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Study on Adsorption and Confinement Factors of Non-polar Amino acids in Alkaline Sandy Loam Soil in the Presence of Fungicide

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ABSTRACT

Amino acids are major sources of nitrogen in plants and soil. Amino acids areone of the most important parameter for growth of plants and health of soil. In the present study, fungicide Zole (tebuconazole 18.3% + Azoxytrobin 11%) has been added in soil and its effect on soil has been studied in presence of selected amino acids. All the selected amino acids are non-polar and different techniques used in current study include soil thin layer chromatography, X-ray diffraction (XRD) and scanning electron microscopy (SEM). It has been found that the mobility of amino acid decrease with the increase in concentration of fungicidezole in the soil. Theresults also confirmed the improvement of soil quality in terms of amino acid adsorption in presence of fungicide Zole.

Keywords: Fungicide zole, Glycine, Alanine, 2-aminobutyric acid, Scanning electron microscopy and X-ray diffraction.

INTRODUCTION

A major factor in agricultural sustainability is the preservation or improvement of the health and quality of the soil. In addition to decision regarding agricultural viability, soil quality also affects environmental quality and the health of plants, animals, and people[1]. In Asia, over fertilization, soil pollution, nutrient imbalances, and the process of soil loss are the main causes of poor soil quality. Nitrogen deficiency can hinder the crop growth and also affects the quality of crop. Addition of inorganic nitrogen fertilizers in soil systems may affect soil pH, cause ammonia emissions[2]. Inorganic fertilizers may increase nitrate concentration in water resources and cause human health implications[3]. It has been highlighted that it may increase the risk of cancer and congenital disorder.

Amino acids present in free form in soil may contribute nitrogen for plants and help to recover from deficiencies. Amino acids contain an amino group bounded to the central carbon (C) atom, a carboxyl group [–C(=O) –OH], a hydrogen ion, and alkyl group. Noroozlo *et al.,* in 2019 confirmed that glycine soil application in sweet basil plant improved the plant growth[4]. Rosa *et al.,* in 2022 studied the impact ofglycine on butterhead lettuce and confirmed the improved growth parameters in post application studies[5]. Characteristics of free amino acids like molecular weight, nitrogen content, and charge have significant role that affect their behaviour in soil. It was found in previous study that amino acids with low molecular weight have more availability in soil for uptake by plants[6]. Soil scientists assessed the effect of amino acids on nitrogen mineralization[7]. Natural amino acids can also contribute towards sustainable agriculture and they can act as green alternatives to different chelating agents[8]. Various studies confirmed that application of amino chelates improve the nutrient uptakes compared to soil application of conventional chemical fertilizers[9-10].

Previous studies confirmed the effect of amino acids lysine, methionine, isoleucine, leucine, valine, tryptophan, phenylalanine and cysteine on food of plant origin and confirmed their significant contribution in enhancing nutritional quality[11-12]. Detailed studies are required to analyse the impact of specific amino acids on soil system and plants. Few studies have discussed the sorption of polar and non-polar free amino acids and confirmed that lysine and glycine exhibited effective sorption capacity as compared to neutral and negativelycharged amino acid[13]. Raza *et al.,* 2023 evaluated the impact of Zinc enriched amino acid on the growth of rice plants under salt stress[14]. It has also been found from literature that lysin, valine and leucine play significant role in interaction mechanism of kaolinite surface with amino acids[15]. The stable hydrogen bond could be observed between kaolinite surfaces with carboxylic groups of amino acids[15]. Study reveals that glycine, glutamic acid and cysteine bring changes in structure of montmorillonite after getting adsorbed on it[16]. They are responsible for bring interlayer space on montmorillonite and forming chemical bond[16]. Hence, soil properties can be altered by the presence of free amino acids which leads to crop productivity[17].

Molecular weight, C: N ratio, polarity and hydrophobicity of amino acids significantly affect their behaviour in soil and in plant growth[18-20]. Amino acids differ significantly in molecular weight with lowest value of 75 for glycine and highest value of 204 for tryptophan. As per literature, organic matter undergoes adsorption to a negatively charged site through a positively charged amino group[21]. However, organic matter can also get adsorbed through a negatively charged amino group if multivalent metal cation acts as a metal bridge. In order to safeguard crops from fungal infections, fungicides are also commonly used in agriculture[21]. Numerous soil processes are known to be impacted by addition of materials including fungicides, bacteriostatic agents etc. either directly or indirectly. Datta *et al.,* in 2017 studied the effect of amino acid and bacteriostatic agent on soil respiration and confirmed the increase in soil respiration. However, the changes were different for different soil types[22].

