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COMPARATIVE EVALUATION OF POULTRY MANURE AND NPK FERTILIZER ON HYDROGEN CYANIDE CONCENTRATION, TOTAL PROTEIN AND RHODANESE ACTIVITY IN TOMATO (*SOLANUM LYCOPERSICUM* L.) LEAVES

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ABSTRACT
Green house experiments were conducted in the Department of Agronomy, Ladoke Akintola University of Technology on Tomato (*Solanum lycopersicum* L.) to evaluate the effect of two selected fertilizers (*Azatobactor* and *Azospirillum*) on total protein, Rhodanese activity and Hydrogen Cyanide concentration. Rhaodanese activity was assayed following the method of Agboola and Okonji (2004) while Hydrogen cyanide and protein concentrations were determined by alkaline picrate colourimeter Bradford methods respectively. The result of this study reveals the response of tomato plant to inorganic fertilizer and manure application. The application of the poultry manure and NPK fertilizers significantly ($p<0.05$) increased total protein, rhodanese activity while no effect was observed on Hydrogen cyanide concentration in tomato leaves. The mineral fertilization increased total protein concentration from 445.15 ± 23.04mg to 664.37mg ± 35.55mg and rhodanese activity from **0.74U/mg** ± 0.03 to 1.75 ± 0.12 while the manure increased the protein concentration to 653.49U/mg ± 20.76 and rhodanese activity to **1.93U/mg** ±0.03. Since there was no correlation between rhodanese activity and cyanide content, the presence of rhodanese activity may suggest other functions other than cyanide detoxification.

KEYWORDS: Rhodanese, tomato, cellular respiration and fertilizer.

INTRODUCTION
The cultivated tomato, *Solanum lycopersicum L*., belongs to the diverse family Solanaceae, which includes more than 3000 species, occupying a wide variety of habitats.[1] The Solanaceae contain many plant species of economic benefit, such as food (tomatoes, potatoes, peppers and eggplants), medicines (deadly nightshade, henbane, datura) and ornamental purposes (petunias). Tomato (*Solanum lycopersicum* L.) is one of the most important horticultural crops of the world and has been widely used as a model in several fields of plant research. This has significantly contributed to the understanding of the relationship between tomato plants and environmental stress, such as high- light[2], salt[3], drought[4], temperature[5] and metal[3],[6] stress as well as pathogens and diseases.[7]

Farmers apply fertilizers, either organic or inorganic, to increase crop yield but beyond that several other factors are influenced by fertilizers. Helping farmers to know these would help in deciding the type of fertilisation regime to employ. Inorganic fertilizer has a faster rate of action.[8] When applied nutrients are released to crop plant for proper growth and development, for example NPK, fertilizer is made up of Nitrogen, Phosphorus and Potassium These are the major nutrients required by plant in large quantity when applied enhances vegetative growth of the plants.[9] On the other hand, Organic fertilizers improves soil fertility at a slower rates but do not easily leach from the soil. It also improves the soil structure and helps in the buildup of soil's organic matter of the soil, among several advantage.[10] Many researchers have documented reports on the increase in yield of various crops through the application of fertilizers.[11] Among the convectional fertilizers, NPK is mostly used by farmers in vegetable production. The nutrients composition of tomato fruit is affected by the levels of manure application either organic or inorganic manure. Based on the findings of Abolusoro[12] organic manure increases some of the nutrients component better than the inorganic but not significantly different in most cases.

