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COMPLEMENTED EFFECT OF GLYCINE BETAINEA AND BIOFERTILIZERS ON GROWTH AND PRODUTIVITY OF SWEET PEPPER (*Capsicum annuum L.*) PLANT UNDER HIGH TEMPERATURE CONDITION.

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ABSTRACT

Temperature is one of the major factors are being controlling and/or limiting growth and development of plants. The effect of plant growth promoting rhizobacteria (PGPR) (mixture of *Azospirillum lipoferum, Paenibacillus polymyxa* and *Bacillus circulans*), mycorrhizae (AM) and foliar application with glycine betaine (GB) at 0, 10 &15mmol/L individually or in combination on some growth aspects, photosynthetic pigments, microbial activities, minerals and some bioconstituents, endogenous phytohormones, flowering, fruiting and fruit quality of sweet pepper cv. California wander was studies during 2011 and 2012 seasons under open field at high temperature condition. Results indicated that, different applied treatments significantly increased most growth parameters as number of branches and leaves per plant, leaf area per plant, leaves and shoot dry weight per plant and leaf area ratio as well. Furthermore, photosynthetic pigments, NPK, total sugars and total free amino acids concentration in leaves recoded the maximum values when plants treated with PGPR, AM and foliar application with GB at 10 mmol/L as compared with those of individual application or untreated ones. Moreover, individual application with PGPR, AM and / or GB at 10 mmol/L enhanced the microbial activities in rhizosphere of the pepper plants compared with untreated ones. Also, biofertilizers and GB treatments increased auxin, gibberellin and cytokinin levels in sweet pepper shoots at 65 days after transplanting during 2012 season whereas abscisic acid was decreased. Moreover, the highest early and total yield were obtained when plants were sprayed with GB at 10 mmol/L and treated with microbial consortium (PGPR & AM). In addition, chemical composition of minerals and some bioconstituents such as total carbohydrates, vitamin C, total soluble solids in sweet pepper fruits were also increased at the same treatments. Hence, it could be recommended that foliar application with GB at 10 mmol/L in the presence of PGPR and AM as biofertilizers can be used to increase yield and fruit quality of sweet pepper plant when grown at high temperature condition.

Keywords: biofertilizers, glycine betaine, arbiscular mycorrhizae photosynthetic pigments, endogenous phytohormones, yield, sweet pepper.

INTRODUCTION

Temperature induced stress is an important environmental factor that influences the growth and development of plants. Each of low and high temperatures affect plant growth and development at intact plant, tissue and cell level and even at sub-cellular level. Temperature variation may affect morphology, anatomy, phenology and plant biochemistry at all levels of organization (Bita and Gerats 2013). Direct injuries due to high temperatures in plants include protein denaturation and aggregation, increased fluidity of membrane lipids, as well. Indirect or slower high temperature injuries include

inactivation of enzymes in chloroplast and mitochondria, inhibition of protein synthesis, protein degradation and loss of membrane integrity (Waraich *et al.,* 2012).

Among the various methods to induce tolerability to high temperature stress in plant, foliar application of, or pre-sowing seed treatment with, low concentrations of inorganic salts, osmoprotectants, signaling molecules (e.g., growth hormones) and oxidants (e.g., H_2O_2) as well as preconditioning of plants are common approaches (Wahid *et al.,* 2007). Glycine betaine (GB) is the low molecular weight organic compound which has been successfully applied to induce heat tolerance in various plant species. Ashraf and Foolad (2007) reported that GB is an important compatible osmolyte in plants and accumulates in cytosol and chloroplast. GB can play an important role in effective protection against drought, high salt concentration and high temperature. Also, GB protects physiological processes such as photosynthesis by protecting the ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco) enzyme and photosystem II under stresses condition. Moreover, plants treated with GB maintain higher antioxidative enzyme activities and minimize oxidative stress (Mahmood *et al.,* 2009 and Bhatti *et al*., 2013). Yang and Lu (2005) reported that, not all plants accumulate GB in sufficient amounts to help averting adverse effects of abiotic stresses. Thus, different approaches have been contemplated to increase the concentrations of these compounds in plants grown under stress conditions to increase their stress tolerance. Exogenous application of GB to low-accumulating or non-accumulating plants may help reduce adverse effects of environmental stresses. Externally-applied GB can rapidly penetrate through leaves and be transported to other organs, where it would contribute to improved stress tolerance.

On the other hand, microorganisms could play an important role in adaptation strategies and increase of tolerance to abiotic stresses in agricultural plants. Plant-growth promoting rhizobacteria (PGPR) are associated with plant roots and mitigate most effectively the impact of abiotic stresses (drought, low temperature, salinity, metal toxicity, and high temperatures) on plants through the production of exopolysaccharates and biofilm formation. When plants are exposed to stress conditions, rhizospheric microorganisms affect plant cells by different mechanisms like induction of osmoprotectors and heat shock proteins. During the crop production, microorganisms can be used for (a) monitoring of biological activity in soil (microbial number, enzymatic activity and biodiversity); (b) as indicators of soil health/quality; (c) for mitigation of negative stress caused in plants by abiotic factors; and (d) as beneficial and effective microorganisms as inoculants (Grover *et al*., 2010). Arbuscular mycorrhizal fungi (AMF), the common soil inhabitant fungi, can form symbiotic associations with the roots of ~90% of terrestrial plants, in which plant photosynthates are exchanged for water and mineral resources acquired by the fungi from the rhizosphere. It was previously reported that AM symbiosis can regulate the responses of plants to temperature stress (Wu and Zou, 2010).

