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Research Article

Changes in biochemical constituents and antioxidant enzyme activity in groundnut (*Arachis hypogaea* L.) by the addition of coated multi-nutrient fertilization in calcareous soil

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Abstract
Sulphur and micronutrients play a vital in the growth and development of plants due to their catalytic effect on many metabolic processes. A field experiment was conducted to explore the changes in various biochemical constituents and antioxidants enzyme activities in response to coated multi-nutrient fertilization. The experiment consisted of five organic acids (citric acid, humic acid, fulvic acid, salicylic acid) and amino acid (glycine) coated multi-nutrient fertilizer sources applied at five different levels (0, 5, 10, 12.5 and 15 kg ha^{-1}). Groundnut leaf samples were collected and analyzed for biochemical constituents such as proline, soluble protein and antioxidant enzymes *viz.,* superoxide dismutase, catalase, peroxidase and carbonic anhydrase activities at harvest stages. The results revealed that, application of fulvic acid coated multi-nutrient fertilizer at 15 kg ha^{-1} registered lesser proline (5.93 µmoles g^{-1}) and higher soluble protein (22.2 mg g^{-1}) content, superoxide dismutase (8.93 EU mg^{-1}), catalase (18.2 µg H_2O_2 min^{-1} g^{-1}), peroxidase (6.11 µg min^{-1} mg^{-1}) and carbonic anhydrase (14.8 EU mg^{-1}) activities at harvest stage followed by 12.5 kg humic acid coated multi-nutrient fertilizer. The lesser response was noted with NPK control in influencing the biochemical constituents and antioxidant enzymes. It was concluded that fulvic coated multi-nutrient fertilizer at 15 kg ha^{-1} was the better source for improving the biochemical constituents and antioxidant enzymes of groundnut in calcareous soils.

Keywords: Antioxidant enzymes, Biochemical constituents, Calcareous soil, Coated multi-nutrient fertilizer, Groundnut

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) serves as the world's largest source of edible oil, and ranks second among oilseed crops (Saranyadevi and Mohideen, 2022) in India. It is the major source of dietary oil (42-52%), protein (25-30%) and an important cash crop for both subsistence and urban dwellers (Manaf *et al.,* 2017). It is grown worldwide at 22.2 million ha and in India, it is grown at 48.5 lakh ha (INDIASTAT 2021-22). About

70% of the world's groundnut cultivation occurs in the semi-arid and tropics, where the soils are predominantly calcareous and alkaline (Manasa *et al.,* 2020). Soil calcareousness is the major growth limiting factor that limits plant growth and yield due to its poor soil organic matter and high pH with lesser availability of essential plant nutrients (Chen *et al.,* 2021). The availability of essential nutrients such as phosphorous, sulphur, boron, zinc, iron, copper and manganese were deficient in calcareous soils due to various chemical reactions like precipitation, fixation and adsorption. The fixation of nutrients is an inevitable problem in calcareous soil which can be abated by the application of controlled release fertilizers by coating with various polymers (Sarkar *et al.,* 2018). Coating fertilizers with organic acids helps improve fertilizer use efficiency and reduces the losses of essential plant nutrients (Li *et al.,* 2021). Amino acids are also an interesting coating agent due to their bio-compatibility and higher stability (Aghazadeh *et al.,* 2017). Under such conditions, organic acids and amino acids coated multi-nutrient fertilizer plays an important role in groundnut and substantially enhances yield and quality on calcareous soils.

Sulphur is considered to be the fourth major plant nutrient which helps in the formation of plant proteins and it is essential for the formation of chlorophyll, improves root growth and plays a vital role in the nutrition of oil seed crop as a constituent of S-containing amino acids (Poonia *et al.,* 2022). Micronutrients are usually required in minute quantities but essential for various activities; they play a vital role in the growth and development of plants due to their catalytic effect on many metabolic processes. Iron is involved in synthesizing chlorophyll and is essential for maintaining chloroplast structure and function (Tardy *et al.,* 2020). Many enzymes require iron as a cofactor for their function, which is involved in oxidative phosphorylation, a metabolic pathway that converts nutrients to energy (Rout *et al.,* 2015). Likewise, Zn activates the electrophile and nucleophiles as a component of plant carbonic anhydrase and many other photosynthetic enzymes, influencing the photosynthetic efficiency, chlorophyll structure and content. It is also involved in sucrose and starch formation, protein metabolism, membrane integrity, auxin and defense mechanism (Suganya *et al.,* 2020). Boron has an inevitable role in cell wall synthesis, membrane stability, nitrogen and carbohydrate metabolism and improves protein and oil content of crops (Lewis *et al.,* 2019). Copper is involved in photosynthesis, cell wall biosynthesis and secondary metabolism (Schulten *et al.,* 2017).

