable and ever notorious charcoal rot disease of mung bean by inducing resistance in the plant against pathogens and improving growth. Zinc (Zn) is an essential micronutrient for the metabolism of the plant, and when present in a lower concentration may cause significant losses in plant yield and increase disease susceptibility. Zn as a main constituent and co-factor in various enzymes activation is needed for nitrogen metabolism, energy transfer, redox reactions and stabilization of ribosomal fractions for protein synthesis in plants (Zhao et al. 2012). Its role has been found vital in triggering of many defense-related enzymes such as SOD, CAT and POX (Suba 2014). The important roles of Zn have increased its significance as a vital and most yield-limiting micronutrient in crop production (Samreen et al. 2013). Different crops required Zn according to their growth pattern but normally the critical value of Zn in plant dry matter ranges from 15 to 50 ppm (Imtiaz et al. 2010). Therefore, many investigations have recommended application of Zn to increase growth and productivity of different crops including wheat, maize, chickpea and cotton. Literature has shown that Zn-based supplements also reduced disease incidence and severity in many crops by inducing resistance against pathogens. Outcomes of Zn benefits to plants could be further improved by using it in combination with different types of organic soil amendments. Organic soil amendments expressed aggressiveness against pathogens through intensifying antagonistic microbial activities (Bonanomi et al. 2007), reducing sclerotial number and minimizing the
inoculum contact with plant roots. Various antifungal compounds such as nitrous acid and ammonia are generally released by the decomposition of nitrogenous amendments that induce systemic resistance in the host plants (Li et al. 2016). Farmyard manure (FYM) is a good source of all major nutrients (N, P, K, Ca, Mg and S) and micronutrients (Fe, Mn, Cu and Zn) essential for plant growth. FYM imparts a positive effect on soil health by improving its physicochemical and biological properties, and hence provides better environment for root development. El-hamid and Mosa (2009) found that foliar spray of micronutrients including Zn and FYM application increased the yield of wheat grains as compared to the application of Zn alone. In other studies, zinc in combination with different fertilizers has been used for improvement in growth and yield in different plants. Although either Zn or FYM has been shown to be promising in managing the different fungal diseases including charcoal rot, literature is scanty for their combined effect on disease management. This experiment was aimed to check the effect of Zn and FYM on crop physiology, growth and yield to manage charcoal rot disease in Zn-deficient soils, Bhakkar, Pakistan.

Materials and methods

The experiment was conducted during May–July 2014 in plastic pots (7″ × 6″ length and width) kept in a tunnel at the experimental research area, Institute of Agricultural Sciences, University of the Punjab. On the basis of our previous pathogenicity trial (Khan et al. 2016), susceptible (MNUYT-7) and highly susceptible (MNUYT-105) genotypes of mung bean were selected for the current study.

Collection and physicochemical analysis of soil

Soil was collected from Alam farm Chak no 13/TDA Tehsil Darya Khan 31°47′12″N 71°06′26″E, District Bhakkar, Punjab, Pakistan, and was analyzed for basic physiochemical and macro–micronutrients analysis (Estefan et al. 2013).

Pathogen inoculation

Soil was sterilized with formalin (2%) solution, filled in pots (5 kg pot−1) and inoculated with 30 mL cultural suspension (40 mL−1 sclerotia) of M. phaseolina (FCBP 0751).

Zinc and FYM amendment

Ten days after inoculation, fully decomposed FYM (1% and 2%) was mixed thoroughly and healthy, uniform surface-sterilized seeds were sown (7 seeds pot−1). Zinc (Zn) fertilizer was supplemented in the form of ZnSO4·H2O (33%) with brand name Zingro was purchased from registered dealers of Engro Fertilizers Limited. Three levels, i.e. 1.25, 2.44 and 5 mg kg−1 of zinc were used. Pots for negative control were without any treatment and for positive control were made by inoculating with pathogen only.

Experimental layout

The experiment was designed for 70 days. A total of 48 pots with 16 treatments for each genotypes of mung bean were arranged in a completely randomized designed. The following 16 treatments were designed with each mung bean genotype (Table 1).

Disease assessment

All plants were visually monitored at regular intervals for disease appearance. Morphological changes due to disease started to appear on the 27th day of inoculation; therefore disease incidence (%) described by Cohen et al. (2000) and plant mortality (%) were calculated on the 35th and 55th days of sowing, respectively,

\[
\text{DI} (\%) = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100
\]

\[
\text{Mortality (}) = \frac{\text{Number of dead plants}}{\text{Total number of plants}} \times 100
\]

Physiological assays

Total chlorophyll content, carotenoids and total protein content of plant leaves were determined on the 35th day of sowing (DOS). Plant leaves (0.5 g) were crushed in 80% ethanol solution and centrifuged at 10,000 rpm for 10 min. The absorbance for chlorophyll A (645 nm), chlorophyll B (663 nm) and carotenoid (270 nm) was taken and calculated according to the equation of Lichtenthaler and Buschmann (2001).

