



Effect of Fungicide Zole on Sustainable Growth and Nutrient Uptake in Wheat Plant

Nagar Ankita¹; Jabin Shagufta^{1*}; Chawla Jyoti¹; Bir Ritu² & Gandhi Sona¹

¹Department of Applied Sciences (Chemistry), School of Engineering and Technology,
Manav Rachna International Institute of Research and Studies, Faridabad, Haryana, India

²Department of Applied Sciences, Greater Noida Institute of Technology, Greater Noida, UP, India

*Correspondence: shagufta.set@mriu.edu.in

ABSTRACT

The extensive application of the fungicide zole over last few decades has led to its accumulation as a toxic residue in agricultural soils and plants. To mitigate its environmental impact and to maintain soil and plant health, optimization of its dosage is essential. The present study aims to examine the role of fungicide zole in conjunction with non-polar and neutral amino acid, namely glycine as a soil additive for the growth of wheat. The experiment is done to explore the growth of wheat plants (*Triticum aestivum*) at varying concentration of fungicide zole in terms of seed germination, number of leaves grown, shoot and root length of the wheat plants while the glycine concentration was held constant. To assess the results on wheat plant development and nutrient uptake, the experiment includes five different combinations in a randomized block design (RBD) in three sets of replications. Each set was labelled as S1 (0.1M glycine and 0% fungicide zole/kg soil), S2 (0.1M glycine and 2% fungicide zole/ kg soil), S3 (0.1M glycine and 4% fungicide zole/kg soil), S4 (0.1M glycine and 6% fungicide zole/kg soil) and S5 (0.1M glycine and 8% fungicide zole/kg soil). The integrated use of fungicide zole and glycine significantly increases the micro and macronutrients (N, Na, Mg, Ca, Cr, Fe, Co, Ni, Zn and Cu) of the wheat plant and reduces the concentration of toxic elements such as cadmium and lead. The number of leaves, seed germination, root and shoot length show maximum growth at S5. Reduced soil pH and increased electrical conductivity improve micro and macro-nutrient solubility. improve seed germination, and biomass accumulation. The finding of this study also indicates that fungicide-amino acid amendments are effective soil conditioners that improve soil fertility and nutrient uptake in wheat plants, although careful dosage optimization is necessary to balance crop protection with nutrient bioavailability.

Key words: Glycine, fungicide zole, wheat plant (*Triticum aestivum*), Scanning electron microscopy (SEM), Energy Dispersive X-Ray (EDX)

INTRODUCTION

Preservation of the health of the soil is most significant factor for agricultural sustainability. Soil quality influences not only agricultural profitability decisions but also environmental quality and the health of human beings, animals and plants (Das *et al.*, 2022; Maharjan *et al.*, 2024; Jabin *et al.*, 2021). The poor quality of soil in Asia is one of the biggest challenges for soil scientists. Weeds, insects, pests and plant diseases are causing huge harm to agriculture fields and 40% of total crops get damaged yearly due to this problem (Dhaliwal *et al.*, 2015; Nayak and Solanki, 2021). So, pesticides are used significantly to target insects, pests and weeds. But as per previous studies, only 0.1% pesticides are being utilized effectively for killing pests, insects and weeds (Vermelho *et al.*, 2024). Remaining pesticides is causing accumulation in the environment, deprivation of healthy ecosystem and bringing soil and water contaminations (Datta *et al.*, 2024; Cui *et al.*, 2024). Fungicide zole is one of the significant pesticides which are being used to

control fungi, bacteria, and viruses adversely affecting plants (Jørgensen and Heick, 2021). Fungicide zole is a mixture of Tebuconazole (18.3%) and Azoxystrobin (11%).

Wheat (*Triticum aestivum* L.) is the member of the Poaceae family and second most important crop in the world after rice. Wheat is the most significant staple food globally and meets requirement of nutrition for human beings, especially for poor and below poverty line population of world as many of them cannot afford the requirement of nutrition by purchasing other foods rich in protein. Hence, it is necessary to get micro and macro-nutrient rich wheat products to meet the protein requirement of vast population.

Previous study concluded that presence of zinc, copper, iron and manganese significantly improves the growth of wheat plant including yield (Hasheminasab *et al.*, 2023). In another study, researchers observed that when zinc, copper, iron and manganese dosage are supplied to wheat plants, the height of the plants increases (Norton, 2013; Liu *et al.*, 2014). The inter-relationship of soil properties with micronutrients plays significant role for wheat plant sustainability and crop production improvement. Hence, balanced amount of micronutrients and its proper adsorption in soil are vital requirements for the health of the wheat plants in terms of plant height, number of seed germination, shoot length and root length.

