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# Native *Trichoderma* isolate suppresses damping off disease of chilli

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A pot experiment was undertaken to study the efficacy of a *Trichoderma* isolate native to Medziphema, Nagaland against damping-off pathogen of chilli at the Department of Plant Pathology, Nagaland University using Completely Randomized Design. The investigation was conducted during the period of December 2017 to April 2018. The treatments applied include seed, soil and seedling dip. No damping-off was observed in *Trichoderma*-treated plants whereas highest per cent damping-off recorded was 14.4% pre-emergence and 62.82% post-emergence with highest plant mortality of 65.82% from control. The maximum per cent germination was observed from seed treatment (80.8%) followed by soil application (75.2%) and minimum from control (59.2%). The treatments with *Trichoderma* also increased plant growth and recorded maximum height in plants (73.06 cm), fresh weight (9.51g), number of leaves (8.75), number of branches (4.25), leaf length (12.03cm), leaf width (5.04cm), leaf area (38.98 $cm^2$) and total yield (17.82g) with soil treatment as compared to control. No yield was recorded from control during the period of investigation. Therefore, the *Trichoderma* isolate under present study effectively reduced damping-off as well as gave positive effect on plant growth and yield of chilli in Nagaland region.

**Key words :** *Trichoderma*, damping-off, *Rhizoctonia solani*, chilli, plant growth, yield

## INTRODUCTION

Damping-off disease causes pre-emergence decay of seed and seedlings. The post-emergence mortality of seedlings at soil level is more prominent. There are quite a number of fungi that contribute to damping-off of various crops. Some of the most common or predominant genera include *Pythium* spp., *Rhizoctonia solani*, *Sclerotium rolfsii*, *Fusarium* spp. and *Erwinia* spp. (Uddin *etal*. 2011). The disease is serious in nurseries when there is over-crowding, over watering, poor ventilation, damp and cloudy weather.

Seedlings during first two weeks of emergence are more susceptible. The ultimate result of damping-off disease is death of seedlings. One of the crops that are greatly affected by this disease is chilli. Chilli seedlings are usually raised in nurseries before transplanting to the main field, which expose seedlings to favourable environmental conditions for damping-off. It is one of the most prevalent diseases in chilli which is responsible for 90 percent of plant death (Zagade*etal*. 2012). It is evident that high mortality rates in seedlings can lead to huge economic loss. Therefore, it is highly recommended that preventive measures should be taken to manage this disease through seed and soil treatments either with chemicals or bio-agents. However, the phytotoxicity problems of fungicide residues have resulted in environmental and human health hazards (Zagade*etal*. 2012). There are also concerns about development of new fungicide-resistant pathogen racesdue to indiscriminate use, which further discourages their use. Also, chemical control may not always be available in every region or if available are much lesser economical than other methods. This has led to the need for an alternative method which is environmentally safer, economical and sustainable and the use of bio-agents is realized to provide the need (Brimmer and Boland, 2003). Similar constraints in India including NE region has led to development of bio-control strategies with employment of *Trichoderma*, the most commonly

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studied fungal BCA which is commercially marketed as a biopesticide (Harman, 2000).

This present study emphasized on evaluating the potential of *Trichoderma* isolate indigenous to Medziphema, Nagaland on damping-off pathogen, particularly *Rhizoctoniasolani* in chilli as well as the beneficial effects on host plant through seed, soil treatments and seedling dip methods.

## MATERIALS AND METHODS

### *Microbial cultures*

*Rhizoctoniasolani* was the pathogen used which wasobtained from the Department of Plant Pathology, School of Agricultural Sciences and Rural Development, Medziphema. The *Trichoderma* isolate used was also locally isolated from the campus and cultured in the laboratory of the department.

