

# Effect of graded levels of Zn in combinations with microbial inoculation on maize grain quality in black and red soils

Suganya Ayyar · Saravanan Appavoo

**Abstract** Field experiment was conducted in 2015 for black and red soils in order to study the maize grain quality viz., Protein, Tryptophan, Starch and Amylose using Arbuscular Mycorrhizal (AM) fungi and Zinc Solubilizing Bacteria (ZSB) in combination with graded levels of  $\text{ZnSO}_4$ . Treatment consisted of two factors viz., microbial inoculation ( $M_1$ : control,  $M_2$ : AM fungi,  $M_3$ : ZSB and  $M_4$ :  $M_2+M_3$ ) and graded levels of  $\text{ZnSO}_4$  ( $S_1$ : 0,  $S_2$ : 12.5,  $S_3$ : 25,  $S_4$ : 37.5,  $S_5$ : 50  $\text{kg ha}^{-1}$  and  $S_6$ : 0.5% foliar spray @ 45 and 65 DAS) replicated three times in RBD. In both the soils, the treatment combinations  $M_4S_5$  had registered highest true protein and crude protein than rest of the treatment combinations. The increases were to the tune of 40.0 and 36.3% over control in black soil while 39.6 and 39.0% in red soil followed by  $M_4S_4$ . Irrespective of the soils, the treatment combinations viz.,  $M_4S_5$  recorded the highest tryptophan values over other treatment combinations. The highest starch and amylase content were recorded in the treatment  $M_4S_5$  followed by  $M_4S_4$ .

**Keywords:** Arbuscular Mycorrhizal (AM) fungi · Graded levels of  $\text{ZnSO}_4$  · Maize (*Zea mays* L.) · Zinc Solubilizing Bacteria (ZSB) · Zn content · Quality of maize grain

## Introduction

Maize (*Zea mays* L.) is one of the most important cereals, next to wheat and rice in the world as well as in India. It is one of the most versatile crops and can be grown over diverse environmental conditions and also diversified uses in human food, animal feed and raw materials for large number of industrial products (Ayyar *et al.*, 2019 and Suganya *et al.*, 2020). Maize is a miracle crop called as “Queen of Cereals” and is grown in more than 130 countries. With the changing food habits and fast growing poultry industry due to increased number of non-vegetarians in the state, demand for maize grain in the form of poultry feed is continuously increasing. In India, at present, about 35 per cent of the maize produced in the country is used for human consumption, 25 per cent each in poultry feed and cattle feed and 15 per cent in food processing (corn flakes, popcorn etc.) and other industries (mainly starch, dextrose, corn syrup, corn oil etc.). Maize is a main source of calories and minerals for most rural populations (Ayyar and Appavoo, 2017).

It is the world's most produced cereal and supplies a large portion of the daily calories for billions of people (Suganya *et al.*, 2021). It also contributes to farmers income and food supply in third – world countries. Various physical properties of maize can be assessed visually. Maize has a very large germ (embryo) compared to other cereals. Another obvious physical attribute is its large seed, composed mostly of the starchy endosperm. The large endosperm is full of starch and protein (the storage protein called zein). In most maize hybrids, the starch is typical of most cereals, with a composition of around 70-80% amylopectin and 20-30% amylose. Since starch is the most abundant component in maize. In the case of maize, the kernel consists of approximately 75% starch

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(dry basis). Starch provides energy, and during germination it would provide a source of sugar for the developing germ. Protein supply amino acids, which are an important part of an animals diet. However, like most cereals, maize is quite low in protein content. The proteins in maize are similar to those found in other cereals, with storage proteins (zein in maize) being the most abundant. In addition, there are structural proteins as well as free and bound proteins within the endosperm, and all other tissue groups. The dominant protein is the zein fraction and, as in other cereals, this storage protein is found in a matrix (Tefera, 2020).

The increase in the protein content might be due to marked influence of Zn treatments on the enzymatic activity in the plants which could bring about significant changes in the crude and true protein contents in maize grains. Zinc is essential for protein synthesis, reduction in RNA-polymerase activity and increase in RNA destruction. Formation of NADPH or NADH depending on the Zn concentration might have involved in tapping and converting the radiation energy for photosynthetic activities and increased formation of sugars and starch (Suganya, 2015). Hence, considering the above facts, the present investigation is aimed to focus on increasing the maize grain quality by using graded levels of  $\text{ZnSO}_4$  and combination of Arbuscular mycorrhizal fungi and zinc solubilizing bacteria.

