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# Study on the effect of *Trichoderma harzianum* on growth parameter of cucumber (*Cucumis sativus* L.) infected with R. Solani

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## Abstract

The aim of investigation was to confirm the suppressive activity of Trichoderma on Rhizoctonia and act as plant growth promoter. The study was carried out on cucumber on different parameter of 7, 14, 21, and 28 day's duration. The parameters are shoot length, root length, leaf area and chlorophyll content. The experiment conducted in sand infested with R. solani and inoculated with T. *harzianum*. A consistence increases of root length, shoot length and leaf area was observed. The chlorophyll content increased 28% on Trichoderma treated plant.

## Introduction

Biocontrol is a promising tool to maintain current level of agricultural production (Junaid et al 2013, Handlesman *et al*. 1996) while reducing the release of polluting chemical pesticide to the environment (Lecomate *et al*., 2015). Trichoderma acts as a biocontrol agent (Naher et al 2014, Saba *et a.l* 2012, Tran N Ha 2010, Nosir 2016) and based on the reduction of inoculum or of pathogenic activity due to natural presence of one or more organism. The anamorphic fungal gens Trichoderma Hypocreals, Ascomycota is cosmopolitan in soil and decaying wood and other form of plant organism (Samuels 1996, Klein and Eveleigh, 1998 ). Trichoderma is inhabitant of rhizosphere contribute to control of many soil borne plant disease caused by fungi (Zhang *et al*., 2012,Chet, 1990, Chet *et al.*. 1991, Sharvan Kumar *et al.,* 2017, Amira *et al.,* 2017). Trichoderma result from different mechanism acting synergistically to achieve disease control (Junaid *et al.,* 2013). these involve competition for nutrient and living space with plant pathogenic organism, the direct attack and distruction of pathogen (antagonism (Abbas *et al.,* 2017), mycoparasitism and promotion of plant beneficial process such as plant growth and induction of systemic and localized resistance (Elsharkawy *et al.,* 2013,Vitti *et al.,* 2017)

Rhizoctonia solani is one of the most important soil borne fungal pathogen which develop both in cultured and non-cultured soil. Rhizoctonia causing disease in different crops such as rice, tomato, cucumber etc. (Sun *et al.,* 2015). It is basidiomycetes fungus and fungus produce the sexual spores basidiospores. In nature Rhizoctonia solani reproduce asexually and exist primarily on vegetative mycelium and or sclerotia. The sexual fruting structure and basidiospores (i.e. teleomorph) were first observed and described in detail by Prilliexus and Delacroiz in 1891. The sexual stage of R. solani is now known as Thanatephorus cucumeris (Hawksworth *et al*. 1995, Alexopoulus et al 1996). R. solani primarily attack below ground part such as the seeds, hypocotyl and roots. Almost Rhizoctonia disease are initiated by mycelium are or sclerotia. Rhizoctonia root rot is an imprtant isease of nursery crop vegetable and bean etc. (Adam 1988, Weller *et al.,* 1986; Hall, 1991, Zheng *et al*., 2011). Initial symptom on plant appears on root hypocotyl as small, elongated sunken reddish-brown lesion (Ajayi *et al*., 2017). The canker enlarges with age, become darker (Wolksi *et al.,* 2005). Trichoderma is cosmopolitan in soil and vegetable and it is a component of an integrated disease management (Naher et al 2014) and effective to control Rhizoctonia root rot (Gveroska *et al.,* 2011, Hicks *et al*., 2014).

## Material and Methods

**Isolation of *Rhizoctonia solani*-** Rhizoctonia solani causal agent of root disease of cucumber (*Cucumis sativus* L.) was isolated from roots of disease cucumber plant. Cucumber plant showing symptoms of root rot were collected from the field situated near by the agricultural farm of S.M.M.Town P.G.College. Infected plant uprooted with the help of sterilized stapula, kept in a poly bag and brought to the laboratory. The root were segmented (10mm) and disease pieces were washed thoroughly four to five times with sterilized distilled water (SDW),surface disinfected with 1 % NaOCl solution and 5% ethanol for 2-3 minute and washed twice in SDW. The root segment were place on acidified potato dextrose agar plates (APDA, pH 5.6, 3 pieces plate) and incubated at 28+-2° C. After 4 days of incubation the developing colonies around the root piece were transferred on the fresh APDA plates. Ten different fungal isolates developing were isolated, purified and identified. On the basis of physiological, biochemical properties five isolates were identified as *Rhizoctonia solani*. With the help of Pathogenecity test of Rhizoctonia solani which showed the positive result, were again tested for pathogenicity to prove Koch's postulates and used for further study. *Trichoderma harzianum* obtained from screening of antagonist was grown on Potato Dextrose Agar (PDA) at 28± 2°C. Conidia were harvested with the help of nylon brush from 7 days old PDA plates flooded with cold phosphate buffer (PB; 0.1 M; pH 7). For growth response experiments inoculum of *T. harzianum* was prepared in synthetic medium (Okon *et. al.,* 1973). One ml of conidial suspension (ca. 10 power 9 conidia) was used as inoculum for 100 ml of SM in 250 ml