### *Multiplication and mass culture of the pathogen*

The damping-off pathogen *Rhizoctonia solani* was mass cultured following Papavizas and Lewis (1985). The pathogen was first multiplied on PDA media. A Maize-Meal-Sand Medium was prepared using 40g maize, 960g of clean sand and 200ml of distilled water. It was divided and filled in conical flasks at 200g per 500ml volume flask (Adan *etal*. 2015) and sterilized by autoclaving. Then, this substrate was allowed to dry and cool down. Inoculation was done by first cutting out 5mm discs from 3-4 days old culture of the pathogenusing a sterilized and dry cork-borer from the culture plate. Finally, with the help of a sterilized inoculation needle the discs were added to flasks @5-7 discs/ flask and the flasks were incubated at 27±1$^{o}$C for 5-7 days.

### *Artificial inoculation*

The soil was inoculated with *R.solani*grown on Maize-Meal-Sand Medium @5g/kg soil seven days before inoculation of the *Trichoderma* formulation or before sowing of the chilli seeds (Khair*etal*. 2010).

### *Nursery raising*

A nursery bed was prepared in a green house. The soil was mixed with sand at a ratio of 1: 2 (w/w) and amended with FYM @5 kg/m$^2$ area. The bed was also raised by 15-20 cm above the ground to prevent water logging. The bed size was maintained at 2 x 1 m$^2$. The seeds were then sown in lines with a spacing of 10cm between each row and at a depth of 1-2cm. Light irrigation was done right after sowing and thereafter daily during evening hours.

### *Mass culture and formulation of Trichoderma isolate*

Liquid mass culture method (molasses 30g, yeast extract 5g, distilled water 1000ml) as followed by Papavizas and Lewis (1989) was used to multiply the*Trichoderma* isolate under study.

The medium was dispensed into 250ml conical flasks @50ml per flask with the help of a measuring cylinder thereby acquiring maximum surface area and autoclaved. After the medium cooled down, the mother culture was inoculated into the flasks by cutting 5mm diameter mycelial discs @5-7 discs/flask and incubated for 7-10days at room temperature (25±2$^{o}$C).

When the mycelia have covered the entire surface and turned dark green in colour(Fig. 1),harvesting was done by taking out the mycelial mat and it was ground in a mixer. Around 4-5 ground mats were mixed with 1kg talcum powder or at the ratio of 1:9 (w/w). This powdered formulation was used in the pot culture experiment under study. The formulation used had more than 2 × 10$^{6}$cfu/ml.

### *Treatments with Trichoderma*

#### Seed treatment

The chilli (*Capsicum annuum*) seeds (*cv*. PusaJwala) were treated with *Trichoderma* formulation usually 24 hours before sowing (Mudyiwa *et al*., 2016) @10g/kg seeds. A paste or slurry of the *Trichoderma* formulation was made with 1g in 1-2ml of water and mixed with the 100g of seeds uniformly. The seeds were soaked in the formulation for at least 30 minutes and then shade dried for 6 hours (Uddin *etal*. 2011).

#### Soil application

Before sowing, the damping-off pathogen (*R. solani*) was inoculated into the soil and kept for

seven days before application of *Trichoderma* formulation and regularly irrigated. Then the antagonist was mixed @10-20g/kg soil (Adan *etal*. 2015) and incubated for seven days with regular watering to allow adequate moisture accumulation and growth of *Trichoderma* in the soil (Uddin *etal*. 2011; Khair*etal*. 2010).

**Seedling dip**

A suspension containing 5g of the *Trichoderma* formulation in 500ml of water was prepared. Secondly, the chilli seedlings of 15-20 days old (Thakur and Tripathi, 2015) were treated by dipping the roots in the suspension for 15 minutes and then transplanted.