Availability of these nutrients in soil not only enhances the growth and yield of the groundnut crop but also plays a major role in improving the biochemical constituents such as proline, soluble protein and antioxidants enzymes *viz.,* catalase (CAT), superoxide dismutase (SOD), peroxidase (POX) and carbonic anhydrase (CA) activities. These enzymes are considered efficient hijackers of reactive oxygen species, which cause damage to various physiological functioning (Soares *et al.,* 2019). The increase in the activity of important enzymes with the application of Fe, Zn, Cu and B was reported by several authors in various crops (Zhu *et al*., 2019). The increased activity of antioxidant metabolism allows plants to avoid accelerated senescence, leaving the plants green for longer. Proline plays an important role in cellular osmotic regulation and plant protection against different abiotic stresses. Proline synthesis is a highly exhaustive process and can be reduced with the application of S and micronutrients, especially Fe and Zn (Saha *et al.,* 2016), thereby benefiting the crops by saving more energy. Sulphur fertilization in crops enhanced nitrogen uptake, thus enhancing protein synthesis. From the literature, it was perused that different forms and levels of S and micronutrients give differential responses to plant physiology, particularly in biochemical constituents and enzymes (Avalos-Llano *et al.,* 2018). Hence the present investigation was carried out to understand the responses of groundnut in improving biochemical constituents and antioxidant enzyme activities by the addition of organic acids and amino acids coated multi-nutrient fertilizer on calcareous soils.

MATERIALS AND METHODS

Experimental details

A field experiment was conducted during January to April, 2022 to understand the antioxidant enzyme activity and biochemical responses of groundnut to coated multi-nutrient fertilization in the farmer's field (11˚16'07.1" N, 77˚06'27.9" E) at Annur village, Coimbatore district. The multi-nutrient fertilizer was prepared using straight commercial fertilizers of sulphur as well as micronutrients (Zinc, Iron, Copper and Boron) based on the weightage of crop demand and coated with various organic acids and amino acids. The experiment consisted of two main factors: different organic acids and amino acids coated multi-nutrient fertilizer sources (Citric acid, Humic acid, Fulvic acid, Salicylic acid and Glycine) and levels (0, 5, 10, 12.5 & 15 kg ha^{-1}). The experiment was laid out in Factorial Randomized Block Design (FRBD) with three replications. The groundnut variety "VRI 8" was sown with 30 cm x 10 cm spacing. The recommended fertilizer nutrients such as Nitrogen, Phosphorous and Potassium (25: 50:75 kg ha^{-1}) were applied as per soil test recommendation. The crop was raised with all the standard package of practices and protection measures as and when required. The changes in the antioxidant enzyme activity and biochemical constituents of groundnut for coated multi-nutrient fertilization were determined and reported.

Physico-chemical properties of the experimental soil

The experimental soil was sandy clay loam in texture, with pH of 8.30 and low in electrical conductivity (0.33 dS m^{-1}) representing Periyanayakenpalayam soil series (Typic ustrochrepts) estimated using potentiometry and conductometry, respectively (Jackson, 1973). The free $CaCO_3$ content was analyzed by the rapid titration method (Piper, 1944). Soil was moderately calcareous with a free $CaCO_3$ content of 13.5%. The soil had low available nitrogen (240 kg ha^{-1}) determined by the alkaline permanganate method (Subbiah and Asija,1956), medium available phosphorous (12 kg ha^{-1}) which was estimated by spectrophotometry outlined by Olsen *et al*. (1954) and high (322 kg ha^{-1}) available potassium (flame photometry method, Stanford and English, 1949). The calcium and magnesium were determined by Versenate titration method given by Jackson (1973) and the sulphur content was analyzed using spectrophotometry (Williams and Steinbergs, 1959). The secondary nutrients were sufficient in availability and the values were 286, 134, 10.2 mg kg^{-1} for calcium, magnesium and sulphur, respectively. The micronutrients content in the soil was estimated by 0.005M DTPA extractant using Atomic Absorption spectrophotometer (Model: GBC Avanta PM, Lindsay and Norvell, 1978). As regards the micronutrients, the soil was sufficient in available Cu (0.73 mg kg^{-1}) and Mn (3.45 mg kg^{-1}) and deficient in available Fe (5.86 mg kg^{-1}), Zn (0.69 mg kg^{-1}) and B (0.32 mg kg^{-1}).