Free amino acids in the soil are also very significant for maintaining the needful concentration of nitrogen in soil. It also leads to healthy crop production. Nitrogen is essential element in amino acids and can be supplied to soil and plants for soil conditioning (Dong *et al.*, 2024; Khan *et al.*, 2021). Methionine acts as a precursor for Sulphur containing metabolic activities in plants (Hildebrandt *et al.*, 2015). However, glycine is responsible for folate metabolism and photorespiration; thereby connecting amino acid to both one-carbon metabolism and photosynthetic efficiency (Han *et al.*, 2024). By the transportation of amino acids in plants, they help to enhance microbial activities of soil. Hence, they improve various nutrient cycling and soil fertility for proper growth of the plants (Heidarzadeh, 2025).

Previous research has been found on usage of fungicide zole in cabbage, onion, radish and papaya for killing fungi and insects (Yang *et al.*, 2011; El-Baky *et al.*, 2021; Yu *et al.*, 2023). But no research could be found for the utilization of fungicide zole for increasing the micro and macro nutrient level of wheat plants. Hence, in present study the authors have explored the effects of amino acid, namely glycine with natural soil and soil amended with fungicide zole on wheat plant's overall growth in terms of number of seed germination, shoot length, root length and multi nutrients uptake. The glycine is non-polar and neutral in nature. The molecular weight of glycine is 75.07 gm/mol. The adsorption behaviour of fungicide zole and glycine with soil has also been studied here by SEM analysis.

MATERIALS AND METHODS

The present study was conducted by collecting soil from Tigaon, a village in Faridabad (28.3516°N, 77.3966°E), Haryana, India. The study was conducted between December 2023 to March 2024 (90 days). The physico-chemical properties of soil were determined. The soil is found to be clayey sand soil. The mechanical properties of the soil have been done by using the hydrometer method (Khan *et al.*, 2000). Seeds of wheat plants were taken from the certified "Bharat seeds and pesticides" of Faridabad district, Haryana, India. Uncharged and non-polar amino acid namely glycine was purchased from Central Drug House (CDH, India). Fungicide zole (tebuconazole 18.3% + Azoxystrobin 11%) were chosen as soil conditioners (also known as soil amendments) and purchased from (CDH, India). Soil pH was determined by Elico LI-10T pH meter, and electrical conductivity (EC) could determine in a 1:5 soil-water suspension using

a Hanna HI 8314 meter. Following their extraction using 1 M ammonium acetate buffered at pH 7, the amount of the exchangeable bases sodium (Na), calcium (Ca), magnesium (Mg), potassium (K) was determined using inductively coupled plasma optical emission spectrometry (Perkin Elmer Optima, Waltham, Massachusetts).

The experiment consists of five pots treatments in a randomized block design (RBD) in three different sets of replications to study the effects of fungicide zole and glycine on the growth of wheat plant (*Triticum aestivum* L.). The soil was mixed with uniform dosage of glycine (0.1M in 1 Kg soil) and different dosages of the fungicide zole (0-8% per kg soil). To analyze the effect of fungicide zole amended soil on the growth and nutrient uptake in the Wheat plant (*Triticum aestivum* L.), the 90-days study was done from December 2023 - March 2024. In order to investigate the impacts of fungicide zole and glycine on plant development and nutrient uptake of wheat plant (*Triticum aestivum* L.), the experiment included five different combinations in a randomized block design (RBD) in three sets of replications. Each set includes five different pots and marked as shown in **Table 1**.

Table 1: Concentration of fungicide zole and glycine in each soil sample

Sample types	Additives
S1	0% fungicide zole + 0.1 M glycine/kg soil
S2	2% fungicide zole + 0.1 M glycine /kg soil
S3	4% fungicide zole + 0.1 M glycine/kg soil
S4	6% fungicide zole + 0.1 M glycine /kg soil
S5	8% fungicide zole + 0.1 M glycine/kg soil

The pots were maintained at 20-25°C and distilled water was mixed regularly to maintain the moisture level. After reaching 90 days' time, plants were manually rinsed with distilled water to remove dirt and dust. The set of experiments has been shown in **Figure 1**. The plant growth in terms of number of leaves, seed germination, root growth, shoot growth, and root length has been determined. The pH and EC have been determined at varying concentration of fungicide zole.

Statistical analysis

The data obtained in terms of nutrient uptake at varying concentration of fungicide zole (0% to 8%) was statistically analyzed by using the Two-Factor ANOVA analysis. Analysis of variance for ANOVA was analyzed in the current work. The analysis determines whether the differences among the variables are significant or non-significant. The data has been presented in **Table 3** and **4**.

RESULTS

The physico-chemical properties of soil have been studied. Mechanical composition in terms of sand, clay and sand, pH, EC, moisture content and exchangeable cations have been determined and tabulated in **Table 2**. The composition of sand, silt and clay are found to be 79%, 9% and 12% respectively. Hence, the soil is classified as clayey sand soil. Among all exchangeable cations, Mg^{2+} is present in maximum quantity and Na^{+} is available in minimum quantity.



