

Effect of the Organic Fertilization by Using Home Tea Residues and the Bio Fertilization with *Aspergillus Niger* Fungus on the Growth and Yield of Chili Pepper (*Capsicum Annuam*)

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Abstract

An experiment was conducted in the greenhouse belong to the Department of Biology - college of Education for Pure Sciences - University of Diyala to study the impacts of home tea residues as (dry tea residues and tea residues extract), *Aspergillus niger* fungus and their synergistic effect on the growth and yield of chili pepper (*Capsicum annuum*). Results showed significant increments in the plant height, fresh and dry weights of shoot and fruits, number of leaves and number of fruits per plant as a result of watering the plants with tea residues extract. On the other hand, the results also showed that the bio fertilization with *Aspergillus niger* fungus caused significant increments in all the studied characteristics of the pepper plant exception the total chlorophyll content of leaves and the number of flowers per plant, the results indicate to noticeable increments in these two characteristics, but they were not significant. As for the synergistic effect between home tea residues (dry tea residues and tea residues extract) and *Aspergillus niger* fungus, the results showed significant increments in the plant height, fresh and dry weights of shoot and fruits and the combination consist of (home tea residues extract + *Aspergillus niger* fungus) recorded the highest averages of (44.573 cm) for the plant height, (30.16 g) for shoot fresh weight, (6.153 g) for shoot dry weights and (18.236 g) for fresh weight of fruits , while the control treatment recorded the lowest averages of (30.017 cm, 20.866 g, 2.38 g, 10.596 g) for the mentioned characteristics respectively.

Keywords: *Aspergillus niger*, *Capsicum annuum*, Tea residues, Bio fertilizer.

1. Introduction

The chili pepper or *Capsicum annuum* comes back to the Solanaceae family. It is regarded as one of the most important vegetable crop after tomato and cucumber (Hassan, 2001). Chili pepper is cultivated in different parts of Iraq. Its fruit is either red or green in color with a very hot taste due to its highly content of Capsaicin alkaloid (Kachoosangi et al., 2008). The pepper fruit is utilized for food industry in addition to the use of it as popular recipes for the treatment of many diseases, as alkaloids are important substances in the pharmaceutical and therapeutic industry.

At the beginning of the human era of agriculture, most of the farmer dependence in crops fertilization was on organic fertilizers, but when the need to increase the agricultural product appeared as a result of the imbalance between the steady increase in population on the one hand and the low productivity of the land for crops on the other hand (USDA,2006 ; Ryan, 2008; Lucani, 2012) humans began using different kinds of chemical fertilizers and pesticides. These additives would consequently play a major role in raising the productive capacity of the land. However, the excessive use of these chemicals caused many of bad effects on the environment and biodiversity as well as its impact on the quality of the product , human health, productivity of land in addition to the low economic return of the unit area due to high prices of these chemicals(Sarhance et al., 2011).

Organic fertilizer is defined as a natural substances result from plants and animals waste which supply the plant with nutrients naturally (Spain et al., 1983). Several studies have indicated that the use of organic fertilizers reduces the environmental pollution and helps to enhance the soil chemical and physical properties due to its content of nutrients as claws (Li et al., 2017). Moreover, Its decomposition produces many organic acids with an acidic act which may work to reduce soil pH and thus improving the physical and chemical characteristics of plants (Canellas and Olivares,2014). Home tea residues are one of the ways that used in organic fertilization due to its contain of many important compounds such as; proteins, carbohydrates, amino acids, lipids, vitamins, minerals, alkaloids and polyphenols (Liang et al., 2003).

Biofertilizer is one of the alternative methods of using chemical fertilizers which it is effective, specialized, cheap and no harmful to the environment (Pandey et al., 2013). *Aspergillus niger* fungus one of this biofertilizers have the ability of increasing the readiness of many nutrients present in the soil in a form that is not ready to be absorbed by plants such as, phosphorus, zinc and potassium (Lopes-Assad et al., 2010; Moreno Quevedo et al., 2015). As well as *A. niger* have the ability to produce some plant growth hormones such as

indole acetic acid (IAA) (Usha and Padmavathi, 2013) . Therefore, the current study aims to: Investigate the effects of the fertilizing with dry tea residues, the watering with tea residues extract and the addition of *Aspergillus niger* fungus on the growth and yield of chili pepper , as well as study the synergistic effects between them on the growth and yield of the chili pepper.