The target of this work was to study the effect of glycine betaine and inoculation with plant-growth promoting rhizobacteria and/or mycorrhizal fungi on growth and productivity of pepper plant under the high temperature stress.

MATERIALS AND METHODS

Two field experiments were carried out at the Experimental Farm Station of Fac. Agric., Moshtohor, Benha University, Egypt, during summer seasons of 2011 and 2012 to study the effect of plant growth promoting rhizobacteria (mixture of *Azospirillum lipoferum, Paenibacillus polymyxa* and *Bacillus circulans*), mycorrhizae (AM) and foliar application with glycine betaine (GB) at 0, 10 &15 mmol/L individually or in combination on growth, flowering, yield and fruit quality as well as photosynthetic pigments, minerals content, total sugars, total free amino acid of sweet pepper (*Capsicum annuum* L.) cv. California wander when grown at high temperature.

Mechanical and chemical analyses of the experimental soil are presented in Table (A). Mechanical and chemical analyses were estimated according to Jackson (1973) and Black *et al* (1982), respectively.

Table A: Mechanical and chemical analyses of the experimental soil.

Mechanical analysis

Soil particles	**Unit**	**Seasons**	
		2011	**2012**
Coarse sand	%	13.17	15.40
Fine sand	%	14.21	14.33
Silt	%	15.55	15.67
Clay	%	57.07	54.60
Textural class		Clay	Clay

Chemical analysis

Parameters	Unit	Seasons	
		2011	2012
Organic matter	%	1.86	2.03
Available N	ppm	60.4	64.3
Available P	ppm	7.2	7.4
Available K	ppm	24.5	29.7
CaCo3	%	0.53	0.58
Iron	ppm	24.8	27.0
Zinc	ppm	3.27	3.75
Manganese	ppm	13.60	14.70
Copper	ppm	2.30	2.26
Boron	ppm	13.0	15.0
pH		7.96	8.04

Climatological data :

Maximum and minimum of air temperature monthly were recorded after Shebeen EL- Kanater weather station during 2011 and 2012 seasons.

Table (B): Mean of Maximum and minimum air temperature during 2011 and 2012 seasons :

Months	Air temperature			
	Seasons			
	2011		2012	
	Maximum	Minimum	Maximum	Minimum
May	34.2	20.5	32.7	19.5
June	36.5	22.0	37.2	23.0
July	37.0	23.4	38.4	24.2
August	40.5	27.2	39.6	25.7
September	36.8	24.6	35.8	23.5

Microbial inocula

Azospirillum lipoferum, Paenibacillus polymyxa and *Bacillus circulans* were provided from the Unit of Biofertilizers, Fac. of Agric., Ain Shams Univ., Cairo, Egypt. While arbuscular mycorrhizal fungus (*Glomus mosseae*) was obtained from Agric. Microbiol. Dept., Soils, Water and Environment Res. Inst., Agric. Res. Center, Giza, Egypt.

A heavy cell suspension of each culture was prepared. *Azospirillum lipoferum* was grown on semi-solid N-free malate medium (Dobereiner, 1978) while *Paenibacillus polymyxa* and *Bacillus circulans* were individually propagated in nutrient broth medium. After 5 days of incubation at 30°C, microbial cells were separately suspended into sterile water to reach 10^8 cfu/ml. The mixed inoculum was prepared by mixing equal volumes of the desired cell suspensions. On the other hand, Micorrhizal inoculum consisted of root, hyphae, spores and growth media from a pot culture of onion plants which was previously infected with *Glomus mosseae* and grown for 4 months in pot culture. The standard inoculum (400 kg/fed.) contained about 270 spores/g. Spores of the fungus were measured by a wet-sieving and decanting technique (Gerdemann and Nicolson, 1963).

With respect to foliar spraying treatments, glycine betaine (MW 117.18) of Sigma Aldrich was used at 0, 10 & 15 mmol/L as well as distilled water as control were applied as foliar application at 30, 45 and 60 days after transplanting.

Experimental design

Four-week-old sweet pepper seedlings were successively washed with water gently dried and then were soaked in heavy cell suspension of mixed cultures of *Azospirillum lipoferum, Paenibacillus polymyxa* and *Bacillus circulans* for 30 min. Arabic gum (10%) was added as an adhesive agent prior to inoculation. In uninoculated treatments, seedlings were treated with uninoculated media. In addition, over-head soil technique was carried out using freshly prepared suspension of the mixed culture, which spread on soil surface adjacent to the seedlings. This application was added three times during the growth period up to the flowering stage.

Seedlings were transplanted to the experiment plots on 5th May in the two seasons. The experiments were arranged in a randomized complete block design with 3 replicates. The plot area was 10.5m^2 (3 x 3.5m) with five rows. Calcium superphosphate (15.5% P2O5) and potassium sulphate (48% K2O) were added at the rates of 300 and 200 kg/fed. respectively before transplanting for all treatments. While ammonium sulphate (20.5% N) was added at a rate of 400 kg/fed. in three equal doses at 30, 45 and 60 days after transplanting for all treatments except PGPR treatments that received half dose of nitrogen fertilizer. Different recommended agricultural practices for this plant were followed by the Ministry of Agric., Egypt. This experiment included NPK fertilizers (control), PGPR, mycorrhizae (AM) and PGPR & AM without GB and with GB at 10 or 15 mmol/L.