Figure 1: The set of experiments for wheat plants with various dosages of fungicide zole and glycine (photograph taken after one month of plantation)

Table 2: Properties of soil used for the investigation:

Parameters	Value
Sand	79%
Silt	9%
Clay	12%
Electrical conductivity (ms/m)	5.54
pH (1:5, soil: water)	8.63
Moisture content	25.58%
Exchangeable cations (Cmol/Kg soil)	
Na ⁺	0.65
K ⁺	5.41
Ca ²⁺	13.90
Mg ²⁺	21.84

The Effect of fungicide zole and glycine on pH and electrical conductivity in soil

The values of pH and EC were determined and recorded at varying concentration of fungicide zole (0-8%) in the soil affecting the amount of dissolved salts and alkalinity in the soil. The data has been plotted in **Figure 2**. The pH was recorded as 8.60, 7.89, 7.85, and 7.61 in sample S1, S2, S3, S4 and S5 respectively.

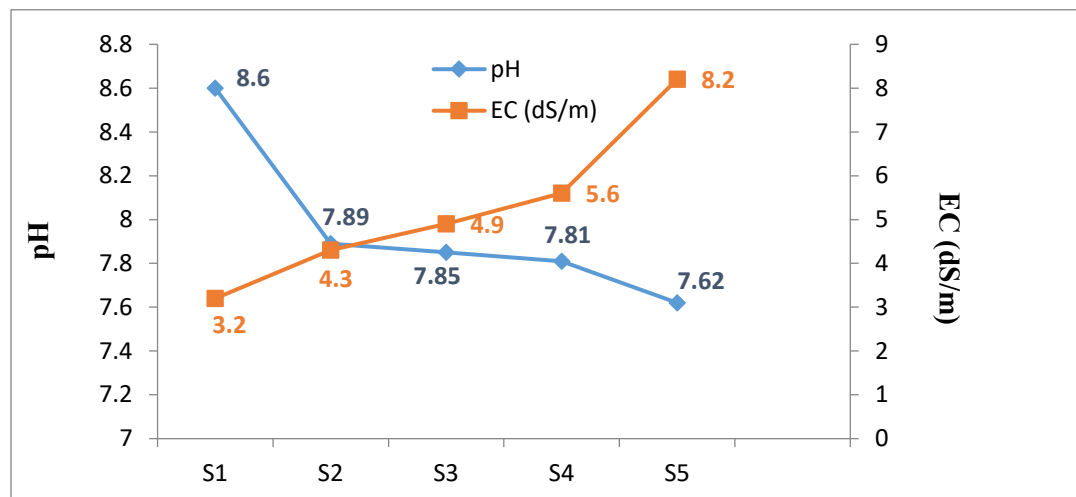


Figure 2: Effect of Fungicide zole) on clayey sand soil's pH and EC.

Effect of fungicide zole-enriched sandy clay loamy soil on wheat plant growth parameters

It can be observed from the results that maximum growth of shoot, root and seed germination could be achieved at the dosage of 8% fungicide zole mixed in natural soil along with 0.1 M glycine/kg soil. Moving from S1 to S5, number of leaves grown also increases (**Table 3**).

Table 3: Effect of varying concentrations of fungicide zole and glycine on growth parameters of wheat (*Triticum aestivum* L.)

Treatment	Seed Germination (%)	Number of Leaves	Shoot Length (cm)	Root Length (cm)
S1	50 ± 2.0 ^{ns}	5.0 ± 0.3 ^{ns}	13 ± 1.0 ^{ns}	8.2 ± 0.3 ^{ns}
S2	55 ± 2.2 ^{ns}	6.0 ± 0.4 ^{ns}	15 ± 1.2 ^{ns}	8.5 ± 0.3 ^{ns}
S3	60 ± 2.5 ^{ns}	6.7 ± 0.4 ^{ns}	18 ± 1.4 ^{ns}	8.7 ± 0.4 ^{ns}
S4	65 ± 2.7 ^{ns}	7.2 ± 0.5 ^{ns}	19 ± 1.5 ^{ns}	9.0 ± 0.4 ^{ns}
S5	70 ± 3.0 ^{ns}	7.5 ± 0.5 ^{ns}	21 ± 1.7 ^{ns}	9.2 ± 0.5 ^{ns}

Note: Values are expressed as Mean ± SD (n = 3). ns = non-significant at p > 0.05.

Scanning Electron Microscopy (SEM) and Energy-Dispersive X-ray Spectroscopy

The images of SEM for pure soil, soil amended with 0.1 M glycine and with varying concentration of fungicide zole has been shown in **Figure 3 (A-F)**. EDX has been applied to identify every element present in a sample. EDX studies provided the details about the element utilization in soil amended with 0.1 M glycine and different dosages of fungicide zole as shown in **Figure 4 (i) A-C** and **(ii) D-F**.