The Lowry method (Lowry et al. 1951) was followed for the estimation of total protein content. Leaves (0.5 g) were crushed in sodium phosphate buffer (10 mL) and centrifuged at 10,000 rpm for 5 min. Supernatant (0.1 mL) was mixed in 1 mL reagent C and blended in 0.1 mL reagent D. Samples were incubated for 30 min and were analyzed for protein measurements by recording absorbance at 650 nm against bovine serum albumin (BSA) as standard.

Antioxidative enzymes activity

The Kumar and Khan (1982) procedure was adopted for the measurement of peroxidase (POX). The enzyme extract (0.5 mL) was mixed with 1 mL phosphate buffer (0.1 M), 1 mL pyrogallol (0.01 M) and 1 mL H2O2 (0.05 M) then incubated (5 min at 25°C). To stop reaction, H2SO4 (2.5 N) was added (5 min at 25°C). The Lowry method (Lowry et al. 1951) was followed for the estimation of total protein content. Leaves (0.5 g) were crushed in sodium phosphate buffer (10 mL) and centrifuged at 10,000 rpm for 5 min. Supernatant (0.1 mL) was mixed in 1 mL reagent C and blended in 0.1 mL reagent D. Samples were incubated for 30 min and were analyzed for protein measurements by recording absorbance at 650 nm against bovine serum albumin (BSA) as standard.

| Zinc dose (mg kg−1) | Negative control | Positive control | FYM + M. phaseolina |
|---------------------|------------------|-----------------|---------------------|
| 0                   | T1               | T2 T3 T4        | T5 T6 T7 T8         |
| 1.25                | T5               | T6 T9 T10       | T11 T12 T13 T14     |
| 2.44                | T9               | T10 T13 T14     | T15 T16             |
| 5.00                | T13              | T14 T15 T16     |                      |

Table 1. Treatments designed during the current experiment.
added and absorbance of the samples were immediately calculated at 420 nm.

For the estimation of polyphenol peroxidase (PPO), enzyme extract was centrifuged at 10,000 rpm for 5 min. To the supernatant (0.1 mL), sodium phosphate buffer (0.1 M, pH 7) and 0.2 mL of catechol (0.01 M) were added. The PPO activity was determined at 30 s intervals three times at 495 nm against a blank sample prepared (Mayer et al. 1966).

The protocol of Maehly (1954) was used to check the catalase (CAT) activity. The CAT reaction mixture (1 mL enzyme extract + 1 mL of 0.01 M H2O2 + 0.1 M of 1 mL phosphate buffer) was incubated for 5 min at 20°C. The reaction was inhibited by adding 10 mL of 1% H2SO4. The mixture was titrated against 0.005 N KMNO4 until pinkish color appeared. CAT activity was expressed at 'n' moles of H2O2 utilized (units min−1 mg−1 protein).

Superoxide dismutase (SOD) was quantified with methods proposed by Maral et al. (1977). Three sets, experimental, blank, and control were prepared. Experimental and blank sets contain 30 mM methionine + 0.07 mM nitroblue tetrazolium (NBT) + 0.1 mM EDTA + 0.002 mM riboflavin + 0.1 mL enzyme extract. Control contains the same reagents except enzyme extract. Experimental and control sets were placed in light for 15 min, whereas blank was placed in dark. SOD activity was noted by taking absorbance at 560 nm.

**Growth and yield assays**

Growth parameters such as plant height and weight (fresh and dry weight) and yield attributes (grain yield, number of matured pods, 100-grain weight and highest harvest index) were recorded after 70 days of sowing to estimate the overall impact of treatments on plant growth. Harvest index was calculated using the following formula:

\[
\text{Harvest index (\%)} = \frac{\text{(Grain yield)}}{\text{(Biological yield)}} \times 100
\]

**Statistical analysis**

All data regarding disease incidence, mortality, physiology, enzymes, growth and yield parameters were analyzed for normality (Kolmogorov–Smirnov’s test (normality test)) then subjected to the analysis of variance (ANOVA) followed by Fisher’s protected least significant (LSD) difference test at the 5% probability level by using software Statistics 8.1. Two-way factorial ANOVA was used to estimate the individual and interactive effects of zinc levels and soil amendments on disease, physiology, plant growth and yield. Pearson’s correlation was applied to check the correlation among different parameters.