Estimation of biochemical constituents

The biochemical constituents, such as proline and soluble protein, were determined at the harvest stage. Five hundred milligram of leaf sample was weighed and macerated with 10 ml of 3 per cent sulpho salicylic acid and centrifuged at 3000 rpm for 10 minutes. Two ml of the supernatant solution was taken in a test tube and added with 2 ml of acid ninhydrin, 2 ml of glacial acetic acid and 2 ml of 6 M orthophosphoric acid. The solution mixture was transferred to a separating funnel and added with 4 ml of toluene. Shaken uniformly for 30 seconds and the colorless bottom layer were discarded. The upper pink colour solution was used for measuring the OD value at 520 nm and proline content was reported as µmoles g^{-1} fresh weight (Bates *et al*., 1973).

Fifty milligrams of leaf sample were weighed and macerated with 10 ml of phosphate buffer solution. The extract was centrifuged at 3000 rpm for 10 min. Supernatant was collected and made up to 25 ml with phosphate buffer. One ml of supernatant was pipetted out into a test tube and added with 5 ml of ACT (Alkaline Copper Tartarate) and 0.5 ml of Folin-Ciocateu reagent. The test tubes were incubated for 30 minutes at room temperature for colour development. The absorbance was recorded at 660 nm and expressed as the amount of protein in mg g^{-1} of the sample (Lowry *et al*., 1951).

Estimation of antioxidant enzymes

The S and micronutrients (Fe, Zn, Cu & B) requiring enzymes in the leaf samples were determined at various growth stages, viz., vegetative, flowering, pegging and harvest to understand the role of applied coated fertilizer in plant nutrition. The superoxide dismutase activity was measured using the Nitro blue tetrazolium (NBT) method (Beau-Champ and Fridovich, 1971). Five hundred milligram of leaf sample was macerated using 10 ml HEPES-KOH buffer containing 0.1mM EDTA and centrifuged at 15000 rpm for 15 min. The supernatant was collected and made up to 50 ml volume. One unit of SOD activity was defined as the amount of enzyme required for 50% inhibition of NBT activity at 560 nm. The result was expressed in units per milligram of fresh leaf weight. Five hundred milligrams of leaf sample were homogenized with a phosphate buffer solution at cold condition and poly vinyl pyrrolidone (PVP) and centrifuged at 5000 rpm. Three ml of 0.1 M phosphate buffer solution was taken and 50 microliter enzyme extract was added to the cuvette. The absorbance was recorded at 240 nm against the standard buffer containing the enzyme. The catalase enzyme activity was calculated and expressed in µg H_2O_2 $min^{-1}g^{-1}$ (Srivastava, 1987).

Peroxidase activity was estimated using leaf samples homogenized in phosphate buffer. Then, one ml of supernatant was taken and to this 3 ml of 0.05 M pyrogallol and 0.5 ml of 30% H_2O_2 were added. The change in absorbance was measured at 430 nm for every 30 seconds up to 180 seconds. The enzyme activity was calculated and expressed as µg $min^{-1}mg^{-1}$ fresh weight of leaf sample (Sadasivam and Manikkam, 1992). Two hundred mg of leaf tissue were weighed and placed in the Petri dish with 10 ml of 0.2 M cysteine solution at 4˚C. The leaf tissue was transferred into a test tube having 4 ml of 0.2 M phosphate buffer (pH-6.8), 4 ml of 0.2M sodium bicarbonate in 0.02 M NaOH and 0.2 ml of 0.002% bromothymol blue (dissolved in ethanol). The test tubes were shaken for 20 seconds and incubated for 120 seconds and the intensity of blue colour development was measured at 600 nm using the spectrophotometer and carbonic anhydrase activity was expressed as EU mg^{-1} (Hatch and Burnell, 1990)

Statistical analysis

Data recorded during investigations are analyzed statistically using Agress software (Snedecor and Cochran, 1967). Critical difference was computed to compare the treatment means. The significant differences between the treatments and the mean comparisons were made using Fisher's least significant difference (LSD) test at P=0.05.