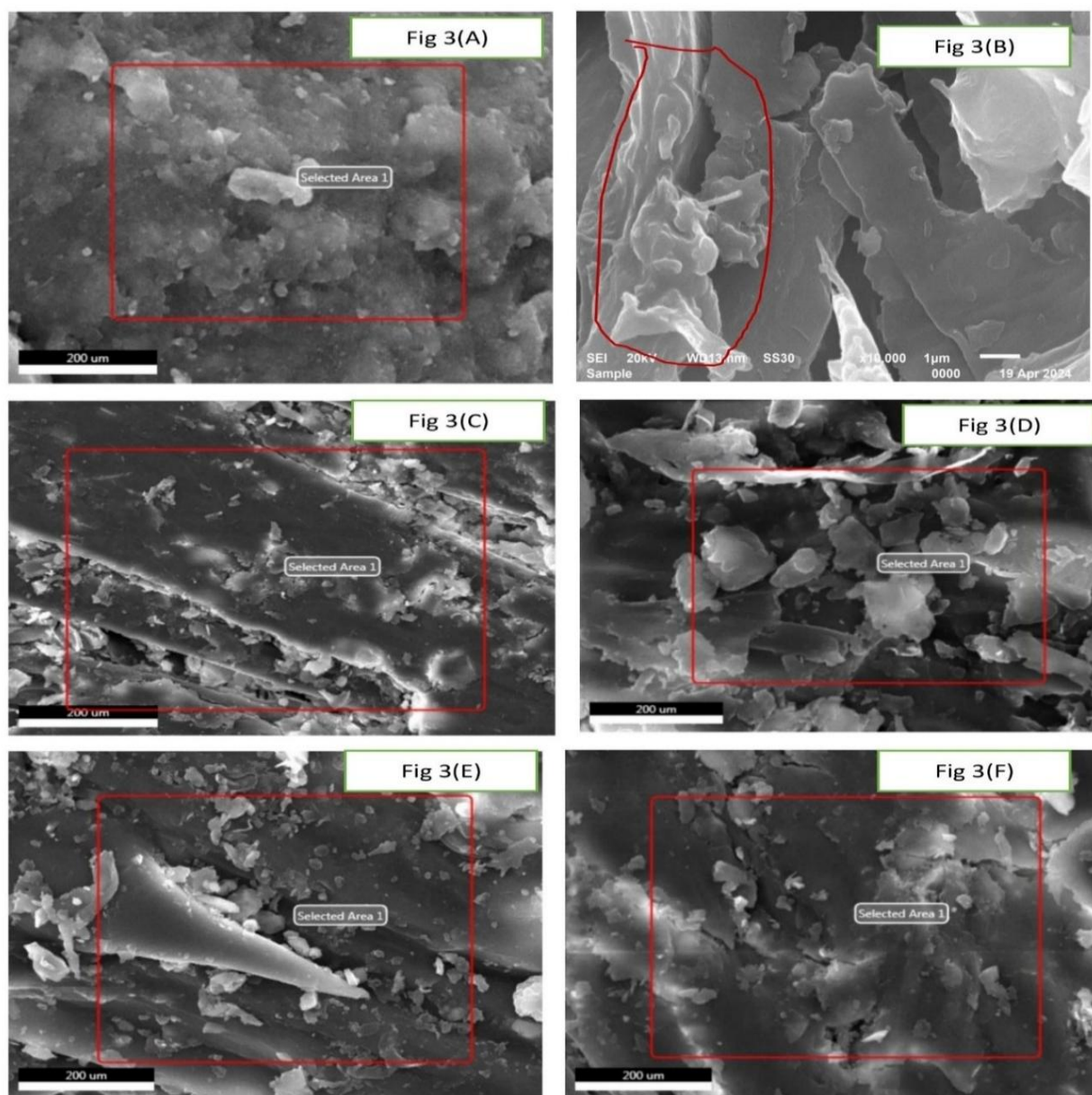


Figure 3: SEM images of (A) Pure soil (B) Pure soil with 0.1M glycine (C) Soil-2% fungicide zole- 0.1M glycine composite (D) Soil-4% fungicide zole- 0.1M glycine composite (E) Soil- 6% fungicide zole-0.1M glycine zole (F) Soil- 8% fungicide zole- 0.1M glycine zole.