Sampling and collecting data:

After 65 days of transplanting, morphological characteristics, photosynthetic pigments, microbial activities, chemical composition, endogenous phytohormones, flowering and fruiting characteristics were estimated as following:

Morphological characteristics i.e. number of branches and leaves/plant, total leaf area (cm^2) using the disk method according to **Derieux *et al*. (1973)**, leaves and shoots dry weight (g) / plant were recorded. Leaf area ratio (LAR) was calculated according to Radford (1967).

$$LAR = \frac{\text{leaf area } (cm^2)}{\text{shoots dry weight /plant (g)}}$$

Photosynthetic pigments: chlorophyll a, b and carotenoids were calorimetrically determined according to the method described by Inskeep and Bloom (1985) and calculated as mg/g fresh weight.

Microbial activities : pepper rhizosphere samples were analyzed for dehydrogenase activity according to the method described by Casida *et al*. (1964). Available nitrogen, available phosphorus according to (A.P.H.A, 1992) and available potassium according to Chapmen and Pratt (1961). Mycorrhizal infection was microscopically estimated on a sample of fresh root as described by Giovannetti and Mosse (1980) after clearing and staining.

Endogenous phytohormones were quantitatively determined in sweet pepper shoots in the second season using High-Performance Liquid Chromatography (HPLC) according to Koshioka *et al*. (1983) for auxin (IAA), gibberellic acid (GA_3) and abscisi acid (ABA) while, cytokinins were determined according to Nicander *et al.* (1993).

Number of flowers/plant, fruit setting and abscission percentage were estimated as flowering characteristics.

$$\text{Fruit setting\%} = \frac{\text{No. of fruits/plant}}{\text{No. of flowers/plant}} \times 100$$

$$\text{Abscission \%} = \frac{\text{Number of flowers/plant - No. fruits/plant}}{\text{No. of flowers/plant}} \times 100$$

Early fruits number/plant was considered as the number of first four pickings. Total fruits number was calculated as number of fruits in all pickings while, fruit yield kg/plant was calculated as fresh weight of fruits in all pickings.

Total nitrogen, phosphorus and potassium were determine in sweet pepper leaves at 65 days after transplanting and fruits at harvest according the methods described by Horneck and Miller (1998), Sandell (1950) and Horneck and Hanson,(1998) respectively. Total carbohydrates, total sugars and total free amino acids were determined according to Dubios *et al.*, 1956, Thomas and Dutcher, 1924 and Rosed, 1957, respectively. Crude protein was calculated according to the following equation: Crude protein = Total nitrogen x 6.25 (A.O.A.C., 1990). Total soluble solid (T.S.S.) was measured using a hand refractometer. Vitamin C and titratable acidity were determined according to the method described by A.O.A.C. (1990**).**

Statistical analysis:

Data obtained in this study were statistically analyzed by using the least significant differences test (L.S.D) according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Vegetative growth

Data in Table (1) indicate that growth parameters of sweet pepper plants as number of branches and leaves, total leaf area, dry weight of leaves and shoots per plant, and leaf area ratio were significantly increased with the application of plant growth promoting rhizobacteria PGPR, mycorrhizae (AM) or foliar application with glycine betaine (GB) treatments than control when grown at high temperature during the two seasons. The interaction effect between biofertilizers, and GB foliar application with all concentration gave the highest values of growth parameters during the two growing seasons as compared with either individual treatments or control plants. Maximum stimulatory effect was excited in plants those treated with biofertilizers, mycorrhizae and (GB) at 10 Mm/L as foliar application during the two seasons. The obtained results could be expected since the applied biofertilizers enhanced plant growth. The effect of inoculation with *Azospirillum lipoferum, Paenibacillus polymyxa* and *Bacillus circulans* on root morphology and development, uptake of nitrogen, phosphorous and other minerals and hormone supply to plants have been suggested as factors are responsible for growth responses (Abou-Aly *et al*., 2006). The obtained results were confirmed by Dobbelaere *et al.* (2003) who reported that PGPR can promote plant growth directly through fixation of nitrogen, facilitation of mineral uptake, solubilization of phosphorus, production of siderophores that solubilize and sequester iron, production of phytohormones, or reduction in soil levels of ethylene. Also, Cagras *et al*. (2000) found that mycorrhizal inoculation of cucumber plants significantly increased leaf and shoot fresh and dry weight, root biomass and leaf area index. On the other hand, Ashraf and Foolad (2007) reported that GB-treated plants exhibited a slower decrease in leaf water potential during drought stress and developed wilting symptoms much later than untreated plants. In addition, GB-treated plants showed a better ability to recover from wilting following the removal of the stress, a very important characteristic for growing plants under water deficit conditions.

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Photosynthetic pigments

Data in Table (2) indicate that different photosynthetic pigments as chlorophyll a, b and carotenoids were positively responded to PGPR, mycorrhizae and GB foliar application than untreated plants during the two assigned seasons. Also, the interaction between biofertilizers and GB gave the highest values in this respect, comparing with individual application or the control plants. Moreover, increase of chlorophylls and carotenoids content may be enhanced photosynthesis efficiency and that is a good explain to the increasing of dry matter production. Also, this enhancement could be indicator for expectable high yielded fruits when plants grown at high temperature. The present results are in agreement with those obtained by Heidari and Golpayegani (2012) who found that inoculation with rhizobacteria could be efficiently used to improve growth, antioxidant status and photosynthetic pigments under water stress. They also reported that a significant increase was found in the inoculation with PGPR, especially combination of the bacterial species. With respect of GB, Wahid and Shabbir (2005) reported that plants treated with GB led to plants with lower membrane damage, better photosynthetic rate, improved leaf water potential and greater shoot dry mass, compared to untreated seeds. Cha-um *et al.* (2006) reported that exogenous application of GB stabilizes pigments and prevents water oxidation and photooxidation. Stabilization of pigments (chlorophyll and carotenoids) aids in light energy capture as required for photosynthesis. Several studies have that application of GB aids in protecting photosynthetic machinery of the plant organelles by stabilizing the ultrastructure of the chloroplast, photosystem-II reaction centers and maintaining the oxygen-evolving machineries (Makela *et al.,* 2000). Moreover, the other physiological parameters such as respiration rate, transpiration rate and injury to leaf membrane decreased with application of GB (Mohammed and Tarpley, 2011).