## Materials and methods

### Experiment I

#### Experimental soil

The field experiment was conducted at Cotton Research Station (TNAU), Perambalur. The soil was clay, deep black in colour belonging to *Typic Chromustert* (Peelamedu series). The details of soil characteristics are given in Table 1. The initial soil was moderately alkaline, non - saline, medium in organic carbon status and low in available N, P and medium in K. With regards to the available micronutrient status of the soil, DTPA-Fe, DTPA-Cu and DTPA-Mn were found in sufficient level but the availability of DTPA-Zn was found deficient.

#### Field experiments

Treatment consisted of two factors viz., microbial inoculation ( $M_1$ : control,  $M_2$ : AM fungi,  $M_3$ : ZSB and  $M_4$ :

**Table 1.** Initial soil characteristic of the experiment

| Particulars                                  | Black soil               |
|----------------------------------------------|--------------------------|
| Taxonomy                                     | <i>Typic Chromustert</i> |
| Textural class                               | Clay                     |
| pH (1:2.5 soil : water)                      | 8.34                     |
| EC ( $\text{dS m}^{-1}$ )                    | 0.30                     |
| Free $\text{CaCO}_3$ ( $\text{g kg}^{-1}$ )  | 90.4                     |
| Organic Carbon ( $\text{g kg}^{-1}$ )        | 5.10                     |
| CEC ( $\text{c mol (p}^+) \text{ kg}^{-1}$ ) | 36.2                     |
| Available nitrogen ( $\text{kg ha}^{-1}$ )   | 221                      |
| Available phosphorus ( $\text{kg ha}^{-1}$ ) | 20.0                     |
| Available potassium ( $\text{kg ha}^{-1}$ )  | 245                      |
| Available Zn ( $\text{mg kg}^{-1}$ )         | 0.80                     |
| Available Cu ( $\text{mg kg}^{-1}$ )         | 2.52                     |
| Available Fe ( $\text{mg kg}^{-1}$ )         | 10.2                     |
| Available Mn ( $\text{mg kg}^{-1}$ )         | 3.45                     |

$M_2 + M_3$ ) and graded levels of  $\text{ZnSO}_4$  ( $S_1$ : 0,  $S_2$ : 12.5,  $S_3$ : 25,  $S_4$ : 37.5,  $S_5$ : 50  $\text{kg ha}^{-1}$  and  $S_6$ : 0.5% foliar spray @ 45 and 65 DAS) replicated three times in RBD. Seeds of maize hybrids (NK 6240) were sown on the sides of the ridges by adopting a spacing of 60 x 25 cm along with vermiculite based mycorrhizal inoculum (*Glomus intraradices* TNAU-03-08) 2 g per hill at a depth of 5 cm. Zinc solubilizing bacteria was applied at 2  $\text{kg ha}^{-1}$  after mixing it with 25 kg each of sand and farm yard manure. The recommended fertilizer for maize viz., 250:75:75 kg N,  $\text{P}_2\text{O}_5$  and  $\text{K}_2\text{O}$   $\text{kg ha}^{-1}$  was followed. The full dose of P and K were applied basally and N was applied at three splits viz., basal (25%), vegetative (50%) and tasselling (25%) stages. Calculated quantities of  $\text{ZnSO}_4$  were applied basally as per the treatment schedule. The soil and plant samples were drawn at vegetative (30<sup>th</sup>), tasselling (60<sup>th</sup>) and harvest stage (110<sup>th</sup>) and analysed for DTPA-Zn and Zn content respectively in soil and plant samples. The grain and stover yields were recorded treatment wise and Zn uptake were computed.

### Experiment II

#### Experimental soil

A field experiment was conducted in red soil during 2014 at farmer's field, Pullanviduthi, near National Pulses Research Centre (TNAU), Vamban, Pudukkottai district. It lies between 8° 30' to 10° 40' North latitude and 78°

24' to 79° 40' East longitude and is at a height of 600 MSL. The soil was Sandy loam, red in colour belonging to *Udic Rhodustalf* - (Mudukulam series). The details of soil characteristics are given in Table 1. The initial soil was neutral in reaction, non- saline, low status of OC and CEC. The available N was low, P and K were found medium. The available DTPA-Fe, DTPA-Cu and DTPA-Mn were found in sufficiency level while the availability of DTPA-Zn was found deficient.

