ARTICLE / INVESTIGACIÓN

Effect of vermicompost, Mycorrhiza and humic acid on some bi-chemical and yield characteristics of pepper *Capsicum annuum L.* under greenhouse conditions

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Abstract: A field experiment was carried out under the conditions of protected agriculture in the agricultural season 2020-2021 to study the effects of three factors: The first is the addition of vermicompost, with three levels: 0, 10 and 20 tons ha^{-1}, the second factor is the addition of Mycorrhiza to the soil with two levels: without and with vaccination of 10 g per plant; and the third factor is spraying humic acid in three concentrations: 0, 2.5 and 5 ml l^{-1} on some biochemical properties and yield characteristics of pepper, according to randomized complete block design (RCBD). Results showed a significant effect on chlorophyll a, b, nitrogen, phosphorus and potassium in leaves, ascorbic acid content in fruit, and yield per plant. The best interaction was when adding the third level of vermicompost (20 tons ha-1) with vaccination of Mycorrhiza and spraying the third level of humic acid (5 ml l^{-1}) on most of the studied characteristics of the plant.

Key words: Vermicompost, humic acid, Mycorrhiza, *Capsicum annuum L*.

Introduction

Agriculture, especially in developed countries, is currently aimed at safe and clean agriculture, which is intended to grow plants without the use of fertilizers and chemical pesticides and to be content with natural sources of fertilizer such as organic fertilizer such as vermicompost and humic acid, as well as biofertilizers such as Mycorrhiza, to avoid the adverse effects of chemicals that affect human health. Vermicompost is a natural organic fertilizer produced by earthworms, and recent studies have shown its positive impact on the physical, chemical and biological characteristics of soils, such as improving soil aeration, increasing water content and fertility, in addition to the fact that this fertilizer is a source of many macro and micro nutrients[1]. Humic acid is an essential organic acid because it contains many nutrients, which led researchers to add it to crops, especially in the method of foliar application, which has proved successful in increasing plant growth and productivity and increasing the development of shoot and root, which is reflected in the quantity and quality of crops[2]. Mycorrhiza is characterized by the hyphae extending into the soil and absorbing nutrients far from the root, making them available for absorption by plant roots[3] and associated with plant roots, making them more efficient in absorbing nutrients, especially phosphorus[4]. Pepper (*Capsicum annuum L.*) belongs to the *Solanaceae* family and grows successfully under protected, unheated farming conditions. Its potassium, calcium, and fluoride content prevents tooth decay and contains vitamins A, C and E[5]. Based on the above trend for safe agriculture and to stay away from the use of chemical fertilizers and because the pepper crop is an important vegetable crop that needs an integrated fertilization program due to the length of its growing season relative to other vegetable crops, this study is aimed to: Studying the effect of vermicompost, and humic acid, and Mycorrhiza on biochemical characteristics and productivity of pepper plant.

Materials and methods

Site of the experiment

A field experiment was carried out in the agricultural season 2020-2021 in a farming field in Baladroz district 40 km northeast of Baquba district of Diyala governorate. Table 1 shows some chemical and physical properties of the soil.

EC dSm^{-1}	pH	OM gkg^{-1}	Carbonate mineral gkg^{-1}	Gypsum gkg^{-1}	Available elements			Texture gkg^{-1} (silty clay)	
					N $mgkg^{-1}$	P $mgkg^{-1}$	K $mgkg^{-1}$		
2.68	7.5	18.7	219.4	5.71	11.7	0.47	210	clay	40.0
								silt	57.2
								sand	2.80

Table 1. Shows some chemical and physical properties of soil.

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Prepare the field before planting

The greenhouse area is 504m^2, 56 meters long, 9 meters wide and 3 m high, divided into three equal blocks, and the distance between one block and another is 1m, each block contains 18 experimental units, and the dimensions of the experimental unit were 2 m*1 m and the distance between one experimental unit and another 80 cm, each block contains two lines for plant cultivation, thus reaching the number of experimental units of 54 experimental units. Each experimental unit includes 10 plants, and the distance between one plant and another is 40 cm.

Experimental design and distribution of treatments

A factorial experiment was carried out following the design of a randomized complete block design (RCBD) as the investigation included a study of three factors: 1- vermicompost at three levels: 0, 10 and 20 Mega gram ha^{-1}, 2- addition of Mycorrhiza to the soil and at two levels: without and with 10 g per plant, 3- spraying of humic acid at three concentrations 0, 2.5 and 5 ml^{-1}, and the results were analyzed using the statistical program SAS, the differences between the averages were tested according to Duncan's multi-border test at a probability level of 0.05.

