

## Maize growth and biomass yield as influenced by diazotroph and mycorrhizae inoculation

Odoh NC <sup>1,\*</sup>, Yakubu C. <sup>1</sup> and Ncho CO <sup>2</sup>

<sup>1</sup> Department of Soil Science, University of Abuja, Abuja FCT-Nigeria.

<sup>2</sup> UFR Sciences Géologiques et Minières, Université de Man, Man, Côte d'Ivoire.

World Journal of Advanced Research and Reviews, 2023, 18(03), 1312–1318

Publication history: Received on 29 April 2023; revised on 14 June 2023; accepted on 16 June 2023

Article DOI: <https://doi.org/10.30574/wjarr.2023.18.3.1082>

### Abstract

The production of maize has significant nutrient needs, notably for nitrogen. Considering the negative impact of chemical fertilizer on the ecosystem, alternative supply of nutrients that is easily accessible and environmentally benign must be sourced. This study looked into the effects of inoculating mycorrhizae and diazotrophs on the growth and biomass output of maize. The treatments included three (3) levels of mycorrhiza (without mycorrhiza, with *Glomus clarum*, and with *Glomus deserticola*), as well as two (2) levels of diazotroph (with and without diazotroph). These treatment combinations were applied to thirty (30) pots, each containing 3 kg of soil. The experiment was laid out in a randomized complete block design (RCBD). Data were collected on growth parameters such as leaf area, number of leaves, stem girth, and plant height at 2-week interval. The trial was terminated at 8 weeks after sowing (WAS), and yield parameters measured included root length, fresh and dry shoot weight, and fresh and dry root weight. The data were subjected to analysis of variance (ANOVA) using the General Linear Model procedure with Minitab statistical software. A significant interactive effect of diazotrophs and mycorrhiza was observed in stem girth at 4 WAS. The highest stem girth (4.02 cm) was obtained under combined application of diazotroph and *G. clarum* while the least stem girth (3.38 cm) was obtained under control (no-diazotroph and no-mycorrhiza treatment). Mycorrhiza application positively influenced root length, fresh root weight, fresh shoot weight, dry root weight and dry shoot weight

**Keywords:** Cereals; Inoculum; Mycorrhizal treatment; Soil environment; Southern Guinea Savanna

### 1. Introduction

Maize (*Zea mays* L.) is the third most commonly consumed cereal and is utilized in three principal ways, namely industrial usages, livestock feed, and human foods [1]. Maize is produced under different ecological conditions owing to its high adaptability, yet the production is faced with challenges of disease, drought, and poor soil fertility. Considering the ever-growing population, the world will need twice as much maize by the year 2050 [1].

Efforts have, however, been intensified to sustain productivity with high chemical fertilizer input, particularly for nitrogen, which is the most crucial element for maize production [2]. Unfortunately, a larger proportion of inorganic N fertilizer applied is lost through gaseous emission, leaching into groundwater, and denitrification. The negative impact of these on the environment is huge, even as the soil remains poor in nitrogen [3].

The overuse of inorganic fertilizers to increase plant yield has been linked to environmental contamination and soil degradation. Finding a source of nutrients that is both easily accessible and environmentally safe is so crucial [4]. In search of sustainable productivity, in the face of the ever-growing world population and the high cost coupled with the environmental impact of chemical fertilizers, inoculants containing plant growth-promoting microorganisms

\* Corresponding author: Odoh NC; Email: [irukaodoh@yahoo.com](mailto:irukaodoh@yahoo.com)

are sourced [5, 6]. Microbial inoculants in the form of diazotrophs and mycorrhizae are good alternatives to pesticides and chemical fertilizers as they serve as phytostimulants, bio-herbicides, and bio-fertilizers [6, 7]. Thus, these inoculants could be useful for meeting the nutritional requirements while tackling other challenges facing maize production [8]. It might be possible to increase the production of maize. Maize production could be improved by these inoculants through the provision of their nutrients, the suppression of plant pathogens, and the inhibition of rhizosphere-based disease organisms [3, 8].

Due to root-associated, nitrogen-fixing bacteria that greatly boost the nitrogen supply to the rhizosphere, less reliance on inorganic fertilizer is possible [9, 10]. Nitrogenase enzymes are used by diazotrophs to convert dinitrogen to ammonium. In root nodules, legumes create specialized symbiotic partnerships with diazotrophic rhizobia, whereas non-legume crops connect with bacteria at the root surface [11].