### Field experiments

Treatment consisted two factors *viz.*, microbial inoculation ( $M_1$ : control,  $M_2$ : AM fungi,  $M_3$ : ZSB and  $M_4$ :  $M_2+M_3$ ) and graded levels of  $ZnSO_4$  ( $S_1$ : 0,  $S_2$ : 12.5,  $S_3$ : 25,  $S_4$ : 37.5,  $S_5$ : 50 kg ha<sup>-1</sup> and  $S_6$ : 0.5% foliar spray @ 45 and 65 DAS) replicated three times in FRBD. Seeds of maize hybrids (NK 6240) were sown on the sides of the ridges by adopting a spacing of 60 x 25 cm along with vermiculite based mycorrhizal inoculum (*Glomus intraradices* TNAU-03-08) 2 g per hill at a depth of 5 cm. Zinc solubilizing bacteria was applied at 2 kg ha<sup>-1</sup> after mixing it with 25 kg each of sand and farm yard manure. The recommended fertilizer prescription for maize *viz.*, 250:75:75 kg N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O kg ha<sup>-1</sup> was followed. The full dose of P and K were applied basally and N was applied at three splits *viz.*, basal (25%), vegetative (50%) and tasselling (25%) stages. Calculated quantities of  $ZnSO_4$  were applied basally as per the treatment schedule.

**Table 2.** Initial soil characters

| Taxonomy                                        | <i>Udic Rhodustalf</i> |
|-------------------------------------------------|------------------------|
| Textural class                                  | Sandy loam             |
| pH (1:2.5 soil : water)                         | 6.69                   |
| EC (dS m <sup>-1</sup> )                        | 0.07                   |
| Free CaCO <sub>3</sub> (g kg <sup>-1</sup> )    | 20.1                   |
| Organic carbon (g kg <sup>-1</sup> )            | 3.53                   |
| CEC (c mol (p <sup>+</sup> ) kg <sup>-1</sup> ) | 12.6                   |
| Alkaline nitrogen (kg ha <sup>-1</sup> )        | 210                    |
| Available phosphorus (kg ha <sup>-1</sup> )     | 15.4                   |
| Available potassium (kg ha <sup>-1</sup> )      | 208                    |
| Available Zn (mg kg <sup>-1</sup> )             | 0.82                   |
| Available Cu (mg kg <sup>-1</sup> )             | 2.57                   |
| Available Fe (mg kg <sup>-1</sup> )             | 14.2                   |
| Available Mn (mg kg <sup>-1</sup> )             | 8.30                   |

### Data collection and analysis

Composite surface (0-15 cm) soil samples were collected from the site before the experiment and analysed for their physical, physio – chemical and chemical properties. Soil samples were also collected from each of the plots at vegetative (30<sup>th</sup> DAS), tasselling (60<sup>th</sup> DAS) and harvesting stage of crop for analyse. The samples were air dried and passed through 2 mm sieve prior to analysis. The pH and EC were determined in 1:2.5 (soil:water) suspension using digital pH meter. Organic carbon (OC) content of soil was determined by chromic acid wet oxidation method (Walkley and Black, 1934); available nitrogen content in soil was determined by alkaline permanganate method (Subbiah and Asija 1956); available phosphorus content in soil was estimated using olsen's extraction method (Olsen *et al.*, 1945); available potassium in soil was analysed neutral normal ammonium acetate method as outlined by Jackson (1973); exchangeable calcium and magnesium in soil was determined by versenate titration – neutral normal ammonium acetate extract (Jackson 1973) and available micronutrient (Zn, Cu, Fe and Mn) in soil was estimated by Atomic Absorption Spectrophotometer (AAS) after extracting soil samples with DTPA (Diethylene Triamine Penta Acetic Acid) extractant (Lindsay and Norwell, 1978).

The crop was harvested plot wise (20 m<sup>2</sup>). The harvested cobs were then dried, dehusked, shelled and cleaned separately. After cleaning, the grains were sun dried to 14 percent moisture content. After threshing, the grains and stover was oven dried and ground in a Willey mill. In the processed grain samples, some quality parameters like crude protein, true protein, tryptophan, starch and amylose content were estimated. True protein in maize grain was estimated by Tri – chloro acid method (Marais and Evenwell, 1983); Crude protein in maize grain was estimated by Micro – kjeldhal method (Humphries, 1956); Tryptophan in maize grain was estimated by Colorimetric method (Sadasivam and Manickam, 1996); Starch in maize grain was estimated by Anthrone reagent method (Hedge and Hofreiter, 1962) and Amylose in maize grain was estimated by Colorimetric iodine assay index method (Juliano, 1971). Data were statistically analysed using ANOVA package method of Gomez and Gomez (1984) and least significant difference (LSD) at 5% probability level was computed to compare the treatments.