Microbial activities in sweet pepper rhizosphere

According to the results obtained in Table (3), significant increases in available N, P and K were observed in sweet pepper plants when inoculated with PGPR individually or when combined with AM or GB compared with control plants. Foliar application of GB with either AM or PGPR exhibited values of available nutrients greater than the treatments of biofertilizers without GB. Also, application of GB with the dual inoculation gave the maximum values of available nutrients. It was also noticed from Table (3) that individual application of GB or biofertilization with PGPR or AM significantly increased dehydrogenase activity in sweet pepper rhizosphere as compared to the control treatment. The combined inoculation with PGPR and AM increased enzymes activity more than the individual inoculation. Also, the highest values of enzymes activity were recorded in rhizosphere of the plants that treated with GB in the presence of biofertilizer especially the dual inoculation.

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This may be due to the mechanisms of plant growth promoting rhizobacteria and AM on soil properties. Moreover, mycorrhizal colonization percentage reached the maximum values in the treatments that received AM. These results are in agreement with Abou-Aly and Mady (2009) who found that inoculation with the biofertilizer agents increased available nutrients and dehydrogenase activity in wheat rhizosphere especially with AM inoculation. The highest values of enzymes activity were observed when the plants were treated with dual inoculation of biofertilizers. GB not only has a beneficial effect on the plant but also can be an effective thermoprotectant for bacteria. Under stress conditions, bacteria accumulate organic compounds, termed compatible solutes. These solutes are nontoxic low molecular that protect some macromolecular structures against denaturation. The main thermoprotectants include polyols and their derivatives, various sugars and zwitterions such as amino acids and betaines. Glycine betaine is reported to serve as the major effective thermoprotectant in gram-negative and gram-positive bacteria (Holtmann and Bremer, 2004).

Minerals and some bioconstituents

With regard to the minerals content in sweet pepper leaves, data in Table (4) clearly indicate that biofertilizers, mycorrhizae and GB foliar application treatments significantly increased N,P and K concentration in sweet pepper leaves compared with those of control plants in both seasons. Similar results with increased contents of minerals due to application of GB were observed in wheat (Mahmood *et al.*, 2009 and Bhatti *et al.*, 2013). Also, it could be noticed that biofertilizers, mycorrhizae and GB at 10 mmol/L gave the best significant values of total sugars, total free amino acids content in leaves of sweet pepper plants at 65 days after transplanting during the two seasons. In this respect, high content of total sugars and some bioconstituents consider as a direct result for high rates of photosynthesis with great efficiency that was preceded with large photosynthetic area (Table 1) and high content of photosynthetic pigments (Table 2). Also, Liu and Huang (2000) suggested that high carbohydrate availability (e.g., glucose and sucrose) during high temperature stress represents an important physiological trait associated with heat stress tolerance. Moreover, accumulation of amino acids and soluble sugars are necessary to regulate osmotic activities and protect cellular structures from increased temperatures by maintaining the cell water balance, membrane stability (Farooq et al.,2008). On the other hand, PGPR promote plant growth directly by facilitating resource acquisition nitrogen, phosphorus and essential minerals (Ahmed and Kibret, 2014).

Endogenous phytohormones

Endogenous phytohormones in sweet pepper leaves as affected by biofertilizers and GB as foliar application treatments are shown in Table (5). According to these results, all promoters (gibberellins, auxins and cytokinins) were increased by using PGPR, mycorrhizae or (GB), while, abscisic acid was decreased.

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Combination of biofertilizers and GB at 10 mmol/L treatment gave the maximum values in auxins and cytokinins while they gave the highest reduction of abscisic acid in leaves of sweet pepper leaves after 65 days from transplanting during the second season. These data could also be of great influence upon different vegetative and reproductive growth. In addition, increasing cytokinin level on the account of auxin could be in favor of increasing the number of formed branches (Table 1) and improvement of photosynthetic pigments content (Table 2) in sweet pepper plants. Larkindale *et al.* (2005) found that several phytohormones including abscisic acid and ethylene were increased under high temperature stress, while others decreased, such as gibberellins, auxins and cytokinins. Moreover, the abscission of reproductive organs an important effect of heat stress is known to be caused by increased abscisic acid and ethylene levels and reduced levels and transport of auxins (Binder and Patterson, 2009). Furthermore, the maintenance of high levels of cytokinins in the plants during heat stress appears to be important in increasing thermotolerance and providing yield stability in crop system, as it is known that cytokinins have potential to reduce oxidative stress in plants (Hare et al., 1997 and Hsu *et al*., 2010).

Flowering and yield

Data in Table (6) indicate that significant increase in number of flowers, early and total fruits dominantly existed with using PGPR and/ or mycorrhizae treatments during the two assigned seasons. The combination treatments gave the highest values especially biofertilizers, mycorrhizae and GB at 10 mmol/L ranked the first in this respect. Concerning fruit setting and total fruit yield per plant data in Table (6) recorded significant increases of the picked fruits during harvest time dominantly existed with the treatments that sprayed with GB at 10 mmol/L in combination with biofertilizers during 2011 and 2012 seasons. These data is being more evident when related to the control. Warrag and Hall (1984) reported that high temperature induced male sterility and excessive floral abscissions in cowpea. Also, Mohammed and Tarpley (2009) found that high temperature can decrease crop yields by decreasing crop growth duration, suppressing floral bud development and decreasing pollen production and viability. Moreover, the suppression of floral bud and flowering under high temperature was also attributed to a shortage of photosynthetic assimilates supplied to the floral buds (Guinn,1974) and / or inability of floral buds to mobilize carbohydrates under heat stress. The decrease in crop yields as a result of high temperature was due to increased respiration and decreased photosynthesis and membrane stability. While, foliar application with GB increased production of photosynthates and decreased consumption of photosynthates and injury to the membrane, thereby increasing crop productivity under high temperature stress.

