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Efficiency of Organic Matter Levels and Bio Fungus *Trichoderma Harzianum* on cucumber Plant

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Abstract: *The experiment was set up to evaluate the effect of Trichoderma harzianum inoculation as combination with organic matter on cucumber plant growth and T.harzianum development. The experiment was conducted in a greenhouse in the agricultural experimental station of the agricultural research directorate in Tewatha 20 Km south east of Baghdad. Organic matterwas applied in 1, 2.5, 5, 10, and 20 % of the weight of the soil to 30 cm depth combination with T.harzianum. A statistical analysis was designed to experimental units according to Duncan's multiple range with three replicates for each treatment. Results of this study showed the interaction between T.harzianum and organic matter addition had significant effect on growth parameters of cucumber plant, T.harzianum population, dry and fresh weight, plant height as well as nitrogen, phosphorus and potassium concentrations.*

Keywords: *Organic matter, Cucumber, T.harzianm, Fresh and Dry weight.*

I. Introduction

Currently the huge increase in agricultural and various industrial residues resulted to use them as sources to improve soil fertility, plant nutrition and protection of the environment, on the other hand can also be used to inhibit plant diseases that attack plant host and study their impact on these diseases (Huper et al., 1993; Huang and Huang, 1993).

Plants residue have been seen their effect to suppress plant diseases for the first time by (Linfod et al., 1938) when it reduced the population of nematodes that causing root-knot to the plant tissue, and the reason for that case related to direct production of poisoning from decomposition of organic materials, especially ammonia (Sagre et al.,1965).Several studies have been emphasized the main role of organic residue such as (plant and animal) to suppress the plant diseases that resulting from fungi, it has been found that the addition of organic material by 2 % to the soil had led to the high reduction in disease incidence of the fungi *Fusarium* spp. and *Scleritium* spp. that caused root rot of rice to 20, 18 % respectively, as compared to control treatment 32 and 31 % respectively. And also the disease severity of the *Fusarium* spp. and *Scleritium* spp. decreased from 52 and 60% to 38 and 42% respectively (Sirry et al., 1973).

Tisdale et al. (1997) point out the importance role of organic materials when added to the soil, and provided nutrients elements and reduce the losing processing as result by washing and irrigation, due to retaining moisture in the rhizoshper area. And also the organic materials work to improve the properties of the clay soil and prevent the cohesion of the blocks and the improvement of ventilation and facilitate the growth and development of the roots movement inside the soil. The fungi use organic matter as food to build their cells and obtain their necessary energy, as average the cells microbial containing 50 % of carbon its obtained from the surrounding medium, fungi are more active than other microbes to represent between 30 to 40 % of the carbonate organic material, and these numbers are considered very important of soil fertility and plant nutrition. The microbes when exploit the carbon from organic material and later representation to build their not need only carbon, but also they need phosphorus and potassium and other major and minor elements, these fungi have taken these elements from the decaying organic material.

Although there have been several reports on the use of *Trichderma* spp. induce systemic resistance mechanisms of plant, this biocontrol agent, is applied to soil as conidia or resting spores which must become active (Hamdia, 2014). To achieve effective biocontrol systems developed of *T.harzianum* in plant we need to test more than one method that may be included into the integrated pest management programs to control crop disease and stimulation plant parameters (Damicone et al., 1993).One other method that is used is the application of organic residue for instance cotton plants, yellow corn and soybeans in eliciting resistance in plants to reduce the disease incidence of the fungus *Sclerotium cepivorum* pathogen that caused rot white on onion, and found the best reduction when adding cotton residue and gave disease incidence 46 % as comparison with 64 % in the treatment without the addition (Mohamed and Fahmy,1985). Also other study found that the addition of yellow corn residue exhibited enhanced resistance to *Fusarium oxysporum* that caused wilt in

tomatoes and reduction the disease severity, while the application of chicken manure resulted to increase the disease severity (Waudo et al., 1995). Also found that the addition of neem cake or plant residue displayed significant levels of protection to reduction in root rot disease caused by *Macrophomina phaseolina* and increased plant growth parameters on cowpea (Ushamalini, 1997).