## Results and discussion

### True and crude protein

True protein content of maize grain significantly varied with the imposed treatments in both the soils. The true protein mean values ranged from 5.00 to 8.34% in both the soils. A significant increase in true protein was observed for the inoculation of AM fungi ( $M_2$ ), ZSB ( $M_3$ ) and their combinations ( $M_4$ ) with graded doses of Zn application in both the soils. Irrespective of soils, among the microbial inoculums  $M_4$  had significantly highest true protein (7.06 and 6.90% respectively for black and red soil) followed by  $M_3$  (6.82 and 6.62% respectively for black and red soil),  $M_2$  (6.09 and 5.86% respectively for black and red soil) and  $M_1$  (5.37 and 5.28% respectively for black and red soil). In both the soils, the Zn levels significantly increased the true protein. Irrespective of soils,  $S_5$  recorded highest true protein (7.26 and 7.14% respectively for black and red soil) followed by  $S_4$ ,  $S_3$ ,

$S_2$ ,  $S_6$  and  $S_6$  and  $S_1$ . Crude protein content of maize grain was significantly increased for the different treatment combinations under both the soils. Crude protein values indicated that the black soil had slightly higher value (8.94%) than red soil (8.65%). The crude protein mean values were ranged from 7.50 to 11.78% for black soil and 7.00 and 11.47% in red soil. Inoculation of AM fungi ( $M_2$ ), ZSB ( $M_3$ ) and their combinations ( $M_4$ ) under graded doses of Zn application significantly increased the crude protein in both the soils. In black soil, highest crude protein was noticed for  $M_4$  (9.96%) followed by  $M_3$  (9.20%) and  $M_2$  (8.76%) while the  $M_1$  registered lower crude protein (7.83%). In red soil, the trend of crude protein values (9.72, 8.90, 8.42 and 7.56% respectively for the  $M_4$ ,  $M_3$ ,  $M_2$  and  $M_1$ ) as influenced by the microbial inoculums was similar as that of black soil. The treatment combinations  $M_4S_5$  had registered highest true protein and crude protein. The increases were to the tune of 40.0 and 36.3% over control in black soil while 39.6 and 39.0% in red soil followed by  $M_4S_4$ . The increase in the

**Table 3.** Graded levels of Zn with Arbuscular Mycorrhizal Fungi and Zinc Solubilizing Bacteria on true protein content (%) of grain

| Treatments | Black soil |       |       |           |       |       |      | Red Soil |       |       |           |       |       |      |
|------------|------------|-------|-------|-----------|-------|-------|------|----------|-------|-------|-----------|-------|-------|------|
|            | $S_1$      | $S_2$ | $S_3$ | $S_4$     | $S_5$ | $S_6$ | Mean | $S_1$    | $S_2$ | $S_3$ | $S_4$     | $S_5$ | $S_6$ | Mean |
| $M_1$      | 5.00       | 5.23  | 5.45  | 5.67      | 5.71  | 5.17  | 5.37 | 4.98     | 5.15  | 5.28  | 5.57      | 5.63  | 5.07  | 5.28 |
| $M_2$      | 5.43       | 5.99  | 6.23  | 6.46      | 6.89  | 5.54  | 6.09 | 5.24     | 5.76  | 6.05  | 6.11      | 6.67  | 5.32  | 5.86 |
| $M_3$      | 5.68       | 6.49  | 6.92  | 7.53      | 8.11  | 6.16  | 6.82 | 5.34     | 6.28  | 6.76  | 7.34      | 8.00  | 6.01  | 6.62 |
| $M_4$      | 6.00       | 6.57  | 7.28  | 7.79      | 8.34  | 6.37  | 7.06 | 5.93     | 6.32  | 7.11  | 7.54      | 8.25  | 6.26  | 6.90 |
| Mean       | 5.53       | 6.07  | 6.47  | 6.86      | 7.26  | 5.81  | 6.33 | 5.37     | 5.88  | 6.30  | 6.64      | 7.14  | 5.67  | 6.17 |
|            | SEd        |       |       | CD (0.05) |       |       |      | SEd      |       |       | CD (0.05) |       |       |      |
| M          | 0.02       |       |       | 0.03      |       |       |      | 0.03     |       |       | 0.06      |       |       |      |
| S          | 0.02       |       |       | 0.04      |       |       |      | 0.03     |       |       | 0.07      |       |       |      |
| M X S      | 0.04       |       |       | 0.08      |       |       |      | 0.67     |       |       | 0.14      |       |       |      |