RESULTS AND DISCUSSION

Biochemical constituents

Proline

The amino acid proline is part of the compatible solutes group and play an important role in cellular osmotic regulation and plant protection against different abiotic stresses. The results revealed that different coated multi-nutrient fertilizers and their levels significantly impacted the proline content of groundnut leaves with the mean proline content of 6.99 to 9.02 µmoles g^{-1}. The lowest proline content (5.93 µmoles g^{-1}) was recorded in plants treated with fulvic acid coated multi-nutrient fertilizer at 15 kg ha^{-1} and the highest proline content (10.2 µmoles g^{-1}) was recorded with NPK control (Table 1). The activity of the proline content reduced with increasing coated multi-nutrient rates from 0 to 15 kg ha^{-1}. These findings are well following Yin *et al.* (2013) who stated, that proline synthesis is a highly exhaustive process and reduced production of proline could benefit the crops by saving more energy. Heidari and Sarani (2012) reported that micronutrients, particularly Fe and Zn deficiency, lead to higher amount of proline accumulation in plants, the same as in our study. The decrease might be attributed to the proline oxidation catalyzed by iron application (Arias-Baldrich et al., 2015) and enhanced availability of S, which may play a role in maintaining the osmotic potential of the cytoplasm of cells which is important for the survival of plants (Saha *et al.,* 2016).

Soluble protein

Data presented in Table 2 shows that coated multi-nutrient fertilizer exhibited a significant effect on soluble protein content and increased with the advancement of crop growth stages and reached maximum at the harvest stage. Increasing multi-nutrient fertilizer rates from 0 to 15 kg ha^{-1} increased the soluble protein content (12.3 to 18.1 mg g^{-1}). Addition of fulvic acid coated multi-nutrient fertilizer @ 15 kg ha^{-1} recorded significantly higher (22.2 mg g^{-1}) soluble protein content. However, it was closely followed by 12.5 kg humic acid coated multi-nutrient fertilizer ha^{-1} (21.0 mg g^{-1}). The NPK control recorded lesser soluble protein content (10.1 mg g^{-1}). The results are following the findings of Krishnaprabu *et al*. (2016), who stated that lesser proline accumulation reduces denaturing of enzymes and is responsible for stabilized protein synthesis. The higher soluble protein in groundnut could be attributed to the fact that S in the plants is known to enhance nitrogen uptake, which might improve protein synthesis (Meena and Shivay, 2010). Application of micronutrients can increase the activity of antioxidant enzymes, which prevents the protein oxidation in crops, increasing the soluble protein content. Similar findings were reported by Abdel Latef and Tran (2016). The fulvic acid and humic acid coated fertilizer reduces dissolution rate and nutrient fixation in soil (Noor *et al.,* 2017), improving crop quality through efficient utilization of soil and fertilizer resources.

Antioxidant enzymes

Superoxide dismutase (SOD)

The generation of reactive oxygen species such as superoxide radicals, hydroxyl radicals and hydrogen peroxide causes oxidative damage to plants. Plants with high levels of antioxidants, either constitutive or induced, have been reported to have greater resistance to oxidative damage. Plants have evolved specific protective mechanisms involving antioxidant molecules and enzymes to defend against oxidants (Silva *et al.,* 2020). The SOD is the first enzyme in line of defense against ROS in the antioxidant system. It is responsible for eliminating O_2^- and converting it into H_2O_2, which in turn converted into water by catalase and peroxidase. The application of coated multi-nutrient fertilizer significantly increased the superoxide dismutase activity in the leaves of groundnut. Coated fertilizer application

Table 1. Effect of various sources and levels of organic acids and amino acids coated multi-nutrient fertilizer granules on plant proline (µmoles g^{-1}) content in groundnut grown on calcareous soil

Sources of coated	Levels of MNF (kg ha^{-1})					
MNF	**0**	**5**	**10**	**12.5**	**15**	**Mean**
Uncoated MNF	10.2^{a}	9.59^{ab}	9.06^{ac}	8.27^{ad}	8.00^{ad}	9.02
Citric acid-MNF	9.28^{ad}	7.41^{bd}	6.92^{cd}	6.87^{d}	6.43^{d}	7.38
Humic acid-MNF	9.53^{ae}	6.98^{be}	6.87^{ce}	6.03^{de}	6.65^{de}	7.21
Fulvic acid-MNF	9.17^{af}	6.87^{bf}	6.44^{cf}	6.52^{df}	5.93^{df}	6.99
Salicylic acid-MNF	9.67^{ab}	8.47^{b}	8.02^{bc}	7.36^{bd}	7.42^{bd}	8.19
Glycine-MNF	9.85^{ac}	8.06^{bc}	7.38^{c}	6.92^{cd}	7.01^{cd}	7.84
Mean	9.62	7.90	7.45	7.00	6.91	7.77
		S	L	S x L		
SEd		0.06	0.05	0.14		
CD (P=0.05)		0.12	0.11	0.28		

