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 Tewathu 20 Km south east of Baghdad. Organic matter was 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.harzianum*, 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 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 *Sclerotium* 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 *Sclerotium* 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 rhizosphere 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 needs 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 *Trichoderma* 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...
Efficiency Of Organic Matter Levels And Bio Fungus Trichoderma Harzianum on cucumber Plant
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.harzianum 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.harzianum 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 °C and pressure of 1.5 kg / cm² 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 °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 10³ 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 °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.

DOI: 10.9790/2380-0861 2834  www.iosrjournals.org  29| Page
Efficiency Of Organic Matter Levels And Bio Fungus Trichoderma Harzianum on cucumber Plant

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 °C for a period of almost 72 hours, where weight did not change after that.

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 × 10^4 unit 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
Efficiency Of Organic Matter Levels And Bio Fungus Trichoderma Harzianum on cucumber Plant
treatment of organic matter118.33×10^4 CFU /1 g of soil. But after three months has been seen a significant
decline 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

<table>
<thead>
<tr>
<th>Treatments</th>
<th>The average² of T.harzianum population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>After one month of addition20/3/2009</td>
</tr>
<tr>
<td>Urea (mineral fertilizer) + T.harzianum</td>
<td>17.33 × 10^c</td>
</tr>
<tr>
<td>(Mineral + organic g / m³) + T.harzianum</td>
<td>86 × 10^a</td>
</tr>
<tr>
<td>(Organic matter g / m³) + T.harzianum</td>
<td>71 × 10^b</td>
</tr>
</tbody>
</table>

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.harzianum,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

<table>
<thead>
<tr>
<th>The levels of organic matter</th>
<th>The average² of population T.harzianum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>After one month of addition 6/3/2010</td>
</tr>
<tr>
<td>1% Organic matter+ T.harzianum</td>
<td>33.33 × 10^4 e</td>
</tr>
<tr>
<td>2.5% Organic matter+ T.harzianum</td>
<td>36.33 × 10^4 d</td>
</tr>
<tr>
<td>5% Organic matter+ T.harzianum</td>
<td>70.33 × 10^4 c</td>
</tr>
<tr>
<td>10% Organic matter+ T.harzianum</td>
<td>84.33 × 10^4 b</td>
</tr>
<tr>
<td>20% Organic matter+ T.harzianum</td>
<td>89.33 × 10^4 a</td>
</tr>
<tr>
<td>Control</td>
<td>27.33 × 10^4 f</td>
</tr>
</tbody>
</table>

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, 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

<table>
<thead>
<tr>
<th>The levels of organic matter</th>
<th>Average of plant growth parameters</th>
<th>%</th>
<th>the length of plant root (cm)</th>
<th>the plant length(cm)</th>
<th>dry weight (g)</th>
<th>fresh weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td></td>
<td></td>
<td>17.1 e</td>
<td>270.33 f</td>
<td>46.3d</td>
<td>465.6 f</td>
</tr>
<tr>
<td>1% Organic matter + <em>T. harzianum</em></td>
<td></td>
<td></td>
<td>17.9 cd</td>
<td>280.6 e</td>
<td>49.6cd</td>
<td>470.7 e</td>
</tr>
<tr>
<td>2.5% Organic matter + <em>T. harzianum</em></td>
<td></td>
<td></td>
<td>20 c</td>
<td>287.2 d</td>
<td>53c</td>
<td>477 d</td>
</tr>
<tr>
<td>5% Organic matter + <em>T. harzianum</em></td>
<td></td>
<td></td>
<td>20.6 c</td>
<td>294.57 c</td>
<td>57.7b</td>
<td>490.5 c</td>
</tr>
<tr>
<td>10% Organic matter + <em>T. harzianum</em></td>
<td></td>
<td></td>
<td>22ab</td>
<td>303.07 b</td>
<td>66.6 b</td>
<td>500.4 b</td>
</tr>
<tr>
<td>20% Organic matter + <em>T. harzianum</em></td>
<td></td>
<td></td>
<td>23.3 a</td>
<td>306.33 a</td>
<td>69.3 a</td>
<td>550 a</td>
</tr>
</tbody>
</table>

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. Salisbury 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. harzianum*, 5% + *T. harzianum*, 10% + *T. harzianum*, 20% + *T. harzianum* gave 2.907, 3.193, 3.457, 3.767 and 4.057 mg/plant, was is higher than control treatment without organic matter 2.613 mg/plant. This shows the viability of organic material + *T. harzianum* used in the study, and influence on the plant also supplied potassium and also won for phosphorus and nitrogen.
Tables 4. The effect of organic matter level and T. harzianum on concentration of phosphorus, potassium and nitrogen in cucumber plant

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

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. harzianum 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 ×104 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 exoenzymes as these enzymes consider hydrolysis enzyme, and digest these materials into simple sugars and turning them into 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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Efficiency Of Organic Matter Levels And Bio Fungus Trichoderma Harzianum on cucumber Plant

Reference


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