Vermicompost was obtained from a local source, and its characteristics are shown in Table 2; the Mycorrhiza were obtained from the laboratories of Baghdad University/ College of Science with a density of 50 spores per gram in the soil before agriculture, but the humic acid was sprayed on shoot three times the first after a month of agriculture, the second after one month of the first spray, and the third spray after one month of the second spray.

Seed cultivation and seedling preparation

On September 1, 2020, pepper seeds cultivar of carizma were grown, and when the seedlings reached 3 leaves that were transported and cultivated on the agricultural lines inside the greenhouse on October 15, 2020, and began the process of harvesting on December 18, 2020 and continued until April 1, 2021.

Irrigation

The irrigation was carried out using the drip irrigation system consisting of the main pipe, a source of irrigation water processing (river) and a pump with a horsepower of 6.5 hp. The diameter of the main pipe is 1.5 inches distributed on the width of the field 9 m, from which the sub-drip pipes were extended as two sub-lines were extended in each block and the distance between dotted and another 40 cm as well as the number of irrigations 30 irrigations.

Chlorophyll estimation in leave

The content of the chlorophyll a and b were determined following the method of (6). 1 g of fresh leaves were taken and cut into small pieces and then mashed in a ceramic mortar using 20 ml of acetone (80%) to extract the pigment and left to the next day in the refrigerator, then completed the volume to 50 ml and placed in a centrifuge for 5 minutes at a speed of 1000 rpm. The clear solution was taken, and the absorbance was measured using a spectrophotometer at 663 and 645 nm wavelengths. The concentrations of chlorophyll a and b were determined as follows:

Chl. a mg g^{-1} = (12.5 × A633 – 2.79 × A645) × V/1000 ×W

Chl. b mg g^{-1} = (21.5 × A645 – 5.68 × A663) × V/1000 ×W

Nutrient estimation in leaves

Samples of the leaves were taken for five random plants per trial unit and placed in the oven at a temperature of 70° until the weight stabilized, after which they were milled and placed in plastic boxes, after which the digestion was performed by taking 0.2 g of dry plant sample and digested according to the method proposed by (7). Using 4 ml of H_2SO_4, sulphuric acid was added, and the model was left for 24 hours, after which 1 ml of perchloric acid was added. Digestion swivels were placed on a hot plate until the appearance of yellow fumes was left for a while until the arrival of white fumes and then heated at a high temperature. When the digestion was completed was, obtained colorless chips then cooled and transferred to a 50 ml cycle and completed with distilled water to the mark and then the elements were estimated as follows:

Nitrogen was estimated after adding sodium hydroxide (10 M) using the microkjildal and abrasion device with sulphuric acid[8]. The phosphorus was calculated using modified ammonium molybdates after adjusting the laboratory's reaction score and measuring the spectrophotometer (882 nm)[9]. Potassium was estimated using a flame photometer and according to the proposed method.

Vitamin C content in fruits

2 g of fresh fruit samples were taken from five random plants, mixed with 2.5 ml of solution (3% phosphoric acid + 8% acetic acid), and placed in a high-speed blender (6000 rpm) for 1 minute. The samples were placed in a centrifuge for 20 minutes and then measured according to Himesh[10].

Results

Chlorophyll's content in leaves

The results of Table 3 indicate the significant effect of vermicompost, Mycorrhiza and humic acid on chlorophyll a and b content. All treatments were increased significantly compared to the control treatment. However, the treatments of interaction between the third level of vermicompost with the addition and non-addition of Mycorrhiza with the third level of humic acid gave the highest increase in the content of chlorophyll a to 2.03 and 1.98 mg g^{-1} fresh weight, respectively. However, they do not differ significantly, but they are superior to all other treatments, in addition to the control treatment, which amounts to 0.54 mg g^{-1} fresh weight, where the treatment of interaction between the third level of vermicompost with the addition of Mycorrhiza with third level of humic acid gave the highest increase in the content of chlorophyll b to 1.10 mg g^{-1} fresh weight and the lowest range of chlorophyll b with a control treatment of 0.21 mg g^{-1} new weight.

OC	N	P	K	Ca	Mg	S	Fe	Cu	Zn
30.8	130	25	54	34	77.7	80	48	1.0	1.2

Table 2. Shows some chemical properties of vermicompost (ppm).