Fruit quality

Data presented in Table (7) show that PGPR, (AM) or foliar application with glycine betaine GB increased NPK, crude protein and total carbohydrates concentrations in marketable stage of sweet pepper fruits.

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Here, it could be noticed that biofertilizers, mycorrhizae and GB at 10 Mm/L gave the highest concentration of total carbohydrates in the ripened sweet pepper fruits. Data in Table (8) showed that all treatments increased the amount of vitamin C, total soluble solids and titratable acidity in sweet pepper fruits during the two seasons. Beside, it could be noticed that the highest increase of vitamin C was existed with biofertilizers, mycorrhizae and (GB) at 10 mmol/L. These data are being important from the view of fruit quality since, that could be prolong the shelf time with different applied treatments specially that of biofertilizers, mycorrhizae and (GB) at10Mm/L . These findings are supported by Abou-Aly and Mady (2009) and Iqbal *et al.* (2008).
Finally, this study clearly indicated that plant growth promoting rhizobacteria and mycorrhizae in the presence of glycine betaine make and/ or enable agricultural plants to increase their tolerance and adaptation to high temperature as one of many abiotic stresses. In addition, interactions between biofertilizers and GB under heat stress conditions could affect not only the growth and productivity of economic plants but also the properties of soil. Furthermore, some microbial species and strains could play an important role for understanding how plant can tolerate and adapt to stress through mechanisms those develop in plants under stress conditions. Thereby, selection of certain microorganisms from stressed ecosystems would insert it to the concept of biotechnology application in agriculture managements.

Table (8): Effect of biofertilizers and glycine betaine on quality of pepper fruits under high temperature condition.

Treatment		Vitamin C. mg/100 F.W.		Total soluble Solids (%)		Titratable Acidity (%)	
		S1	S2	S1	S2	S1	S2
NPK-fertilization	GB (Zero)	52.65	53.76	3.02	3.06	0.315	0.314
PGPR		61.52	61.73	3.25	3.24	0.362	0.359
Mycorrhizae (AM)		60.25	60.48	3.22	3.23	0.355	0.347
PGPR + AM		62.34	62.84	3.27	3.28	0.371	0.368
NPK-fertilization	GB (10 mmol/ L)	65.35	63.47	3.26	3.32	0.368	0.376
PGPR		77.50	77.96	3.48	3.46	0.386	0.392
Mycorrhizae (AM)		76.15	76.64	3.43	3.44	0.384	0.387
PGPR + AM		79.14	79.54	3.52	3.50	0.396	0.397
NPK-fertilization	GB (15 mmol/ L)	62.80	61.73	3.35	3.37	0.354	0.351
PGPR		65.17	65.74	3.39	3.46	0.368	0.372
Mycorrhizae (AM)		64.44	64.62	3.36	3.38	0.364	0.369
PGPR + AM		67.61	66.40	3.40	3.42	0.375	0.370
LSD at 5%		3.35	4.47	0.11	0.12	0.02	0.03

PGPR: Mixture of *Azospirillum lipoferum*, *Paenibacillus polymyxa* and *Bacillus circulans*
GB: Glycine betaine

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التأثير المتكامل للجليسين بيتاين و الأسمدة الحيوية علي نمو وإنتاجية نبات الفلفل تحت ظروف الحرارة المرتفعة.

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تعتبر درجة الحرارة واحد من العوامل المحددة لنمو وتطور النباتات . لذلك أجريت تجربتين حقليتين لدراسة تأثير استخدام البكتريا المشجعة للنمو وهى خليط من (*Azospirillum lipoferum, Paenibacillus polymyxa and Bacillus circulans*) والميكوريزا الداخلية (*Glomus mosseae*) و التفاعل بينهم في وجود الرش بالجليسين بيتاين بتركيز (صفر، ١٠ و١٥ مللي مول/لتر) على بعض قياسات النمو المورفولوجية و الأنشطة الميكروبية وصبغات البناء الضوئي والتقديرات الكيميائية والمحتوي الداخلي من الهرمونات النباتية و كذلك التزهير و معدل إنتاج وجودة ثمار الفلفل الحلو خلال الموسم الصيفي لعامي ٢٠١١ و ٢٠١٢ تحت ظروف الحرارة المرتفعة في الحقل المفتوح.

