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Promotion of maize growth by a yellow morel, *Morchella crassipes*

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Abstract

Fungal species of morel (genus *Morchella*) have recently been found to form a symbiotic relationship with grasses. Our previous study documented that *M. crassipes* from Shaanxi, northwest China, increased growth of sweet corn *Zea mays var. saccharata* and suppressed *Fusarium* infections. In the present study, we examined the effect of *M. crassipes* inoculation on dent corn, *Zea mays var. indentata* cv. Plant growth response indexes and variables and soil variables were used to demonstrate how *M. crassipes* inoculation stimulates maize growth. Three suspensions of *M. crassipes* mycelium (50, 100, 150 mL) were inoculated into *Zea may* var. *indentata***.** The results showed that *M. crassipes* inoculation significantly affected growth of all the inoculated maize plants and influenced some variables and indexes that are related to tissue specificity and dose dependence. Soil moisture, available K and P accumulation by *M. crassipes* were affected in inoculated plants and resulted in growth enhancements that were equal to that of the plants treated with urea. Our findings reveal that inoculation with *M. crassipes* had a positive effect on maize yield, making the crop system more sustainable. Thus *M. crassipes* has the potential to become a supplement or an alternative to urea fertilizers.

Keywords Sweetcorn (*Zea mays var. saccharata*) · *M. crassipes* (yellow morel) · Nutrients · Soil moisture · Antioxidant enzyme activities (SOD · CAT · POD · PAL)

1 Introduction

Plants are globally diverse, with approximately 500,000 species globally (Corlett 2016). Fungi that can form symbiotic relationship with plants are even more abundant and estimated at 1.5 million (Hawksworth 2001). A proportion of these fungi are not pathogens but promoters of plant growth (Castro et al. 2009). Some promote growth by producing growth hormones or suppressing the infection of fungal species like *Fusarium verticillioides* (Yu et al. 2016). Others improve water and nutrient relations (Asrar et al. 2012). Mutualism involves exchanges that benefits both partners in the symbiosis. Nutrient exchanges, for example, have been demonstrated for many mycorrhizal fungi (Jakobsen et al. 1992; Kheyrodin 2014), and other symbiosis as endophytes result in improved root biomass and root branching (Bossuyt et al. 2001; Denef et al. 2001; Harman and Uphoff 2019).

Species of *Morchella* (family Morchellaceae, order Pezizales, Phylum Ascomycota), commonly called morels, are among the most recognized edible mushrooms (Baynes et al. 2012). *Morchella* spp. have been documented to form mycorrhizae with trees (Dahlstrom et al. 2000; Rossbach et al. 2017) but they can also be saprotrophs (Liu et al. 2017). It has also been shown that *Morchella* spp. can form *endophytic* relationships with an invasive grass, *Bromus tectorum* (Baynes et al. 2012). Studies show that inoculation with *Morchella elata* improves the growth of *B. tectorum* and its fecundity (seed yield per plant). Inoculation experiments have shown that it is possible to promote growth and pathogen defense in commercial crops such as *Zea mays var. saccharata*, or sweet corn (Yu et al. 2016). Inoculation with *M. crassipes* improves maize plant height, biomass, and root development and suppresses *F. verticillioides* infection in mature corn ears, which can be attributed to phytohormones SA, ABA and IAA secreted by *M. crassipes* (Yu et al. 2016; Yu et al. 2017).

The objective of the present study was to examine the possible symbiotic relationship between *M. crassipes* and dent corn, *Zea mays var. indentata*. cv. 'Shaandan 985, which is widely grown in the Shaanxi Province (China).

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2 Materials and methods

2.1 Soil sample and mycelium suspension preparation

Soil was purchased from Xintiandi Organic Farm Co. Ltd., Yangling, China, which contained organic material >50%, sapropel >20%, glimmerite >20%, at pH 5.5–6.5 with low moisture content. Soil was autoclaved at 121 °C for 40 min before being used to fill pots.

Two-week old mycelium (with sclerotia) of *M. crassipes*, grown on potato dextrose agar medium (PDA), was scraped down and immediately suspended in 100 ml sterilized deionized water per dish. Mycelia biomass was approximately 1 g per dish (Yu et al. 2016), which contains nitrogen 4.6%, phosphorus 1.38%, potassium 3.51%, and carbon 37.1% (Rossbach et al. 2017). The *Morchella* mycelium suspension (MMS) was then applied to plant pots 2 times in the first 20 days of seedling growth.