2.Materials and Methods

Isolation and diagnosis of *Aspergillus niger* fungus : Pure *Aspergillus niger* isolates were obtained by exposing a petri dish containing the PDA medium to air for several minutes. After that, the petri dish was placed in the incubator at 25 ± 2 ° C for three days (Collee et al., 1996). The fungus was then isolated and examined under a microscope to confirm the diagnosis based of cultural and morphological characters as described by Kwon and Bennett (1992) , as shown in (Fig..1). After the confirmation of diagnosis, several dishes of *A. niger* fungus were prepared for use in the experiment.

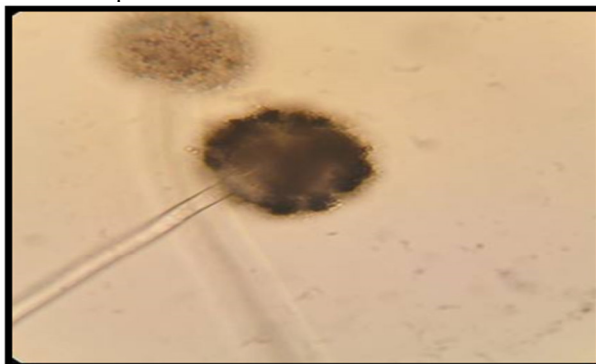


Figure .1: *Aspergillus niger* fungus at 40 X magnification

Execute the experiment : Plastic pots were filled with two kilos of sandy - loam soil then *Aspergillus niger* fungus was add to the soil (quarter of a petri dish for each pot) by mixed fungus mycelium with the soil surface a week before sowing. As for the organic fertilization with home tea residues , tea residues were added to the soil in two ways, the first one was by mixing the dry tea residues with soil at 10 g tea / kg soil, while the second one was by watering the plant with a hot water extract of residues tea. Harborne (1984) method was used to prepare the extract by taking 20 g of the dry tea residues in a conical flask and the distilled water was then added until the volume up to 200 ml. After the boiling of solution for 20 minutes, the conical flask was then placed in a horizontal shaker with medium speed for half an hour. The solution was left to settle for an hour, then filtered with three layers of gauze cloth to separate the residual and take the leachate which was used later in watering according to the need of the plant.

Chili Pepper seedlings at the age of one week were planted in the pots (2 seedlings per pot). The experiment was carried out by using Randomized Complete design. The design included (6) treatments and each treatment was replicated three times. Total number of treatments was 18, as shown in Table (1).

Table (1) Experimental treatments design .

T1	Control
T2	Fertilization by using dry tea residues
T3	Fertilization by using <i>Aspergillus niger</i>
T4	Fertilization by using dry tea residues+Fertilization by using <i>Aspergillus niger</i>
T5	Watering by using tea residues extract
T6	Watering by using tea residues extract+Fertilization by using <i>Aspergillus niger</i>

Measurement of some growth and yield parameters of chili plant : At maturity, shoot growth and yield traits of chili pepper were measured, such as: The plant height, which was measured by a ruler from the soil level (base of the plant) up to the top of the plant. Both of the fresh and dry weights of shoot and fruits were measured by using the sensitive balance, The number of leaves, number of floral branches, number of flowers and number of fruits were counted in addition to the total chlorophyll which was estimated in leaves by using (SPAD) meter .

Statistical analysis : Data were assessed using the Statistical Package for Social Science (SPSS Statistics, version 18) to determine the least significant difference between the treatments using the LSD test at a significant level of 0.05 (Steel and Tourie, 1960).

3.Results

3.1 shoot growth parameters

The results in Table (2) showed the effect of the organic fertilization by using home tea residues (tea dry residues and tea residues extract) and the bio fertilization with *Aspergillus niger* fungus, in addition to them synergistic effects on some parameters of shoot growth for chili pepper that growing under greenhouse conditions.

Plant height (cm) : The results in Table (2) pointed to a significant increment in pepper height due to the addition of home tea residues weather the addition was in the form of dry residues or extract. The highest average of this characteristic (42.716 cm) was obtained as a result of the watering the plant with tea residues extract with increments of (17. 245 and 42.306 %) compared to dry tea residues treatment and control treatment respectively.

