

The effect of nitrogen fixation and phosphorus solvent bacteria on growth physiology and vitamin C content of *Capsicum annum* L.

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Abstract

In order to study the effects of biofertilizers on yield quantity and quality of *Capsicum annuum* L., an experiment was conducted in completely randomized block design with three replications in 2011. All plants were treated before transplanting in pots in greenhouse condition by biological fertilizers including nitroxin and biophosphates. Treatments included 3 concentrations of nitroxin (1:3, 1:5 and 1:7) diluted by water, 2 levels of biophosphate fertilizer (inoculated and non- inoculated plants) and NPK (2:6:1.5 g per pot) fertilizer. The control group had no treatment of chemical and biofertilizer. Results revealed that the highest yield (3177.85 g/plant) dry weight and fresh weight (5.43 g and 120 g) respectively, were obtained by nitroxin 1:5 combined with phosphate solubilizing bacteria treatment. This combination of biofertilizers (nitroxin 1:5 and biophosphate) significantly improved leaf phosphorous and nitrogen content, 0.27% and 2.16%, respectively. Calcium content of fruits treated by nitroxin and biophosphate also showed considerable increase (0.33%). Vitamin C content was enhanced in combined treatments of nitroxin 1:5 and 1:3 with biophosphate. Application of low concentrations of nitroxin and biophosphate made no significant difference with control plants. Based on this investigation the best fertilizer concentration and combination for sweet pepper production in greenhouse condition was nitroxin 1:5 together with biophosphate fertilizer.

Keywords: biological fertilizers; Capsicum annum; nitrogen; phosphorus; physiology, vitamin C

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Introduction

Sweet pepper (*Capsicum annum* L.) is a valuable vegetable product of eggplants (Solanaceae) family. Importance and value of this plant lies on its properties such as appetizing and

*Corresponding author *E-mail address*: pjmoradi@iau-saveh.ac.ir Received: October, 2012 Accepted: December, 2012 digestive characteristic, carotene content and specially vitamin C. Sweet pepper with significant vitamin C and minerals is a highly nutritious and healthy vegetable. It alsocontains high amount of antioxidant vitamins A, C, and E. Many researchers are studying on anticancer antioxidants and pepper is considered as one of fourteen treatment sources for cancer (Bosland and Votava, 1999).

In recent years, efforts to increase the product in unit area and population growth have resulted in a high and unbalanced use of chemical fertilizers which in turn has led to natural environment pollutions especially of water, soil, and human nutrition sources. The consequence is threats to human health. Expansive efforts have started in order to find suitable ways to improve soil and agricultural products quality. Using biofertilizers in sustainable agricultural systems especially in poor soils with low nutrients are particularly important in improving soil quality increasing the yield (Sharma, 2004).

Bio-fertilizer is mainly defined as a production which includes one or several terricolous organisms and stimulates host plant growth through increasing nutrition and/or providing the plant with main nutrients, when applied with seed or in soil (Vessey, 2003).

Nitroxin bio-fertilizer includes a set of most effective nitrogen stabilizing strains of Azospirillum and Azotobacter genus. Owing to the importance of nitrogen for plants, application of Nitroxin is recommended to increase the yield in unit area, promote the quality of products and provide for the needs of crop such as cereals, industrial and oily plants, vegetables, greenhouse productions, fruit trees, green spaces, flower and ornamental plants in different climatic conditions and in all regions plants.

Phosphate solubilizing bacteria belong to Bacillus, Pseudomonas genus. Phosphorus is an essential and highly needed element for plants ranking the most vital element for plant growth after nitrogen. Phosphorus plays several key roles in plant as it participates in energy transfer interactions and photosynthesis, converts sugar into starch, and transfers genetic properties in plants. It also helps the plant to produce deeper and more intense roots. Moreover, phosphorous is reported to hasten the plant growth, decrease seed humidity and improve product quality (Vessey, 2003).