2.2 Experimental design and inoculation

Plant pots (10 cm × 15 cm size) were filled almost full of autoclaved soil samples. The experiments had five treatments: control (CK), urea solution and three different volumes of *Morchella* inoculum (50-, 100- and 150-ml MMS). Each treatment had five replicates and each experiment was repeated three times for a total of 75 pots. Maize seeds(*Zea mays var. indentata* cv 'Shaandan 985') were incubated in wet-cheese cloths at 25 °C for 40 h. Germinating seeds were then carefully placed in plant pots (5 seeds per pot). 100 ml deionized water was added for control treatments; 100 ml solution of 100 ppm urea was added for urea treatments; and MMS was added for inoculation treatments. After one week of growth, the tallest seedling was kept while the others were removed. Plants were watered every 4 days. Urea solution and MMS were added to plant pots three times on 1st day, 15th day and 30th day during the experiment period. Experiments were performed in a greenhouse at 30/20 °C (day/night) with an alternation of 10-h light and 14-h darkness.

2.3 Root staining following inoculation

After harvesting, root samples were randomly selected from each plant and washed in running tap water to remove particles of soil. They were cut into 1 cm sizes and transferred to 1.5 ml centrifuge tubes for staining (Kiheri et al. 2017), softened by adding 1 ml of 10% KOH and subsequently heated to 90 °C for 40 min in a Thermostat Plus. After being washed in 10% H_2O_2 and again in distilled water twice, the roots were acidified in 5% KCL solution for 5 min, followed by immersion in 2% Trypan Blue for 10 min. Specimens were examined in Lacto-phenol using an Olympus CX41 microscope.

2.4 Measurement of plant growth promotion

To assess the growth index, plant height was measured on the 7th day and every four days thereafter for the remaining of the experimental period. The collar-leaf method (Nielsen 2003; Abendroth et al. 2011) was used to make the measurement of stem height. Stem diameter was measured twice for each plant at 10 mm above ground with a digital measuring device. The length and width of the latest visible collar leaf were measured to determine the leaf area. The width of leaf was measured at the widest point. Plants were harvested for biomass assessment after 50 days. Fresh weights of roots and shoots were separately noted and plants were dried at 70 °C for 50 h.

2.5 Plant elemental analyses (N, P, K)

Plant parts (i.e., roots, stems, and leaves) were washed, dried and ground. Extraction was performed with the H_2SO_4 and 30% H_2O_2 digestion method (Idera et al. 2014). The digestion solution was used to determine the total nitrogen (N), phosphorus (P), and potassium (K) contents. Total N, P, K of plant parts (root, stem, leaf) were analyzed separately, following the methods described by Chapman and Parker (1961) and Razi et al. (2011). Total N was determined using the Branne Luebbe Auto Analyzer 3 (AA3) system (Branne and Luebbe AA3, Norderstedt, Germany). Total P was determined with the Molybdenum Antimony Colorimetric Method by using a spectrophotometer (Adam et al. 2015). Total K was determined with K standard by using a flame photometer.

2.6 Assessment of photosynthetic activity and chlorophyll

Photosynthesis was measured in the greenhouse at 30 °C on a sunny day by using the Li-6400 portable photosynthesis system (LI-COR *Inc.*, Lincoln, NE, USA). Photosynthetic rates (P_N), stomatal conductance (Gs), intercellular CO_2 concentration (Ci), and transpiration rates (Trmmo) were measured for each plant leaf and the measurement repeated four times. The light response curve was made with a CO_2 flow rate at 500 $\mu molm^{-2}\ s^{-1}$ and a PFD range of 0–1800 $m^{-2}\ s^{-1}$ (Chang et al. 2017). For the chlorophyll content of maize leaves, a Konica Minolta Chlorophyll Meter (SPAD, Japan) was used on the 40th day (Khan et al. 2015; Ling et al. 2011). Each plant was measured 10 times on three recently-formed leaves.

2.7 Assessment of antioxidant enzyme activities

Extraction Maize leaves were collected on the 40th day and samples were immediately immersed in liquid nitrogen and stored at −80 °C. To obtain leaf extractions, 5-mg pieces of fresh leaf were cut and placed in tubes followed by addition of 300 μl extraction solution (containing 100 mM Tris, pH 7.4;

150 mMNaCL; 1 mM EGTM; 1 mM EDTA; 1% Triton x-100 and 0.5% sodium deoxycholate) and homogenized in an electric homogenizer at 4 °c for 2 min. Inner walls of test tubes were washed twice with 300 μl extraction solution. Test tubes were placed at 4 °C for 2 h in an orbital shaker and spun at 4 °C at 13000 x g for 20 min. Supernatants were transferred into new tubes and stored at −80 °C for antioxidant enzyme assays.