The aim of this study is to provide a substitute to chemical treatment through the application of eco-friendly solution such as biocontrol agents while adding agronomic value such as growth induction and improvement through more efficient nutrient absorption. Here we report the results of *T.harzianum* in combination with different concentration of organic material in cucumber as a model plant. We believe that the efficacy shown by any of these concentrations may be tested later on other economically important crops.

II. Materials And Methods

Preparation of *T.harzianm* Inoculation and Greenhouse Experiment

This research was conducted under greenhouse condition which was not received any agriculture chemical one. The entire area was sterilized to ensure successful an integrated management of disease and insect. This study was used *T.harzianm* isolate 26 which was used as the active substance in the production of the biopesticide (department of biopesticide, Agricultural Research Directorate). And used simple component as medium to growth this fungus which include: wheat bran, corn cob and water (3: 7: 3 / weight / weight /size) (Hafedh, 2001), this medium putted inside glass bottles 50 g / vial capacity, and after that the media sterilized at a temperature 121 ^{0}C and pressure of 1.5 kg / cm^2 for 20 minutes for three times. After that a 5 mm disc of mycelium from *T. harzianm* was placed inside glass bottles. Later the bottles that include *T.harzianm* incubated at a temperature (28 ± 2 ^{0}C) for 5 days to become ready to use. *T.harzianm* inoculation used by 6 g / m² where includes per gram of fungus Trichoderma on 10^8 spore /1 g of the inoculation. The soil was inoculated with *T.harzianm*, watered and kept moisturized and mixed thoroughly every other day for duration of 1 week.

Preparation of Organic Material

One type of organic material used in this study, which include the stalks of maize that brought from Tuwaitha area, where it was moistened with water, and later 1% weight of urea ($N(NH)_2CO$, 46 %) was added. After that the combination was covered by polyethylene with left a suitable distance for ventilation and left for a period of three months with stirring and moving the waste each week to increase ventilation in order to full decomposition of the residue (Al-Mustafa, 1993).Different levels of organic matter 1%, 2.5 %.,5 %,10 %,20 % were used in this study.

Cucumber Seeds

Seeds of Cucumber (*Cucumis sativus*) kind Lahluba were chosen in this study, obtained from Agricultural Research Directorate / Ministry of Science and Technology. The soil was inoculated with *T.harzianm* combination with organic matter, there were holes made aseptically, and cucumber seeds were planted inside hole, distance between every hole was 30 cm planted on both sides of the lines containing a single line on the length of the refined 3.5 per m², then was conducted drip irrigation process. A pH degree of soil was calculated in extract of ex-saturated soil paste by using a pH meter (Radiometry). The degree of electrical conductivity in the E.C. as well as the estimated in saturated paste extract using conductivity meter device (Radio Meter DM8) and readings were adjusted to 25 ^{0}C.

Figure 1: Cucumber plants in the first stage of growth under greenhouse conditions

Pesticides and Mineral Fertilizers Used

This study used pesticide 5 g /m² of 50 % Methyl 1- (butyl carbamoyl benzimidazol-2-carbamate) (Hafedh, 2001). Also was used mineral fertilizer urea ($N(NH)_2CO$,46 %) at a concentration 6 g / m².

Phenotypic Traits (Morphological) Plant Option

The rate of root length and plant height

The length of each of the root and plant height (shoot) to three plants per treatment was measured by using a tape measure; length has been calculated for each replicate rate by dividing the total number of plants on the lengths and then analyzed the results statistically.

The rate of fresh and dry weight

Fresh weight was estimated of shoots to three plants per treatment using a sensitive balance type (Mettler pc 4000). The dry weight was appreciated after drying vegetative aggregates in the oven type (Memmert) at a temperature of 65 ^{0}C for a period of almost 72 hours, where weight did not change after that.