Application of bio-fertilizers such as Azotobacter, Azospirillum, phosphate solubilizing bacteria, nitrogen fixation bacteria and their mixture in *Ocimum Sanctum* and *Withania Somniferum* led to improved germination indexes like percent and speed of germination, seed vigor index and also length of rootlets and stems (Krishna et al., 2008). An experiment performed on Brassica species showed that the oil yield decreased with successive increase in nitrogen level and application of Azotobacter (Sharma and Singh, 1997). The same study also showed that protein and oil production increased significantly when nitrogen was applied through Azotobacter (Sharma and Singh, 1997)

Azotobacter promotes plant growth, percent of seeds germination, rooting and development of root through synthesis of growth stimulus hormones such as indole acetic acid, gibberellins and cytokinins (Vessey, 2003). Interaction between mycorrhiza Glomus mosseae and Azotobacter, chroocooum and Azospirillum brasilense was studied in Azadirachat indicia and results showed that combined inoculation with mycorrhiza, and Azotobacter Azospirillum increased biomass and the absorption of plant nitrogen and phosphorus (Sumana and Bagyaraj, 2002). In another study, wheat physiological response to Azospirillum and Azotobacter was investigated and the results showed that application of 50 mg/L zinc with Azospirillum and Azotobacter led to absorption of nitrogen, magnesium, manganese, carbohydrate and total soluble protein in stem (Ebrohim and Al, 2004).

The most important plant growth stimulus bacteria are Azotobacter, Azospirillum and pseudomonas which in addition to biologically stabilizing nitrogen and solubilizing soil phosphate, affect the yield performance of the plants through production of a significant amount of growth stimulus hormones especially auxins, gibberellins and cytokinins (Sumana and Bagyaraj, 2002).

Wheat seedlings inoculated with Azospirillum bacteria had better growth than non-inoculated ones and inoculation increased absorption. Moreover, inoculation of plants with growth stimulating bacteria resulted in increased root surface (Subba Rao et al., 1979). The effect of fluorescent pseudomonas was also studied on exuding materials of tomato such as glucose, fructose and sucrose (Mozafar and Oertil, 1992). The inoculation of sugar beet with fluorescent pseudomonas increased wet and dry weight of plant root and shoot 20% to 25% in greenhouse condition (Suslow et al., 1982). The same researchers in experiments carried out on the a farm in three consecutive years reported 21% to 77% increase in sugar beet bushes' weight and also an increase in root weight from 1.6 to 8.6 tons per hectare in comparison with control (Suslow and Schroth, 1982).

The use of biologic fertilizers has been studied on many agricultural plants and outstanding have been achieved. However, few research studies have been done on vegetables. Therefore, an experiment was carried out on the effect of bio-fertilizers on quality and quantity of sweet pepper properties. An overview of the findings of research studies suggests that application of bio-fertilizers results in favorable effects on quantity and quality of different agricultural products. In this line, the present study was carried out to investigate the effect of biofertilizers including nitroxin, phosphate solubilizing bacteria and their interaction on quantity and quality of sweet pepper yields under greenhouse conditions

Material and Methods

This study was conducted in the Research Greenhouse of the Faculty of Agriculture in 2011 - 2012. The experimental design was arranged in complete random blocks with 3 replicates and 9 treatments in pot. Treatment included control (without any fertilizer), nitroxin bio-fertilizer at1:3, 1:5 and 1:7 concentrations, phosphate solubilizing bacteria (presence or absence of bacteria), combination of nitroxin and phosphate solubilizing bacteria and the chemical fertilizer NPK (2:6:1.5 g per pot). Biofertilizers included Azotobacter and Azospirillum (nitroxin) and Bacillus pseudomonas and (phosphate solubilizing bacteria). The greenhouse soil nutrients, pH and EC were measured prior to the experiment. The plastic pots selected for the experiment were 22 cm in diameter, 30cm in height and approximate mass of 14 kg soil. Forty pots were placed at 30 cm from one another in each replicate. Based on the recommendations of producers, bio-fertilizers were inoculated through transplantation method. Seedlings were placed in bio- fertilizers for 5-10 minutes before they were transferred to soil bed pots. In the subsequent

stages the plants were provided with sufficient amount of chemical fertilizers while the control plants did not receive any chemical or biofertilizers.

The percent of root wet and dry weight were obtained at 60 °C when these parameters were constant. Harvested fruits were sliced and their wet weight was measured using a digital scale with ± 0.001 g measurement error. Fruit slices were then oven dried at 70 °C for 48 hours and the percent of fruit dry matter was obtained using the difference between dry and wet weight.