Arbuscular mycorrhizal fungi (AMF) are symbiotic relationships between soil plants and fungi that allow them to adapt to their surroundings and thrive. The adoption of AMF is reckoned to be beneficial to soil fertility in natural and agricultural ecosystems. Mycorrhizae are thus a key factor for agricultural sustainability. It stimulates plant development and increases N, P, and Z content, particularly in cereals. The availability of AMF contributes to the improvement of the efficiency of nutrient uptake by plants and limits the waste of nutrients in the environment [12].

The introduction of diazotrophs and AMF as inoculants may benefit rural farming communities. These beneficial soil microorganisms, besides being eco-friendly, could enhance crop yield. The introduction of both AMF and diazotrophs as consortia could be of greater benefit to plants and the environment than their use as single inoculants. This investigation was carried out to determine how the inoculation of mycorrhizae and diazotrophs affected the growth and biomass production of maize.

---

## 2. Materials and methods

### 2.1. Experimental site description

The experiment was carried out at the Teaching and Research Farm, University of Abuja, in the Federal Capital Territory, in the Southern Guinea Savanna of Nigeria. The climate of the area is characterized by moderate to high rainfall and a temperature of about 26 – 30 °C. The rainfall is between 1000 mm and 1800 mm per year, with abundant sunshine. The vegetation of the area is characterized by wooded land, grass, and shrubs. It falls within a basement complex.

### 2.2. Soil sampling and experimental set-up

Using a soil auger, soil was sampled at random from eight spots at the University of Abuja's Teaching and Research Farm. In order to create a composite sample from the soil, it was well mixed together. A subsample of this composite sample was then taken to the laboratory for physical and chemical examination of the soil.

Soil of 3 kg weight was filled into thirty (30) pots. Each pot was watered to field capacity and allowed to drain for 24 hours. Compost was used to amend the soil at 120 g/3kg pot. Maize seed (Sammays-52) was sown at 4 seeds per pot and at two (2) weeks after sowing (WAS) it was thinned to 1 stand per pot. The maize variety used was collected from the Institute for Agricultural Research (IAR) at Ahmadu Bello University, Zaria, Nigeria. The treatments involved two (2) levels of diazotroph (with and without diazotroph) and three (3) levels of mycorrhiza (without mycorrhiza, with *Glomus clarum*, and with *Glomus deserticola*). The strain of diazotroph used was isolated from Laboratoire des Symbioses Tropicales et Méditerranéennes (LSTM) in Montpellier, France, from soil that originated in Nigeria. Diazotroph was inoculated at 10 ml per plant while mycorrhiza was sourced from the Soil Microbiology Laboratory at the University of Ibadan and inoculated at 20 g pot<sup>-1</sup>, at 2 WAS.

Six (6) treatment combinations were obtained and replicated five times. The experiment was laid out in a randomized complete block design (RCBD). No inorganic fertilizer was applied. Routine manual weeding was done by handpicking throughout the experiment.

### 2.3. Collection of data and statistical analysis

Growth parameters such as leaf length, leaf width, number of leaves, stem girth, and plant height were assessed at 2-week intervals. Destructive sampling was done at 8 WAS, and parameters measured included root length per plant, fresh and dry shoot weight per plant, and fresh and dry root weight per plant. Harvested roots were carefully picked after sieving the soil and washed. Fresh root and shoot were dried for 21 days, and their dry weight recorded.

Analysis of variance (ANOVA) was performed on the data using the General Linear Model technique and analysis of means. The Fisher test was used to separate significant means at a p-value of 0.05. The Minitab statistical software was used to run all analyses.

### 3. Results and discussion

#### 3.1. The soil's physical and chemical characteristics

Table 1 displays the outcomes of soil's physical and chemical properties before treatment application. Soil pH in water (H<sub>2</sub>O) was neutral at 7.21, while pH in KCl was moderately alkaline at 8.31. These pH values were within the required pH range for maize (5–8), as earlier recommended [13]. The total nitrogen and electrical conductivity of the soil were low. Relatively moderate amounts of exchangeable bases were present in the soil. Organic carbon and available P in the soil were in a medium range. Organic matter in the soil was high [14]. The soil texture class was loamy sand.