**Results and discussion**

**Soil physicochemical properties**

The electrical conductivity of soil was 2.0 mS L−1 with pH 8.1 having 32% saturation, 0.43% organic matter, 0.044% nitrogen, 79 mg kg−1 potassium, 4.6 mg kg−1 phosphorus, 5.4 meq L−1 chloride, 0.4 meq L−1 carbonates, 6.2 meq L−1 bicarbonates, 6.2 meq L−1 sulphate, 2.1 meq L−1 calcium + magnesium and 17.9 meq L−1 sodium. Iron, copper, zinc, manganese, magnesium and boron found were 4.2, 1.52, 1.24, 3.2, 8.6 and 0.03 mg kg−1, respectively.

**Disease and growth**

Highly susceptible (MNUYT-105) and susceptible (MNUYT-7) mung bean genotypes showed the highest disease incidence (53% and 47%) and plant mortality (40% and 27%) in positive control (with M. phaseolina), respectively. However, when only zinc (1.25–5 mg kg−1) was supplemented to pathogen-inoculated soil, disease incidence and mortality significantly decreased to 34 ± 6% and 17 ± 3%, respectively. The interactive effect of Zn + FYM significantly reduced the disease incidence and plant mortality from 62% to 30% in both genotypes. Among all the treatments, the maximum charcoal rot disease was managed up to 70% when Zn (2.5 mg kg−1) was applied with 2% FYM (Figure 1).

Similarly, in both genotypes the growth attributes (length and weight) were drastically and significantly decreased by 20–80% in positive control and Zn (2.44 or 5.00 mg kg−1) + FYM (2%) improved the growth attributes up to 145 ± 15% in pathogen-inoculated soil. Zn supplement exclusively in negative control also proved beneficent in increasing growth attributes by 200 ± 25%, and data showed harmony in both mung bean genotypes (Tables 2 and 3).

**Yielding components**

Infection of M. phaseolina in highly susceptible and susceptible genotypes significantly decreased grain yield by 80% and 68%, 100-seed weight by 70% and 40%, respectively. Mean values of biological yield (160%) and grain yield

![Figure 1. Effect of different levels of zinc and farmyard manure on disease incidence (%) and mortality (%) in the highly susceptible genotype (MNUYT-105) (A) and in the susceptible genotype (MNUYT-7) (B).](image-url)
increased number of mature pods plant$^{-1}$ and grain yield (5.20 and 6.32 g plant$^{-1}$) in both genotypes, respectively (Figure 2(a–d); Table 4).

**Correlation among different plant traits**

Generally, in both genotypes, all studied parameters were found in highly strong positive correlation except disease parameter. Disease incidence and mortality were found strongly in negative correlation with all other growth and yielding components with magnitude ($r = -0.55$ to $-0.94$, $P < .01$) and ($r = -0.55$ to $-0.85$, $P < .01$), respectively (Tables 5 and 6).

**Plant physiology**

In positive control of the highly susceptible genotype, total chlorophyll content, carotenoids, CAT and PPO were decreased by 19%, 5%, 3% and 9%, respectively, whereas total protein content, SOD and POX were significantly increased by 213%, 31% and 32%, respectively, as compared to negative control. Benefits of zinc with or without FYM on disease management were indicated by reduction in the level of protein and enhancement in activities of all the defense-related enzymes to variable extents over positive control.

In the susceptible genotype, infection of *M. phaseolina* insignificantly affected total chlorophyll and carotenoids, significantly decreased the total protein content by 30% and
significantly accelerated activities of all enzymes by 23–91% over negative control. When plants in inoculated treatments were grown with Zn, FYM and Zn + FYM, the total protein content was significantly enhanced, and activities of all enzymes were gradually and significantly reduced over positive control. Incorporation of Zn (1.25–5.00 mg kg\(^{-1}\)) in negative control of either genotype increased total protein content and decreased enzymes activities in highly susceptible and susceptible genotypes with few exceptions (Tables 7 and 8).