S- Sources; L-Levels; MNF- Multi-nutrient fertilizer

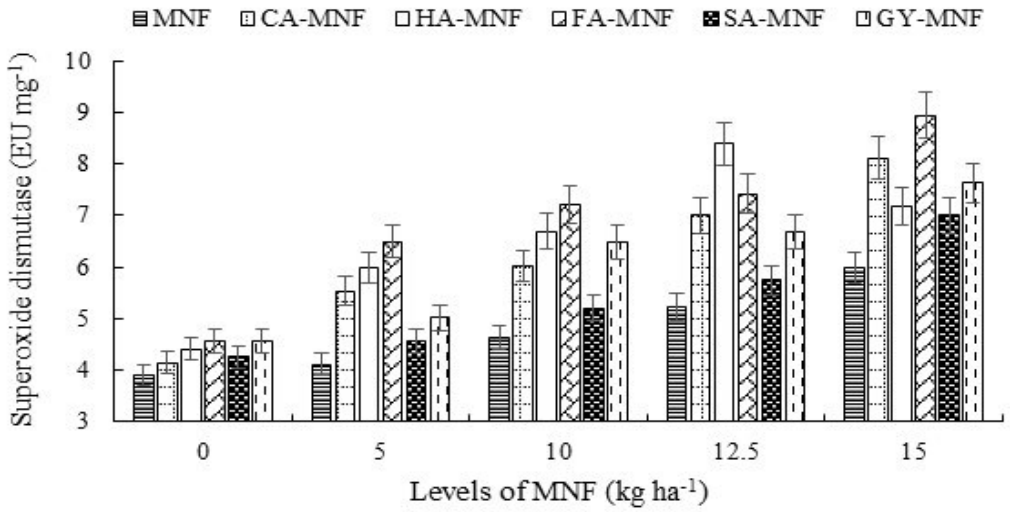

Fig. 1. *Effect of various sources and levels of organic acids and amino acids coated multi-nutrient fertilizer (MNF) on superoxide dismutase activity of groundnut grown on calcareous soil (CA-Citric acid, HA-Humic acid, FA- Fulvic acid, SA -Salicylic acid, GY-Glycine, Error bars indicate significance at 5%)*

Table 2. Effect of various sources and levels of organic acids and amino acids coated multi-nutrient fertilizer granules on plant soluble protein content (mg g^{-1}) content in groundnut grown on calcareous soil

Sources of coated MNF	Levels of MNF (kg ha^{-1})					
	0	**5**	**10**	**12.5**	**15**	**Mean**
Uncoated MNF	10.1^{ef}	11.3^{df}	12.2^{cf}	13.5^{bf}	14.2^{af}	12.3
Citric acid-MNF	12.5^{ce}	14.9^{cd}	17.0^{c}	18.0^{bc}	20.3^{ac}	16.5
Humic acid-MNF	11.5^{be}	15.6^{bd}	17.8^{bc}	21.0^{b}	18.5^{ab}	16.9
Fulvic acid-MNF	12.8^{ae}	16.8^{ad}	18.9^{ac}	20.0^{ab}	22.2^{a}	18.1
Salicylic acid-MNF	12.0^{e}	13.3^{de}	14.4^{ce}	15.8^{be}	17.3^{ae}	14.5
Glycine-MNF	12.3^{de}	14.1^{d}	15.6^{cd}	16.6^{bd}	$19.0^{a\,d}$	15.5
Mean	11.9	14.3	16.0	17.5	18.6	15.6
		S	L	S x L		
SEd		0.15	0.14	0.34		
CD (P=0.05)		0.31	0.28	0.69		