Assay procedures Activities of antioxidant enzymes (Superoxide Dismutase (SOD), Catalase (CAT), Peroxidase (POD), Phenylalanine Ammonia-Lyase (PAL)) were measured using an ELISA assay according to the protocol by Gill & Tuteja (2010): 10 μl of sample extraction, 40 μl of sample diluent and 100 μl of horseradish peroxidase (HRP-conjugate) reagents were added into each sample tube. Tubes were then incubated at 37 °C for 60 min and washed five times with wash solution. Next, 50 μl of Chromogen Solution A and B were separately added into sample tubes which were then shaken several times carefully. Samples were then incubated at 37 °C for 15 min (samples were kept from light) and finally, 50 μl of stop solution was added to tubes. When sample color changed from blue to yellow in a spectrophotometer, a standard curve was plotted and enzyme activities were calculated.

2.8 Soil moisture and pH value

One week before plant harvesting, plant pots were irrigated with 100 ml deionized water. Five days later, soil moisture content was measured using a TDR 100/200 Soil Moisture Meter (Spectrum Tech. Inc., USA). Each plant pot was measured three times. pH (H_2O) value was measured according to procedures described by Adam et al. (2015): dried soil was ground, 0.2 g of dried soil was transferred to a beaker and 5 ml of distilled water was added. The mixture was stirred every 10 min for one hour and then the pH value was measured with a Sartorius-PB 10, pH meter (Sartorius AG, Germany).

2.9 Soil assessment of available NO_3^-, NH_4^+, P, K

The effect of *M. crassipes* on essential macro-nutrients (N, P, K) was analyzed. Sample soil from each pot was divided into subsamples. For available mineral nitrogen (NO_3^-, NH_4^+) measurement, fresh soil was collected and stored immediately at 4 °C. For available P and K contents, soil was air dried for one month and ground. Measurements of N, P, K in soil were obtained, as per method in section 2.5 (following Adam et al. 2015).

2.10 Statistical analysis

Raw data was arranged in excel and all pairwise comparisons were tested for variability using Two-way ANOVAs and Tukey HSD's multiple comparisons tests. All analyses were conducted in IBM SPSS Statistic software, version 25. Mean differences were significant ($p < 0.05$).

3 Results

3.1 Root staining following inoculation

Confirmation of *M. crassipes* colonization of maize plant roots was demonstrated (Fig. 1). Hypha developed along with longitudinal axis of the maize roots (Fig. 1c), and intercellular hyphae of *M. crassipes* appeared in the epidermal cells, cortical cells and even pericyclic cells of maize roots (Figs. 1a and b). However, no hypha and colonization of non-*M. crassipes* were found in the maize roots (Fig. 1d). *M. crassipes* formed endophytic colonization in the roots of dent corn, as is in sweet corn roots (Yu et al. 2016).

3.2 Effects of *M. crassipes* inoculation on plant growth

Inoculation with *M. crassipes* had positive effects on maize plant (Table 1, Fig. 2). Fresh and dried biomasses of maize plants were substantially increased by the higher MMS volumes. Both the 100- and 150-MMS treatments increased green biomass when compared to the control (shoot: $p = 0.000$, $p = 0.000$; root: $p = 0.012$, $p = 0.000$, respectively) and urea treatment (shoot: $p = 0.015$; $p = 0.000$; root: $p = 0.018$, $p = 0.003$, respectively). For dry biomasses, both the 100- and 150-MMS treatment had promoted more growth than the control (shoot: $p = 0.000$, $p = 0.000$; root: $p = 0.019$, $p = 0.002$, respectively) and urea treatment (shoot: $p = 0.024$; $p = 0.001$; root: $p = 0.036$, $p = 0.015$, respectively). The 50-MMS treatment had a lesser impact on maize plant growth when compared to the control and urea treatments ($p < 0.05$). Effects on index of growth were similar to effects on biomass. The stem height of the plants inoculated with 100-MMS and 150-MMS were significantly improved when compared to controls ($p = 0.000$, $p = 0.004$) and the stem height of 100-MMS inoculated plants was significantly improved as compared to urea treatment ($p = 0.029$) (Table 1). Stem diameters of the plants inoculated with 100- and 150-MMS on the 40th day were significantly improved as compared to the controls ($p = 0.011$, $p = 0.012$). Leaf areas were sharply increased by 100- and 150-MMS inoculation compared to control ($p = 0.001$, $p = 0.000$) and urea treatments ($p = 0.025$, $p = 0.016$). The density of the maize root systems was greater with increasing amount of *Morchella* inoculum. The crown root lengths of the plants inoculated with 50-, 100- and 150-MMS were significantly increased when compared to the controls ($p = 0.033$, $p = 0.004$, $p = 0.004$, respectively) and only the crown root lengths of the plants inoculated with 100-MMS and 150-MMS were significantly improved as compared with urea treatment ($p = 0.013$, $p = 0.013$) (Table 1).