Vermicompost	Mycorrhiza	Humic acid	Chlorophyll a mg g-1 fresh weight	Chlorophyll b mg g-1 fresh weight
V_1	M-	H_1	0.54 I	0.21 j
		H_2	0.82 h	0.23 j
		H_3	0.98 g	0.30 I
	M+	H_1	0.77 h	0.22 j
		H_2	0.94 g	0.27 i
		H_3	0.99 g	0.34 h
V_2	M-	H_1	0.99 g	0.40 g
		H_2	1.30 e	0.77 d
		H_3	81.1 c	0.91 c
	M+	H_1	1.00 g	0.70e
		H_2	1.31 e	0.78 d
		H_3	1.91 b	0.97 b
V_3	M-	H_1	1.10 f	0.51 f
		H_2	1.46 d	0.79 d
		H_3	1.98 a	1.00 b
	M+	H_1	1.32 e	0.71 e
		H_2	1.80 c	0.88 c
		H_3	2.03 a	1.10 a

*Transactions with similar characters do not differ morally between them at the probability level of 0.05, according to the Dunkin' multi-border test.

Table 3. Effect of vermicompost, Mycorrhiza and humic acid on chlorophyll a and chlorophyll b content in leaves.

Concentration of nutrients in leaves

Table 4 shows the effect of vermicompost, Mycorrhiza and humic acid on Nitrogen, Phosphorus and Potassium concentration in the leaves. The results indicate that all treatments increased significantly in the concentration of Nitrogen, Phosphorus and Potassium in the leaves compared to the control treatment. However, treating the interaction between the third level of vermicompost with the addition of Mycorrhiza with the third level of humic acid gave the highest increase in the concentration of N and P in leaves, which reached 4.36 and 0.50 %, respectively. The lowest nitrogen and phosphorus concentration in the leaves with a control treatment was 1.76% and 0.21%, respectively. The results in the same table indicate that all treatments significantly increased the concentration of K in leaves of pepper compared to the control treatment. However, it reached the highest K concentration in leaves in treating the interaction between the third level of vermicompost with adding Mycorrhiza with humic acid spraying at the third level, which reached 3.81%. The lowest potassium concentration in the leaves with the control treatment was 3.44%.

Ascorbic acid content in fruits

Table 5 results show the effect of vermicompost, Mycorrhiza, and humic acid, indicating that there are moral effects in the fruit content of ascorbic acid, the treatment of the interaction between the third level of vermicompost with the addition and non-addition of Mycorrhiza with the third level of humic acid gave the highest increase in the content of ascorbic acid, which leads to 65.33 mg g^{-1} fresh weight. The lowest ascorbic acid content with a control treatment was 44.00 mg g^{-1} fresh weight.

Yield per plant

Table 5 results show the effect of vermicompost, Mycorrhiza, and humic acid, indicating that all treatments were significantly increased compared to the control treatment. However, treating the interaction between the third level of vermicompost with the addition of Mycorrhiza and the third level of humic acid gave the highest increase in yield per plant, reaching 2.64 Kg $plant^{-1}$. This is superior to all other treatments and the control treatment that reached 1.07 kg $plant^{-1}$.

Vermicompost	Mycorrhiza	Humic acid	N %	P %	K %
V_1	M-	H_1	1.76 m	0.21 j	3.44 f
		H_2	2.00 kl	0.26 hi	3.58 def
		H_3	2.30 hij	0.31 fg	3.57 def
	M+	H_1	1.87 lm	0.24 ij	3.59 de
		H_2	2.16 ijk	0.31 fg	3.64 cde
		H_3	2.50 fgh	0.34 of	3.71 abcd
V_2	M-	H_1	2.10 jkl	0.25 i	3.49 ef
		H_2	2.40 fghi	0.31 fg	3.73 abcd
		H_3	2.96 de	0.37 d	3.63 cde
	M+	H_1	2.30 hij	0.28 gh	3.71 abcd
		H_2	2.63 f	0.36 de	3.72 abcd
		H_3	3.13 cd	0.40 c	3.72 abcd
V_3	M-	H_1	2.33ghij	0.31 fg	3.62 bce
		H_2	2.90 e	0.41 c	3.65 bcde
		H_3	3.83 b	0.47 b	3.80 ab
	M+	H_1	2.56 fg	0.35 de	3.71 abcd
		H_2	3.23 c	0.42 c	3.75 abc
		H_3	4.36 a	0.50 a	3.81 a

*Transactions with similar characters do not differ morally between them at the probability level of 0.05 according to the Dunkin' multi-border test.