Moreover, the result showed that the addition of *Aspergillus niger* fungus caused increment in plant height and recorded the heights average of (38.833 cm) with an increment of (29.37%) compared to control treatment which recorded the lowest average of (30.017 cm). However, these increment was not significant. Significant increment in plant height has been obtained as a result to the synergistic effect of home tea residues and *Aspergillus niger* fungus and the results showed that the combination consist of (home tea residues extract + *Aspergillus niger* fungus) gave the highest average of (44.573 cm) with increments of (48.492 and 13.610 %) compared to the combination consist of (dry home tea residues + *Aspergillus niger* fungus) and control treatment, respectively. While the lowest average of (30.017 cm) has been obtained from the control treatment, as shown in (Table . 2).

Shoot fresh weight (g) : The results indicated in Table (2) shows that the addition of home tea residues to the soil resulted in a significant increment in the shoot fresh weight. The highest average was due to the watering the plant with tea residues extract which reached (29.203 g) with increments of (16.101 and 39.954%) compared with other averages (25.153 g) and (20.866 g) that obtained from soil treatment with tea dry residues and control treatments respectively.

Table (2) Effect of home tea residues (dry tea residues and extract), *Aspergillus niger* fungus and them synergistic effect on the shoot growth parameters of chili pepper plant grown under greenhouse conditions .

Treatments	Plant height (cm)	shoot fresh weight (gm)	shoot dry weight (gm)	number of leaves (plant)	number of branches (plant)	total chlorophyll (mg/g)	
T1	30.017	20.866	2.38	12	7	51.43	
T2	38.833	27.196	4.593	14.67	9.33	62.730	
T3	36.433	25.153	4.836	13.67	10.33	61.570	
T4	39.233	29.086	5.180	15.33	12.67	69.486	
T5	42.716	29.203	5.294	12.67	9	66.863	
T6	44.573	30.16	6.153	15.67	11.67	69.853	
LSD at p ≤ 0.05	Tea	2.384	1.598	0.336	0.691	2.254	N.S.
	Fungus	2.919	1.957	0.411	0.846	N.S.	N.S.
	Interaction	4.129	2.768	0.582	N.S.	N.S.	N.S.

N.S. = Not Significant

As shown in Table (2) a significant difference in the shoot fresh weight was obtained as a result of the addition of *Aspergillus niger* to the soil. The addition of fungus recorded the highest average of (27.196 g) with an increment of (30.336%) as compared to control treatment. The results of the statistical analysis showed significant differences obtained from the synergistic effect of home tea residues and *Aspergillus niger* fungus. The highest average was obtained as a result of the combination consist of (tea residues extract + *Aspergillus niger* fungus), with increments of (3.692 and 44.541%) as compared to the combination of (dry tea residues + *Aspergillus niger* fungus) and control treatment respectively .

Shoot dry weight (g) : A significant increment was obtained in the dry weight of shoot as a result of treatment the plant with home tea residues (dry tea residues and extract). Watering the plant with tea residues extract gave the highest average of (5.294 g) with increments of (15.262 and 122.394%) as compared to treatment the plant with dry tea residues and control treatment respectively, as shown in (Table . 2). On the other hand, the treatment of chili pepper with *Aspergillus niger* fungus, caused insignificant differences in the shoot dry weight. Table (2) shows that treatment the plant with *Aspergillus niger* fungus gave the highest average of (4.836 g) with an increment of (103.193%) compared to control treatment which recorded the lowest average of (2.38 g).

Moreover, The results of this experiment that depicted in Table (2) refers to significant differences in shoot dry weight of chili pepper due the synergistic effects between home tea residues (dry tea residues and extract) and *Aspergillus niger* fungus. It is clear from the same Figure that the highest average of (6.153 g) in shoot dry weight was from the combination consist of (tea residues extract + *Aspergillus niger* fungus) with increments of (18.783 and 158.529 %) as compared with the combination consist of (tea dry residues + *Aspergillus niger*

fungus) and control treatment respectively.

Number of leaves per plant : The results of the statistical analysis that shown in Table (2) indicate that there are significant differences in the number of leaves per plant of chili pepper as a result of the organic fertilization using the residues of home tea (dry residue and extract). Moreover, the treatment of chili pepper with dry tea residues recorded the highest average of (13.67 leaves) with increments of (7.892 and 13.916%) compared to the treatment of plant with tea residues extract and control treatment respectively.