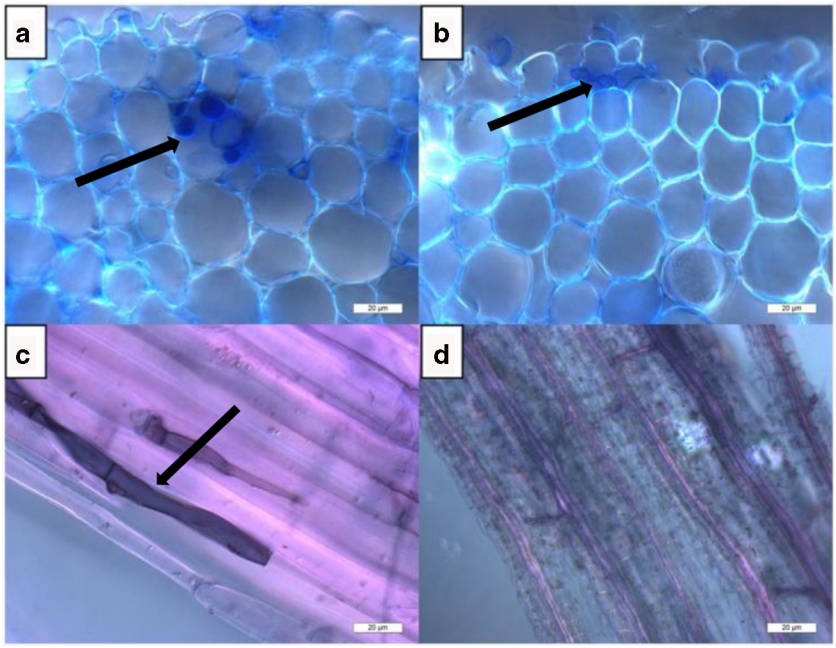

Fig. 1 Maize roots sections (40th day) stained with Trypan blue showing hyphae of *M. crassipes,* growing in air channels around cells (Figs: 1a and b, cross sections, 1c longitudinal). Control root cells were devoid of colonization (Fig. 1d longitudinal section)

3.3 Plant elemental analyses (N, P, K)

N, P, and K contents in plant parts were assessed after inoculation with *M. crassipes*. For all the treatments with and without *M. crassipes*, the plants had highest N level in leaves, second highest N level in stems, and lowest N level in roots. The only exception found was that the N levels were higher in roots than in stems in the plants with the urea treatment. Potassium level in roots was found to be much lower than that in leaves and stems in all plants. The total N level in plants inoculated with *M. crassipes* was not significantly different from those in the control and urea-treated plants ($p > 0.05$). The same was true for the total K level in all the plants with treatments ($p > 0.05$). Increases in the total P content were seen in leaves of plants inoculated with the biggest volume of *M. crassipes* (Table 2).

3.4 Assessment of photosynthesis and chlorophyll

Both *M. crassipes* inoculation and urea treatment significantly increased the chlorophyll content in maize plant leaves when compared to control at $p = 0.000$. However, the chlorophyll content in MMS-inoculated maize leaves was not significantly improved when compared to urea ($p > 0.05$) (Table 3). Photosynthetic activities were not generally improved by *M. crassipes* inoculation treatments. A simple explanation is that *M. crassipes* inocula had no positive influence on the photosynthetic parameters. The only exception we found was that intercellular CO_2 concentration (Ci) in the plants treated with 50-MMS was higher than in 100- and 150-MMS treated plants (Table 3). It indicated that increasing amounts of MMS inocula had reduced photosynthetic activities.