**Table 4.** Graded levels of Zn with Arbuscular Mycorrhizal Fungi and Zinc Solubilizing Bacteria on Crude protein content (%) of grain

| Treatments | Black soil |       |       |           |       |       |      | Red Soil |       |       |           |       |       |      |
|------------|------------|-------|-------|-----------|-------|-------|------|----------|-------|-------|-----------|-------|-------|------|
|            | $S_1$      | $S_2$ | $S_3$ | $S_4$     | $S_5$ | $S_6$ | Mean | $S_1$    | $S_2$ | $S_3$ | $S_4$     | $S_5$ | $S_6$ | Mean |
| $M_1$      | 7.50       | 7.76  | 7.91  | 8.07      | 8.23  | 7.51  | 7.83 | 7.00     | 7.43  | 7.67  | 7.98      | 8.02  | 7.28  | 7.56 |
| $M_2$      | 7.89       | 8.45  | 8.98  | 9.54      | 9.82  | 7.90  | 8.76 | 7.49     | 8.12  | 8.76  | 9.03      | 9.54  | 7.58  | 8.42 |
| $M_3$      | 7.97       | 8.78  | 9.62  | 10.30     | 10.58 | 7.98  | 9.20 | 7.71     | 8.56  | 9.22  | 10.00     | 10.13 | 7.77  | 8.90 |
| $M_4$      | 8.34       | 9.36  | 10.52 | 11.43     | 11.78 | 8.34  | 9.96 | 8.11     | 9.23  | 10.14 | 11.03     | 11.47 | 8.36  | 9.72 |
| Mean       | 7.93       | 8.58  | 9.26  | 9.84      | 10.10 | 7.93  | 8.94 | 7.58     | 8.34  | 8.95  | 9.51      | 9.79  | 7.75  | 8.65 |
|            | SEd        |       |       | CD (0.05) |       |       |      | SEd      |       |       | CD (0.05) |       |       |      |
| M          | 0.02       |       |       | 0.03      |       |       |      | 0.04     |       |       | 0.07      |       |       |      |
| S          | 0.02       |       |       | 0.04      |       |       |      | 0.04     |       |       | 0.10      |       |       |      |
| M X S      | 0.04       |       |       | 0.07      |       |       |      | 0.09     |       |       | 0.18      |       |       |      |

protein content might be due to marked influence of Zn treatments on the enzymatic activity in the plants which could bring about significant changes in the crude and true protein contents in maize grains. Zinc is essential for protein synthesis, reduction in RNA- polymerase activity and increase in RNA destruction (Ziaeyan and Rajaie, 2009). Moreover, the protein content is directly related to N content in the materials present and the role of Zn on the enzymatic activity in plants which could bring about significant changes in the protein content in grains. The results are in consonance with the findings of Mishra (2012).

### Tryptophan

Tryptophan content of maize grain was significantly increased for the different treatment combinations under both the soils. Tryptophan values indicated that the red soil had higher value (77.5 mg g<sup>-1</sup>) than the black soil (69.8 mg g<sup>-1</sup>). The tryptophan mean values were ranged

from 65.3 to 76.0 mg g<sup>-1</sup> for black soil and 73.2 to 81.8 mg g<sup>-1</sup> in red soil. Irrespective of soils, inoculation of AM fungi (M<sub>2</sub>), ZSB (M<sub>3</sub>) and their combinations (M<sub>4</sub>) under graded doses of Zn application significantly increased the tryptophan content of maize grain. Highest tryptophan was recorded for M<sub>4</sub> (71.9 and 79.2 mg g<sup>-1</sup> for black and red soil, respectively) followed by M<sub>3</sub> (70.6 and 78.0 mg g<sup>-1</sup> for black soil and red soil, respectively), M<sub>2</sub> (69.0 and 76.9 mg g<sup>-1</sup> for black and red soil, respectively) while M<sub>1</sub> registered lower tryptophan content (67.9 and 75.8 mg g<sup>-1</sup> for black soil red soil, respectively). In both the soils, graded levels of Zn application increased the tryptophan content significantly. In both the soils higher doses of ZnSO<sub>4</sub> application S<sub>5</sub> had highest tryptophan content of maize grain (72.9 mg g<sup>-1</sup>) followed by S<sub>4</sub> (71.8 mg g<sup>-1</sup>), S<sub>3</sub> (70.4 mg g<sup>-1</sup>) and S<sub>2</sub> (68.8 mg g<sup>-1</sup>). The spray treatment (S<sub>6</sub>) had significantly higher tryptophan content (68.4 mg g<sup>-1</sup>) over control (66.9 mg g<sup>-1</sup>) but the values were low as compared to soil application ZnSO<sub>4</sub>. Irrespective of the soils, the