وقد أظهرت النتائج أن البكتريا المشجعة للنمو والميكوريزا الداخلية سواء بمفردها أو مجتمعة في وجود الرش الورقي بالجليسين بيتاين بتركيز (١٠ مللي مول/لتر) أدى إلى تحسين صفات النمو الخضري مثل عدد الأفرع والأوراق للنبات و مساحة الأوراق للنبات الوزن و الجاف للأوراق والمجموع الخضري للنبات، وكذلك صبغات البناء الضوئي وهى كلوروفيل أ ، ب والكاروتينويدات والعناصر المعدنية وبعض المكونات الكيماوية مثل السكريات الكلية والأحماض الامينية الحرة الكلية مقارنة بالنباتات غير المعاملة. أيضا أظهرت النتائج وجود تأثيرات ايجابية معنوية بالنسبة للنشاط الميكروبى في التربة متمثلا في نشاط إنزيم الديهيدروجينيز و كذلك زيادة في النيتروجين و الفوسفور و البوتاسيوم الميسر في منطقة الريزوسفير، وأيضا أدى إلى زيادة نسبة إصابة الميكوريزا للجذور. أظهرت النتائج أيضا أن البكتريا المشجعة للنمو والميكوريزا الداخلية في وجود الرش بالجليسين بيتاين بتركيز (١٠ مللي مول/لتر) أدت إلى زيادة محتوى النباتات من الجبر يللين والأكسين والسيتوكينين بينما أدى إلي تقليل حمض الابسيسيك. كذلك أدى إضافة البكتريا المشجعة للنمو والميكوريزا الداخلية في وجود الرش بالجليسين بيتاين بتركيز (١٠ مللي مول/لتر) إلى تحسين واضح في التزهير وعقد الثمار وتقليل التساقط للأزهار والثمار والتبكير في إنتاج الثمار وكذلك زيادة في الإنتاج الكلى مع تحسن في جودة الثمار.

ولقد أكدت هذه النتائج التأثير الفعال للتلقيح المزدوج من الأسمدة الحيوية مع الميكوريزا الداخلية في وجود الرش الورقي بالجليسين بيتاين بتركيز (١٠ مللي مول/لتر) أدي إلي تحسين نمو و إنتاجية وجودة نباتات الفلفل الحلو تحت ظروف الحرارة المرتفعة.

قام بتحكيم البحث

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Table (1): Effect of biofertilizers and glycine betaine on vegetative growth of pepper under high temperature condition.

Treatment		No. of Branches/ plant		No. of l eaves/ plant		Leaf area / plant (cm^2)		leaves dry weight g/ plant		Total shoots dry weight g/ plant		Leaf area ratio LAR cm^2/g	
		S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
NPK-fertilization	GB (Zero)	17.18	18.22	60.40	67.27	401.18	418.26	5.08	5.23	10.37	10.79	38.69	38.76
PGPR		26.73	28.77	98.45	102.11	575.34	584.50	8.29	8.42	14.20	14.35	40.52	40.73
Mycorrhizae (AM)		23.40	24.80	90.96	92.35	515.85	540.67	6.84	7.10	12.84	13.32	40.18	40.59
PGPR + AM		30.60	29.25	110.50	107.18	669.45	637.70	9.65	9.19	16.01	15.29	41.81	41.71
NPK-fertilization	GB (10 mmol/L)	22.60	24.76	93.45	95.15	620.75	645.15	8.12	8.09	14.56	15.27	42.36	42.25
PGPR		34.25	34.15	134.11	131.24	768.70	760.70	11.15	11.60	17.99	17.80	42.72	42.74
Mycorrhizae (AM)		32.70	32.95	122.90	125.36	718.15	721.46	10.35	10.40	16.89	16.85	42.52	42.81
PGPR + AM		35.83	36.68	136.40	138.15	815.21	841.70	11.75	12.35	18.29	18.83	44.57	44.69
NPK-fertilization	GB (15 mmol/L)	21.74	23.88	88.43	86.19	556.16	568.20	7.28	6.46	13.36	13.59	41.63	41.81
PGPR		32.90	33.17	124.80	126.70	725.75	741.83	10.45	10.69	17.33	17.65	41.76	42.03
Mycorrhizae (AM)		31.85	32.70	118.43	123.70	718.70	723.50	10.36	10.43	17.01	17.17	42.25	42.13
PGPR + AM		34.70	33.45	131.17	130.70	781.40	751.65	11.26	10.83	17.86	17.53	43.75	42.88
LSD at 5%		3.11	3.42	15.25	17.33	102.40	104.73	1.11	1.12	2.04	2.15	1.25	1.32

PGPR: Mixture of *Azospirillum lipoferum*, *Paenibacillus polymyxa* and *Bacillus circulans*
GB: Glycine betaine

Table (2): Effect of biofertilizers and glycine betaine on photosynthetic pigments (mg/g F.W.) of pepper leaves under high temperature condition.

Treatment		Chlorophyll (a)		Chlorophyll (b)		Chlorophyll (a+b)		Carotenoids		Chlorophyll a+b / Carotenoids	
		S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
NPK-fertilization	GB (Zero)	0.324	0.335	0.211	0.217	0.535	0.552	0.286	0.298	1.871	1.852
PGPR		0.526	0.524	0.346	0.336	0.872	0.860	0.368	0.370	2.369	2.324
Mycorrhizae (AM)		0.517	0.520	0.322	0.331	0.839	0.851	0.362	0.366	2.317	2.325
PGPR + AM		0.530	0.534	0.373	0.378	0.903	0.912	0.386	0.389	2.339	2.344
NPK-fertilization	GB (10 mmol/ L)	0.521	0.532	0.350	0.354	0.871	0.882	0.426	0.432	2.045	2.042
PGPR		0.637	0.655	0.484	0.487	1.121	1.142	0.538	0.544	2.084	2.099
Mycorrhizae (AM)		0.617	0.622	0.462	0.472	1.079	1.094	0.518	0.523	2.083	2.092
PGPR + AM		0.688	0.692	0.496	0.498	1.184	1.190	0.562	0.568	2.107	2.095
NPK-fertilization	GB (15 mmol/ L)	0.431	0.426	0.370	0.368	0.801	0.794	0.385	0.396	2.081	2.005
PGPR		0.628	0.636	0.446	0.452	1.074	1.088	0.476	0.480	2.256	2.266
Mycorrhizae (AM)		0.620	0.624	0.431	0.435	1.051	1.059	0.463	0.471	2.269	2.248
PGPR + AM		0.646	0.650	0.457	0.464	1.103	1.114	0.484	0.492	2.279	2.264
LSD at 5%		0.04	0.06	0.02	0.03	0.11	0.13	0.07	0.09	0.12	0.14

PGPR: Mixture of *Azospirillum lipoferum*, *Paenibacillus polymyxa* and *Bacillus circulans*
GB: Glycine betaine

Table (3): Effect of biofertilizers and glycine betaine on microbial activities of pepper rhizosphere under high temperature condition.