In order to measure fruit calcium and leaf phosphorus contents, 1 g dry fruit and leaf were put in stove and digested with acid HCl 2 N to get 1 liter of solution. Calcium content was recorded using an atomic absorption apparatus (WFX 130B). Phosphorus was measured through colorimetry method using a UV spectrophotometer (T 80).

Also to measure nitrogen content, 0.3 g plant leaves were weighed and digested in hydrogen peroxide and measured using Kejeldal apparatus.

To measure Vitamin C content, 3-4 ripe fruits were selected from each plant. They were then chopped and homogenized with a mixer. Vitamin C content was measured through titrimetry in the presence of starch (mg per 100 g fresh matter).

Finally, total number of fruits per plant was recorded and weighed using a digital scale with ± 0.001 g measurement error. SPSS and Excel software were used for statistical analysis of data and drawing the graphs. Means were compared using Duncan's multiple range test (P ≤ 0.01 and P ≤ 0.05).

Results

Plant performance

Analysis of variance of the obtained data (Table 1) shows that treatments of nitroxin and phosphate the solubilizing bacteria had meaningful effects on fruit performance (P≤0.01). Also the interaction of these treatments was meaningful (P≤0.01). Maximum mean fruit vield recorded in combined was nitroxin and solubilizing phosphate bacteria treatment measuring 3177.85 g per plant and there was no meaningful difference between nitroxin treatment 1:5 without application of phosphate solubilizing bacteria and nitroxin 1:3 with bacteria. Lower concentrations of nitroxin 1:7 with no application of phosphate solubilizing bacteria did not show a meaningful difference compared with chemical fertilizer treatments. Minimum fruit yields per plant were observed in control plants (587.58 g) and also in the treatment including nitroxin 1:7 and phosphate solubilizing bacteria (782.19 g). In this study it was observed that nitroxin and phosphate solubilizing bacteria treatments had meaningful effect on fruit yield at P≤0.01. Also the interaction of these treatments was meaningful at P≤0.01. Furthermore, the treatments of nitroxin 1:5 and phosphate solubilizing bacteria significantly increased the fruit yield compared with other treatments. It is noteworthy that at low concentrations of nitroxin such as combination of nitroxin 1:7 and phosphate solubilizing bacteria, plant performance was reduced even compared with than the chemical fertilizer treatment. This suggests that for each particular plant nitroxin can affect performance factors at specific concentrations. Also biologic fertilizers alone cannot improve the performance of pepper; therefore, sweet obtaining appropriate proportion of biologic fertilizers is one of the main requirements in horticulture and agricultural production. Findings of this study suggest that the treatment including а combination of nitroxin 1:5 and phosphate solubilizing bacteria is more suitable to improve the yield.



Fig. I. Effects of different compounds of biological fertilizers and chemical treatments on plant yield



Fig. II. Effect of different compounds of biological fertilizers and chemical treatments on fruit fresh weight

Table 1

Effect of different compounds of biological fertilizers and chemical treatments on fruit performance

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The average of squares				
Property	nitroxin	Bacteria	Interaction of nitroxin* bacteria	
Root wet weight	174.1**	42.7*	25.7*	
Root dry weight	54.6**	1.9 ^{ns}	4.5 ^{ns}	
Fruit performance	255726.8**	35.1**	115064.1**	
Vitamin C	1234.6**	758.2 ^{ns}	534.3*	
Fruit wet weight	351.7**	290.2*	21.7**	
Fruit dry weight	54.6**	7.7**	0.03 ^{ns}	
Leaf nitrogen	0.41**	0.00 ^{ns}	0.05 ^{ns}	
Calcium	0.06**	0.01*	0.01 ^{ns}	
Phosphor	0.21*	0.00 ^{ns}	0.00 ^{ns}	

*: significant at the 1% probability level; **: significant at the 5% probability level; ns: non significant



Fig. III. Effect of different compounds of biological fertilizers and chemical treatments on fruit dry weight

Fruit wet and dry weight

Analysis of variance (Table 1) shows that fruit wet weight was meaningfully changed under nitroxin treatments (P≤0.01) and phosphate solubilizing bacteria (P≤0.05). Also, the interaction of these treatments had meaningful effect on these parameters ($P \le 0.01$). As Fig. (II) suggests, nitroxin 1:5 without phosphate solubilizing bacteria (115.86 g) and also nitroxin 1:5 with phosphate solubilizing bacteria (124.61 g) had the highest effect on fruit wet weight showing no meaningful difference. Also nitroxin treatments 1:5 and 1:3 with or without phosphate solubilizing bacteria were not meaningfully different in comparison with the treatment involving phosphate solubilizing bacteria alone. The lowest fruit wet weight was observed in control plants (49.17 g) and the combined treatment of low concentration of nitroxin 1:7 and phosphate solubilizing bacteria (57.43).