Fungicides are being used in soil and plants from longer time for protection from fungi[23]. So, it is important to study the behaviour of free amino acids in naturalsoil in the presence of commonly used fungicides.

Several studies concluded that charged amino acids can be adsorbed easily on soil surface[24]. However, trend is not same with neutral and non-polar amino acids. Hence, to understand the mechanism of adsorption behaviour of neutral and non-polar amino acids including glycine, leucine, methionine, alanine, and 2-aminobutyric acid in natural soil and soil amended with fungicide Zole (tebuconazole 18.3% + Azoxytrobin 11%) has been investigated in this study. The aim of the study is to understand the effect of fungicide zole (tebuconazole 18.3% + Azoxytrobin 11%) on the mobility and adsorption of amino acid through stationary soil phase. All five amino acids are non-polar and neutral in nature. The molecular weight of selected amino acids ranges from 75.07 (glycine) to 149.21(methionine). Fungicide Zole is used in a number of different popular fungicide products and effectively control fungi, bacteria, and viruses affecting plants. Soil-TLC method has been adopted to study the effects of five amino acids in natural soil. These amino acids are non-polar in nature. Soil-TLC is one of the most efficient and useful technology for study of adsorption/mobility of amino acids[25-26]. The extent of contact and mobility behaviour between soil and fungicide is done by microstructural analysis such asX-ray diffraction study (XRD) and scanning electron microscopy (SEM).

MATERIALS AND METHODS

Materials

Five amino acidsviz;glycine, leucine, methionine, alanine and 2-aminobutyric acid have been purchased from Sigma Aldrich. Ninhydrin and fungicide zole have been taken from CDH (India). Glass plates and chromatographic jars have been purchased from Sisco, India. Solution of amino acid with 0.01 M was prepared in distilled water.

Soil sample was collected from village Tigaon, Faridabad district of Haryana, India (28.3516°N, 77.3966°E). Soil type was alkaline sandy loam soil in nature. Dried Soil sample was then crushed in a mortar and sieved by 150 μL size sieve so that homogenous soil particles can be obtained.

The mechanical analysis of the soil was done using the standard analytical procedure by Beretta *et al.,* in 2014[27]. pH meter-Elico model L1-10T was used for pH studies. Electrical conductivity of soil was determined by preparing 1:5 soil suspension (Soil: water ratio) with the help of electrical conductivity meter -Hanna HI 8314. The Jackson method was used to calculate cation exchange capacity (CEC) of soil sample[28]. Soil TLC has been employed for the identification and separation of some of the essential inorganic and organic chemicals via stationary soil bed.

Thin Layer Chromatographic Studies

The slurry of soil was prepared in deionised water with specific concentration of zole. The resulting uniform slurry was transferred on a glass plate. The prepared plateswere dried and stored in an airtight chamber. 15 μL of 0.01 M solution of selected amino acids was addedatthe base line of the chromatographic plate. The prepared glass plates were fixed in chromatographic jar with distilled water as developer. The distance of 10 cm was travelled by selected amino acids on pure soil as well as on soil mixed with Zole.

The dried soil plates were used for marking the amino acids with 0.2% solution of ninhydr in (distinguished visualizing agent, W/V). They were all placed in an oven for 20 min at the temperatures ranging from 70°C to 80°C until coloured spots appeared. After the spots had been stable for next few days, the R$_f$ values of amino acids were calculated.

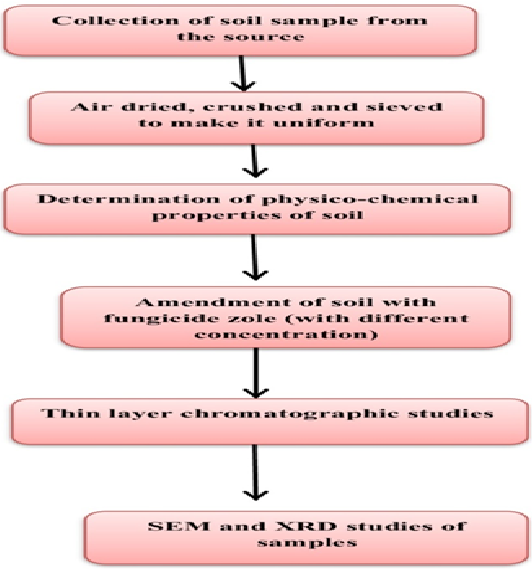
X-ray Diffraction Analysis

The X-ray diffraction studies all the soil samples under study were performed by using X-ray diffractometer (Rigaku D/Max-2500) to get an idea of interaction of natural soil and soil amended with fungicidewith five different amino acids.