Earlenmyer flasks. The flasks were incubated for 24 h at 28±2 °C on a rotatory shaker at 150 rpm. The mycelia were collected by centrifugation (10000 g; 15 min), washed twice with sterilized distilled water and used as inoculum.

**Growth response measurement**

Effect of T. harzianum on growth of cucumber was studied under greenhouse. Inoculum of T. harzianum was applied a wheat bran preparation (Sivan et al.,1984).The wheat bran mixture (1:1 v/v)was adjusted to 40% moisture (w/w) and autoclaved in autoclavable polythene bags (50 × 50 cm) for 1 h at 121° C on three selective days. The sterilized wheat bran mixture was then inoculated in growth chamber for 14 days at 30 °C (Inbar and Chet, 1994). The inoculum was mixed with raw red sandy soil (ph 7.3, 78.3% sand, 5.8% silt, 15% clay, 0.3% organic matter, 0.02% N, 0.06 % K, 0.01% P and 0.03% extractable Fe) to a final inoculum density of $10^4$, $10^5$, $10^6$ CFU g-1. The inoculated soil mixture was transferred to pot (15 cm dia $^{-1}$; 500 g pot$^{-1}$). Controlled pot was treated with equal quantity of autoclaved wheat bran preparation. Surface disinfected wheat bran were sown in pot (6 pot$^{-1}$) containing T. harzianum inoculated sandy soil and kept under greenhouse condition ( 28-30 ° C day and 24°C night). The pots were waterded daily (25 ml SDW and once in a week poured Knop's nutrient solution (20 ml). Each treatment was sampled from 6 pots and 6 plants were chosen at random from each pot. Growth response of plant were measured for shoot and root length, leaf area and chlorophyll content of each plant 15 days after incubation with *T. harzianum* isolates. The plants were also assayed for the development of root system determining complete root length per plant by computer software Delta Scan™. The chlorophyll content in leave was estimated by the method given by Arnon (1949).

## Results

Increase in plant vigour and growth of cucumber plants grown in soil inoculated with Trichoderma was recorded in green house experiment. A consistent increase in shoot length and root length, leaf area was noticed throughout the experiment. The average shoot length of plant grown in inoculated soil was 15.4, 39.5, 49.4 & 54.6 after 7, 14, 21 and 28 days seedling emergence. Where it was 7.6, 21.1, 26.3, and 39.1 cm in controlled plants. The leaf area of first and second leaf after 28 days was 8.51, 7.1 in treated plant while 5.7, 4.82 cm$^2$ in control plant respectively.