**Table 1** Physical and chemical properties of soil used before planting

Properties	Values	Properties	Means
PH (H <sub>2</sub> O)	7.21	K (cmol kg <sup>-1</sup> )	0.042
PH (KCl)	8.31	Na (cmol kg <sup>-1</sup> )	0.216
EC (dS/m)	0.21	Mg (cmol kg <sup>-1</sup> )	3.36
OC (%)	1.9	Ca (cmol kg <sup>-1</sup> )	4.2
OM (%)	3.28	Ex. Acidity	1.8
TN (%)	0.08	ECEC	9.618
Available P (mg kg <sup>-1</sup> )	30.66	Exchangeable Al <sup>3+</sup> (cmol kg <sup>-1</sup> )	0.61
		Base Saturation (%)	81.28
Particles size			
Sand (g kg <sup>-1</sup> )	809.6		
Silt (g kg <sup>-1</sup> )	632.0		
Clay (g kg <sup>-1</sup> )	127.2		
Textural class	Loamy sand		

#### 3.2. Growth parameters

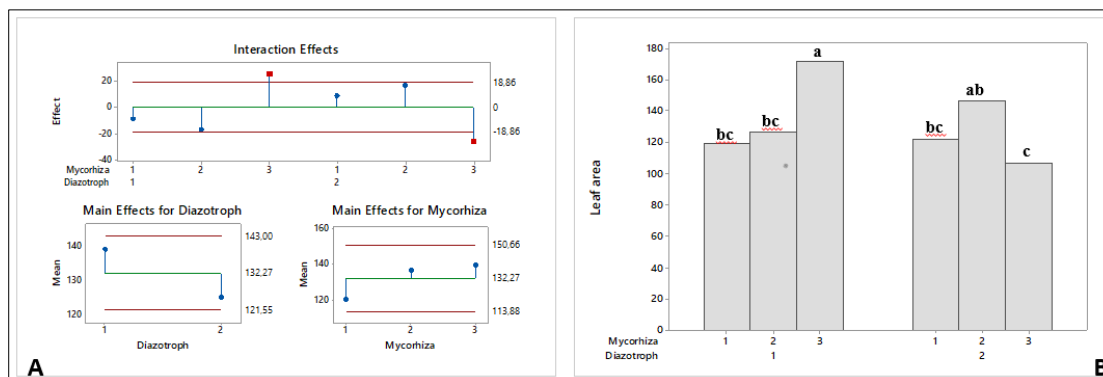
**Table 2** Effect of diazotroph and mycorrhizal application and their interaction on plant height and leaf area of maize

	Adj SS	P-Value		Adj SS	P-Value		Adj SS	P-Value
	Plant height (cm)				Leaf area (cm <sup>2</sup> )			
	2 WAS			4 WAS			2 WAS	
Diazotroph	0.117	0.924		161.01	0.127		0.80	0.98
Mycorrhiza	18.314	0.491		93.05	0.496		3183.30	0.16
DPH*MYC	23.497	0.491		57.32	0.646		1024.20	0.54
	6 WAS			8 WAS			4 WAS	
Diazotroph	27.65	0.479		91.88	0.451		2992.50	0.19
Mycorrhiza	21.20	0.822		420.62	0.279		949.40	0.76
DPH*MYC	78.5	0.491		199.95	0.536		950.90	0.76

WAS = weeks after sowing, DPH\*MYC = interaction diazotroph-mycorrhiza, Adj SS = adjusted sum of square

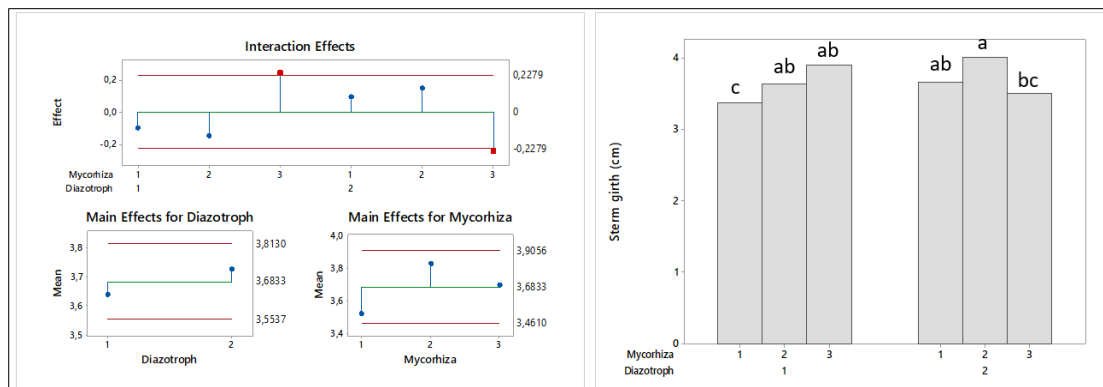
The main factors and their interactions had no significant effect on plant height throughout the experiment and on the leaf area at 2 and 4 WAS. However, the analysis of variance showed a significant interaction effect of diazotroph and mycorrhiza on leaf area at 6 WAS. The analysis of means also showed significant differences; the largest leaf area was obtained with the application of *G. deserticola* with 139.48 cm<sup>2</sup>.