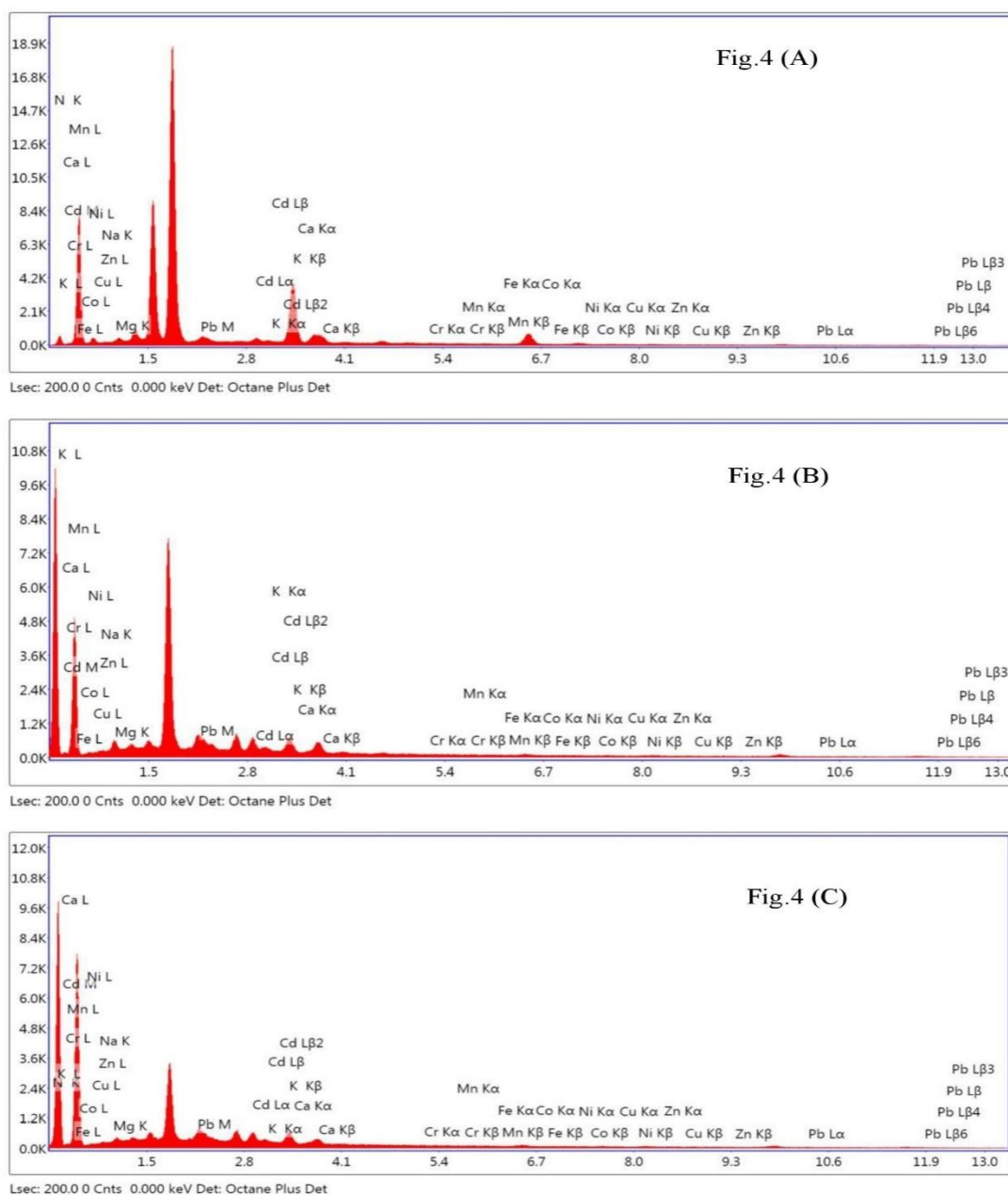


Figure 4 (i): Elemental Analysis for (A) S1: pure soil (B) S2: Soil and 0.1M glycine per kg soil (C) S3: Soil, 0.1M glycine and 2% fungicide zole per kg soil