Treatment		Available-N ppm		Available-P ppm		Available-K ppm		Dehydrogenase activity (µl DHA/g soil/day)		Mycorrhizal colonization %	
		S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
NPK-fertilization	GB (Zero)	148.60	150.70	61.45	63.70	44.20	47.35	23.70	24.93	10.20	11.15
PGPR		166.20	168.15	98.50	97.44	56.12	57.20	44.21	46.28	19.80	22.45
Mycorrhizae (AM)		165.42	169.74	69.44	71.20	53.75	55.80	37.24	39.14	68.23	66.50
PGPR + AM		171.50	174.15	104.73	108.20	58.42	60.40	45.70	47.66	74.15	76.40
NPK-fertilization	GB (10 mmol/ L)	169.53	172.15	85.50	87.31	60.42	62.70	36.50	38.44	18.46	17.75
PGPR		196.40	191.55	121.50	118.70	80.24	82.70	50.64	52.77	23.50	25.30
Mycorrhizae (AM)		201.77	198.20	101.70	100.20	76.14	77.45	46.70	48.52	72.25	74.50
PGPR + AM		215.70	221.83	134.15	131.70	84.52	87.20	56.12	54.70	84.35	86.45
NPK-fertilization	GB (15 mmol/ L)	167.77	165.34	74.70	76.53	55.17	58.96	32.70	34.40	16.70	15.95
PGPR		173.25	170.15	115.36	113.22	73.25	75.46	46.80	44.76	20.65	22.70
Mycorrhizae (AM)		182.33	185.44	98.60	96.55	69.71	71.54	43.55	42.76	70.55	72.80
PGPR + AM		188.50	192.70	117.18	119.26	78.44	81.70	51.77	50.20	81.20	83.77
LSD at 5%		11.75	12.05	6.77	7.22	3.27	3.38	2.68	2.84	3.26	3.57

PGPR: Mixture of *Azospirillum lipoferum*, *Paenibacillus polymyxa* and *Bacillus circulans*
GB: Glycine betaine

Table (4): Effect of biofertilizers and glycine betaine on some biochemical and nutrients content of pepper leaves under high temperature condition.

Treatment		N mg/g D.W.		P mg/g D.W.		K mg/g D.W.		Total sugars mg/g F.W.		Free amino acids mg/g F.W.	
		S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
NPK-fertilization	GB (Zero)	30.70	30.21	3.12	3.17	40.52	40.56	16.18	18.95	9.15	10.27
PGPR		34.52	35.65	4.52	4.55	41.74	41.79	26.50	27.60	12.60	12.54
Mycorrhizae (AM)		32.44	33.50	4.23	4.22	41.22	41.56	24.70	25.52	12.25	12.47
PGPR + AM		36.17	36.88	4.50	4.54	41.80	41.87	28.85	28.44	12.75	12.86
NPK-fertilization	GB (10 mmol/	33.55	34.48	4.44	4.57	42.82	42.88	32.77	30.50	13.26	13.52
PGPR		38.62	37.50	4.64	4.65	45.64	45.72	35.75	33.46	16.50	16.62
Mycorrhizae (AM)		35.46	34.71	4.45	4.48	43.71	44.50	34.90	31.74	15.35	15.48
PGPR + AM		38.91	38.96	4.66	4.67	45.92	45.98	38.24	36.84	18.57	19.12
NPK-fertilization	GB (15 mmol/	32.35	33.27	4.30	4.39	41.67	41.71	27.15	25.44	12.45	12.47
PGPR		35.91	35.88	4.37	4.39	42.34	42.44	32.65	33.28	14.65	14.75
Mycorrhizae (AM)		34.67	34.84	4.28	4.26	42.25	42.31	30.70	31.45	13.75	13.68
PGPR + AM		36.21	36.52	4.57	4.60	42.70	42.79	34.55	36.84	15.42	15.60
LSD at 5%		1.10	1.12	0.11	0.14	0.24	0.27	1.88	1.94	1.34	1.47

PGPR: Mixture of *Azospirillum lipoferum*, *Paenibacillus polymyxa* and *Bacillus circulans*
GB: Glycine betaine

Table (5):L Effect of biofertilizers and glycine betaine on endogenous phytohormones of pepper shoots under high temperature condition.