As shown in the same Table, a significant difference was found in the number of leaves per plant from the treatment of chili pepper with *Aspergillus niger* fungus which recorded an average of (14.67 leaves) with an increment of (22.25%) compared to control treatment. However, insignificant increments in the number of leaves per plant has been obtained as a result of the synergistic effect between home tea residues and *Aspergillus niger* fungus. The results showed that the highest average of (15.67 leaves) was obtained from the combination of (dry tea residues + *Aspergillus niger* fungus) while the lowest average of (12 leaves) was a result of control treatment .

Total chlorophyll (SPAD unit) : The results in Table (2) shows insignificant increment in the total chlorophyll content of chili pepper leaves that growing under plastic house conditions as a result of treatment the plant with home tea residues (dry residue and extract). The highest average of (66.683 Spad unit) was from treatment the plant with tea residues extract with an increments of (8.296% and 30.007%) compared to the treatment of plant with dry tea residue and control treatment respectively. Moreover, non-significant increment was obtained in total chlorophyll content of leaf due to the addition of *Aspergillus niger* fungus to the soil. This treatment recorded an average of (62.73 SPAD unit) with an increment of (21.971%) compared to the control treatment. Non-significant differences were also obtained from the synergistic effect between home tea residues and *Aspergillus niger* fungus and the combination of (tea residues extract + *Aspergillus niger* fungus) gave the highest leaves content of total chlorophyll (69.853 Spad unit) with increments of (0.528 and 35.821%) compared to the combination of (dry tea residues + *Aspergillus niger* fungus) and control treatment respectively .

Number of branches : Significant differences in the number of branches per plant were obtained as a result of the addition of home tea residues whether it added in the form of dry tea residues or tea residues extract. Table (2) shows that the treatment of the plant with dry tea residues recorded the highest average of (10.33 branches) with increments of (1.430 and 47.571%) compared to the treatment of the plant with tea residue extract and control treatment respectively.

On the other hand, non-significant differences have been indicated by the statistical analysis in this characteristic as a result of the addition of *Aspergillus niger* fungus. Moreover, the results showed that the treatment of plant with *Aspergillus niger* fungus caused noticeable increments in the number of branches per plant, but these were not significant and gave the highest average of (9.33 branches) with an increment of (33.285%) compared to control treatment, which recorded the lowest average of (7 branches).

On the other hand, insignificant increments have been obtained in the number of floral branches per plant from the synergistic effect of home tea residues and *Aspergillus niger* fungus and the combination of (dry tea residue + *Aspergillus niger*) gave the highest average of (12.67 branches) with increments of (8.568% and 81%) as compared to the combination of (tea residues extract + *Aspergillus niger*) and the control treatment respectively .

3.2 yield parameters

Number of flowers per plant : The results of the addition of home tea residues and *Aspergillus niger* fungus on the number of flowers per plant are depicted in Fig. (2). The results shows that there are non-significant differences in the number of flowers per plant of chili pepper and for all the treatments. It is also shown by the same Figure that the treatment of plant with dry tea residues recorded the highest average of (10.33flowers) with increments of (24.009 and 93.808%) compared to the treatment of the plant with tea residues extract and control treatment respectively.

The statistical analysis showed insignificant differences between treated plant using *Aspergillus niger* fungus and the control treatment in the number of flowers per plant and the highest average of (9.67 flowers) has been obtained as a result of *Aspergillus niger* fungus treatment with an increment of (81.425%) compared to the control treatment which recorded the lowest average of (5.33 flower) for the number of flowers per plant. Moreover, non-significant differences were obtained due to the synergistic effect of home tea residues (dry tea residues and tea residues extract) and *Aspergillus niger* fungus. The results of this study that depicted in Fig.(2) showed that the combination of (dry tea residues+ *Aspergillus niger*) gave the highest average of (13.67 flowers) with increments of (18.744 and 156.472%) compared to the combination of (tea residues extract + *Aspergillus niger*) and the control treatment respectively.