Figure 2: Cucumber plants in the end of season under greenhouse conditions.

The Effect of Organic Matter inside Plant to Absorb the Elements

The concentration of nitrogen, phosphorus and potassium

Nitrogen N was estimated in soils by using a Kueldahl Appartus according to the method of Bremner and Mulvaney (1982) described in (Page, 1982). Phosphorus was appreciated by using distilled water depending on the method of Mohammed (2002). The amount of potassium also has been evaluated as suspension by digestion the plants with distilled water according to the method of Mohammed (2002).

Figure 3: Cucumber plants in the end of season under greenhouse conditions.

III. Results

The result of effect fertilizer type on the population and development of *T.harzianum* showed in two types of fertilizer (organic matter and mineral) on the population *T.harzianum* under greenhouse conditions. Data presented in Table 1 showed a significant differences between the treatments ($p < 0.05$) Table 1. The different fertilizer treatments as following: (mineral alone, organic alone and mineral + organic) in combination with *T.harzianum* gave a significant differences between them after one month of addition processing. The fertilizer treatment (mineral + organic) achieved high population density of *T.harzianum* 86×10^4 unit consisting of the colony (CFU) /1 g of soil. However, the lowest number of *T.harzianum* population when applied with mineral fertilizer treatment 17.33×10^4unit consisting of the colony (CFU)/1 g of soil was showed after one month of the application processing.

Clearly, a significant differences of *T.harzianum* population were observed between the treatments testing, where the population of *T.harzianum* increased in the treatment of mineral fertilizer after two months 55×10^4 unit consisting of the colony (CFU) /1 g of soil, while decreased slightly in the treatment of fertilizer (organic + mineral) 72.67×10^4 CFU /1 g of soil, whereas increased to become double increasing in the

treatment of organic matter118.33× 10^4 CFU /1 g of soil. But after three months has been seen a significant decrease in the treatment of mineral fertilizer 13.33× 10^4 CFU/1 g of soil as compared to the two treatments (Mineral + organic + *T.harzianum* and organic + *T.harzianum*), which revealed 55.7 × 10^4 and 59.33 × 10^4 CFU/1 g of soil respectively.

Table 1: The effect of fertilizer on the growth and development of the fungus *T. harzianum* population under greenhouse conditions

Treatments	The average* of *T.harziauum* population		
	After one month of addition20/3/2009	After two months of addition20/4/2009	After three months of addition20/5/2009
Urea (mineral fertilizer) + *T.harzianum*	17.33 × 10^4c	55 × 10^4c	13.33 × 10^4c
(Mineral + organic6 g / m²) + *T.harzianum*	86 × 10^4a	72 × 10^4b	55.7 × 10^4b
(Organic matter6 g / m²) + *T.harzianum*	71 × 10^4b	118.33 × 10^4a	59.33 × 10^4a

Mean* of three replicate of each treatment.
Numbers in each column that have same letter do not differ significantly from each other at p<0.05 according to Duncan's multiple range test.

The Effect of Different Concentrations of Organic Matter on the Growth and Development of *T. harzianum* Population

The results of different levels of organic matter1% + *T.harzianum*, 2.5 % + *T.harzianum*, 5 % + *T.harzianm*,10 % + *T.harzianum*, 20 % + *T.harzianum* besides the control treatment (*T.harzianum* alone) under the greenhouse conditions showed a significant differences between the treatments (p < 0.05) Table 2.The treatments were differed significantly between them in the first reading after one month of *T.harzianum* addition; the treatment 1% + *T.harzianum* had significantly differences from treatment 2.5 % + *T.harzianum* 33.33 × 10^4 and 36.33 × 10^4 unit consisting of the colony (CFU)/1 g of soil respectively. The second reading which was taken after two months of *T.harzianum* addition, the treatment that included the concentration 20 % + *T.harzianum* gave a high significant difference as compared with all treatments. In the third reading which was recorded after three months after the process of *T.harzianum* addition, the *T.harzianum* population had been declined in all the concentrations tested of organic matter levels.