Discussion

Disease, growth and yield

Results revealed that the highly susceptible mung bean genotype showed the highest disease incidence (53%) and plant mortality (40%), along with the maximum reduction in biological attributes by 20–80% in positive control (inoculated with \(M.\) phaseolina only) over negative control. Disease incidence and plant mortality in the susceptible mung bean genotype were 47% and 27%, respectively, while biological attributes were declined by 70% in infected plants over negative control. Infected plants showed the typical symptoms of charcoal rot disease like brown lesions on the stem and leaves 27 days after sowing (DAS). Later (40 DAS) these lesions turned black and finally the plants wilted and died after 55 DAS. The plants that survived till 70 days under pathogen stress were observed with stunted growth, shuttering of flowers, immature and dry pods without grain that with passage of time turned into white to gray color, became narrow, thin and deformed. The pathogen stress negatively affected the physiological processes in plants with the elevation of reactive oxygen species (ROS) production at the membranes of bioenergetics organelles (thylakoid and mitochondria) (Schaller et al. 2005). The fungi extracellular enzymes degraded the cytoskeleton and walls of the cells which resulted in plant death (Figures 3 and 4).

The application of zinc (1.25–5 mg kg\(^{-1}\)) decreased disease incidence and mortality to 34 ± 6% and 17 ± 3%, increased biological attributes (20–125 ± 15%) and, HI (mean: 38%) and grain yield (mean: 7 g plant\(^{-1}\)) in both

![Figure 2. Effects of different levels of zinc (Zn) and farmyard manure (FYM) on 100-seed weight (g) (A–B) and no. of pods plant\(^{-1}\) (C–D) in highly susceptible (MNUYT-105) and susceptible (MNUYT-7) mung bean genotypes inoculated with \(M.\) phaseolina (MP), respectively.](image)

| Treatments | SS | HS | Mean | SS | HS | Mean |
|------------|----|----|------|----|----|------|
| **Grain yield** | g plant\(^{-1}\) | g plant\(^{-1}\) | g plant\(^{-1}\) | g plant\(^{-1}\) | g plant\(^{-1}\) | g plant\(^{-1}\) |
| T1 | 4.56 | 3.59 | 4.07 | 14.33 | 13.27 | 13.80 |
| T2 | 0.88 | 0.39 | 0.64 | 6.16 | 4.57 | 5.36 |
| T3 | 1.08 | 0.79 | 0.93 | 9.74 | 9.36 | 9.55 |
| T4 | 1.41 | 0.96 | 1.18 | 11.10 | 9.55 | 10.33 |
| T5 | 5.91 | 4.10 | 5.01 | 17.82 | 14.93 | 16.37 |
| T6 | 3.20 | 1.83 | 2.52 | 12.93 | 10.48 | 11.71 |
| T7 | 3.34 | 2.82 | 3.08 | 13.11 | 12.50 | 12.80 |
| T8 | 3.35 | 3.60 | 3.48 | 14.17 | 13.32 | 13.74 |
| T9 | 7.62 | 5.59 | 6.61 | 19.59 | 16.48 | 18.04 |
| T10 | 3.96 | 2.77 | 3.37 | 14.78 | 12.50 | 13.64 |
| T11 | 4.49 | 4.70 | 4.59 | 16.40 | 15.53 | 15.96 |
| T12 | 5.11 | 5.90 | 5.50 | 17.07 | 16.77 | 16.92 |
| T13 | 8.18 | 8.52 | 8.00 | 20.16 | 16.72 | 18.44 |
| T14 | 4.87 | 2.82 | 3.85 | 15.73 | 12.59 | 14.16 |
| T15 | 5.15 | 4.98 | 5.06 | 17.13 | 15.87 | 16.50 |
| T16 | 5.20 | 6.32 | 5.76 | 17.18 | 17.21 | 17.19 |

Note: T1: Negative control; T2: Positive control; \(M.\) phaseolina (MP); T3: 1% FYM + MP; T4: 2% FYM + MP; T5: Zn (1.25 mg kg\(^{-1}\)) + MP; T6: 1% FYM + MP + Zn (1.25 mg kg\(^{-1}\)) + MP; T7: Zn (1.25 mg kg\(^{-1}\)) + MP; T8: 2% FYM + MP + Zn (1.25 mg kg\(^{-1}\)) + MP; T9: Zn (2.44 mg kg\(^{-1}\)) + MP; T10: 1% FYM + MP + Zn (2.44 mg kg\(^{-1}\)); T11: 2% FYM + MP + Zn (2.44 mg kg\(^{-1}\)); T12: 2% FYM + MP + Zn (2.44 mg kg\(^{-1}\)); T13: Zn (5 mg kg\(^{-1}\)) + MP; T14: Zn (5 mg kg\(^{-1}\)) + MP; T15: 1% FYM + MP + Zn (5 mg kg\(^{-1}\)); T16: 2% FYM + MP + Zn (5 mg kg\(^{-1}\)).
genotypes (Figure 1; Tables 2–4). An increase in biological attributes in negative control might be endorsed to the essential role of zinc in the biosynthesis of growth-promoting hormone (e.g., gibberellins and indole-3-acetic acid), along with the activation of many enzymes involved in cell elongation and cell division, nitrogen fixation and nodule metabolism, which has been confirmed (Ali et al. 2010); therefore, it seems that soil supplementation with zinc facilitates the