Number of fruits per plant : The results that depicted in Fig.(2) pointed to significant increments in the number of fruits per plant of chili pepper as a result of the treatment the plant with home tea residues. Moreover, the results showed that the addition of home tea residues weather it added in the form of dry residues or extract

gave the same average of (4.33 fruits) with an increment of (85.836%) as compared to the control treatment. On the other hand, the results of the statistical analysis that shown in Fig.(2) refers to significant differences in the number of fruits per plant obtained as a result of the addition of *Aspergillus niger* fungus to the soil and recorded the highest average of (3.67 fruits) with an increment of (57.510%) compared to the control treatment which gave the lowest average of (2.33) for the number of fruits per plant.

The synergistic effect of home tea residues with *Aspergillus niger* fungus didn't cause any significant differences in the number of fruits per plant. The results that presented in the same Figure showed that the highest average of (7.67 fruits) has been obtained from the combination that consist of (tea residues extract+ *Aspergillus niger* fungus) with increments of (53.4 and 229.184%) compared to (dry tea residues + *Aspergillus niger* fungus) and control treatment respectively.

Fresh weight of fruits (g) : Significant differences in the fresh weight of fruits have been obtained as a result of the treatment the chili pepper with home tea residues. The results of this study shows that the highest average of (17.72 g) for the fresh weight of shoot has been obtained as a result of the treatment with tea residues extract with increments of (21.569 and 67.232%) compared to the treatment of the plant with dry tea residues and control treatment respectively, as shown in (Fig. 2)

As for the bio fertilization using *Aspergillus niger* fungus and its effect on chili pepper characteristics, the results presented in Fig.(2) showed significant differences in fresh weight of fruits as a result of the addition of *Aspergillus niger* fungus which recorded the highest average with increment of (39.731%) compared to control treatment. On the other hand, significant increments in the fresh weight of fruits have been obtained due to the synergistic effect of home tea residues and *Aspergillus niger* fungus. Moreover, The results shows that the combination of (tea residues extract + *Aspergillus niger* fungus) gave the highest average of (18.236 g) for the fresh weight of fruits with increments of (23.970 and 65.884%) compared to the combination that consist of (dry tea residues + *Aspergillus niger* fungus) and control treatment respectively, (Fig.2)