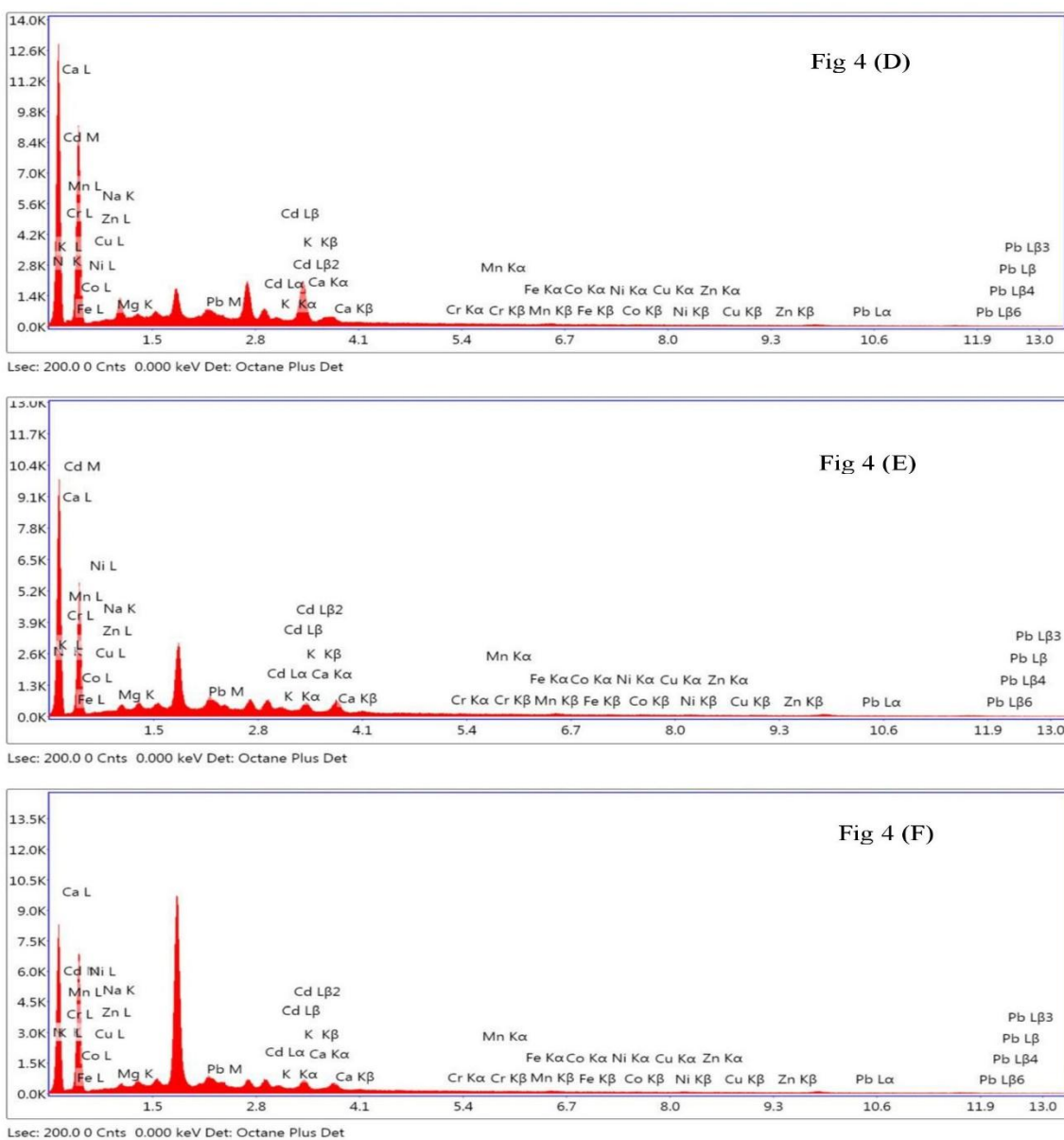


Figure 4 (ii) (D) S4: Soil, 0.1M glycine and 4% fungicide zole per kg soil (E) S5: Soil, 0.1M glycine and 6% fungicide zole per kg soil (F) S6: Soil, 0.1M glycine and 8% fungicide zole per kg soil obtained during energy-dispersive X-ray spectroscopy – EDS

DISCUSSION

Effect of fungicide zole on sandy clay loamy soil's pH and EC

The pure soil sample collected from village Tigaon, Faridabad, Haryana, India is found to be alkaline in nature and has pH value of 8.63. However, after the addition of 0.1M glycine, the pH attainment is 8.60. However, pH was found to be 8.60, 7.89, 7.85, 7.80 and 7.61 at the dosage of 0%, 2%, 4%, 6% and 8% fungicide zole amended in soil respectively. Hence, increase in the concentration of the fungicide zole resulted in a corresponding decrease in soil pH. The results

also explored that EC is found to be 5.4 for pure soil as shown in table 1. The addition of 0.1M glycine per kg of soil increased the EC to 5.60. Subsequent amendment with fungicide zole further increased EC values, with the exception of the 6% zole treatment, which showed a decrease. Increase in value of EC may be due to presence of soluble salts present in the soil.

The study also concluded that there is a strong negative correlation found between pH and EC across all samples. As the pH of soil sample increases, the EC tends to decrease across all samples. The soil samples are found to be alkaline in nature. As concentration of fungicide zole increases in soil, the salinity increases and pH decreases (Tewatia *et al.*, 2025).