Table 5. Correlation matrix of different phenotypic and genotypic traits of mung beans which were treated at different levels of zinc and FYM with and without and in combination to manage charcoal rot disease in the highly susceptible genotype.

| Traits | DI | SDW | RDW | PP | SW | GY | BIO | MOR | HI |
|--------|----|-----|-----|----|----|----|-----|-----|----|
| Ger    | -0.66** | 0.68** | 0.72** | 0.82** | 0.86** | 0.88** | 0.83** | -0.60** | 0.87** |
| DI     | -0.67** | -0.67** | -0.55** | -0.83** | -0.68** | -0.71** | -0.67** | 0.94** | -0.67** |
| SDW    | 0.98** | 0.85** | 0.79** | 0.80** | 0.94** | -0.68** | 0.81** | 0.86** |
| RDW    | 0.89** | 0.84** | 0.96** | -0.70** | 0.86** |
| PP     | 0.87** | 0.94** | 0.95** | -0.55** | 0.97** |
| SW     | 0.96** | 0.93** | 0.95** | -0.78** | 0.95** |
| BIO    | 0.95** | -0.64** | 0.98** |
| MOR    | 0.70** | 0.95** |

Note: DI: disease incidence; Ger: germination; MOR: mortality; SDW: shoot dry weight; RDW: root dry weight; RL: root length; PP: number of mature pods plant⁻¹; SW: 100-seed weight; GY: grain yield; BIO: dry biomass; HI: harvest index.

**Significant at P ≤ 0.01; *significant at P ≤ 0.05; ns: non-significant.

Table 6. Correlation matrix of different phenotypic and genotypic traits of mung beans which were treated at different levels of zinc and FYM with and without and in combination to manage charcoal rot disease in the susceptible genotype.

| Traits | DI | SDW | RDW | PP | SW | GY | BIO | MOR | HI |
|--------|----|-----|-----|----|----|----|-----|-----|----|
| Ger    | -0.71** | 0.64* | 0.67** | 0.55* | 0.70** | 0.64* | 0.68** | -0.77** | 0.60* |
| DI     | -0.69** | -0.72** | -0.88** | -0.78** | -0.89** | -0.84** | -0.88** | 0.92** | -0.88** |
| SDW    | 0.98** | 0.76** | 0.85** | 0.70** | 0.94** | -0.78** | 0.74** |
| RDW    | 0.77** | 0.88** | 0.81** | 0.94** | -0.81** | 0.77** |
| PP     | 0.81** | 0.96** | 0.94** | 0.91** | -0.82** | 0.97** |
| SW     | 0.92** | 0.95** | -0.83** | 0.99** |
| GY     | -0.83** | 0.95** |
| BIO    | 0.95** |
| MOR    | 0.85** |

Table 7. Effects of different levels of zinc (Zn) and farmyard manure (FYM) on physiological attributes in the highly susceptible (MNUYT-105) mung bean genotype inoculated with Macrophomina phaseolina (MP).