A significant difference in stem girth was observed at 4 WAS. The highest stem girth (4.02 cm) was obtained under combined application of diazotroph and *G. clarum* while the least stem girth (3.38 cm) was obtained under absolute control (no-diazotroph and no-mycorrhiza application). At 6 and 8 WAS, the main factors and their interactions did not significantly influence the stem girth (Table 2).



A: Means analysis result. B: Fisher means comparison, bars with same letter are not statistically different. Mycorrhiza (1= no mycorrhiza, 2 = *G. clarum*, 3 = *G. deserticola*), Diazotroph (1 = no diazotroph, 2 = with diazotroph).

**Figure 1** Effect of combined diazotroph and mycorrhizal inoculation on maize leaf area at 6 weeks after sowing



A: Means analysis result of leaf area. B: Fisher means comparison, bars with same letter are not statistically different. Mycorrhiza (1= no mycorrhiza, 2 = *G. clarum*, 3 = *G. deserticola*), Diazotroph (1 = no diazotroph, 2 = with diazotroph).

**Figure 2** Effect of combined application of diazotroph and mycorrhizae on maize stem girth at 4 weeks after sowing

The number of leaves at 8 WAS showed a significant difference from the primary factor "mycorrhiza"; *G. clarum* had the highest number of leaves (11.9) while the treatment without mycorrhiza had the least number of leaves (10.4) (Figure 3). Despite the application of diazotroph, there was no discernible alteration in the interaction between these two organisms during the course of the experiment (Table 2). The use of diazotrophs, mycorrhiza, and their interactions did not result in a substantial increase in root length (Table 2). Plant growth improvement by diazotroph and mycorrhiza inoculation is abundant in the literature. Thus, previous reports specified that diazotrophs increased plant height in cereal crops [15, 3]. Contrarily, the observations from the present study did not confirm these findings. This could be due to multiple reasons, mainly soil pH. For most diazotrophs, cell growth and biological N<sub>2</sub> fixation are optimal near neutral pH [16], while the soil used in this experiment was neutral.

During the experimentation, mycorrhiza significantly influenced the leaf area and stem girth of the plant. A positive effect of mycorrhizal application on plant growth parameter had earlier been reported [17].

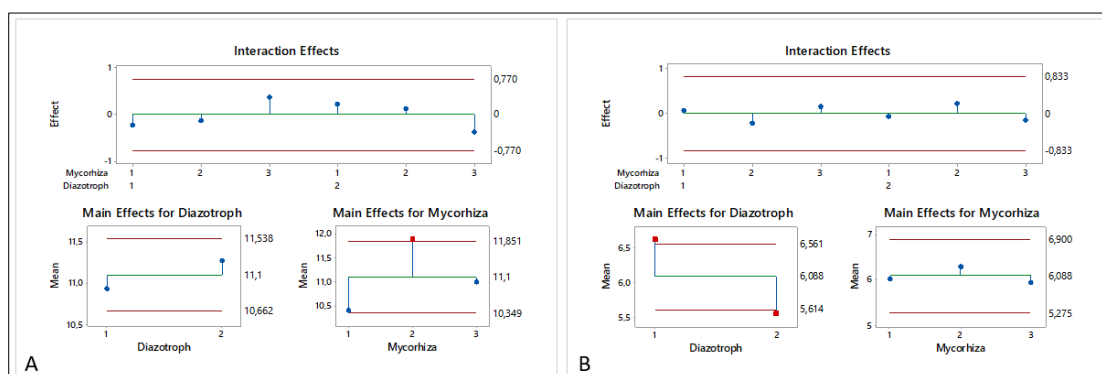
**Table 3** Effect of combined application of diazotroph and mycorrhiza on stem girth, number of leaves and root length of maize.