Treatment		Gibberellins (GA3)		Auxins (IAA)		Cytokinins		Abscisic acid (ABA)	
		µg/g fresh weight	%(±) control	µg/g fresh weight	%(±) control	µg/g fresh weight	%(±) control	µg/g fresh weight	%(±) control
NPK-fertilization	GB (Zero)	30.70	0.00	19.62	0.00	5.22	0.00	0.842	0.00
PGPR		33.88	+10.35	23.80	+21.31	6.24	+19.54	0.730	-13.30
Mycorrhizae (AM)		33.26	+8.34	22.71	+15.75	6.12	+17.24	0.754	-10.45
PGPR + AM		33.96	+10.62	25.09	+27.88	6.35	+21.64	0.748	-11.17
NPK-fertilization	GB (10 mmol/ L)	32.42	0.00	34.14	0.00	14.07	0.00	0.675	0.00
PGPR		36.70	+13.20	46.44	+36.03	17.73	+43.82	0.531	-21.33
Mycorrhizae (AM)		35.66	+9.99	45.72	+33.92	15.54	+40.69	0.544	-19.41
PGPR + AM		36.86	+13.70	48.60	+42.36	19.25	+85.34	0.523	-22.52
NPK-fertilization	GB (15 mmol/ L)	31.70	0.00	31.65	0.00	11.61	0.00	0.725	0.00
PGPR		34.62	+9.21	40.55	+28.12	13.26	+18.82	0.646	-10.90
Mycorrhizae (AM)		34.55	+8.99	38.17	+20.60	12.84	+11.95	0.657	-9.38
PGPR + AM		34.68	+9.40	41.70	+31.75	14.31	+19.64	0.632	-12.83

PGPR: Mixture of *Azospirillum lipoferum*, *Paenibacillus polymyxa* and *Bacillus circulans*
GB: Glycine betaine

Table (6): Effect of biofertilizers and glycine betaine on yield and yield components of pepper under high temperature condition.

Treatment		No. of flowers/ plant		Total fruits No./plant		Fruit setting (%)		Abscission (%)		Early fruits No./ plant		Total yield kg/plant	
		S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
NPK-fertilization	GB (Zero)	84.25	85.12	29.51	29.85	35.02	35.07	64.98	64.93	15.23	16.14	0.82	0.83
PGPR	GB (Zero)	96.16	95.40	37.57	38.28	39.07	40.12	60.93	59.88	19.73	19.80	1.04	1.06
Mycorrhizae (AM)	GB (Zero)	91.50	93.70	34.80	36.63	38.03	39.10	61.97	60.90	19.40	19.66	0.97	1.02
PGPR + AM	GB (Zero)	101.11	104.83	39.59	41.76	39.15	39.82	60.85	60.18	20.44	20.86	1.10	1.16
NPK-fertilization	GB (10 mmol/ L)	98.34	100.70	41.25	42.83	41.95	42.59	58.05	57.47	20.43	21.80	1.15	1.18
PGPR	GB (10 mmol/ L)	115.80	116.74	50.80	52.29	43.37	44.79	56.63	55.21	26.12	26.38	1.41	1.45
Mycorrhizae (AM)	GB (10 mmol/ L)	113.73	114.45	46.25	48.64	42.43	42.50	57.57	57.50	25.75	25.87	1.29	1.35
PGPR + AM	GB (10 mmol/ L)	122.55	125.40	54.40	56.49	44.39	45.02	55.61	54.98	27.17	28.18	1.51	1.57
NPK-fertilization	GB (15 mmol/ L)	98.60	99.55	40.53	40.80	41.11	40.98	58.89	59.02	19.25	20.07	1.13	1.14
PGPR	GB (15 mmol/ L)	111.43	113.65	46.09	47.55	41.36	41.84	58.64	58.16	28.15	23.73	1.28	1.32
Mycorrhizae (AM)	GB (15 mmol/ L)	107.40	109.77	44.56	45.36	41.49	41.32	58.51	58.68	22.50	22.62	1.24	1.26
PGPR + AM	GB (15 mmol/ L)	114.26	118.94	48.93	50.12	42.82	42.41	57.18	57.59	24.52	24.80	1.36	1.39
LSD at 5%		4.43	5.15	2.53	2.75	2.34	2.85	1.96	1.84	1.12	1.18	0.06	0.08

PGPR: Mixture of Azospirillum lipoferum, Paenibacillus polymyxa and Bacillus circulans
GB: Glycine betaine

Table (7): Effect of biofertilizers and glycine betaine on quality of pepper fruits under high temperature condition.

Treatment		N (%)		P (%)		K (%)		Crude protein (%)		Total Carbohydrates mg/g D.W	
		S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
NPK-fertilization	GB (Zero)	1.11	1.12	0.315	0.318	2.20	2.22	6.94	7.00	526.14	528.46
PGPR		1.25	1.29	0.445	0.450	2.55	2.57	7.81	8.06	564.22	561.73
Mycorrhizae (AM)		1.26	1.27	0.430	0.438	2.51	2.50	7.88	7.94	563.54	568.18
PGPR + AM		1.31	1.33	0.456	0.468	2.65	2.67	8.19	8.31	573.25	572.54
NPK-fertilization	GB (10 mmol/ L)	1.30	1.32	0.450	0.453	2.56	2.58	8.13	8.25	570.60	575.77
PGPR		1.45	1.47	0.574	0.579	2.64	2.72	9.06	9.19	585.75	592.44
Mycorrhizae (AM)		1.43	1.44	0.553	0.561	2.63	2.66	8.94	9.00	580.84	582.50
PGPR + AM		1.48	1.50	0.581	0.588	2.74	2.77	9.25	9.38	597.38	595.86
NPK-fertilization	GB (15 mmol/ L)	1.26	1.28	0.436	0.442	2.53	2.55	7.88	8.00	568.12	532.25
PGPR		1.36	1.38	0.561	0.569	2.60	2.62	8.50	8.63	581.38	583.72
Mycorrhizae (AM)		1.31	1.34	0.547	0.553	2.53	2.58	8.19	8.38	572.42	575.40
PGPR + AM		1.40	1.41	0.572	0.578	2.71	2.76	8.75	8.81	583.55	588.24
LSD at 5%		0.11	0.12	0.06	0.09	0.13	0.14	0.27	0.32	14.55	17.72

PGPR: Mixture of Azospirillum lipoferum, Paenibacillus polymyxa and Bacillus circulans
GB: Glycine betaine

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