| Parameters | Zinc dose (mg kg⁻¹ FW) | Negative control | Positive control (MP) | FYM + MP | 1% | 2% | Mean (F) |
|------------|------------------------|------------------|-----------------------|----------|----|----|----------|
| Total chlorophyll content (mg g⁻¹ FW) | 0 | 0.51b | 0.41c | 0.44e | 0.29f | 0.41C |
| 1.25 | 0.42c-e | 0.46b-d | 0.37a | 0.45e | 0.43A |
| 2.44 | 0.44b-e | 0.46b-d | 0.42c-e | 0.42de | 0.44b |
| 5 | 0.47bc | 0.46b-d | 0.48f | 0.49f | 0.48C |
| Mean (F) | 0.46A | 0.45AB | 0.43B | 0.41C |
| Carotenoids (mg g⁻¹ FW) | 0 | 2.40b | 2.27bc | 2.05d-f | 1.69bc | 2.10A |
| 1.25 | 2.38b | 2.11b-d | 1.89a | 2.64g | 2.25A |
| 2.44 | 1.65g | 1.74a-g | 1.99h | 1.39c-e | 1.69B |
| 5 | 2.13b-d | 2.19b-d | 1.90h | 1.64h | 1.96B |
| Mean (F) | 2.14A | 2.08A | 1.96B | 1.84B |
| Total protein content (mg g⁻¹ FW) | 0 | 0.04g | 0.13a | 0.10bc | 0.08de | 0.09A |
| 1.25 | 0.07d-f | 0.11b | 0.09cd | 0.08de | 0.08AB |
| 2.44 | 0.08c-e-d | 0.08de | 0.08de | 0.08de | 0.08B |
| 5 | 0.09cd | 0.07ef | 0.07ef | 0.06f | 0.07C |
| Mean (F) | 0.07C | 0.09A | 0.08B | 0.07C |
| Polyphenol oxidase activity (units mg⁻¹ protein) | 0 | 0.18b | 0.06g | 0.11d | 0.05g | 0.10C |
| 1.25 | 0.09de | 0.16bc | 0.09de | 0.08ef | 0.11BC |
| 2.44 | 0.09de | 0.17b | 0.09de | 0.08ef | 0.11B |
| 5 | 0.10de | 0.24a | 0.14c | 0.06af | 0.14A |
| Mean (F) | 0.11b | 0.16A | 0.11B | 0.07C |
| Catalase activity (units mg⁻¹ protein) | 0 | 3.19b | 2.96b | 13.58h | 23.34d | 20.77B |
| 1.25 | 2.35e-g | 27.98bc | 18.31h | 19.40e-h | 21.76B |
| 2.44 | 19.02f-h | 29.99g | 16.18h | 25.82cd | 22.75B |
| 5 | 18.60gh | 38.05a | 22.70f-d | 25.82cd | 22.75B |
| Mean (F) | 22.72b | 27.00A | 18.26C | 24.53B |
| Superoxide dismutase activity (units mg⁻¹ protein) | 0 | 0.08h | 0.10f-h | 0.11e-g | 0.09gh | 0.09D |
| 1.25 | 0.15d | 0.20c | 0.09f-h | 0.12ef | 0.14C |
| 2.44 | 0.20c | 0.24ab | 0.13de | 0.19c | 0.19B |
| 5 | 0.24ab | 0.25a | 0.21c | 0.21bc | 0.22A |
| Mean (F) | 0.17b | 0.20A | 0.13C | 0.15B |
| Peroxidase activity (units mg⁻¹ protein) | 0 | 14.34b | 18.91a | 17.88a | 12.25c | 15.84A |
| 1.25 | 11.73c | 11.18cd | 9.47d | 7.11f-h | 9.87B |
| 2.44 | 9.35de | 8.02eg | 6.95f-h | 7.00f-h | 7.83C |
| 5 | 8.25f | 6.34gh | 5.87h | 6.10h | 6.64D |
| Mean (F) | 10.92AB | 11.11A | 10.04B | 8.11C |

Note: Values with same lower case and upper case show an insignificant difference (P ≤ 0.05) as determined by the LSD Test.
Table 8. Effects of different levels of zinc (Zn) and farmyard manure (FYM) on physiological attributes in the susceptible mung bean genotype (MNUYT-7) inoculated with Macrophomina phaseolina (MP).