Rhodanese has been confirmed to exist in plants and the enzyme has been proposed to have a role in cyanide detoxification. Rhodanese (thiosulphate:cyanide sulphurtransferase, EC 2.8.1.1) is a sulphur transferase (Str) that catalyses the formation of thiocyanate from

free cyanide and a sulphur donor. The vast distribution and abundance of rhodanese as well as its subcellular localization in the matrix of liver and kidney mitochondria suggest additional functions. It is now recognized that rhodanese is part of a system that provides sulfane sulfur for use in the formation of the characteristic prosthetic group of iron–sulfur proteins[13], [14] Rhodanese has been proposed to serves as a converter enzyme which directly alters the rate of the respiratory chain and, thus, ATP production by the reversible sulfuration of key iron-sulfur centers.[15] The model, when expanded to include signal pathways initiated by hormones or neurotransmitters, represents a mechanism by which mitochondria can recognize and meet changing energy demands.[15] There is also evidence indicating that, by virtue of this role, rhodanese may be involved directly in the modulation of mitochondrial respiratory activity.[16] This study attempts to observe the effect of NPK fertilizer and poultry manure on total protein, Rhodanese activity and Hydrogen Cyanide concentration in tomato leaves.

MATERIALS AND METHODS

Seeds of the tomato (*Solanum lycopersicum* L.) were sown in nursery trays maintained in a greenhouse at the Department of Agronomy, Ladoke Akintola University of Technology, Ogbomoso. Fifteen days after germination, plants were transferred to plant bags and assigned A, B or C. Group A is the unfertilised control while group B and C were fertilized with NPK fertilizer and poultry manure respectively. Three tomato seedlings were transferred to each bag. Fertilization was done after 50 days of transplant. Four to five tomatoes leaves were collected on the 5th day after fertilizer and manures application for analysis of protein, rhodanese activity and Hydrogen cyanide.

Preparation of Crude Extract.

The tomato leaves were washed with saline water to remove dirt. The leaves were homogenised in three volumes of 0.02 M phosphate buffer, pH 7.2. This was followed by filtration using cheese cloth. The filtrate was then centrifuged at 5000 rpm (IEC, DPR 6000) for 30 min. The supernatant was used for the final analysis.

Protein and Enzyme Assays

Protein Assays: Protein concentration was determined by Bradford method[17] by extrapolated each protein concentration from standard curve using bovine serum albumin (BSA) as standard.

Rhodanese activity: was assayed by the method of Agboola and Okonji.[18] The reaction mixture contained 50 mM sodium thiosulphate, 50 mM potassium cyanide, 0.25 mM borate buffer, pH 9.4 and 10 µl of enzyme solution in a final volume of 1.0 ml. The reaction was carried out for 1 min at 37°C and stopped by adding 0.5ml 15% formaldehyde and 1.5 ml of Sorbo reagent (which is made up of ferric nitrate solution containing 0.025 g $Fe(NO_3)$ 3,9 H2O in 0.74 ml water and 0.26 ml concentrated nitric acid). Absorbance was measured at 460 nm. The unit of enzyme activity was defined as micromoles thiocyanate formed per minute at 37°C and pH 9.2.

Determination of Hydrogen Cyanide (HCN)

This was determined by alkaline pikrate colourimeter method using 1.02g of the sample which was dispersed in 50ml of distilled water in a 25.0ml conical flack. An alkaline pikrate paper was hung over the sample mixture and the blank in their respective flasks. The set up were incubated overnight and each pikrate paper was eluted (or dipped) into a 60ml of distilled water. A standard cyanide solution was prepared and diluted to a required concentration. The absorbance of the eluted sample solution and the standard were measured spectophotometrically at 540nm wavelength with the reagent blank at zero.

The cyanide content was determined by the formula shown below:

HCN mg/kg = 1000/w x au/as x c x D

Where
W= weight of sample analyzed
au = absorbance of standard HCN solution
c = concentration of the standard in mg (d)
D = dilution factor where applicable.