Table 4. Effect Of vermicompost, Mycorrhiza and humic acid on nitrogen, phosphorus and potassium concentration in leaves of pepper.

Vermicompost	Mycorrhiza	Humic acid	Ascorbic acid (mg g^{-1}fresh weight)	Yield per plant (Kg plant $^{-1}$)
V_1	M-	H_1	44.00 n	1.07 m
		H_2	46.66 ml	1.30 kl
		H_3	48.33 kl	1.47 hi
	M+	H_1	45.33 nm	1.24 l
		H_2	48.00 kl	1.41 ij
		H_3	49.33 Jk	1.57 gh
V_2	M-	H_1	50.66 ij	1.36 jk
		H_2	53.00 gh	1.60 fg
		H_3	56.66 de	1.82 e
	M+	H_1	52.00 ih	1.52 gh
		H_2	54.33 fg	1.69 f
		H_3	57.00 cde	2.12 c
V_3	M-	H_1	55.66 fg	1.31 ghi
		H_2	59.00 bc	1.81 e
		H_3	64.00 a	2.23 b
	M+	H_1	58.66 cd	1.68 f
		H_2	61.00 b	1.96 d
		H_3	65.33 a	2.64 a

*Transactions with similar characters do not differ morally between them at the probability level of 0.05 according to the Dunkin' multi-border test.

Table 5. Effect of vermicompost, Mycorrhiza and humic acid on the content of ascorbic acid in fruits and yield per plant.

Discussion

These results agree with the results of (11) also showed an increase in chlorophyll a and b in the leaf of pepper by adding different types of vermicompost compared to the control treatment. These results also agree with result[12], which noted an increase in chlorophyll a and b in the leaves of the tomato plant when adding the Mycorrhiza. The reason for the rise in the concentration of nutrients in the leaves of the plant is due to the richness of vermicompost in bacteria, fungi, and their microbes' decomposition of organic matter, which leads to increased acidity of the medium, leading to the readiness of nutrients and thus increased absorption by the plant[13]. In addition, vermicompost contains a high amount of nutrients, which increases its concentration in the plant leaves. These findings agree with the results of the (14), who found an increase in chlorophyll a and b content and the concentration of nutrients N, P and K in the eggplant leaf plant by adding vermicompost. Mycorrhizal addition showed moral differences in the concentration of nutrients in the leaves of plants where Mycorrhiza acts as a biofertilizer with the ability to convert nutrients from the unready-made form to the ready-to-absorb form through their vital activities, and radical fungi interact with a wide range of microbes in the rhizosphere region. This attributed the reason to the symbiotic relationship between Mycorrhiza and plants, which enhanced the movement of elements and absorbed them by plant roots as they observed increased activity of phosphate enzyme in the plant[12]. Earlier, it was found that the spraying of humic acid has led to moral differences in most of the plant's chemical traits, which may be caused by foliar application of humic acid because of its apparent effects on plant growth and increased carbon representation activity[15]. Humic acid stimulates proton release through the plasma membranes of the roots by activating the H^+ -Atpase. In addition, humic acid transports nutrients within the plant, increasing the level of photosynthesis and absorption of nutrients[16].

On the other hand, humic acid spraying has a direct role in the construction of chlorophyll as one of the components of porphyrins in the composition of chlorophyll, which has led to an increase in carbon representation activity represented by increasing the content of carbohydrates in the leaves. The proliferation of yield per plant when adding vermicompost may be because vermicompost is one of the most suitable non-chemical plant nutrition sources. It positively affects plant growth and yield characteristics, as it stimulates and increases the absorption of nutrients. These results are in agreement with and (17) from the increase in pepper fruit yield when using vermicompost, as well as with the results of who obtained the highest growth in potato tuber yield with an increase in yield when they added vermicompost (0, 3, 6 and 9 tons ha^{-1}) attributed the reason to the fact that vermicompost works to increase the porosity and aeration of the soil and increase its ability to retain water, which leads to an increase in the yield of sweet pepper and tomato plants.

Conclusions

Through the results obtained in this study, we recommend adding 20 t ha^{-1} with vaccination of Mycorrhiza with spraying of 5 ml l^{-1} of humic acid. This is to give it the best results in the studied characteristics of pepper plants under protected cultivation conditions.