| Parameters | Zinc dose (mg kg⁻¹) | Negative control | Positive control (MP) | 1% | 2% | Mean (F) |
|------------|---------------------|-------------------|-----------------------|----|----|----------|
| Total chlorophyll content (mg g⁻¹ FW) | 0 | 0.32cd | 0.35a–c | 0.31cd | 0.37ab | 0.34A |
| | 1.25 | 0.22f | 0.35a–c | 0.21f | 0.21ef | 0.26C |
| | 2.44 | 0.24ef | 0.39a | 0.24ef | 0.33b–d | 0.30B |
| Mean (F) | 3 | 0.36–a | 0.36–a | 0.29de | 0.33b–d | 0.33A |
| Carotenoids (mg g⁻¹ FW) | 0 | 1.714b–e | 1.79b–d | 1.81b–d | 1.11g | 1.60B |
| | 1.25 | 1.30g | 1.87bc | 1.43ef | 1.52d–f | 1.53B |
| | 2.44 | 1.47ef | 2.68a | 1.59c–f | 1.59c–f | 1.83A |
| | 5 | 1.03g | 2.39a | 1.99b | 1.88bc | 1.82A |
| Total protein content (mg g⁻¹ FW) | 0 | 0.05h | 0.04i | 0.03i | 0.10a | 0.06D |
| | 1.25 | 0.06gh | 0.09e–b | 0.07g | 0.06fg | 0.07C |
| | 2.44 | 0.08de | 0.08ef | 0.08e–e | 0.09b–e | 0.08B |
| Mean (F) | 5 | 0.09–d | 0.08de | 0.10 –c | 0.10ab | 0.09A |
| Polyphenol oxidase activity (units mg g⁻¹ protein) | 0 | 0.074b | 0.07b | 0.07b | 0.09A | 0.12A |
| | 1.25 | 0.09de | 0.13b | 0.19a | 0.05sh | 0.12A |
| | 2.44 | 0.07f | 0.05gh | 0.09de | 0.08f | 0.07C |
| Mean (F) | 5 | 0.077C | 0.09b | 0.11A | 0.07C | 0.07D |
| Catalase activity (units mg g⁻¹ protein) | 0 | 30.04b | 44.05a | 32.74b | 16.74a | 30.88A |
| | 1.25 | 24.83c | 21.33cd | 21.99c | 23.92cd | 22.63B |
| | 2.44 | 22.05cd | 8.68f | 19.43d | 21.96cd | 18.03C |
| Mean (F) | 5 | 11.13f | 19.35de | 10.57f | 18.81de | 14.96D |
| Superoxide dismutase activity (units mg g⁻¹ protein) | 0 | 0.074b | 0.13ab | 0.09cd | 0.10cd | 0.09B |
| | 1.25 | 0.08d–f | 0.13ab | 0.10c | 0.07f | 0.10B |
| | 2.44 | 0.09–e | 0.14ab | 0.12b | 0.06f | 0.10AB |
| Mean (F) | 5 | 0.09cd | 0.14a | 0.15ab | 0.07f | 0.11A |
| Peroxidase activity (units mg g⁻¹ protein) | 0 | 13.20b | 16.24a | 12.21bc | 7.97g | 12.40A |
| | 1.25 | 9.23ef | 12.50b | 11.77bd | 7.00gh | 10.13B |
| | 2.44 | 8.47fg | 10.54cd | 9.25ef | 6.00h | 8.57C |
| Mean (F) | 5 | 5.3 4h | 10.25de | 8.45fg | 5.46h | 7.37D |

Note: Values with the same lower case and upper case show an insignificant difference \(P < 0.05\) as determined by the LSD Test.

mung bean to fix nitrogen, increase the number of productive nodules, pods plant⁻¹ and grain pod⁻¹. Amanullah and Inamullah (2016) concluded that the application of zinc may affect crop productivity and disease incidence by affecting resistance indirectly. In many studies, the antagonistic effect of zinc was linked with its toxicity to pathogens (Dordas 2008). Besides protection against diseases Zn could cause the formation of the physical barrier in plant roots against invading pathogens.

Zn along with FYM ameliorated the plant growth attributes (length and biomass) by 20–140% and 20–110% by managing disease incidence (25–63%) and plant mortality (33–67%) in highly susceptible and susceptible genotypes, respectively, over positive control. Besides managing charcoal rot disease zinc (2.44 and 5 mg kg⁻¹) + FYM (2%) pronouncedly improved grain yield by mean 5.76 g plant⁻¹ and HI (mean 34%) in both genotypes. Nasredeen et al.’s (2016) findings showed increased growth and yield in different crops due to the application of FYM. FYM decomposition releases organic acids and lowers pH, which promotes the increase in Zn availability and other nutrients’ uptake in rhizosphere. Similarly, a positive interaction between organic matter and leguminous plants tends to enhance the availability of phosphorus by solubilizing it and decomposition of FYM helps to promote soil biological activities. The synergetic effect of FYM + Zn was found to be more beneficial for mung bean health probably owing to easy retention of nutrients by adsorbing on its active binding site. Therefore, improving biological, physical and chemical properties of soil, FYM decomposition produced humic acid and other intermediate compounds which lower the soil pH level, which likely retains Zn in the available form for plants or releases Zn-mobilizing compounds (phytosiderophores) from roots, and may induce polypeptides involved in Zn uptake and translocation to shoots. The aforesaid physiochemical process in soil might produce such chemical compounds that induced resistance in mung beans and another possibility is that the chemical reaction produced a prohibitive effect on the M. phaseolina growth (Figure 4).

**Plant physiology**

Pathogenic infection in mung bean genotypes (MNUYT-105 and MNUYT-07) showed a significant increase in total protein content and activities of antioxidant enzymes raise in the following order SOD ≥ POX ≥ PPO ≥ CAT. Host defense mechanism and pathogen attack could be responsible for enhancement in total protein content by synthesizing several different proteins in the host cell and nitrogenous compounds.