Scanning Electron Microscope (SEM) Study

Scanning electron microscopy studies of soil samples were performed by Jeol, JSM 6510 v SEM apparatus at the accelerating voltage of 15KV and images were collected to explore and understand the results.

The complete methodology adopted in this study has been shown in Scheme 1.



Scheme 1: Complete experimental process adopted in the study

RESULTS AND DISCUSSION

Physicochemical Investigation

Physicochemical properties of soil samples were determined and the results are represented in Table 1. Soil isalkaline (pH=8.6) in nature with very low electrical conductivity. Electrical Conductivity indicates the salinity of soil. The cation exchange capacity of soil was found to be 0.82 cmol Kg^{-1}. The soil has maximum composition of sand (76%) and minimum composition of silt (9%).

Table 1: Physico-chemical properties of natural soil

Characteristics	Values
Sand;	76%
Silt;	9%
Clay.	15%
Electrical Conductivity	0.56ms/m
pH	8.6
Moisture	27.58%
Cation exchange capacity	0.82 cmol Kg^{-1}
Exchangeable cations (Wtpercentage)	
Na^+	2.88
K^+	1.82
Ca^{2+}	17.3
Mg^{2+}	13.37
Zn^{2+}	2.53

Study of Mobility of Amino acids with Natural and Amended Soil

Variation in retention (R_f) values of amino acids through stationary soil phase in presence and absence of fungicidezole is depicted in Fig. 1. It indicates the maximum value for leucine (0.97) and minimum for glycine (0.70). The same behaviour in mobility of different amino acids was also noted in soil amended with 2%, 4%, 6% and 8% of fungicidezole (Fig. 1). However, R_f value/movementof each amino acid in soil decreases significantly with increase inconcentration of fungicidezole. At 8% concentration of fungicide, the decrease in retention factor for leucine was 0.17 (0.97 to 0.8) and for glycine was 0.16 (0.7 to 0.54) Thechange in R_f values/movement of all other amino acids such as alanine, 2-aminobutyric acid, methionine from 0% zole to 8% zole was 0.12-1.13. Maximum change was observed in case of leucine and minimum variation was concluded for 2-aminobutyric acid during change in concentration of fungicide zole from 0-8%. The retention of amino acids depends on the stationary phase-amino acid interaction, mobile phase-amino acid interaction and stationary phase-mobile phase intermolecular interaction. Results for all amino acids indicate that there is stronger interaction between amino acids and components of the soil amended with fungicide zole at concentration 2% or more. The mobility of studied amino acids is found to be directly proportional to themolecular weight of amino acids (glycine; alanine, 2-aminobutyric acid; and leucine) for all the soil samples under investigation but in case of methionine with sulphur group (Maximum molecular weight 149) variation in trend was observed as there is less increase in R_f value. It may be attributed due to stronger intermolecular forces between soil and methionine with sulphur element. It is noticeable that magnitude of amino acid-soil intermolecular forces for alanine and methionine are almost similar in spite of significant difference in their molecular weight. It can be concluded that the presence of fungicide in soil significantly decreases the mobility of all the amino acids.

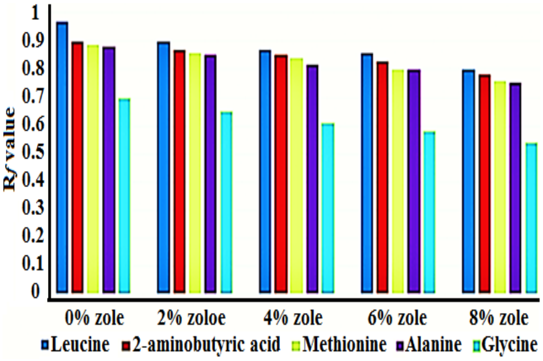


Fig. 1. R_f values of amino acids through pure soil in presence and absence of fungicidezole