**Table 5.** Graded levels of Zn with Arbuscular Mycorrhizal Fungi and Zinc Solubilizing Bacteria on tryptophan content (mg g<sup>-1</sup>) of grain

| Treatments     | Black soil     |                |                |                |                |                |      | Red Soil       |                |                |                |                |                |      |
|----------------|----------------|----------------|----------------|----------------|----------------|----------------|------|----------------|----------------|----------------|----------------|----------------|----------------|------|
|                | S <sub>1</sub> | S <sub>2</sub> | S <sub>3</sub> | S <sub>4</sub> | S <sub>5</sub> | S <sub>6</sub> | Mean | S <sub>1</sub> | S <sub>2</sub> | S <sub>3</sub> | S <sub>4</sub> | S <sub>5</sub> | S <sub>6</sub> | Mean |
| M <sub>1</sub> | 65.3           | 67.5           | 68.3           | 69.6           | 70.2           | 66.5           | 67.9 | 73.2           | 75.7           | 76.5           | 77.3           | 78.1           | 74.1           | 75.8 |
| M <sub>2</sub> | 66.1           | 68.1           | 69.6           | 70.5           | 71.8           | 68.0           | 69.0 | 74.2           | 76.3           | 77.3           | 78.6           | 79.2           | 75.9           | 76.9 |
| M <sub>3</sub> | 67.9           | 69.2           | 71.6           | 72.4           | 73.7           | 69.0           | 70.6 | 75.6           | 77.4           | 78.1           | 79.7           | 80.4           | 77.0           | 78.0 |
| M <sub>4</sub> | 68.3           | 70.2           | 72.0           | 74.7           | 76.0           | 70.0           | 71.9 | 76.3           | 78.5           | 79.7           | 81.0           | 81.8           | 77.9           | 79.2 |
| Mean           | 66.9           | 68.8           | 70.4           | 71.8           | 72.9           | 68.4           | 69.8 | 74.8           | 77.0           | 77.9           | 79.1           | 79.9           | 76.2           | 77.5 |
|                | SEd            |                |                | CD (0.05)      |                |                |      | SEd            |                |                | CD (0.05)      |                |                |      |
| M              | 0.08           |                |                | 0.2            |                |                |      | 0.038          |                |                | 0.1            |                |                |      |
| S              | 0.10           |                |                | 0.2            |                |                |      | 0.046          |                |                | 0.1            |                |                |      |
| M X S          | 0.19           |                |                | 0.4            |                |                |      | 0.092          |                |                | 0.2            |                |                |      |

**Table 6.** Graded levels of Zn with Arbuscular Mycorrhizal Fungi and Zinc Solubilizing Bacteria on starch content (%) of grain

| Treatments     | Black soil     |                |                |                |                |                |       | Red Soil       |                |                |                |                |                |       |
|----------------|----------------|----------------|----------------|----------------|----------------|----------------|-------|----------------|----------------|----------------|----------------|----------------|----------------|-------|
|                | S <sub>1</sub> | S <sub>2</sub> | S <sub>3</sub> | S <sub>4</sub> | S <sub>5</sub> | S <sub>6</sub> | Mean  | S <sub>1</sub> | S <sub>2</sub> | S <sub>3</sub> | S <sub>4</sub> | S <sub>5</sub> | S <sub>6</sub> | Mean  |
| M <sub>1</sub> | 49.79          | 50.12          | 50.67          | 51.25          | 51.99          | 49.85          | 50.61 | 48.15          | 49.34          | 50.12          | 50.99          | 51.46          | 48.93          | 49.83 |
| M <sub>2</sub> | 50.32          | 50.91          | 52.45          | 52.98          | 53.21          | 50.49          | 51.73 | 49.78          | 50.67          | 51.23          | 51.96          | 52.45          | 49.94          | 51.00 |
| M <sub>3</sub> | 50.76          | 51.13          | 52.46          | 53.75          | 54.01          | 50.83          | 52.16 | 49.89          | 50.54          | 51.67          | 52.83          | 53.42          | 50.00          | 51.39 |
| M <sub>4</sub> | 51.58          | 52.57          | 54.89          | 55.34          | 57.46          | 51.95          | 53.96 | 50.79          | 51.43          | 53.65          | 54.89          | 55.56          | 51.07          | 52.89 |
| Mean           | 50.61          | 51.18          | 52.62          | 53.33          | 54.17          | 50.78          | 52.12 | 49.65          | 50.50          | 51.67          | 52.67          | 53.22          | 49.99          | 51.28 |
|                | SEd            |                |                | CD (0.05)      |                |                |       | SEd            |                |                | CD (0.05)      |                |                |       |
| M              | 0.07           |                |                | 0.14           |                |                |       | 0.06           |                |                | 0.13           |                |                |       |
| S              | 0.08           |                |                | 0.17           |                |                |       | 0.08           |                |                | 0.16           |                |                |       |
| M X S          | 0.16           |                |                | 0.34           |                |                |       | 0.15           |                |                | 0.32           |                |                |       |