*M. phaseolina* is a necrotrophic pathogen and it cannot be detected by the pathogen-associated molecular patterns (PAMPs) – a protein system for the detection of an elicitor released by the pathogen – present on the plasma membrane of the pathogen. The identification of the pathogen elicitor causes the activation of a signaling protein known as mitogen-activated protein 3 kinase (MAPKKK) which sends a signal to the nucleus for activation of the oxidative burst gene.
the rboh gene (Sagi et al. 2004). The rboh gene has a promoter at the Cis-regulatory element and this promoter is activated by its transcriptional binding protein, the WRKY protein (Eulgem et al. 2000). It is clear from Figure 2 that the plant has not detected the pathogen and there is no signal transduction for the oxidative burst which allowed the spread of the pathogen from the first cell attacked to other surrounding cells and this invasion of the pathogen caused biotic stress to the plant. The biotic stress resulted in a decrease of water uptake and increase in CO2 which caused closure of the stomata. The production of ROS was increased at the mitochondria and chloroplast due to disturbance in electron flow at the membranes of these organelles due to biotic stress (Joshi et al. 2016). This could be the reason that our statistics showed an increase in the activity of antioxidants such as SOD, CAT and PPO. The pathogen also released hydrolytic enzymes which degraded the organelles and cytoskeleton of the cells, leading to plant death (Figure 3).

Both mung bean genotypes (negative control) were grown with Zn application, which resulted in a gradual increase in total protein content and POX while the remaining enzymes’ activities (SOD, PPO and CAT) were significantly decreased. Total protein content and POX activity were decreased with zinc application in the highly susceptible genotype (pathogen inoculated), while the remaining attributes were increased to variable extents. In contrast, in the susceptible genotype (pathogen inoculated), zinc application showed a significant increase in total protein content, while SOD activity was decreased due to less ROS production on cellular organelles. When the pathogen-inoculated soil was amended with Zn or FYM, the disease was managed but Zn and FYM in combination produced more effective results in both genotypes. FYM provided essential nutrients and facilitated beneficial microbial growth; these microbes release proteins that trigger the defense system of the plant and released antimicrobial proteins (AMPs) as well. Most probably, the activation of defense gene release the AMPs which might suppress the pathogen in soil thus develops SAR in whole plant (Che et al. 2011). The activities of antioxidant enzymes are well regulated under normal conditions but altered under a stress environment (Dehgahi et al. 2015). Production of ROS due to zinc deficiency or after pathogen infection could result in alteration in health markers (photosynthetic pigment and carotenoids). It appears that toxins of M. phaseolina damaged chlorophylls (health markers) by disturbing photosystems (Dehgahi et al. 2015). The importance of zinc in cell wall development, respirational membranes, photosynthetic membranes, chlorophyll formation and rate of maturity is well reported (Samant 2000). Benefits of zinc may result in increased chlorophyll contents due to its function as structural and catalytic component of proteins, enzymes and as a co-factor of SOD (Imtiaz et al. 2010) for normal development of pigment biosynthesis (Samreen et al. 2013). Plants may have increased sensitivity to oxidative damage when grown under zinc deficiency (Cakmak and Marschner 1993) (Figure 4).
Figure 4. The biochemical pathway induced by Zn and FYM. The soil amended with Zn and FYM plays a vital role in the suppression of pathogens. FYM activated the soil-beneficial microbes (SBMs), which improved the soil physical–chemical properties (PCP) by secreting different substances. The proteins released by SBM in soil sent an upstream signal to the genes in the plant and activated the defense gene. The plant in response released antimicrobial proteins (AMP) in the soil via root exudates. The systemic acquired resistance (SAR) was also developed in surrounding cells due to the activation of defense genes and downstream signaling proteins. SAR enhanced the immunity of the whole plant and the plant become healthy. Zn$^+$ entered into root cells via protein channels on the plasma membrane which resulted in improved activity of antioxidants as it is the co-factor and structural part of most of the antioxidant enzymes. As the plant managed the biotic stress, the ROS production at cellular organelles was less and the activity of antioxidant enzymes was also decreased (Table 1).

Conclusion

*M. phaseolina* infection disturbed normal plant growth, physiology (chlorophyll, sugar and carotenoid contents) but precision use of zinc (2.4 and 5 mg kg$^{-1}$) combined with FYM 2% proved most effective in managing disease by improving antioxidative enzymes’ activities (POX, PPO, CAT and SOD). Moreover, these improved yield components such as mature pods, grain yield, 100-seed weight, total biological dry biomass and HI.

Disclosure statement

No potential conflict of interest was reported by the authors.

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