X-ray Diffraction Analysis

X-ray diffraction can be utilized to check the composition and quality of soil. In XRD Patterns of soil low angle region diffraction line at 21.02 (d=0.42nm) attributed to SiO_2. A prominent peak at 26.8 (d=0.33nm) indicates $CaSiO_3$ where as peak at 39.6 (0.23nm) corresponds to Al_2SiO_3. Interplanar spacind d have been calculated by Bragg's law. For soil amended with glycine, changes in dhave been noticed. Significant change in peak height has also been observed for few peaks and some additional peaks were observed. This is due to change in no/type of atoms in soil sample amended with glycine. In soil amended with glycine and fungicidezole, significant changes in d value have been observed in low angle region (2-theta up to 40) due to further change in composition of soil sample. The XRD of soil sample in presence of other amino acids in presence and absence of fungicidezole is depicted in Fig. 2 (a-b). Similarly, shifts have been observed in soil-methionine-fungicide zole peaks as compared to pure soil. The observations are supported by change in d spacing of soil crystalline plane. These variations in peaks are clearly pointing towards the change in the soil structural arrangements due to the presence of methionine and fungicide zoleboth (Figure 2b).

d-valuescorresponding to observed peaksfor glycine and methionine are depicted in

Table 2 and 3 respectively. The similar behaviour was also observed for other amino acids indicating the change in interlayer spacing after adsorption of amino acid and addition of fungicide zole.

Table 2: 2-Theta and d spacing and values during XRD studies for soil in presence of glycine and fungicidezole

Soil		Soil with Glycine		Soil, glycine and fungicide	
2-Theta	d (nm)	2 Theta	d (nm)	2 Theta	d (nm)
21.02	0.42	20.82	0.43	20.87	0.43
26.79	0.33	23.59	0.38	26.69	0.33
27.70	0.32	26.62	0.33	26.87	0.33
27.89	0.32	27.98	0.32	27.84	0.32
28.43	0.31	36.52	0.25	30.55	0.29
36.70	0.24	39.45	0.23	36.55	0.25
39.59	0.23	42.44	0.21	37.08	0.24
45.90	0.20	45.77	0.20	39.47	0.23
50.27	0.18	50.12	0.18	40.33	0.22
55.03	0.17	54.79	0.17	42.51	0.21
60.09	0.15	59.94	0.15	50.20	0.18
64.11	0.15	64.03	0.15	51.35	0.18
67.87	0.14	67.70	0.14	55.40	0.17
68.29	0.14	73.41	0.13	59.99	0.15
68.41	0.14	75.59	0.13	68.38	0.14
75.75	0.13	79.84	0.12	75.73	0.13

Table 3: 2-Theta and d spacing and values during XRD studies for soil in presence of Methionine and fungicidezole

Soil		Soil with Methionine		Soil, methionine and Fungicide	
2-Theta	d (nm)	2 Theta	d (nm)	2 Theta	d (nm)
21.02	0.42	20.77	0.43	20.65	0.43
26.79	0.33	24.15	0.37	21.84	0.41
27.70	0.32	26.58	0.34	26.92	0.33
27.89	0.32	27.39	0.33	27.93	0.32
28.43	0.31	27.96	0.32	29.67	0.30
36.70	0.24	36.46	0.25	36.38	0.25
39.59	0.23	39.40	0.23	39.32	0.23
45.90	0.20	40.21	0.22	40.16	0.22
50.27	0.18	42.39	0.21	45.64	0.20
55.03	0.17	45.62	0.20	50.00	0.18
60.09	0.15	50.08	0.18	54.74	0.17
64.11	0.15	59.90	0.15	59.84	0.15
67.87	0.14	67.69	0.14	67.62	0.14
68.29	0.14	68.05	0.14	68.01	0.14
68.41	0.14	73.34	0.13	68.20	0.14