**Table 7.** Graded levels of Zn with Arbuscular Mycorrhizal Fungi and Zinc Solubilizing Bacteria on amylose content (%) of grain

| Treatments     | Black soil     |                |                |                |                |                |       | Red Soil       |                |                |                |                |                |       |
|----------------|----------------|----------------|----------------|----------------|----------------|----------------|-------|----------------|----------------|----------------|----------------|----------------|----------------|-------|
|                | S <sub>1</sub> | S <sub>2</sub> | S <sub>3</sub> | S <sub>4</sub> | S <sub>5</sub> | S <sub>6</sub> | Mean  | S <sub>1</sub> | S <sub>2</sub> | S <sub>3</sub> | S <sub>4</sub> | S <sub>5</sub> | S <sub>6</sub> | Mean  |
| M <sub>1</sub> | 15.11          | 15.45          | 15.99          | 16.23          | 16.67          | 15.22          | 15.78 | 15.00          | 15.23          | 15.56          | 16.11          | 16.34          | 15.15          | 15.56 |
| M <sub>2</sub> | 15.37          | 16.01          | 16.84          | 17.45          | 17.98          | 15.73          | 16.56 | 14.98          | 15.76          | 16.24          | 17.12          | 17.47          | 15.67          | 16.20 |
| M <sub>3</sub> | 15.48          | 16.23          | 16.94          | 17.79          | 18.45          | 15.72          | 16.77 | 15.23          | 16.06          | 16.48          | 17.27          | 18.21          | 15.25          | 16.42 |
| M <sub>4</sub> | 16.12          | 17.35          | 18.17          | 19.38          | 20.23          | 16.69          | 17.99 | 15.98          | 16.97          | 17.65          | 18.54          | 19.12          | 16.23          | 17.42 |
| Mean           | 15.52          | 16.26          | 16.99          | 17.71          | 18.33          | 15.84          | 16.78 | 15.30          | 16.01          | 16.48          | 17.26          | 17.79          | 15.58          | 16.40 |
|                | SEd            |                |                | CD (0.05)      |                |                |       | SEd            |                |                | CD (0.05)      |                |                |       |
| M              | 0.02           |                |                | 0.05           |                |                |       | 0.03           |                |                | 0.07           |                |                |       |
| S              | 0.03           |                |                | 0.06           |                |                |       | 0.04           |                |                | 0.09           |                |                |       |
| M X S          | 0.06           |                |                | 0.12           |                |                |       | 0.08           |                |                | 0.17           |                |                |       |

treatment combinations *viz.*, M<sub>4</sub>S<sub>5</sub> recorded the highest tryptophan values over control followed by M<sub>4</sub>S<sub>4</sub>. Mycorrhizal plants assimilated greater amounts of Zn and translocated to grains which assisted in enrichment of tryptophan in grains. Mycorrhizal symbiosis enhanced the tryptophan concentration in grains, which commensurate with improved Zn status of the inoculated plants as Zn is required for synthesis of tryptophan (Cakmak *et al.*, 1989; Brown *et al.*, 1993; Ahmed *et al.*, 2012).