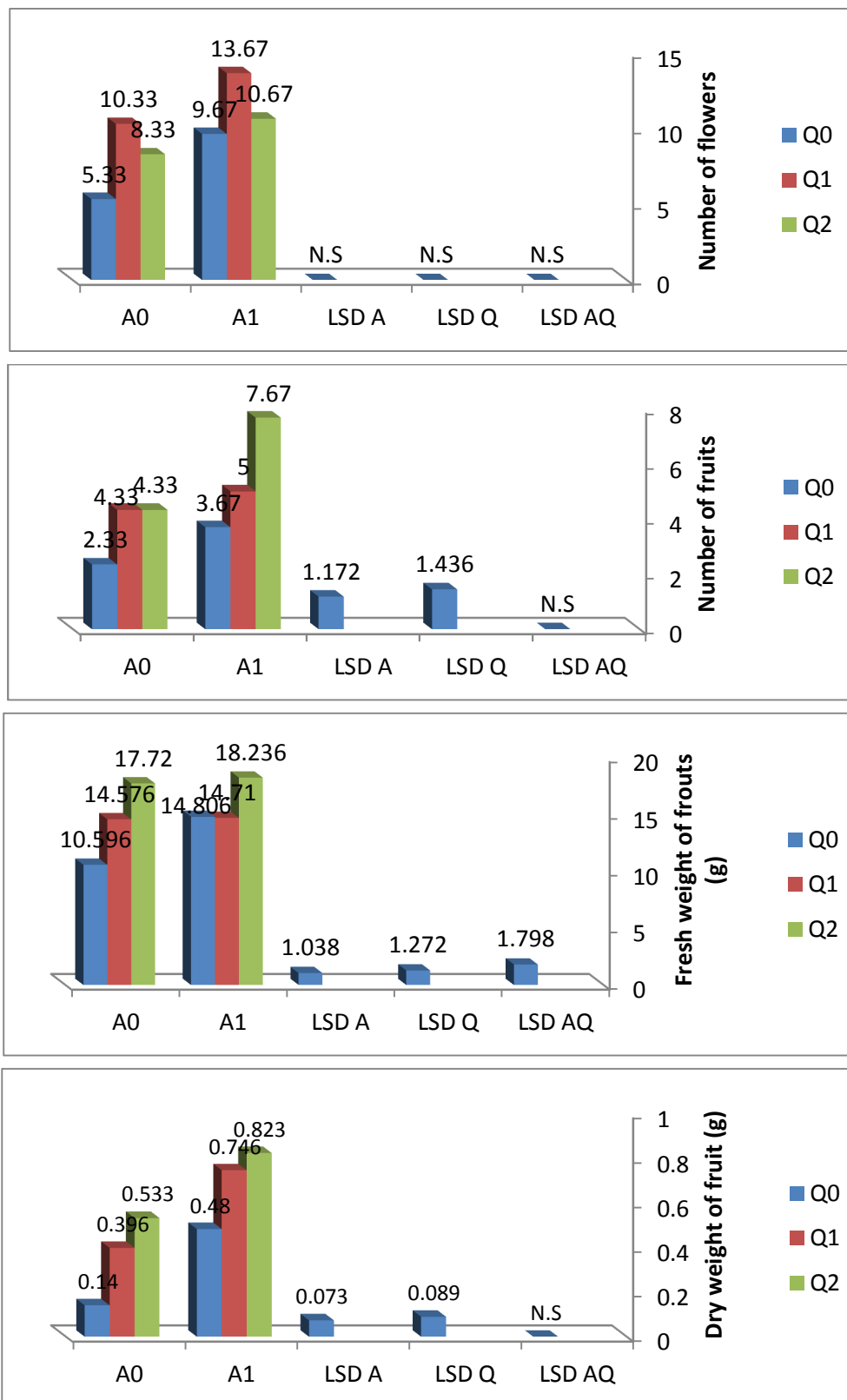


Figure (2) Effect of home tea residues as (dry tea residues and extract), *Aspergillus niger* fungus and them synergistic effect on some of yield parameters for chili pepper plant grown under greenhouse conditions.

A0 = without *Aspergillus niger* ; A1= with *Aspergillus niger*; Q0= without home tea residues; Q1= with dry home tea residues ; Q2= with home tea residues extract.

Dry weigh of fruit (g) : The results of the statistical analysis that shown in Fig. (2) indicate significant

differences in the dry weight of the chili pepper fruits as a result of organic fertilization using the home tea residues (dry tea residues or tea residues extract). The highest average of (0.533 g) was due to the treatment the plant with tea residues extract with increments of (34.595 and 280.714%) compared to the treatment of plant with dry tea residues and control treatment respectively.

As shown in the same Figure, significant differences in the dry weight of chili pepper fruits were found from the treatment of *Aspergillus niger* which recorded an average of (0.48 g) with an increment of (242.857%) compared to the control treatment.

The synergistic effect of home tea residues and *Aspergillus niger* fungus did not show any significant differences in this characteristic. The results showed that the highest average of (0.823 g) was obtained from the combination of (tea residue extract + *Aspergillus niger*), while the lowest average of (0.14 g) for the dry weight of fruits has been obtained from the control treatment, as shown in (Fig.2).

4. Discussion

Tea residues are organic matter rich in nutrients that necessary for plant growth and development. Several studies have indicated that the use of tea residues, whether in the form of dry tea residues or tea residues extract, have played an important role in improving the growth of plants.

Some studies have shown that the compost made from the tea residues is characterized by its highly content of sulphate, chloride, phosphorus, phosphorus, magnesium and organic matter Gurav and Sinalkar (2013). Its absorption by plants increase its concentration in leaves and cause an increment in leaves content of chlorophyll which intern leads to increase the leaves content of carbohydrate (Obeidi, 2008) and this what was reflected in the growth and productivity of the plants. The results of current research pointed to significant increments in most of studied characteristics due to the addition of home tea residues, weather its added in the form of dry tea residues or tea residues extract. However, watering with tea extract has a greater effect than the use of tea residues in the form of fertilizer.

The reason behind this could be attributed to that the hot water extraction process dissolves the various chemical compounds found in the tea residues and increases its concentration in the extract and thus increases its readiness to be absorb by the roots of the plant. This result was in agreement with previous findings from Morikawa and Saigusa (2008) where they found that tea leaves retained a good proportion of nutrients even after extraction with hot water. They found that the addition of tea residues to radish increase its leaves content of iron and improved its growth and this was in line with the results of the current study as the results shows noticeable development in the vegetative and productive characteristics of the chili pepper was obtained from the addition of home tea residues to the plant, especially when it was added in the form of extract.