***Pot experiment***

A pot experiment was conducted following Completely Randomized Design (CRD) taking eight treatments with five replications each. Twenty-five number of chilli seeds (*cv*. PusaJwala) were sown in each pot of dimension 22cm (diameter) X 19cm (height). For seedlings, 15 of them were planted in each pot. While filling the pots, dried and well decomposed FYM was mixed with soil @ 1: 1 (w/w) ratio. Cultural practices such as weeding, irrigation, thinning was done as often as required throughout the planting season. Two plants were tagged as 1 and 2 for observation purposes.The number of seedlings/pot was recorded and compared with number of seeds sown to obtain the germination percentage. Disease incidence was recorded at different intervals such as 10 DAS, 15 DAS and 20 DAS by counting the number of seedling stand and seedling death per treatment and control and mortality percentage was worked out. The fruits were harvested at maturity by judging their colour of dark green to tainted red. The fruits were harvested by handpicking with clean hands at different intervals for different treatments since their maturity varies with time.

Observations were recorded at 15 days interval at 30 DAS, 45 DAS, 60 DAS, 75 DAS, 90 DAS and 105 DAS.Observations on growth parameters like fresh weight of plants, plant height, number of leaves, number of branches, leaf length, leaf width, leaf area, yield per plant were recorded. The data collected were analysed by the ANOVA method (Panse and Sukhatme, 1995) and the significance of different sources of variation were tested at 5% level of significance.

**RESULTS AND DISCUSSION**

It has been observed that *Trichoderma* performed better when isolated from and used locally (Bunker and Mathur, 2001), that is, higher efficacy is brought about by isolates that were obtained natively and applied in the same region. The efficacy of *Trichoderma* depends on the crop and the mode of application (Harman, 2000). Therefore, an effort to exploit the native potency of *Trichoderma*isolated from Medziphema, Nagaland was carried out in this study in respect of antagonistic potential on *R. solani* as well as beneficial effects on chilli plant. The results of this investigation are presented and discussed here.

***Characters of the Trichoderma isolate***

The colony characters of the *Trichoderma*sp. isolatedon Potato Dextrose Agar (PDA) in the present investigation were observed. The upper surface of the colony grown on Petri dish was dark green coloured and the lower surface was bright yellow. Initially the colour was white which later turned pale yellowish green and finally dark green due to conidia formation. The fungus formed 2-3 concentric rings with a cluster of green conidia around the point of inoculum. Conidia are produced in abundance and covered the entire plate within five days(Fig.2).

Microscopic characters include hyaline mycelium from which hyaline and branched conidiophores of 4-5µ wide arise. Phialides of two to three were borne terminally on conidiophores and were non-verticillate. Each phialide measured about 4.8-6.7 × 2.4-3.2µ. The conidia were borne singly at the tip of phialides. The size of conidia was 3.5 × 1.7-2.2µ.

***Suppression of damping-off of chilli plant***

The observations on the pre-emergence and post-emergence damping-off are presented in Fig 3a and b.As seen clearly, no damping-off was seen in *Trichoderma*-treated plants and therefore no death occurred. The highest percent damping-off was observed in untreated-plants with 14.1% and 62.82% as pre-emergence and post-emergence damping-off respectively (Figs. 4 and 5)which resulted in high rate of mortality of about 65.82% (Fig 3c).