3.5 Assessment of antioxidant enzyme activities

Antioxidant enzyme activities of maize leaf tissues on the 40th day indicated that activities of urea-treated plants were generally increased when compared with control (Figs. 3a–d). The *M. crassipes* inoculation showed positive effects on SOD activity in maize leaves. Increase of SOD activity in the MMS-treated plants was significant at 270.11 Unit L^{-1}, 318.72 Unit L^{-1}, 342.57 Unit L^{-1} of 50-, 100-, 150-MMS, respectively (Fig. 3a). The SOD activity registered higher in plants treated with more MMS inoculum. The SOD activity in 100- and 150-MMS inoculated maize leaves showed significant difference when compared to control at $p = 0.000$. The PAL activity in leaves was enhanced in plants treated with low MMS volumes (Fig. 3b) and PAL activity tended to decrease in the plants with high MMS treatments. However, the plants treated with different amount of *M. crassipes* inocula showed significantly different level of increase in PAL activity in leaves compared to control ($p < 0.05$) but showed no significant change in PAL activity compared to the urea. CAT and PAL have similar antioxidant activities. A larger dose of MMS inocula may have less positive effect on their antioxidant activities in the plants as showed in (Fig. 3c). The POD activities in leaves were significantly decreased in the plants treated with increasing amount of MMS inocula as showed in Fig. 3d. An increase of POD activity was found in urea-treated plants and control.

3.6 Soil assessment of available NO_3^-, NH_4^+, P, K

M. crassipes inoculation improved the soil moisture as shown in Table 4. The water holding capacity (WHC) was positively

Table 1 Effects of urea and increasing amounts of *M. crassipes* inocula on maize growth

treatment	Stem height (cm)	Stem diameter (mm)	leaf area (mm^2)	Crown root length (cm)	Green weight (g)		Dry weight (g)	
					Root	Shoot	Root	Shoot
control	14.234 ± 1.09a	3.32 ± 0.16a	5032.15 ± 0.71a	8.3 ± 0.73	0.323 ± 0.15a	3.324 ± 1.26	0.065 ± 0.04	0.453 ± 0.29a
Urea	16.861 ± 0.54*	3.48 ± 0.10a	6292.71 ± 0.83a	12.4 ± 0.53*	0.686 ± 0.31a	5.773 ± 1.28*	0.171 ± 0.69*	0.788 ± 0.15a
50 MMS	16.226 ± 0.76a	3.69 ± 0.19a	6057.75 ± 0.80a	12.1 ± 0.67*	0.663 ± 0.30a	6.151 ± 1.53*	0.130 ± 0.03*	0.838 ± 0.16a
100 MMS	19.152 ± 2.61* (**)	4.47 ± 0.34*	8298.85 ± 1.72*(**)	16.9 ± 0.48*(**)	1.588 ± 0.43*(**)	8.750 ± 1.53*(**)	0.258 ± 0.07*(**)	1.102 ± 0.21*
150 MMS	18.159 ± 1.54*	4.55 ± 0.11*	8694.20 ± 1.43*(**)	16.5 ± 0.74*(**)	1.851 ± 0.45*(**)	9.357 ± 2.68*(**)	0.284 ± 0.06*(**)	2.230 ± 0.67*(**)

Mean of five replicates ($n = 5$) and ± standard error (SE). Same letter following means difference is not significant at $p < 0.05$ level

*A significant difference between MMS treatment and control at $p < 0.05$ level

**A significant difference between MMS treatment and the urea treatment at $p < 0.05$ level

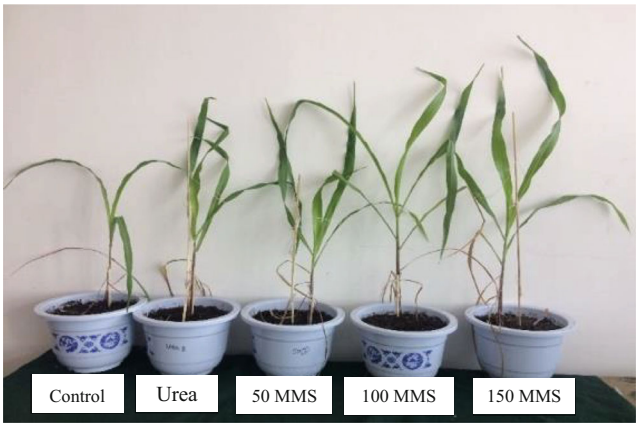

Fig. 2 Maize plant height

increased by higher amount of inocula (100- and 150-MMS). Soil moisture in the pots with both 100- and 150-MMS treatments were significantly improved when compared to control ($p = 0.009$, $p = 0.012$) but soil moisture in the pots treated with urea and 50-MMS showed no significant difference. Therefore, *M. crassipes* inoculation may have the potential to increase water-holding capacity and nutrient storage in soil. The pH (H_2O) was not significantly affected by *M. crassipes* inoculation (Table 4). A mean difference was not significant among all plants, which indicated *M. crassipes* had no effects on soil pH value during the short experimental period in a greenhouse, but pH values were higher in the *M. crassipes*-treated soil than in control.