Table 2: The effect of different concentrations of organic matter on the growth and development of the population of *T.harzianum*

The levels of organic matter	The average* of population *T.harziauum*		
	After one month of addition 6/3/2010	After two months of addition 6/4/2010	After three months of addition 6/5/2010
1% Organic matter+ *T.harzianum*	33.33 × 10^4 e	48 × 10^4 e	24 × 10^4 e
2.5% Organic matter+ *T.harzianum*	36.33 × 10^4 d	80.33 × 10^4 d	45 × 10^4 d
5% Organic matter+ *T.harzianum*	70.33 × 10^4 c	92.33 × 10^4 c	50.67 × 10^4 c
10% Organic matter+ *T.harzianum*	84.33 × 10^4 b	101.33 × 10^4 b	60.67 × 10^4 b
20% Organic matte + *T.harzianum*	89.33 × 10^4 a	128.67 × 10^4 a	62.67 × 10^4 a
Control	27.33 × 10^4 f	42.33 × 10^4 f	19.33 × 10^4f

Mean* of three replicate of each treatment
Numbers in each column that have same letter do not differ significantly from each other at p<0.05 according to Duncan's multiple range test.

The Efficiency of Organic Matter Levels on Plant Growth Parameters (Morphological) Plant Root Length, Plant Height (Shoot), Dry and Fresh Weight

The effect of different concentrations of organic matter combination with *T.harzianum* as following: 1% + *T.harzianum*, 2.5 % + *T.harzianum*, 5 % + *T.harzianum*, 10 % + *T.harzianum*, 20 % + *T.harzianum* were added to the soil on the root length of the cucumber plant Table 3. The results showed the root length was increased with increased of organic matter concentrate. The beneficial effect of these concentrations was shown in the highest concentration of organic matter 20 % + *T.harzianum* gave root length 23.3 cm as compared to the organic matter concentration 1% + *T.harzianum* which gave 17.1 cm. Table 3 shows the relationship between the concentrations of organic matter 1% + *T.harzianum*, 2.5 % + *T.harzianum*, 5 % + *T.harzianum*, 10 % + *T.harzianum*, 20 % + *T.harzianum* were added to the soil and the cucumber plant height after three months of agriculture. The concentrations of organic matter levels induced parameters progression of cucumber plant. As the plant height is very important of plant growth average and development as to estimate the height of the plant after the agriculture, and it is very important to give a clear picture of the effect of different concentrations of organic matter that added to the soil. Statistical analysis in tables 3 shows that the

compatibility between the fungus *T.harzianum* and organic matter. This is very high degree of statistically significant at level of probability of ($p < 0.05$).These results is clear that the rate of increasing in plant height were 280.6, 287.2, 294.57, 303.07 and 306.33 cm respectively of concentrations 1% + *T.harzianum*, 2.5 % + *T.harzianum*, 5 % + *T.harzianum*,10 % + *T.harzianum*, 20 % + *T.harzianum* respectively, compared with non-treated control which was valued at 270.33 cm.Table 3 shows the results of the effect of different concentrations of organic matter 1% + *T.harzianum*, 2.5 % + *T.harzianum*, 5 % + *T.harzianum*,10 % + *T.harzianum*, 20 % + *T.harzianum* were added to the soil on dry weight of the cucumber plant after the end of the season as they gave 49.6,53,57.7,60.6 and 69.3 g respectively compared to the control treatment that achieved 46.3 g. As for dry weight results showed increased in dry weight of the cucumber plant with increasing of organic matter concentrations, i.e. the increasing in organic matter levels that added to the soil gave clear evidence of the importance of organic matter in efficacy on plant growth, which later reflected positively on plant productivity. The results had been shown in Table 3 also showed increased in fresh weight of the plant with increasing of organic material concentrations which achieved 470.7, 477, 490.5, 500.4 and 550 g, respectively, compared to the treatment comparison which gave 465.6 g.