Combined effect of soil- glycine and Fungicide zole composite on wheat plant and nutrients uptake

Table 3 demonstrates the effects of fungicide zole in soil in conjunction with glycine, affecting wheat plant's capability to absorb nutrients. Glycine has been chosen to add in soil for proper growth of wheat plants. Because glycine is responsible for folate metabolism and photorespiration; thereby connecting amino acid to both one-carbon metabolism and photosynthetic efficiency (Han *et al.*, 2024). Glycine also coordinates plant physiological processes by linking primary and secondary metabolism. Field examination data revealed sets of values in terms of seed germination, number of leaves grown, shoot length and root length. The study revealed that number of leaves and seed germination is maximum in wheat plants grown in soil amended with 8% fungicide zole. It has also been observed that shoot length and root length are also maximum in soil mixed with 8% fungicide zole. An application of zole with glycine leads to the consistent and dose-dependent improvement in all growth parameters ranging from S1 to S5. But these differences were not statistically significant ($p > 0.05$). Although improvements were consistent and meaningful. Results show that fungicide zole in combination with glycine may enhance plant growth, the variability within treatments limits statistical confirmation at the current sample size.

The nitrogen content in samples from S1 to S5 shows substantial variation, with the lowest value in S1 (5.6%) and highest in S5 (91.28%) as shown in **Table 4**. The trend may indicate higher organic matter in S5 value due to conjunction of 8% fungicide zole. The fungicide zole improved the soil properties and leads to the proper development of the wheat plants. Nitrogen is an essential component for various metabolic activities of the plants including photosynthetic activities. Photosynthetic activities lead to proper growth of the wheat plants in terms of seed germinations, number of leaves, shoot and root length. The S1 sample has minimum nutrient uptake in terms of nitrogen, sodium, magnesium, calcium, chromium, iron, cobalt, nickel, zinc and copper. However, it has also been observed that nutrient uptake and plant height is not directly proportional to each other. But at high concentration of micro and macronutrient, plants showed appreciable growth. However, Na rise from 16.09 (S1) to 22.15 (S4), followed by a sharp drop in S2 (4.21). This trend recommends possible accumulation of salt in wheat plants. Fe concentration enhances sharply from 3.44 (S1) to 24.01 (S4), showing strong positive correlation with Cr. It may be because of mineral dissolution or Fe oxide-bound elements. Mg values rise from 12.56 (S2) to 28.45 (S4), highest in S4, shows enhanced mineralization in plants due to clay-cation interactions. Both nickel and cobalt are significant micronutrients required for critical enzymatic functions in wheat plants, but they are required in trace amounts. But they were available in appreciable amount in sample "S5" also. Overall results S3 to S5 show rich nutrient uptake by plants and indicate high level of organic and microbial activities as shown in **Table 4**. The reduced amount of toxic metals such as Cd and Pb

shows healthy plant growth in the presence of fungicide zole. Although, the differences are not statistically significant.

Table 4: Effect of fungicide zole and glycine on nutrient uptake of wheat plants

%	N	Na	Mg	Pb	Cd	Ca	Cr	Fe	Co	Ni	Cu	Zn
S	5.6 ±	16.1 ±	14.8 ±	24.4 ±	10.3 ±	13.2 ±	3.6 ±	3.4 ±	1.9 ±	1.9 ±	3.2 ±	1.9 ±
1	0.5 ^{ns}	1.2 ^{ns}	1.0 ^{ns}	1.5 ^{ns}	0.8 ^{ns}	1.0 ^{ns}	0.3 ^{ns}	0.3 ^{ns}	0.2 ^{ns}	0.2 ^{ns}	0.3 ^{ns}	0.2 ^{ns}
S	89.9 ±	4.2 ±	12.6 ±	4.7 ±	2.1 ±	14.1 ±	7.1 ±	8.2 ±	0.0 ±	4.1 ±	5.2 ±	5.1 ±
2	2.0 ^{ns}	0.5 ^{ns}	0.8 ^{ns}	0.4 ^{ns}	0.3 ^{ns}	1.1 ^{ns}	0.5 ^{ns}	0.6 ^{ns}	0.0 ^{ns}	0.3 ^{ns}	0.4 ^{ns}	0.4 ^{ns}
S	90.0 ±	14.9 ±	18.6 ±	2.6 ±	1.0 ±	19.8 ±	22.0 ±	13.2 ±	1.0 ±	8.0 ±	9.0 ±	7.1 ±
3	2.2 ^{ns}	1.1 ^{ns}	1.3 ^{ns}	0.3 ^{ns}	0.1 ^{ns}	1.3 ^{ns}	1.5 ^{ns}	1.0 ^{ns}	0.1 ^{ns}	0.6 ^{ns}	0.7 ^{ns}	0.5 ^{ns}
S	87.7 ±	22.2 ±	28.5 ±	2.6 ±	1.3 ±	26.9 ±	18.1 ±	24.0 ±	2.0 ±	11.0 ±	12.4 ±	10.1 ±
4	2.1 ^{ns}	1.5 ^{ns}	1.8 ^{ns}	0.2 ^{ns}	0.2 ^{ns}	1.6 ^{ns}	1.3 ^{ns}	1.7 ^{ns}	0.2 ^{ns}	0.8 ^{ns}	0.9 ^{ns}	0.8 ^{ns}
S	91.3 ±	18.6 ±	20.9 ±	3.4 ±	1.8 ±	16.6 ±	10.6 ±	20.5 ±	2.1 ±	11.2 ±	14.0 ±	11.0 ±
5	2.3 ^{ns}	1.3 ^{ns}	1.4 ^{ns}	0.3 ^{ns}	0.2 ^{ns}	1.2 ^{ns}	0.8 ^{ns}	1.5 ^{ns}	0.2 ^{ns}	0.9 ^{ns}	1.0 ^{ns}	0.9 ^{ns}