Statistical analysis

The results are presented as means ± SEM. Data were analysed by one-way ANOVA by using SPSS software to examine whether there was any statistical difference among the groups. Tukey post hoc test was used to for pairwise comparison when ANOVA was significant. A *P* value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Hydrogen cyanide concentration

The result of the concentration of hydrogen cyanide as presented in table 1 showed that hydrogen cyanide is not present in tomato leaves and that the fertilization regime has no effect on the availability of Hydrogen cyanide. 'Hydrogen cyanide is a chemical compound that can be released from cyanogenic glycosides that are natural constituents of some plants such as: bitter almonds, sorghum, cassava, lima beans, stone fruits and bamboo shoots'.[19] Cyanogenic glycosides are ubiquitous in a number of plant species. These compounds serve as a defence system against predators e.g. herbivores, insects etc. Only a few of these plants are known to be of significant risk to livestock. They include grasses cultivated as forage for livestock and horses and others are ornamentals, commercial fruit trees, shrubs, weeds and range plants.[20] Every method aimed at reducing or removing hydrogen cyanide in plants must be targeted at cyanogenic glycosides which is the precursor for hydrogen cyanide, otherwise the intervention may prove futile.[19] It is obvious in our study that fertilization does

not have any effect on cyanide content in tomato leaves. Our result is in tandem with that of Omodamiro and Amechi[21] whose result of the phytochemical screening of tomato revealed the absence of hydrogen cyanide. Also, Musa *et al*., 2010[22] reported that nitrogen fertilizer has no effect on cyanide level in *Corchorus olitoriu* leaves regardless of growth stage. In contrast, Rolinda and Ma[23] reported that application of nitrogen fertilizer significantly increased cyanide content in vegetables. Nitrogen fertilizer is believed to stimulate the enzymatic conversion of tyrosine to p - hydroxymandelonitrile which ultimately leads to increase in the biosynthesis of cyanogenic glycoside.[24] The variations observed in this study from the reports of these authors suggest that the influence of nitrogen fertilizer on cyanide may depend on other factors beyond the scope of this study.

Cassava (Manihot esculenta) grown on organic fertilizer had lower cyanide concentration in both leaves and tuber than inorganic fertilizer grown.[25] The results of Imakumbili, 2018[26] revealed that varieties and soil type but not fertilizer type come into play in altering cyanide level in cassava. HCN concentration in sorghum increases as the dose of chicken manure increases. This increase in HCN content did not reach the toxic level with the highest dose of chicken manure used (7.5 tons/ha.). Poultry manure decreased the hydrogen cyanide level in passion fruit juice with increased rate of application at the second season of life.

The endogenous source of cyanide in plants is mainly the conversion of 1-amino-cyclopropane-1-carboxylic acid to ethylene that produces cyanide in equimolar amounts as ethylene and is drastically increased during fruit ripening and senescence.[27] Various enzymes, such as superoxide dismutase, catalase, glutathione reductase, and glutathione *S*-transferase (GST) work in concert to control the oxidative damage by scavenging ROS[28] and militate against the ROS generation due to the electron transport leaks. Plant GSTs have a crucial role to remove cytotoxic or genotoxic compounds. They have been found in maize, soybean, and *A. thaliana*. GSTs have been noticed to reduce peroxides by the assistance of GSH and yield scavengers of cytotoxic and genotoxic compounds.[29] Str might play a role in the control of redox homeostasis in the different subcellular compartments in a protein-protein interaction with thioredoxin. In this process, Str might act as a thioredoxin peroxidase with the intermediate formation of a sulfenate at the active-site cysteine.[30] Str activities are present in many living organisms, but their physiological roles are still ambiguously. Their ubiquity suggests additional physiological functions.[31]

The result presented in table 2 showed the total protein concentration in the tomato leaves of the various groups. Here we employed Bradford's Method.[17] This method is a rapid, simple and sensitive method for estimation of proteins in a sample extract. The method was preferred to Lowry's method[32] because metal ions such as NH_4^+ , Na^+ , K^+ , phenol and carbohydrate such as sucrose do not interfere in the assay. The procedure is based on interaction of a dye, Coosmassic Brillaint Blue with protein. Group B which is NPK fertilizer treated group had the highest protein concentration while the control group had the lowest. In similar study, Ammonium nitrate and well rotted cattle manure tested on Lucerne (*Medicago sativa l.*) had positive influence on protein content of the legume. It was found that the both types of fertilizers had strong influence on the crude protein yield in the first year of development of lucerne compared with unfertilized control. With increasing age of the plant the effect of the mineral fertilization significantly decreased while manure had positive effect all through the experimental period.[33]