#### Starch and amylose

The starch content of maize grain recorded under black and red soils showed significant variations for the imposed treatments. The mean values were ranged from 49.79 to 57.46% for black soil while 48.15 to 55.56% in red soil. In both the soils, the treatment M<sub>4</sub> registered significantly higher starch content of maize grain (53.96%) followed by the treatment M<sub>3</sub> and M<sub>2</sub> in black soil. Irrespective of soils, graded levels of Zn application correspondingly increased the starch content significantly. Application of ZnSO<sub>4</sub> at 50 kg ha<sup>-1</sup> (S<sub>5</sub>) had registered highest starch content (54.17 and 53.22% respectively for black and red soil) followed by S<sub>4</sub> (53.33 and 52.67% respectively for black and red soil), S<sub>3</sub> (52.62 and 51.67% respectively for black and red soil) and S<sub>2</sub> (51.18 and 50.50% respectively for black and red soil). Amylose content significantly varied among the treatment combinations in both the soils. The mean amylose content values ranged from 15.11 to 20.23% for black soil while 15.00 to 19.12% in red soil. A significant increase in amylose content for AM fungi (M<sub>2</sub>), ZSB (M<sub>3</sub>) and their combination (M<sub>4</sub>) was recorded in both the soils.

Irrespective of soils, highest amylose content was observed for M<sub>4</sub> (17.99 and 17.42% respectively for black and red soil) followed by M<sub>3</sub> (16.77 and 16.42% respectively for black and red soil) and M<sub>2</sub> (16.56 and 16.20% respectively for black and red soil) while M<sub>1</sub> (15.78 and 15.56% respectively for black and red soil) had lower amylose content. Graded doses of ZnSO<sub>4</sub> soil application increased the amylose content significantly in both the soils. Irrespective of soils, the treatment S<sub>5</sub> registered highest amylose content followed by the treatment S<sub>4</sub>, S<sub>3</sub>) and S<sub>2</sub>. However, the treatment S<sub>6</sub> (15.84 and 15.58% for black and red soil, respectively) had significantly lower amylose content than S<sub>2</sub> but was superior to S<sub>1</sub>. Studies on starch and amylose content in grains showed that, starch is a polymer of glucose and amylose is a linear polymer of glucose. In the present investigation, application of different treatments resulted in significant marked variation in the starch and amylose content. The highest content of starch and amylose were recorded in the application of M<sub>4</sub>S<sub>5</sub> followed by M<sub>4</sub>S<sub>4</sub>, the lowest content was recorded with control (M<sub>1</sub>S<sub>1</sub>). Formation of NADPH or NADH depending on the Zn concentration might have involved in tapping and converting the radiation energy for photosynthetic activities and increased formation of sugars and starch. The increase in grain quality with application of micronutrients may be due to nutrient availability and its uptake by crop. This might have lead to accumulation of higher quantities of seed components like calcium carbonate and the increased metabolism which helps in increasing the carbohydrate and protein content in seeds. This result was in accordance with the results of Harish and Devasenapathy (2010).

## Conclusion

This study aimed to provide insights into understanding the relationship among quality characteristics of maize grain. Application of graded levels of Zn in combination with Arbuscular Mycorrhizal (AM) fungi and Zinc Solubilizing Bacteria (ZSB) increased the protein, tryptophan, starch and amylose. In black and red soil  $M_4$  had highest protein, tryptophan, starch and amylose followed by  $M_3$ ,  $M_2$  and  $M_1$ . In both the soils higher dose of  $ZnSO_4$  application  $S_5$  registered highest protein, tryptophan, starch and amylose content followed by  $S_4$ ,  $S_3$  and  $S_2$ . The spray treatment ( $S_6$ ) had significantly higher tryptophan content over control but the values were low as compared to soil application  $ZnSO_4$ . Irrespective of the soils, the treatment combinations viz.,  $M_4S_5$  recorded the highest protein, tryptophan, starch and amylose values over control followed by  $M_4S_4$ . The protein content is directly related to N content in the materials present and the role of Zn on the enzymatic activity in plants which could bring about significant changes in the protein content in grains. Mycorrhizal plants assimilated greater amounts of Zn and translocated to grains which assisted in enrichment of tryptophan in grains. Mycorrhizal symbiosis enhanced the tryptophan concentration in grains, which commensurate with improved Zn status of the inoculated plants as Zn is required for synthesis of tryptophan. Formation of NADPH or NADH depending on the Zn concentration might have involved in tapping and converting the radiation energy for photosynthetic activities and increased formation of sugars and starch. The findings highlight the relationship between Zn levels in combination with arbuscular mycorrhizal fungi and zinc solubilizing bacteria on quality attributes of maize grain.

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