Table 3. The effect of organic matter level and *T.harzianum* on percentage of the root length rate, plant height, dry weight and fresh weight of cucumber plant

The levels of organic matter	Average*of plant growth parameters			
	the length of plant root (cm)	the plant length(cm)	dry weight (g)	fresh weight (g)
0%	17.1 e	270.33 f	46.3d	465.6 f
1%Organic matter + *T. harzianum*	17.9 cd	280.6 e	49.6cd	470.7 e
2.5%Organic matter + *T. harzianum*	20 c	287.2 d	53c	477 d
5%Organic matter + *T. harzianum*	20.6 c	294.57 c	57.7b	490.5 c
10%Organic matter + *T. harzianum*	22ab	303.07 b	60.6 b	500.4 b
20%Organic matter + *T. harzianum*	23.3 a	306.33 a	69.3 a	550 a

Mean* of three replicate of each treatment
Numbers in each column that have same letter do not differ significantly from each other at $p<0.05$ according to Duncan's multiple range test.

The Effect of Organic Matter on Plant Absorption the Elements

The evaluation of nitrogen, phosphorus and potassium concentration in cucumber plant

Table 4 shows the concentrations of organic matter added to the soil as following: 1% + *T. harzianum*, 2.5 % + *T. harzianum*, 5 % + *T. harzianum*, 10 % + *T. harzianum*, 20 % + *T. harzianum* have been achieved significant increasing ($p<0.05$) in the concentration of nitrogen at all concentrations 3.00, 3.15, 3.54, 3.64 and 3.86 mg / plant respectively, as compared to the treatment comparison which gave 2.79 mg / plant after three months of agriculture. The results showed a continuous increasing in the nitrogen concentration in the plant also in high levels of organic matter. The nitrogen concentration very important to plant activity to form the fruit and floral buds and growth, and the compatibility between *T. harzianum* and organic matter led to a steady increase in nitrogen concentration in plant.

The phosphorus concentration in the plant was influenced by levels of organic matter was also increased as is the case in nitrogen. The concentrations of organic matter 1% + *T. harzianum*, 2.5 % + *T. harzianum*, 5 % + *T. harzianum*, 10 % + *T. harzianum*, 20 % + *T. harzianum* gave increased in phosphorus concentrations as following: 0.46, 0.497, 0.53, 0.567 and 0.607 mg / plant respectively, compared to the control treatment 0.393 mg / plant after three months of agriculture. Salisburry and Ross (1985) reported that the reason may be due to assemble phosphorus in plant configuration for the production fruits, and agree with the nature of the plant to absorb phosphorus especially in the first weeks of growth, and this result as well as the importance of plant physiology to uptake the phosphorus, and impact on plant growth and production (Mohammed, 2002).

The phosphorus concentration increased inside the plant occurred after three months from sowing at any level of organic matter added that probably explains a large part of the phosphorus in the plant was transferred to the fruit to achieve production during the stages of growth above. These results are agree with Mohammed et al.,(2012), who pointed out that the plant accumulates a large part of the phosphorus uptake in fruits and seeds and this fact indicated to move phosphorus element easily inside plant (Highly Mobil).Table 4 shows the concentration of potassium in the cucumber plant, as in the same case of nitrogen and phosphorus results showed that the potassium uptake by the plant at different levels of organic matter 1% + *T.harzianum*, 2.5 % + *T.harzianm*, 5 % + *T. harzianum*,10 % + *T.harzianm*, 20 % + *T.harzianm* gave 2.907, 3.193, 3.457, 3.767 and 4.057mg / plant, was is higher than control treatment without organic matter 2.613mg / plant. This shows the viability of organic material + *T.harzianm* used in the study, and influence on the plant also supplied potassium and also won for phosphorus and nitrogen.