Based on variance analysis of the data (Table 1), under nitroxin treatments and phosphate solubilizing bacteria (P≤0.01) fruits dry weight was affected meaningfully but the interaction of these treatments was not meaningful. Nitroxin treatment 1:5 and phosphate solubilizing bacteria (5.43 g) resulted in the highest fruit dry weight which was meaningfully different from all other treatments (Fig. III). While nitroxin treatments 1:5 without bacteria, 1:3 with bacteria and 1:7 with bacteria increased fruit dry weight, the difference



Fig. IV. Effect of different compounds of biological fertilizers and chemical treatments on vitamin C

between these treatments was not meaningful. Also no meaningful difference was observed between the treatment involving no application of phosphate solubilizing bacteria and the treatment of chemical fertilizer alone. The lowest fruit dry weight (1.9 g) was recorded in the control plants without application of biofertilizers and chemical fertilizers.

Root wet and dry weight

Root wet weight was significantly different under nitroxin treatments (P≤0.01), treatments of phosphate solubilizing bacteria (P≤0.05) and the interaction of effects of these treatments at P≤0.01 (Table 1). As Fig. (VIII) shows, combined treatment of nitroxin 1:5 with phosphate solubilizing bacteria produced the highest root wet weight (30 g). Nitroxin treatments at higher concentrations with or without bacteria, phosphate solubilizing bacteria treatment alone and the application of chemical fertilizers resulted in the development of root and increased its wet weight while there was no significant difference between the effects of these treatments on root wet weight. The application of nitroxin with 1:7 concentration with or without phosphate solubilizing bacteria did not have a meaningful effect on the wet weight of sweet pepper root. Minimum observed wet weight of root was 13.54 g in control plants without biologic and chemical fertilizers.

Root dry weight was significantly different under nitroxin treatment at P≤0.01 but





phosphate solubilizing bacteria and its interaction with nitroxin did not result in meaningful difference in this parameter (Table 1). On the other hand, combined nitroxin treatment with 1:5 concentration and phosphate solubilizing bacteria had the highest root dry weight (12.53 g) (Fig. IX). No significant difference in root dry weight was observed between biological fertilizer treatment of nitroxin 1:5 without phosphate solubilizing bacteria and nitroxin 1:3 with phosphate solubilizing bacteria. Minimum root dry weight was observed in nitroxin 1:7 with phosphate solubilizing bacteria (4.54 g).

Vitamin C content

Nitroxin treatments had meaningful effect on vitamin C content of fruits (P≤0.01) but the treatment of phosphate solubilizing bacteria was not meaningful (Table 1). The interaction of these treatments had meaningful effect at $p \le 0.05$. Nitroxin 1:3 combined with phosphate solubilizing bacteria resulted in maximum vitamin C content ,i.e., 148 mg in 100 g wet weight of fruit (Fig. IV). Minimum vitamin C content was recorded in control treatment (90.24 mg in 100 g wet weight of fruit) without using biologic and chemical fertilizers. Lower concentrations of nitroxin combined with phosphate solubilizing bacteria do not meaningfully increase fruit vitamin C content; these treatments produced higher vitamin C in fruits compared with the



Fig.VI. Effect of different compounds of biological fertilizers and chemical treatments on leaf phosphor

treatment. In fact, combination of nitroxin fertilizer 1:3 and phosphate solubilizing bacteria had better effects on fruit vitamin C content; likewise, nitroxin concentration of 1:5 and phosphate solubilizing bacteria and also treatment of nitroxin 1:5 without phosphate solubilizing bacteria increased vitamin C in fruits. These findings emphasize the importance of appropriate combination both of biologic fertilizers to increase this important factor in sweet pepper. In sum, combinations of nitroxin 1:3 and phosphate solubilizing bacteria and also nitroxin 1:5 and phosphate solubilizing bacteria are found to increase vitamin C contents in sweet pepper.