Positive effects have been obtained in the growth characteristics of basil plant as a result of mixing the soaked tea with soil (Al-Kaisy et al. 2011). They found significant increments in germination rate, plant height, stem diameter, root diameter, shoot dry weight and the absolute growth rate of plant due to this treatment. Abd Al-Rasul (2012), indicated in his study that the addition of tea residues to soil planted with tomatoes improved the plant's productive properties by improving the soil physical properties.

On the other hand *Aspergillus niger* ability in increase plant growth and improve its productivity is attributed to its role in convert organic material in the soil into its initial compounds that are easily absorbed by the plant (Varenyam & Reddy, 2007; Richa et al., 2007; Wang et al., 2018). Moreover, the ability of *Aspergillus niger* to dissolve phosphate it's either through the production of some organic acids and reduce soil pH such as (Citric acid, Oxalic acid, formic acid and maleic acid) (Kumari et al., 2008) or through the secretion of phytase and phosphatases enzymes, which increase the level of dissolved phosphorus in the soils and increase plant resistance to inappropriate surrounding conditions (Panda et al., 2008).

However, the improvement that occurred in the growth of chili plant may be due to the ability of *Aspergillus niger* to produce some compounds that contribute to increasing plant growth, such as: 2-Carboxymethyl-3-n-hexylmaleic acid), which plays an important role in accelerating the process of seed germination and increasing the total vegetative growth and (Cexylbutadioic acid-3-2-Methylen), which increases the root growth and Stimulates the flowering process (Mondal et al., 2000), taking into consideration the growth hormones of the Gibberellic acid and Indole acetic acid that produced by these fungus in large quantities and their effective role in improving the vegetative characteristics of plants and thereby increasing their productivity by stimulating the elongation of cells and the internodes, enhancing the growth of floral branches, promoting the flowering, accelerating the germination of seeds as well as its role in overcoming the state of genetic dwarfism in some plants (Qureshi et al., 2013). Moreover, *Aspergillus niger* is characterized by its ability to produce phynolic and flavonoid compounds inhibiting the growth of pathogenic fungi of plants (Nayak and Pandey, 2017) which include Gibberella, Fusarium, Monographella, Bipolaris and Volutella. (Wang et al., 2018),

The results of this research were in consistent with many previous studies such as: Mondal et al. (2000), who found in their study on the *Aspergillus niger* and its role in the growth and development of cauliflower plant, significant increments in both root and shoot lengths and dry mass of cauliflower seedlings treated with

metabolic compounds isolated from this fungus. Significant increments in the dry weight of the *Brassica chinensis* (Linn) in addition to its contents of phosphorus and nitrogen was the result of research conducted by Chuang et.al., (2007) to study the role of the *Aspergillus niger* in dissolving inorganic phosphorus and developing plant growth.

This results are in agreement with previous findings from (Anwer and Khan 2013), where they found that *Aspergillus niger* significantly improved the tomato fruits quality and caused significant increments of 54% and 59.8% in yield and dry matter. Moreover, a significant increment in total chlorophyll content of leaves has been obtained in treated plant with *Aspergillus niger* compared to control treatment. Significant increments in plant height, shoot fresh and dry weights and chlorophyll content of leaves in have been obtained in cucumber as a result of the treatment with *Aspergillus niger* isolated from some plant residues(Altaie & Alwan, 2017).

5. Conclusion

From the results of this research we conclude that the organic fertilization by using home tea residues and bio fertilization with *Aspergillus niger* fungus played an effective role in promoting the pepper growth and increase its productivity by increasing the plant height, fresh and dry weights of shoot in addition to increase the number of leaves per plant which were reflected positively on the number of fruits and the fresh and dry weights of fruits and thus increase its productivity . We can also conclude from the results that the addition of home tea residues and *Aspergillus niger* fungus as simultaneously did not have an effective effect on the pepper development compared to their addition as individually, although adding them as simultaneously caused noticeable increments in all the studied characteristics, but these were not significant

6. Acknowledgments

The authors are thankful to the department of biology in diyala university for providing supplies needed to complete the search , and we would like to thanks Mr. Mohammed Hussein for his help to complete this research .

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