	Adj SS	P	Adj SS	P	Adj SS	P
	Stem girth at 2 WAS		Stem girth at 8 WAS		Number of leaves at 6 WAS	
Diazotroph	0.51	0.18	0.26	0.42	0.03	0.81
Mycorrhiza	0.99	0.18	0.01	0.99	0.80	0.50
DPH*MYC	0.26	0.62	1.48	0.17	1.07	0.40
	Stem girth at 6 WAS		Number of leaves at 2 WAS		Root length	
Diazotroph	0.13	0.44	0.83	0.27	149.63	0.20
Mycorrhiza	0.05	0.89	2.07	0.23	290.12	0.20
DPH*MYC	0.91	0.14	0.47	0.70	43.12	0.78

WAS = weeks after sowing, DPH\*MYC = interaction diazotroph-mycorrhiza, Adj SS = adjusted sum of square.

### 3.3. Maize biomass yield

The biomass yield was estimated using maize fresh root weight, fresh shoot weight, and dry shoot weight. It was observed that the application of diazotroph, mycorrhiza, and their interaction did not significantly influence fresh and dry shoot weight (Table 3). However, the main factor "diazotroph" had a significant effect on dry root weight (Figure 3); without diazotroph, 6.62 g and with diazotroph, 5.55 g.



**Figure 3** Effect of diazotroph, mycorrhizal application and their interaction on number of leaves at 8 weeks after sowing (A) and dry root weight of maize (B).

The effect of diazotroph application in the field vary from negative to significantly positive. In fact, biomass yield of maize was increased by diazotroph inoculation [18, 19]. However, previous investigation did not observe significant effect of selected diazotroph on maize dry weight [3].

Mycorrhiza application influenced root length, fresh root weight, fresh shoot weight, dry root weight and dry shoot weight. This agreed with past reports showing that mycorrhiza inoculation increased biomass yield of cereals [20, 21].

### 4. Conclusion

Improvement of maize growth and yield using biofertilizer is an important step to boost its production and soil sustainability particularly in sub-Saharan Africa. The inoculation of diazotroph and mycorrhiza, in this study, revealed some relevant results in the early growth stage of maize in terms of leaf area, number of leaves and biomass yield. Further investigation would be conducted to evaluate the grain yield. Biofertilizer utilization will reduce inorganic fertilizer application and farming cost.

---

## Compliance with ethical standards

### *Acknowledgments*

The authors are grateful to African Women in Agricultural Research and Development (AWARD) for funding part of this research (Trip and stay in Montpellier, France).