S- Sources; L-Levels; MNF- Multi-nutrient fertilizer

exhibited higher superoxide dismutase activity than uncoated fertilizer and NPK control. The highest superoxide dismutase activity was recorded with the addition of 15 kg fulvic acid coated multi-nutrient fertilizer ha^{-1} (8.93 EU mg^{-1}) followed by humic acid coated multi-nutrient fertilizer at 12.5 kg ha^{-1}. The lowest (3.90 EU mg^{-1}) SOD activity was measured with NPK control (Fig. 1) which could be attributed to the significant influence of Fe, Zn, Cu & B, which can increase the defense systems of plants (Tavanti *et al.,* 2021). Zinc has an essential role on enzymes (Cu/Zn-SOD) and the enhanced enzyme activity is due to the involvement of Zn as a co-factor for the functioning of SOD (Taran *et al.,* 2016). Rout *et al.* (2015) reported that the application of various concentrations of iron increased the SOD activity to mitigate the oxidative damage caused by ROS. An increase in the activity of SOD, CAT and POX corresponded with the application of B was observed by Keles *et al.* (2011). Lesser SOD activity was registered in uncoated multi-nutrient fertilizers since they had less benefit to controlling nutrient release for a longer period, which could result in insufficient nutrient supply in the latter period of plant growth (Cruz *et al.,* 2017).

Catalase (CAT)

Reactive oxygen species are generated through photosynthetic process in the chloroplast or during the respiratory process in the mitochondria (Sies *et al.,* 2017). They can react with various cellular constituents, such as proteins, DNA, RNA and lipids and oxidize them (Foyer, 2020). Catalase scavenges H_2O_2 generated during mitochondrial electron transport, β- oxidation of the fatty acids and most importantly respiratory oxidation. It plays an important role in plant defense, ageing and senescence of crops. The catalase activity was

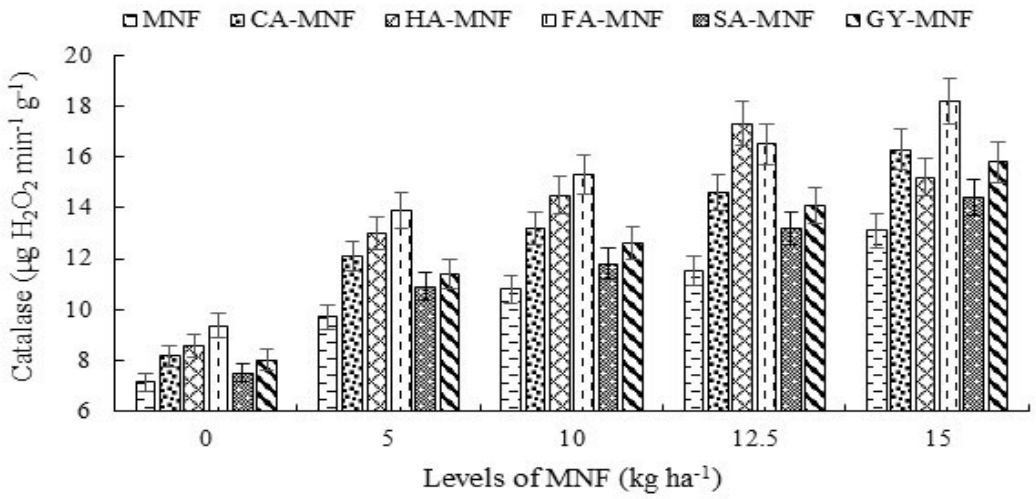

Fig. 2. *Effect of various sources and levels of organic acids and amino acids coated multi-nutrient fertilizer (MNF) on catalase (µg H_2O_2 min^{-1} g^{-1}) activity of groundnut grown on calcareous soil (CA-Citric acid, HA-Humic acid, FA- Fulvic acid, SA-Salicylic acid, GY-Glycine, Error bars indicate significance at 5%)*

higher with the coated multi-nutrient fertilizer application than with other treatments (10.5 to 14.7 µg H_2O_2 min^{-1} g^{-1}). The higher catalase activity was registered with the application of 15 kg ha^{-1} fulvic acid coated multi -nutrient (18.2 µg H_2O_2 min^{-1} g^{-1}) followed by humic acid coated multi-nutrient fertilizer @ 12.5 ka ha^{-1} (17.3 µg H_2O_2 min^{-1} g^{-1}). Lesser catalase activity in the leaves was noticed with NPK control (7.15 µg H_2O_2 min^{-1} g^{-1}, Fig. 2). The present findings are in close agreement with the results obtained by Zhu *et al.* (2019). Positive correlation between Zn, Fe, B, Cu application and catalase activity in the leaves was reported by Sarkar *et al.* (2020), thus increasing the ability to develop antioxidant defense mechanisms. Lesser nutrient release from coated fertilizers is presumably because of the reduction in effective surface area available for contact between the core of coated granules and soil. This might be the reason for coated granules to slow down the movement of nutrients into the soil, resulting in higher nutrient use efficiency and enzyme activity, which is in agreement with the findings of Sarkar *et al.* (2018).