Furthermore, this study has demonstrated *M. crassipes* inoculation improved available nutrients (NH_4^+, NO_3^-, P, K) in soil as shown in Table 4. The exchangeable NH_4^+ was not affected significantly by MMS inoculation. According to the mean differences at 95%, the NH_4^+ content shows no difference in any treated plants. The lowest NH_4^+ content is found in control at 7.221 mg kg^{-1} and the highest NH_4^+ content is found in the urea-treated soil at 8.62 mg kg^{-1}. The exchangeable NO_3^- is increased in both the urea and the MMS-treated plants. As indicated by the mean difference analyses ($p < 0.05$), the NO_3^- content in 100-MMS and urea-treated plants show significant improvement when compared to control ($p = 0.021$, $p = 0.032$). Exchangeable P is not affected by *M. crassipes* inoculations, but available P in soil is decreased over time (Tables 2 and 4). In contrast, the exchangeable P in the control plants is higher than in other treated plants (Table 4). The exchangeable K is positively increased by increasing amounts of MMS inocula (Table 4), and the mean difference for exchangeable K in inoculated soils is significant when compared to both the urea and control treatments ($p = 0.000$, $p = 0.001$).

Table 2 Plant element analyses (N, P, K)

Treatments	Nitrogen (N) (mg kg^{-1})			Phosphorus (P) (mg kg^{-1})			Potassium (K) (mg kg^{-1})		
	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
Control	46.67 ± 0.42a	50.14 ± 1.88a	53.78 ± 0.92a	2.96 ± 0.35a	2.71 ± 0.14a	2.589 ± 0.13b	5.73 ± 0.48a	11.88 ± 1.17a	14.14 ± 1.26a
Urea	46.91 ± 0.89a	43.21 ± 2.88b	55.35 ± 0.49a	2.41 ± 0.69b	2.57 ± 0.49a	2.60 ± 0.37b	4.24 ± 1.47b	10.52 ± 1.70b	12.22 ± 1.39b
50 MMS	44.78 ± 0.44a	46.58 ± 1.15a	55.51 ± 1.09a	2.77 ± 0.77a	3.01 ± 0.22a	3.06 ± 0.41a	5.34 ± 0.30a	10.97 ± 1.14ab	12.25 ± 1.31b
100 MMS	45.85 ± 0.58a	47.34 ± 1.33a	55.02 ± 1.18a	3.11 ± 0.29a	3.00 ± 0.46a	3.08 ± 0.42a	5.24 ± 0.61a	11.57 ± 1.07a	13.66 ± 1.08a
150 MMS	44.09 ± 0.73a	49.58 ± 3.8a	54.58 ± 1.13a	3.24 ± 0.20a	3.15 ± 0.22a	3.28 ± 0.29a	4.93 ± 0.40a	11.51 ± 1.01a	12.28 ± 1.36b

Mean of five replicates (n = 5) and ± standard error (SE)

Same letter following means difference is not significant at $P < 0.05$ level

Table 3 Assessment of photosynthesis and chlorophyll

Treatments	phot (μmol CO_2 m^{-2} s^{-1})	Gs (mol H_2O m^{-2} s^{-1})	Ci (μmol CO_2 $emol^{-1}$)	Trmmo (mmol H_2O m^{-2} s^{-1})	Chlorophyll
Control	9.28 ± 0.455a	0.069 ± 0.003a	214 ± 12.42a	2.650 ± 0.092a	31.223 ± 2.39
Urea	9.592 ± 0.516a	0.073 ± 0.009a	183 ± 8.105a	2.536 ± 0.274a	37.837 ± 2.25*
50 MMS	9.76 ± 0.261a	0.075 ± 0.005a	243 ± 17.491(**)	3.230 ± 0.255a	36.055 ± 2.19*
100 MMS	11.42 ± 1.389a	0.079 ± 0.011a	178 ± 17.869a	2.824 ± 0.419a	37.316 ± 1.36*
150 MMS	11.72 ± 0.565a	0.072 ± 0.004a	191 ± 19.732a	3.080 ± 0.180a	38.582 ± 1.29*

Mean of five replicates (n = 5) and ± standard error (SE). Same letter following means difference is not significant at $p < 0.05$ level