### *Disclosure of conflict of interest*

There is no conflict of interest

---

## References

- [1] Hossain F, Muthusamy V, Bhat SJ, Jha KS, Zunjare R, Das A, Saraki K, Kumar R. 2016. Maize. In: Singh M, Kumar S, eds. Broadening the genetic base of grain cereals. New Delhi, India, Springer; 2016; p. 67-88. Doi 10.1007/978-81-3223613-9\_4.
- [2] Ikeda AC, Zecchin VJS, Savi DC, Kava V, Glienke MH, Hungria M, Terasawa LVG. 2018. Bioprospecting plant Growth promoting bacteria isolated from Maize (*Zea mays* L.) root. J Biotech Res Biochem. 2018; 1: 002. DOI: 10.24966/BRB-0019/100002
- [3] Kifle HM, Laing DM. Effect of selected diazotrophs on Maize growth. Front. Plant Sci. 2016; 7:1429. doi: 10.3389/fpls.2016.01429.
- [4] Mvumi C, Tagwira F, Chiteka AZ. Effect of moringa extract on growth and yield of maize and common beans. Greener Journal of Agricultural Sciences. 2013; 3 (1): 055-062.
- [5] Hungria M, Campo R, Souza EM, Pedrosa FO. Inoculation with selected strains of *Azospirillum brasilense* and *A. Lipoferum* improves yield of Maize and wheat in Brazil. Plant and soil. 2010; 331: 413-425.
- [6] Alori ET, Babalola OO. Microbial Inoculants for Improving Crop Quality and Human Health in Africa. Front. Microbiol. 2018; 9: 2213. doi: 10.3389/fmicb.2018.02213
- [7] Babalola OO, Glick B R. The use of microbial inoculants in African agriculture: current practice and future prospects. J. Food Agric. Environ. 2012; 10: 540–549.
- [8] Silva K, Person L, Gomes M de L, Barauna CA, Pereira GMD, Mosqueira AC, Costa da BI, O'Hara G, Zilli EJ. Diversity and capacity to promote maize growth of bacteria isolated from the Amazon region. Acta Amazonica. 2016; 46 (2): 111-118.
- [9] Mus F, Crook MB, Garcia K, Costas AG, Geddes BA, Kouri ED, Paramasivan P, Ryu MH, Oldroyd GED, Poole PS, Udvardi MK, Voigt CA, Ané JM, Peters JW. Symbiotic nitrogen fixation and the challenges to its extension to nonlegumes. Applied and Environmental Microbiology. 2016; 82(13): 3698-3710. doi: 10.1128/AEM.01055-16.
- [10] Batista MB, Dixon R. Manipulating nitrogen regulation in diazotrophic bacteria for agronomic benefit. Biochemical Society Transactions. 2019; 47: 603–614.
- [11] Bloch SE, Clark R, Gottlieb SS, wood LK, Shah N, Mak S, Lorigan GJ, Johnson J, Austine GD, Williams L, McKellar M, Soriano D, Petersen M, Horton A, Smith O, Wu L, Tung E, Broglie R, Tamsir A, Temme K. Biological Nitrogen fixation in Maize: optimizing nitrogenase expression in a root-associated diazotrophs. Journal of experimental botany. 2020; 71(15): 4591-4603. doi:10.1093/jxb/ersa177.
- [12] Cozzolino V, Di Meo V, Piccolo A. Impact of arbuscular mycorrhizal fungi applications on maize production and soil phosphorus availability. Journal of geochemical exploration. 2013; 129: 40-44.
- [13] Kamara AY, Kamai N, Omogui LO, Togola A, Ekeleme F, Onyibe JE. Guide to maize production in North Nigeria: Ibadan, Nigeria. 2020, 18 p.
- [14] Ravikumar P, Somashekar RK. Evaluation of nutrient index using organic carbon, available P and available K concentrations as a measure of soil fertility in Varahi River basin, India. Proceedings of the International Academy of Ecology and Environmental Sciences. 2013; 3(4): 330-343.
- [15] Jalal A, Azeem K, Filho MCMT, Khan A. Enhancing soil properties and maize yield through organic and inorganic nitrogen and diazotrophic bacteria. In: Hasanuzzaman M, Fujita M, Filho MCMT, Nogueira TAR, eds. Sustainable crop production 2014. DOI: 10.5772/intechopen.92032

- [16] Van Dommelen A, Vanderleyden J. Associative nitrogen fixation. In: Bothe H, Ferguson SJ, Newton WE, eds. *Biology of the Nitrogen Cycle*. Elsevier, 2007; p.179-192. <https://doi.org/10.1016/B978-044452857-5.50013-8>.
- [17] Assogba S, Noumovo AP, Dagbenonbakin DG, Agbodjato AN, Akpode C, Koda DA, Aguegue MR, Bade F, Adjanohoun A, Falcon AR, Novapons MB, Baba ML. 2017. Improvement of Maize productivity (*Zea mays* L.) by Mycorrhizal Inoculation on ferruginous soil in center of Benin. *International journal of sustainable Agricultural Research*. 2017; 4(3): 63-76
- [18] Hadi F, Bano A. Effect of diazotrophs (Rhizobium and Azatebactor) on growth of maize (*Zea mays* L.) and accumulation of lead (PB) in different plant parts. *Pakistan Journal of Botany*. 2010; 42: 4363-4370
- [19] Sandhya A, Vijaya T, Sridevi A, Narasimha G. Influence of vesicular arbuscular mycorrhiza (VAM) and phosphate solubilizing bacteria (PSB) on growth and biochemical constituents of *Marsdenia volubilis*. *African Journal of Biotechnology*. 2013 ; 12(38): 5648-5654
- [20] Chen Y, Liu RJ, Bi YL, Feng G. Use of mycorrhizal fungi for forest plantations and mine site rehabilitation. In: Solaiman ZM, Abbott LK, Varma A, eds. *Mycorrhizal Fungi: Use in Sustainable Agriculture and Land Restoration*. *Soil Biology* 41; 2014. p. 325–355.
- [21] Shi Z, Zhang J, Lu S, Li Y, Wang F. Arbuscular Mycorrhizal Fungi Improve the Performance of Sweet Sorghum Grown in a Mo-Contaminated Soil. *J Fungi (Basel)*. 2020; 31;6(2): 44. doi: 10.3390/jof6020044. PMID: 32244390; PMCID: PMC7344874.