Leaf nitrogen

While nitrogen content of leaves showed meaningful difference under nitroxin treatments (P≤0.01), phosphate solubilizing bacteria treatments and interaction of these the treatments did not result in meaningful difference in leaf nitrogen (Table 1). AS Fig. (V) suggests, higher mean leaf nitrogen was observed under nitroxin 1:5 concentration and phosphate solubilizing bacteria (2.16%). On the other hand, nitroxin 1:5 without phosphate solubilizing bacteria and nitroxin 1:3 with phosphate solubilizing bacteria did not cause a meaningful difference in leaf nitrogen content. Also the absorbed nitrogen was high in the plants treated with a combination of nitroxin 1:5 and

phosphate solubilizing bacteria. Low concentrations of nitroxin (1:7) with and without phosphate solubilizing bacteria were not meaningfully different and also phosphate solubilizing bacteria treatment alone was not meaningfully different from chemical fertilizer treatment. Finally, minimum leaf nitrogen (1.2%) was recorded in control plants without chemical and biological fertilizers.

Leaf phosphorus

Nitroxin meaningfully (P≤0.05) affected leaf phosphorous content in the treated plants (Table1). However, phosphate solubilizing bacteria and their interaction with nitroxin did not make meaningful difference in leaf phosphorous content. Generally, biofertilizers meaningfully increased leaf phosphorus more than chemical fertilizer treatment and control (Fig.VI). Furthermore, combined treatment of nitroxin 1:5 and phosphate solubilizing bacteria had a better effect and increased leaf phosphorus content compared with the other treatments of the study.

Fruit calcium

The effect of nitroxin treatments on the fruit calcium content was meaningful at P≤0.01 (Table 1). Also, phosphate solubilizing bacteria had a meaningful effect on this parameter at P≤0.05. However, the interaction of these treatments did not result in a meaningful difference on the calcium content of fruits. Maximum fruit calcium (33%) was observed in combined treatment of nitroxin 1:5 and solubilizing phosphate bacteria (Fig. VII). Minimum fruit calcium on the other hand was obtained in control plants without chemical and biofertilizers (0.01%). Finally, no meaningful difference was observed between chemical treatment alone, biofertilizer treatment alone and also nitroxin alone.

Discussion

In this study it was found that the combined treatment of nitroxin 1:5 and phosphate solubilizing bacteria meaningfully increased the fruit performance of sweet pepper



Fig. VII. Effect of different compounds of biological fertilizers and chemical treatments on fruits calcium



Fig. VIII. Effect of different chemical and bio-fertilizers on root wet weight



Fig. IX. Effect of different chemical and bio-fertilizers on root dry weight

plants as compared with other treatments. In low concentrations of nitroxin, e.g., the combined treatment of nitroxin 1:7 and phosphate solubilizing bacteria, plant performance was decreased even compared with chemical fertilizer treatment. This suggests that in each plant, specific concentration of nitroxin may improve performance factors. Also biologic fertilizers alone cannot improve the performance of sweet and therefore, finding pepper, suitable combination of biologic fertilizers is a main pursuit in horticulture in an attempt to increase yields. Base on the findings of the present study, the combined treatment of nitroxin 1:5 and phosphate solubilizing bacteria are more suitable to increase the performance in sweet pepper plants. A study on the effect of plant growth promoting rhizobacteriacontaining Azospirillum and Azotobacter on sunflower found that the application of growth promoting bacteria increased the performance of the seeds, the amount of seed oil and protein content (Shehata EL-Khawas. 2003). Increase in and the performance of tomato after inoculation with bacteria was also reported between 5.6% - 9.6% in autumn harvest (Gagne et al., 1993). This same research study which involved two experiments during spring and autumn planting showed that the performance in spring harvest was better than that of autumn (13.3% -18%). Also the percent of non-saleable fruits decreased in both harvest from 23% (control) to 12% in inoculated plants with bacteria (Gagne et al., 1993). Herman et al. (2008) reported that inoculation with rhizosphere bacteria improves growth and performance of rice and at the same time protects the crop against pathogens and pests. The researchers argue that the improved growth and performance of rice in the presence of these bacteria is probably through induced production of IAA (Herman et al., 2008).