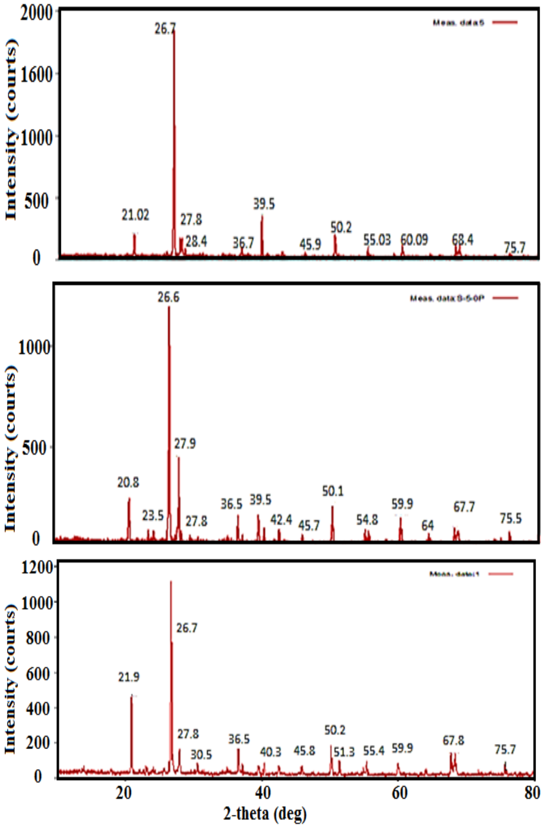


Fig. 2. (a) XRD Results of (i) pure soil (ii) soil and glycine (iii) soil, glycine and Fungicide zole

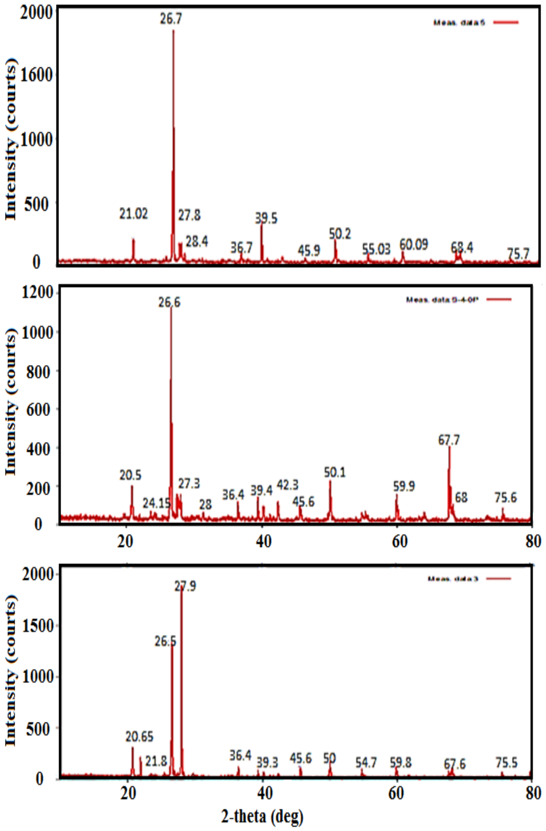


Fig. 2. (b) XRD Results (i) pure soil (ii) soil and methionine (iii) soil, methionine and fungicide zole

SEM Analysis

SEM studies of pure soil, soil amended with 8% fungicide, soil-fungicide-amino acid complex has been studied at 5000X magnification scale. SEM images of natural soil without any amendment indicates the microstructure of pure soil in Fig. 3a. The unadjusted non uniform space is visible on the surface. It has also been observed that when the fungicide is amended to the natural soil then, fungicide penetrates in the soil's internal section and covers up thevoids which results in fortification of the forces that bound all the particles together as shown in Figure 3(b).

When amino acid molecules were integrated in soil amended with fungicide, they penetrate and filled up voids indicating the formation of soil-fungicide-amino acid composite (Fig. 3c to 3g). High amino acids concentration enhance theirtendency to combine with soilthat results in increase in the formation of giant molecules.

The strong bonding in soil-fungicide-methionine complex has been found with unique and well-defined features when studied under the SEM as shown in Fig. 3(c). It may be because of presence of sulphur which occupies the vacant spaces and visible in Fig. 3(c) Increase in concentration of fungicide leads to decrease in movement of amino acid in thesoil. Thisis quite obviousin SEM images. The assimilationof amino acids on soil surface leads to larger aggregates and formation of macromolecules (Figure 3c to 3g).