*A significant difference between MMS treatment and control at $p < 0.05$ level

**A significant difference between MMS treatment and the urea treatment at $p < 0.05$ level

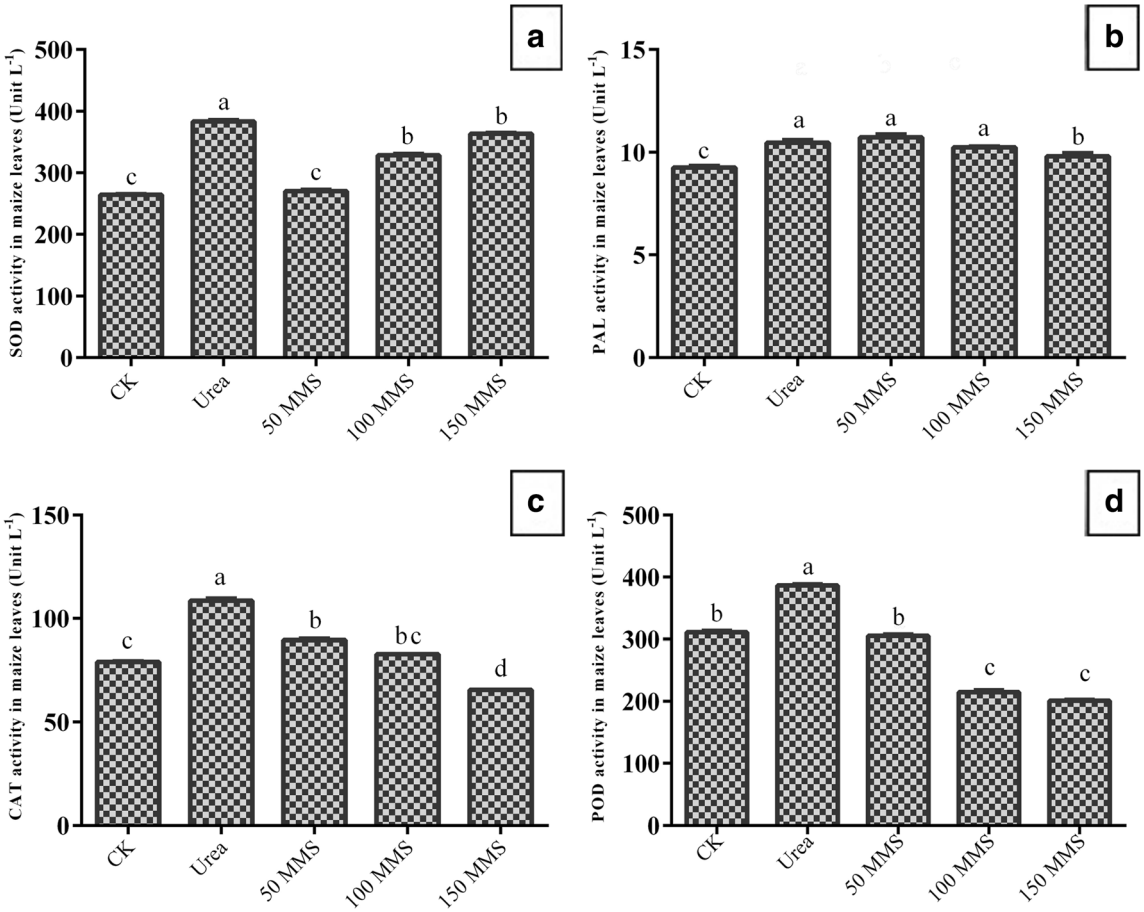

Fig. 3 Effects of urea and increasing volumes of *M. crassipes* on antioxidant enzyme activities in maize plants. (**a**) Superoxide dismutase (SOD) content, (**b**) Phenylalanine ammonia-lyase (PAL) content, (**c**) Catalase (CAT) content, (**d**) Peroxidase (POD) content of maize plant leaf. Bars showing by the same letter are not significantly different at $p < 0.05$ level

4 Discussion

Our findings support the positive effects of *M. crassipes* on maize plant production. The inoculation with *M. crassipes* successfully increases maize plant growth, biomass, plant height, crown root length and leaf area. It was clear that the 100- and 150-MMS treatments significantly enhanced maize plant growth (Table 1), similarly to the findings of Yu et al. (2016) who tested a different corn cultivar in a greenhouse setting with the same *Morchella* isolate. Baynes (2012) has also reported that *M. elata* complex improves growth and fecundity (i.e., seed yield per plant) of *B. tectorum*. Mycelium of *M. crassipes* colonizes the root and forms an ectendomycorrhizal-like symbiosis with dent corn, similar to sweet corn.