Table 3 and figure 1 showed the rhodanese activity of the tomato leaves of the various groups. Group C (poultry manure applied) had the highest specific activity, followed by group B (NPK fertilizer applied) while the control group had the least specific activity. Both group B and C had a significant ($p<0.05$) increase in rhodanese specific activity when compared with the control. Our research design sought to investigate the level of rhodanese activity in response to inorganic fertilizer (NPK) and organic manure application under a greenhouse condition. It was observed that the group C (poultry manure) plants rhodanese activity was significantly higher to that of group A (control) and group B (inorganic fertilizer) in experimental plants (table 3 and figure 1). Although, the control group showed the lowest level, the relatively higher rhodanese activity level may be indicative of its possible role as part of cellular antioxidant system. The higher level of rhodanese activity in the experimental groups plants could be as a result of the ease of availability of the nutrients to the plant through its roots compared to that of group A. The uptake of these nutrients propels varying metabolic pathways in the plant. Shirai and Kurihara (1991)[34] studied the distribution rhodanese in 13 cyanogenic plants and 12 non cyanogenic plants species and concluded with the hypothesis that the enzyme is widely distributed in nature. According to Volini and Ogata (1991)[15] rhodanese in plant is located in the mitochondria and chloroplast which is suggesting its role as a modulator of heme energy production enzymes.

Plants absorbs available mineral nutrient like Nitrogen, sulphur, potassium, calcium, magnesium and utilise then for the production of various metabolic products . During the synthesis, storage and degradation of the metabolic products, chemical energy is expended which is under enzymatic control. As demonstrated in various research works that the availability and absorption of nutrient dictates its utilisation for the production of plants secondary metabolites such as carotenoids, alkaloids, flavonoids, phenols, and cyanogenic glycosides (CNglcs) that in turn affects the growth, development and (fruit) yield. The speculation of rhodanese activity as a

converter in the mitochondria in the modulation of cellular[15] might be triggered by the level of nutrient utilisation of the plant in requirement to metabolic energy. Also from the view of rhodanese functioning as a component of cellular enzymatic anti-oxidation, an increase in cellular energy production might trigger an increase in reactive oxygen species which may explain the rise in rhodanese activity in order to militate against the ROS generation due to the electron transport leaks.

Selmar and Kleinwachter[35] propose that enhanced synthesis of plant secondary products under stressful conditions provides a mechanism for dissipating excess excitation energy. Neilson *et al.*,[36] Shows that plants reduce defence costs through the use of shared biosynthetic pathways and integration into the primary metabolism. The reduced rate of photosynthesis in stressed plants makes it economically attractive for plants to store nitrogen in a reduced form, ready to be remobilized when conditions improve, rather than as photosynthetic proteins or nitrate.[37] In *H. brasiliensis*, the carbon and nitrogen resources in CNglcs can, for example, be mobilized and used for growth and latex production[38], which may likewise be similar with other plant secondary metabolites such lycopene a carotenoid found in tomatoes.

The ability of plants to remobilize the sugar and nitrogen stored in CNglcs, combined with the ability to transport CNglcs to specific parts of the plants guided by the generation of differently glycosylated transport forms, illustrates how turnover of CNglcs may play an important role in balancing primary metabolism, especially during major changes in plant ontogeny, and thereby improve plant plasticity.[35], [39], [40]

In plants, proteins with single rhodanese domains are associated with the process of leaf senescence, for example in *Arabidopsis thaliana*, *Nicotiana tabacum and Raphanus sativus*.[41] However, the mode of action in response to stress or senescence processes is not yet known. The proteins, composed of two rhodanese domains with the catalytic cysteine in the C-terminal rhodanese domain, are represented by the bovine mitochondrial rhodanese[42] and the *Azotobacter vinelandii* rhodanese (RhdA).[43] The amino acid composition of the active site loop containing the active cysteine residue affects the substrate recognition and specificity.[13] The high abundance of Str proteins in *A. thaliana* and other plant species [30] in different cellular compartments is speculated to pave the way for several specific biological functions, especially in abiotic and biotic stress defence.[31]

Table 1: Hydrogen cyanide concentration in the tomato leaves of the various groups.