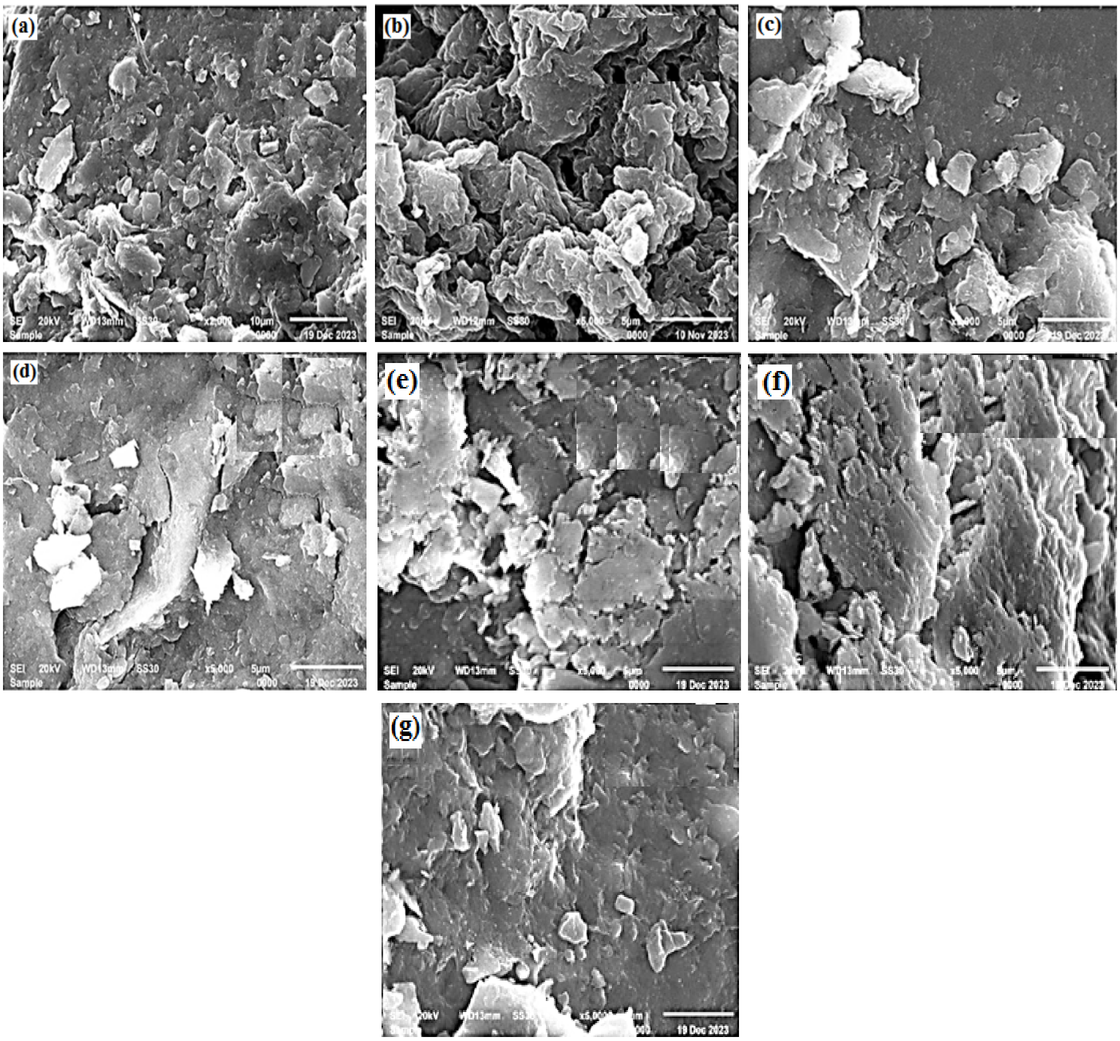


Fig. 3. SEM images of (a) Pure soil (b) Soil amended with fungicide (without any amino acid) (c) Soil amended with 8% methionine and fungicide zole (d) Soil amended with 8% leucine and fungicide zole (e) Soil amended with 8% glycine and fungicide zole (f) Soil amended with 8% alanine and fungicide zole (g) Soil amended with 8% 2-aminobutyric acid and fungicide zole

CONCLUSION

Physicochemical studies of soil indicated high soil compaction propensity with 76% of sand, 15% of clay, and 9% silt. The movement of amino acids increased with increase in molecular weight of four amino acids (glycine, alanine, 2-aminobutyric acid, and leucine) for all the soil samples under investigation but in case of methionine with sulphur group (Maximum molecular weight 149) variation in trend was not observed as per molecular weight. It may be attributed due to stronger intermolecular forces between soil and methionine with sulphur element. It is noticeable that magnitude of amino acid-soil intermolecular forces for alanine and methionine are almost similar in spite of significant difference in their molecular weight. It can be concluded that the presence of fungicide in soil significantly decreases the mobility of all the amino acids. Variations in XRD and SEM images after addition of amino acid and fungicide further support the change in magnitude of intermolecular forces and minor changes in morphological characteristics of soil sample. The results may be utilized to improve the crop quality and yield with addition of optimized dose of amino acids to sandy loam alkaline soil in presence of commonly used fungicide zole.

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Conflict of interest

The authors do not have any kind of conflict (financial or non-financial) to disclose.

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