Bibliographic references

1. Padamanabhan, V. Effect of Vermicompost on Growth and Flowering of Chrysanthemum. Annals of the Romanian Society for Cell Biology. 2021. 25(4): 5068-5077.
2. Kteczek, A. and Anielak, A. M. Humic substances and significance of their application–a review. Journal Technical Transactions. 2021. 12 (12):1-14.
3. Salman, D. N. and Hameed S. S. The role of mycorrhiza inoculum and potassium fertilizer on the growth of Zea mays L. under water stress. Diyala Agricultural Sciences Journal. 2017. 9(2):240-255.
4. Issa, F. H. and Mhaibes, N. H. Effect of mycorrhiza and licorice root extract on growth and yield of okra (abemoschus esculentus L. CV. Hasinawi. Diyala Agricultural Sciences Journal. 2020. 12, special issue: 731-740.
5. Al-Salami H. J. A. and Abbas J.A. Sweet pepper (capsicum annum L.) plant response to spraying with nano-NPK and antioxidants. Annals of the Romanian Society for Cell Biology, 2021. 25(6):7031-7038.
6. Arnon, D.I. Copper enzymes in isolated chloroplast. Polyphenyle oxidase in Beta vulgaris. J. Plant Physiol., 1949. 24(1): 1-5.
7. Cresser, M.S. and Parsons, J.W. Sulphuric-perchloric acid digestion of plant material for determination of nitrogen, phosphorus, potassium calcium and magnesium. Analytical Chemical. Acta. 1979. 109:431-436.
8. Haynes, R.J. A comparison of two modified Kjeldahal digestion techniques for multi-element plant analysis with conventional wet and dry ashing methods. Communications in Soil Sciences and Plant Analysis. 1980. 11(5): 459-467.
9. Olsen, S.K. and L. E. Sommers. Phosphorus in : Methods of soil analysis Page, A. L. et al . 1982. pp: 403-430.
10. Himesh, S., Singhai A. and Sharma, S., Quantification of ascorbic acid in leaves of Annona squamosa. Int. J. Pharm. Pharm. Sci., 2012. 4(3): 144-147.
11. Chatterjee, D., Dutta, S. K., Kikon, Z. J., Kuotsu, R., Sarkar, D., Satapathy, B. S., Deka, B. C. Recycling of agricultural wastes to vermicomposts: Characterization and application for clean and quality production of green bell pepper (Capsicum annuum L.). Journal of Cleaner Production. 2021. 315(1):1-14.
12. Hashem, A.; Akhter, A.; Alqarawi, A. A.; Singh, G.; Almutairi, K. F.and Abd_Allah, E. F. Mycorrhizal fungi induced activation of tomato defense system mitigates fusarium wilt stress. Saudi Journal of Biological Sciences. 2021. 28 (10): 5442-5450.
13. Prasad, K. Effect of Dual inoculation of arbuscular mycorrhiza fungus and cultivar specific Bradyrhizobium Japonnicum on the growth yield chlorophyll, nitrogen and phosphorus contents of Soybean (Glycine Max (L.) Merrill.) Grown on alluvial soil. Journal of Innovation in Applied Research. 2021. 4(1):1-12.
14. Ebrahimi, M., Souri, M. K., Mousavi, A. Sahebani, N. Biochar and vermicompost improve growth and physiological traits of eggplant (Solanum melongena L.) under deficit irrigation. Chemical and Biological Technologies in Agriculture. 2021. 8 (19): 1-14.
15. Souza, A. C.; Zandonadi, D. B.; Santos, M. P.; Canellas, N. O. A.; Soares, C., Irineu, L. E. S.,Canellas, L. P. Acclimation with humic acids enhances maize and tomato tolerance to salinity. Chemical and Biological Technologies in Agriculture. 2021. 8 (40): 1-13.
16. Mahdi, Z. A. H., and Hussein, J. K. Effect of Humic Acid, Gibberellin and Tryptophan on the Growth and Flowering Characteristics of Goldenrods Plant Tolidago Canadensis. In IOP Conference Series Earth and Environmental Science. 2021. 910(1).1-10.
17. Sohel, M. H. and Ghosh, M. K. Effect of compost, vermicompost, trichocompost and NPKS fertilizers on the growth, yield and yield components of capsicum (Capsicum annum L.). EBAUB Journal. 2021.3(1):29-35.
18. Mostofa, M., Roy, T. S., and Chakraborty, R. Interactive Effects of Vermicompost and Seed Tuber Size On the Production of Potato (Solanum Tuberosum L.). Bangladesh Journal of Botany. 2021. 50(3): 695-700.