Treatment Groups	Hydrogen Cyanide Concentration
GROUP A (CONTROL)	NP
GROUP B (NPK FERTILIZER)	NP
GROUP C (MANURE)	NP

NP means not present

Table 2: Total protein concentration in the tomato leaves of the various groups. Values are in mean ± SEM of five replicate.

Treatment Groups	Total Protein (mg)
GROUP A (CONTROL)	445.15 ± 23.04
GROUP B (NPK FERTILIZER)	664.37 ± 35.55
GROUP C (MANURE)	653.49 ± 20.76

Tabale 3: Rhodanese activity in the tomato leaves of the various groups. Values are in mean ± SEM of five replicate.

Treatment Groups	Rhodanese specific activity (U/mg)
GROUP A (CONTROL)	**0.74** ±0.03
GROUP B (NPK FERTILIZER)	**1.75** ±0.12
GROUP C (MANURE)	**1.93** ±0.03

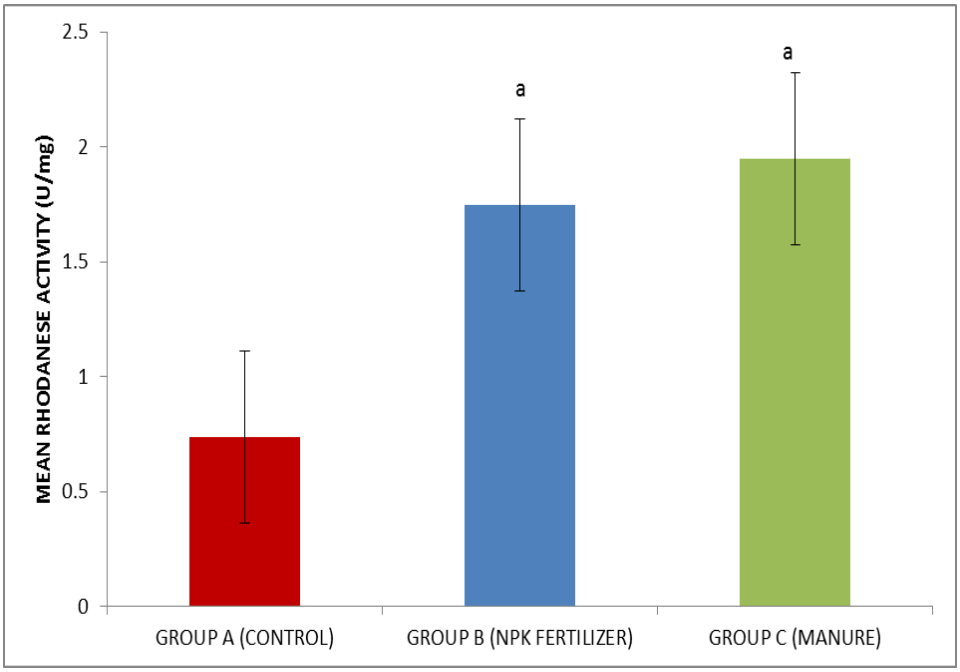

Figure 1: Bar graph of rhodanese activity in the tomato leaves of the various groups. Values are in mean ± SEM of five replicate. Superscript "a" denotes a significant mean difference when compared with to group A (control group) at P value < 0.05.

CONCLUSION

Both Mineral (NPK) and Organic (manure) fertilizers has positive effect on the parameters tested in tomato leaves, meanwhile, poultry manure proved to be more beneficial. The fact that rhodanese is present in tomato leaves with absence of Hydrogen cyanide could be indicative of other role other than cyanide detoxification.

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