Peroxidase (POX)

Reactive oxygen species generation and membrane lipid peroxidation which are responsive to injury and a product of senescence. Peroxidase enzyme has a protective effect and is involved in chlorophyll degradation (Cao *et al.,* 2018). Increasing coated multi-nutrient fertilizer rates from 0 to 15 kg ha^{-1} substantially increased the peroxidase activity in the leaves of groundnut (Fig. 3). The observation also depicted that, coated multi-nutrient fertilizer application had a higher impact on peroxidase activity than NPK control (2.83 to 4.93 µg min^{-1} mg^{-1}). Higher peroxidase activity was observed with fulvic acid coated multi-nutrient fertilizer @ 15 kg ha^{-1} (6.11 µg min^{-1} mg^{-1}) followed by 12.5 kg humic acid coated multi-nutrient fertilizer addition. Lesser peroxidase activity was observed with NPK control (2.08 µg min^{-1} mg^{-1}). The Fe is a constituent of enzymes associated with the cellular antioxidant system, such as CAT, POX and Fe-SOD. Similarly, an increase in POX activity in the plants treated with Zn and Cu was also reported by Kaya *et al.* (2018). Application of B can favor the increase of important enzymes such as CAT, POX and SOD in legumes (Ardic *et al.,* 2009). Hence, plants exposed to multi-nutrient application significantly increase these enzyme activities. Steady nutrient availability from coated fertilizers caused an increase in peroxidase activity, whereas loss of nutrients from uncoated fertilizer is more due to unfavorable environmental conditions, which led to poor performance and a similar result was reported by Yasmeen *et al.* (2021).

Carbonic anhydrase (CA)

Carbonic anhydrase enzyme is mostly located in chloroplast stroma and facilitate photosynthesis in C_3 plants. It is a Zn-containing enzyme which catalyzes the reversible hydration of carbon dioxide to carbonic acid. In C_3 plants, dissolved CO_2 is the substrate for the enzyme RuBisCO, which feeds inorganic carbon into the Calvin cycle (Hines *et al.,* 2021). The different organic acid and amino acids coated multi-nutrient fertilizer and levels significantly affected carbonic anhydrase activity of groundnut leaves, ranging from 7.25 to 11.3 EU mg^{-1} (Fig. 4). The carbonic anhydrase activity was higher (14.8 EU mg^{-1}) when fulvic acid coated multi-nutrient fertilizer was applied at 15 kg ha^{-1} followed by humic acid coated multi-nutrient fertilizer @ 12.5 kg ha^{-1}. The CA activity consistently increased with increasing

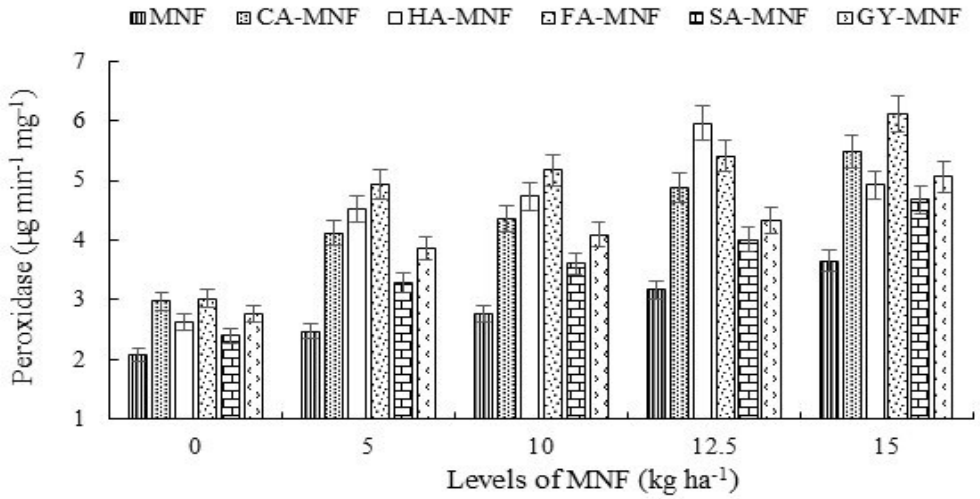

Fig. 3. *Effect of various sources and levels of organic acids and amino acids coated multi-nutrient fertilizer (MNF) on peroxidase (µg min^{-1} mg^{-1}) activity of groundnut grown on calcareous soil (CA-Citric acid, HA-Humic acid, FA- Fulvic acid, SA-Salicylic acid, GY-Glycine, Error bars indicate significance at 5%)*