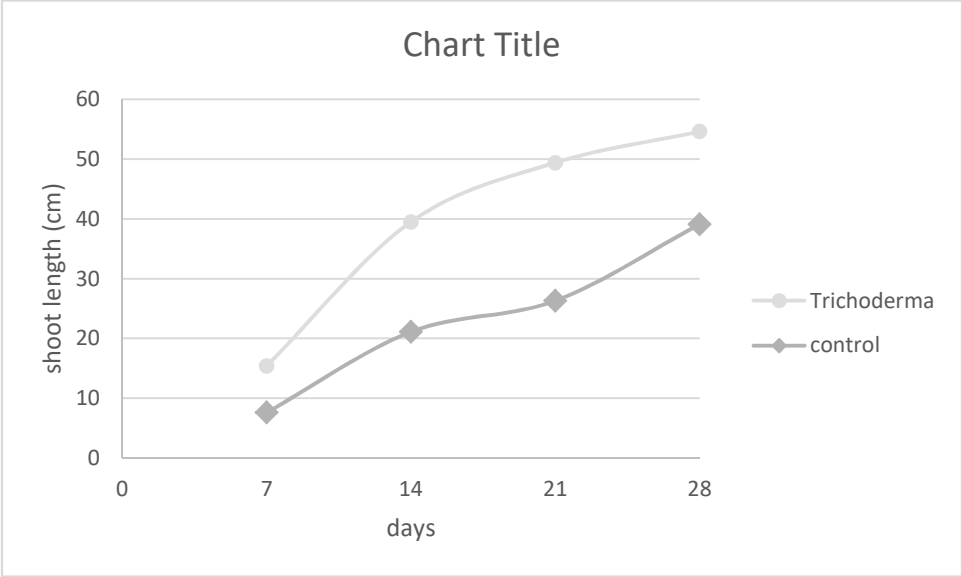


**Fig.:** Growth response of *Tricoderma harzianum* in cucumber plants grown in green house. Shoot length values are mean of 15 replicates of S.D. all values differ significantly (p=0.01) compared to their respective control.

**Table 1: Growth response (Leaf area) in cucumber plants grown in greenhouse**

| **Days** | Treatment | Leaf area($cm^2$) First leaf | Second leaf |
|---|---|---|---|
| 7 | Control | 2.4±0.9 | 1.6±0.4 |
| | Trichoderma | 3.59±2.1 | 2.7±0.4 |
| 14 | Control | 3.19±1.6 | 4.3±0.5 |
| | Trichoderma | 4.71±3.5 | 5.6±1.3 |
| 21 | Control | 3.5±1.0 | 4.57±1.1 |
| | Trichoderma | 7.51±0.7 | 6.5±3.4 |
| 28 | Control | 5.7±2.5 | 4.82±3.5 |
| | Trichoderma | 8.51±5.0 | 7.1±4.9 |

The development of root system was evaluated after one month of emergence measuring cumulative root length (Table-2). The plant grown in Trichoderma inoculated soil exhibited a 38% increase in cumulative root length.

**Table 2: Measurement of development of roots of cucumber plants**

| Treatment | Measurement |
|---|---|
| Trichoderma | 20.5±5.3 |

The measurement was taken after 28 days, values are mean of 20 replicates,± S.D. All values differ significantly (p=0.05) compared to their respective controls. A significant increase (p=0.05) increase in chlorophyll content was also recorded in the plant grown in Trichoderma inoculated soil 28% in total chlorophyll was recorded.

**Table 3: Measurement of Chlorophyll in the leaves of cucumber**

| S .No. | Treatment | Chlorophyll |
|---|---|---|
| 1. | Control | 168±3 |
| 2. | *T. harzianum* | 215±2 |

**Discussion**

Increased growth response in cucumber (Saba et al 2012) has been demonstrated following application of Trichoderma spp. Under greenhouse / field trial condition ( Baker 1989; Kleifeld and Chet, 1992 ;0usley *et al*.,1994; Inbar *et al*., 1994 ; Yedidia *et al*.,2001) The result presented here also demonstrated the significant increase in growth of cucumber plants for each of the parameter i.e. plant height. chlorophyll content, under green-house condition ( Khan *et al.,* 2005,Snjezana *et al,* 2012, Ahmed *et al,* 2017) ). The cumulative root length increased by 2- 2.5 fold. It has been suggested that T. harzianum might affect plant growth as a result of their ability to influence plant harmones and vitamin (Baker 1989, Kleifeld and Chet 1992, Sarvankumar *et al,* 2017). Such substance could influence the early stage of plant growth with better development of plant roots. The enhancement in root total area and growth rate enables the plants to explore a greater volume of soil due to an increase in no. of active site of uptake per unit area.Thus they might be able to sequester more phosphate and other mineral ions liberated as a result of solubilization by microorganism. They colonize plant roots they invade the superficial layers of the root, but do not penetrate further, at least in the part because they elicit plant defence reactions. Therefore, although Trichoderma spp.Probably have an intrinsic ability to attack plants, they are usually avirulent.The plant defence reactions can become systemic and protect the entire range of pathogens and diseases, even when Trichoderma spp. grow only on the roots(Yedidia *et al.,* 2000). This root colonization also increases the growth of roots and of the entire plant. Thereby increasing plant productivity and the yield of reproductive organs.

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