Urea as a fertilizer is widely used in agricultural systems around the world, and is usually not efficiently used. Cereals

Table 4 Chemical and physical characterizations of soil

Treatments	NO_3^- (mg kg^{-1})	NH_4^+ (mg kg^{-1})	K (mg kg^{-1})	P (mg kg^{-1})	soil Moisture	pH
Control	7.703 ± 2.09a	7.221 ± 0.41a	16.706 ± 0.46a	2.794 ± 1.41a	10.88 ± 1.08a	4.17 ± 0.05a
Urea	13.339 ± 3.28*	8.621 ± 0.13a	20.301 ± 2.01a	1.986 ± 0.95a	12.91 ± 0.48a	4.28 ± 0.08a
50 MMS	8.930 ± 3.31a	8.531 ± 0.29a	25.454 ± 1.45*(**)	1.284 ± 0.31a	11.32 ± 0.76a	5.54 ± 0.06a
100 MMS	12.935 ± 1.61*	8.249 ± 0.18a	26.824 ± 2.07*(**)	1.758 ± 0.51a	17.71 ± 1.38*	5.40 ± 0.02a
150 MMS	9.793 ± 1.95a	5.591 ± 0.29a	28.472 ± 3.04*(**)	1.964 ± 0.57a	16.53 ± 1.26*	5.43 ± 0.04a

Mean of five replicates (n = 5) and ± standard error (SE). Same letter following means difference is not significant at $p < 0.05$ level

*A significant difference between MMS treatment and control at $p < 0.05$ level

**A significant difference between MMS treatment and the urea treatment at $p < 0.05$ level

can take up to 20% to 50% of N of urea. A large proportion of N that does not get uptaken by the crop can create environmental problems, such as eutrophication of surface waters. The impact on human health remains controversial (Mosier et al. 2005). In contrast, *M. crassipes* inoculation has the potential to be a supplement or an alternative to urea application. The total P accumulation in *M. crassipes*-treated plants was equal to that in urea-treated plants. The results show that available P was decreased in MMS-inoculated pots but that the total P accumulations in plants treated with 150-MMS was significantly greater than in plants treated with 50- and 100-MMS (Table 2). Under limiting conditions, the mycelium of *M. crassipes* may help plant roots absorb soil P. Although inoculation with *M. crassipes* suspensions has no effects on N and K uptake in maize plant, the availability of soil N and K are enhanced by MMS inocula; N and K are redistributed in plant organs and their levels are highest in leaves, and lowest in roots (Table 2). The available K concentration was increased because *M. crassipes* inoculation may help K dissolve from glimmerite in the tested soil (Table 4, Kheyrodin 2014), which helps root development and nutrient absorption in dent corn.

The key factors influencing photosynthetic parameters were light, temperature, and position in a greenhouse and chlorophyll level in maize leaves (Wang et al. 2017). Inoculated maize plants grew well, and had significantly higher level of chlorophyll (Pinior et al. 2005; Fan and Liu 2011; Zhang et al. 2018). This improves energy absorbance and photosynthetic activity at early plant growth stages (Heinonsalo et al. 2012). *M. crassipes* inoculation also had positive effects on the SOD, CAT and PAL enzyme activities as shown in Fig. 3a, and these antioxidant enzymes in MMS-treated plants work as ROS scavengers more actively than in the urea-treated plants (Gill and Tuteja 2010; MacDonald and D'Cunha 2007).

Soil moisture increased due to receiving more *Morchella* inoculum. It seems that the extraradical mycelium of the fungus is capable of retaining more water compared with controls. An increase in soil moisture content is beneficial for photosynthetic activity and soil nutrient uptake (Slatyer 1967; Kozlowski 1968; Richard 1970; Nadeem et al. 2014). *M. crassipes* had no significant effects on the pH of soil for the short experimental period but pH value goes up with added *M. crassipes* inoculum, as is found in other fungi (Li et al. 1991; Mashela 2002). *Morchella* may change the capacity of K^+ and Na^+ absorption in maize which could lead to a change of pH (Martinez et al. 2004), but the cause remains unknown.

In conclusion, *M. crasssipes* inoculation increases growth without increasing plant stress which is reflected by antioxidant enzyme (SOD, CAT, PAL, and POD) activities. It is clear that *M. crassipes* and perhaps other species of *Morchella* sp. promote growth of maize. It is potentially a greener, safer, and more sustainable bio-fertilizer that can be used as an alternative to urea in farming.

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