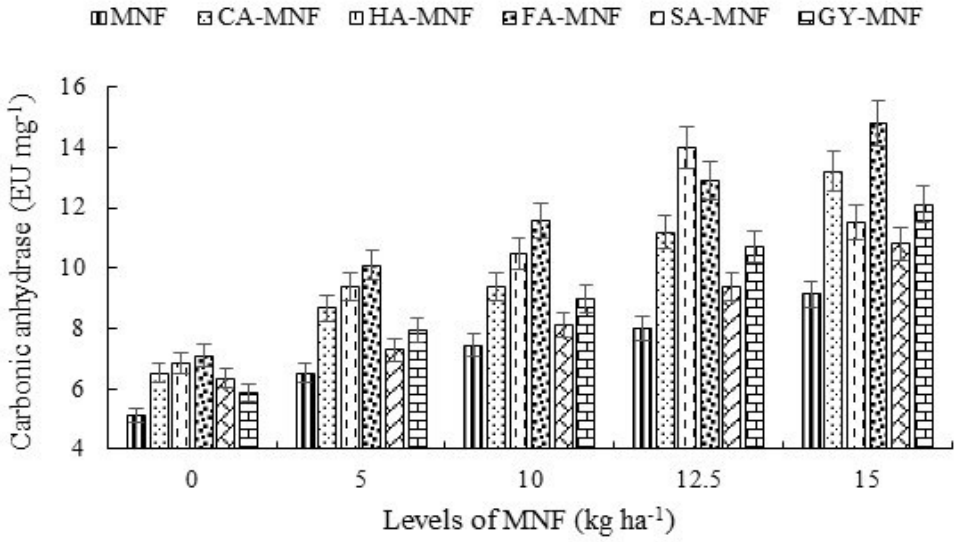

Fig. 4. *Effect of various sources and levels of organic acids and amino acids coated multi-nutrient fertilizer (MNF) on carbonic anhydrase (EU mg^{-1}) activity of groundnut grown on calcareous soil (CA-Citric acid, HA-Humic acid, FA- Fulvic acid, SA-Salicylic acid, GY-Glycine, Error bars indicate significance at 5%)*

coated multi-nutrient fertilizer. The NPK control recorded the lesser activity of carbonic anhydrase (5.12 EU mg^{-1}). These results are in conformity with the findings of Ahmadinejad *et al.* (2017). The balance between the ROS generation and the activities of antioxidant enzymes determines whether oxidative damage and signalling occur. The increase in CA might be attributed to improvement in the soil nutritional environment where coated fertilizers maintain a higher amount of bioavailable fraction in soil solution, thus supplying nutrients over a larger temporal frame (Feng *et al.,* 2015).

Conclusion

The experiment concluded that applying different organic acids and amino acids coated multi-nutrient fertilizer at various levels greatly influenced groundnut's biochemical constituents and antioxidant enzyme activities. Among all coated multi-nutrient fertilizer sources and levels, organic acid-coated multi-nutrient fertilizer was found to be very effective in improving the soluble protein and antioxidant enzymes and reducing the proline content in groundnut grown on calcareous soils. Application of fulvic acid coated multi-nutrient fertilizer @ 15 kg ha^{-1} registered lesser proline (5.93 µmoles g^{-1}) and higher soluble protein (22.2 mg g^{-1}) content, superoxide dismutase (8.93 EU mg^{-1}), catalase (18.2 µg H_2O_2 min^{-1} g^{-1}), peroxidase (6.11 µg min^{-1} mg^{-1}) and carbonic anhydrase (14.8 EU mg^{-1}) activity at harvest stage. Lesser efficiency was observed with NPK control, which was evident from higher proline and lesser soluble protein content and antioxidant enzyme activi-

ties in groundnut. Based on the study, it can be concluded that groundnut's biochemical constituents and antioxidant enzymes were improved by fertilizing fulvic acid coated multi-nutrient fertilizer @ 15 kg ha^{-1} and found to be a better source of fertilizer in calcareous soils.

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Conflict of interest

The authors declare that they have no conflict of interest.

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