According to the obtained results, low concentrations of nitroxin with bacteria and also biologic fertilizers alone did not increase fruit vitamin C contents; however, more vitamin C was obtained compared with the control plants without biologic and chemical fertilizers. This emphasizes the importance of finding suitable combination of both biologic fertilizers to improve this important parameter in sweet pepper. Combinations of nitroxin 1:3 and phosphate solubilizing bacteria and also nitroxin 1:5 and phosphate solubilizing bacteria are suggested to increase vitamin C contents in sweet pepper. A study on chili found that increase in nitrogen led to a partial increase in vitamin C content of fruit (Panchal et al., 2008). Even though no particular trend was observed in vitamin C content through increase in nitrogen levels, vitamin C level in fruits treated with maximum amount of nitrogen was higher in comparison with control plants. The probable reason was attributed to the increase in acids metabolism in the plants. Vitamin C in dry weight decreased with the increase in fruits' maturity but based on the wet weight, vitamin C increased with an increase in fruit maturity (Panchal et al., 2008). The studies on celery (Migahed et al., 2004) and artichoke (Hassan et al., 2006) showed that the maximum amounts of nitrogen in plant tissues were obtained from Azotobacter and Azospirillum inoculation. The effects of growth promoting bacteria and inoculation with Azotobacter were also reported on basil and tomato biomass (Gul et al., 1996).

Although leaf phosphorus contents in biofertilizer treatment with phosphate solubilizing bacteria were expected to be more than other treatments, the combined treatment of nitroxin with phosphate solubilizing bacteria had a better effect on this parameter. Phosphorous content in leaves was measured in the present study as phosphate releasing biofertilizers were applied in the treatments to obtain an estimate of phosphorus absorption in the plant under study. Phosphorus is an important factor in growth and development of roots as well as flowering and fruits. Application biofertilizers increased absorption of of phosphorous more than treatments with chemical fertilizers. The supply of calcium is also an important factor in the fruit tissue which increases its strength and durability. Based on the obtained results, application of combined biofertilizers including nitroxin 1:5 and phosphate solubilizing bacteria was more effective in increasing calcium contents of sweet peppers. In fact, application of each biofertilizer alone had lower effect on the calcium content of fruit tissue. There are research studies on tomato and spearmint under greenhouse condition showing that the performance and absorption of nutrients significantly improved in the plants inoculated with mycorrhiza and Azotobacter (Al-karaki and Hammad, 2001; Gupta et al., 2002).

Conclusion

This research study suggested that nitroxin at 1:5 concentration combined with phosphate solubilizing bacteria resulted in the highest performance in sweet pepper. Measurement of nitrogen and phosphorus contents in shoots of sweet pepper reveals that nitroxin speeds up the shoot growth through providing sufficient nitrogen for the plant. Azotobacter and Azospirillum can be effective in solubilizing inorganic and insoluble phosphates through production and secretion of organic acids such as Malic, Succinic, Propionic, Lactic, Citric, Ketoglonice acids. Also many of these bacteria can trigger releasing phosphorous from organic phosphorous compounds through production of phosphatase enzyme and therefore play an important role in keeping the balance in plant hormones and cell division. Consequently, the plant growth increases and the phosphorus produced from bacterial activities improves florescence and increases fruit formation. This can explain the improvement in fruit wet and dry weight in the plants treated in the present study. The treatment, on the other hand, caused an increase in vitamin C content which is considered as an important index of quality in sweet pepper. Phosphorus as the main component of sugar phosphates and nitrogen as a necessary element, play an important role in production of enzymes effective in biosynthesis of acidic sugars particularly ascorbic acid and their provision through the treatments of this study increased vitamin C contents in the plants under study. Likewise, the key role of nitrogen is also prominent in the study. Regarding overuse of chemical fertilizers and their negative and destructive side effects on human health which is а consequent of application of high concentrations of chemical fertilizers and their concomitant pollution, alternative methods of fertilizing should be used to improve the quality and quantity of crops. In organic agricultural system a solution is application of biofertilizers

which can complement chemical fertilizers and, as a result, reduce application of chemical fertilizers. Based on the findings of the present study, using specific combination of chemical and biofertilizers is suggested for production of sweet pepper during transferring the seedlings. Also, it is necessary to carry out further studies to investigate the effects of various combinations of fertilizers at other stages of plant growth in an attempt to reduce application of chemical fertilizers.

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