Table4. The effect of organic matter level and *T. harzianum* on concentration of phosphorus, potassium and nitrogen in cucumber plant

The level of organic matter+ *T. harzianum**	The concentration of nitrogen mg/kg in the plant	The concentration of phosphorus mg/kg in the plant	The concentration of potassium mg/kg in the plant
0%	2.793 f	0.393 f	2.613 f
1% organic matter + *T. harzianum*	3.003 e	0.463 e	2.907 e
2.5% organic matter + *T. harzianum*	3.153 d	0.497 d	3.193 d
5% organic matter + *T. harzianum*	3.543 c	0.53 c	3.457 c
10% organic matter + *T. harzianum*	3.643 b	0.567 b	3.767 b
20% organic matter + *T. harzianum*	3.86 a	0.607 a	4.057 a

Mean* of three replicate of each treatment.
Numbers in each column that have same letter do not differ significantly from each other at $p<0.05$ according to Duncan's multiple range test.

IV. Discussion

Several investigations have been find that *T.harzianm* species with high ability to release different kinds of enzymes with simple materials, and have activity protection to the plant from pathogens and increased growth standards Fig. 1, 2 and 3 (Meraj-ul et al., 2012; Kalaivani et al., 2014;Wua et al., 2012). The results may be due to organic matter concentration was very appropriate under the conditions after one month from agriculture that encourage the growth of *T.harzianum* population. The compatible between *T.harzianum* population and organic matter was declined in treatment of mineral fertilizer 13.33×10^4 to more than half, probably indicates that the *T.harzianum* growth in the month (May) under the greenhouse conditions was probably not compatible or that the mineral fertilizer may only succeed under moderate temperatures, and may did not give positive results in high temperatures.

T.harzianum colonize plant waste quickly and soil organic compared to other organisms through release secreted enzymes such as internal endoenzymes and external exoenzymesas these enzymes consider hydrolysis enzyme, and digest these materials into simple sugars and turning them into a soluble materials in the water (Burns, 1983; Spalding, 1977, 1978). The population of *T.harzianum* had been increased with high percentage of organic matter, may be due to the use of the high concentration of inoculation led to increase the population of *T.harzianum* with availability of organic matter, moreover, carbon sources, carbohydrates and proteins, and this leads to compete between microorganisms for food, space biology, and secretion of enzymes with requires a high level in order to analyze these organic materials.

The increasing in plant growth gave us evidenced and confirmation about abundance of suitable micro-nutrients to provide the plant as prefabricated ((Mohammed, 2002). The results of this study were agreed with several studies that point out to important role of organic matter to increase plant growth and height (Mohammed, 2010, 2012). Sherry et al. (1984) was found that addition of nutrition mushroom residues such as spent mushroom compost (SMC) to the soil at depth of 30 cm led to an increase in plant height of seedlings by 10.3 cm to 27.7 cm. Navarro and Locasica (1974) also found that the addition of organic matter to the field soil (Silt loam Soil) led to increased vegetative growth of plant from 50 % to 75 %. Yedidia et al. (2001) found the inoculation of *T.harzianum* spores increased the production of peroxidase activity and chitinase activity in cucumber plant. This finding is important in terms to regular plant growth for the root and shoot ratio also give clear indicator of the importance of organic matter and *T.harzianum* on impact in the growth and development of the root system, which will reflect positively on the growth parameters and development of the shoot and then increase of production (Fig. 1 and 2).

V. Conclusions

From this study it was determined that the effect of level of organic matter additions and the application of biological control agent *T.harzianum* soil could be more successful at inducing cucumber plant growth parameters as well as the concentrations of potassium, phosphorus and nitrogen increased logarithmically with the increase of organic matter combination with *T.harzianum* addition. It is clear that organic matter combination with *T.harzianum* is practical proposition for sustainable agriculture.

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