Notes: Values are expressed as Mean ± SD (n = 3). ns = non-significant at p > 0.05; %, Percentage of nutrient uptake

The interpretation of two-factor ANOVA is presented in **Table 5**. The results along with interpretation are summarized here. According to two –factor Anova interpretation, If F-value > F-crit, then the result is statistically significant. If F-value < F-crit, then result is not statistically significant. In the current research work, the computed F-value is 2.028 which is lower than the critical value of 2.584, and the corresponding P-value is 0.107. If the P-value > 0.05 then the result is not statistically significant. Therefore, the variations observed among the soil samples were statistically non-significant. indicating that the apparent differences in nutrient concentrations are likely due to fungicide zole variability rather than any other natural effect.

Table 5. Two-Factor ANOVA without Replication for Elemental Concentrations in plant Samples

Parameters	Value	Interpretation
Df (rows)	4	Number of independent sample groups minus one
F-value	2.028	Indicates how much group means differ relative to within-group variation
P values	0.107	Probability that differences occurred by chance; >0.05 → not significant
F-crit	2.584	Threshold for statistical significance

Notes: DF, degree of freedom; F-value, Variance within groups; F-crit, is a pre-determined threshold value

Scanning Electron Microscopy (SEM) Analysis

The morphological changes in soil could be observed through the scanning electron microscopy (SEM) analysis as shown in figure 3. The vacant sites/voids present in pure soil's surface were observed in **Figure 3 (A)**. However, the spaces are well occupied by the glycine and fungicide zole as shown in **Figure 3 (B-F)**. The image of SEM shows the well-developed interactions between the soil and fungicide zole, therefore, improving the soil's characteristics and adsorption of glycine throughout the soil.

The glycine occupied the void of soil and has been observed clearly in **Figure 3 (B)**. The bonding between soil component and glycine could be observed here. However, soil, 0.1M glycine, and fungicide zole composite (2-8%) have been shown in **Figure 3 (C-F)**. It could be concluded from the results that composite with strong electrostatic force of attraction could be

seen especially in **Figure 3 (E)** (6% zole). It has been concluded from the results that most of nutrient's uptake by wheat plants have also been maximum at 6% fungicide zole per kg soil. The micrographs revealed larger aggregates with dense packing, confirming the synergistic role of fungicide and amino acids in altering soil surface properties.

Energy Dispersive X-Ray (EDX) Analysis

EDX has been applied to identify every element present in a sample. When a high-energy electron beam (HEEB) encounters with a sample under inquiry, X-rays are released. The energy of this X-ray is particular to the element and transition. A detector detects emitted X-rays, and each X-ray has properties associated with each element in the sample. EDX was performed using the Oxford INAX-ACT surface area and porosity analyser. The chemical composition of sample can be visualized by elemental mapping or line scars. XRD patterns of natural soil exhibited distinct crystalline peaks corresponding to quartz, feldspar, and other silicate minerals at $2\theta = \sim 21^\circ, 26.5^\circ, 39.6^\circ, 50.2^\circ, \text{ and } 68.4^\circ$ (figure 4 (i)- A). Soil, glycine and fungicide zole altered peak intensities and generate minor shifts in d-spacing, particularly in the low-angle region ($2\theta \leq 40^\circ$). This suggests partial intercalation of fungicide molecules within soil mineral layers. The combined treatments produced pronounced changes in peak intensity and spacing. Soil with glycine interaction showed additional variations in peak height, indicating strong amino acid adsorption onto mineral surfaces (**Figure 4B**).

CONCLUSION

The study confirms that fungicide zole combined with glycine, significantly enhances wheat (*Triticum aestivum*) performance in clayey sand soil. Reduced soil pH and increased EC increase micro and macro-nutrient solubility. improve seed germination, shoot and root length. These results also conclude that fungicide-amino acid amendments are effective soil conditioners/ additives and improve soil fertility and nutrient uptake by wheat plants. However, careful optimization in terms of dosage is required to balance fungicide application for crop protection while ensuring nutrient bioavailability. The limited statistical significance may be due to varying concentration of fungicide zole in different samples and small sample size. However, directional increase in nutrients and decrease in toxic elements across all samples signify the present work and make it different from conventional work. These findings present potential soil remediation and nutrient-enrichment effects that may reach statistical significance with increased replication with multiple types of plants.

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