**Fig. 1 :** Mass multiplication of native *Trichoderma* isolates in conical flasks

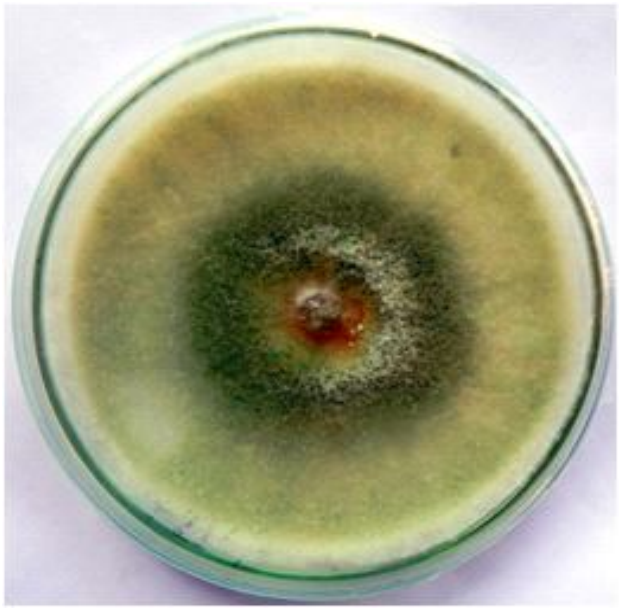

**Fig. 2 :** Growth of native *Trichoderma* isolate under study (5 days after inoculation)

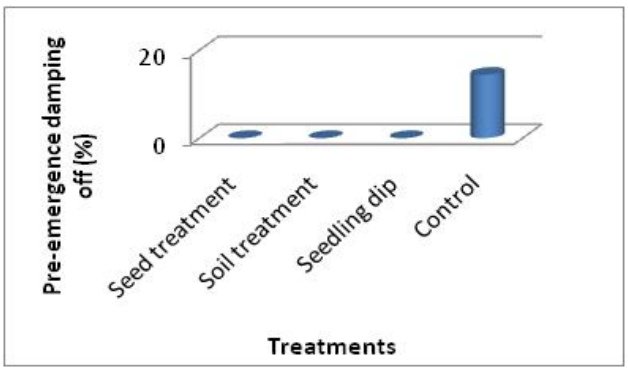


**Fig 3a :** Effect of *Trichoderma* on pre-emergence damping-off (%) pathogen in chilli seedlings

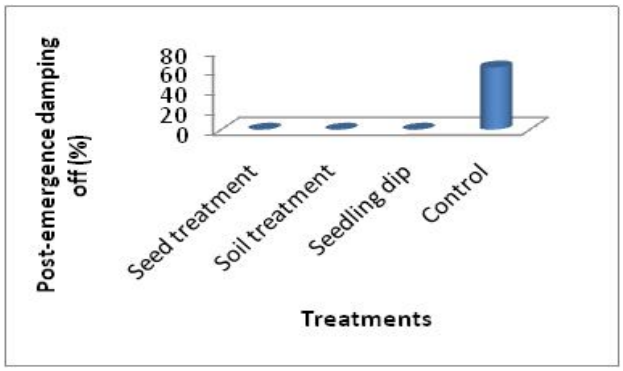


**Fig. 3b:** Effect of *Trichoderma* on post-emergence damping-off (%) pathogen in chilli seedlings

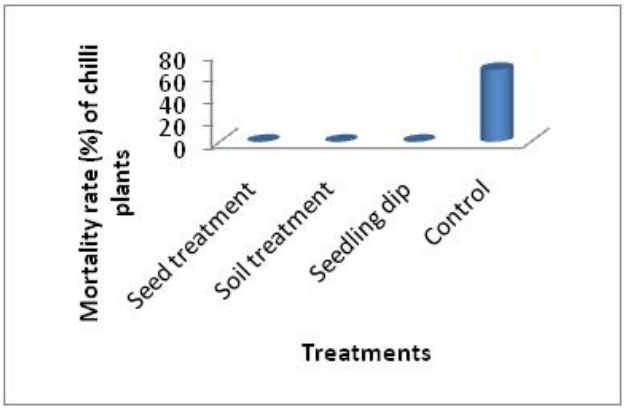


**Fig. 3c:** Effect of *Trichoderma* on mortality rate (%) of chilli plants

**Fig. 4 :** Pre-emergence damping off of chilli

**Fig. 5 :**Post-emergence damping off of chilli

This result confirms the findings of Uddin *et al.* (2011) and Singh *et al*. (2014) who reported the control of damping-off in chilli and tomato by *Trichoderma* spp. This may be due to the production of various chemicals responsible for the antagonistic effect of *Trichoderma* which successfully controlled *R. solani* or the direct killing of the pathogen by *Trichoderma* as reported by Rini (2005). It may also be related with increased protection against diseases by increasing the content of macro-elements like potassium, magnesium and calcium and micro-elements of iron which play important roles in plant defense mechanisms as observed by Khair *et al*. (2010). Harman *et al*. (2004) reported that *Trichoderma* can also induce systemic resistance in plants.

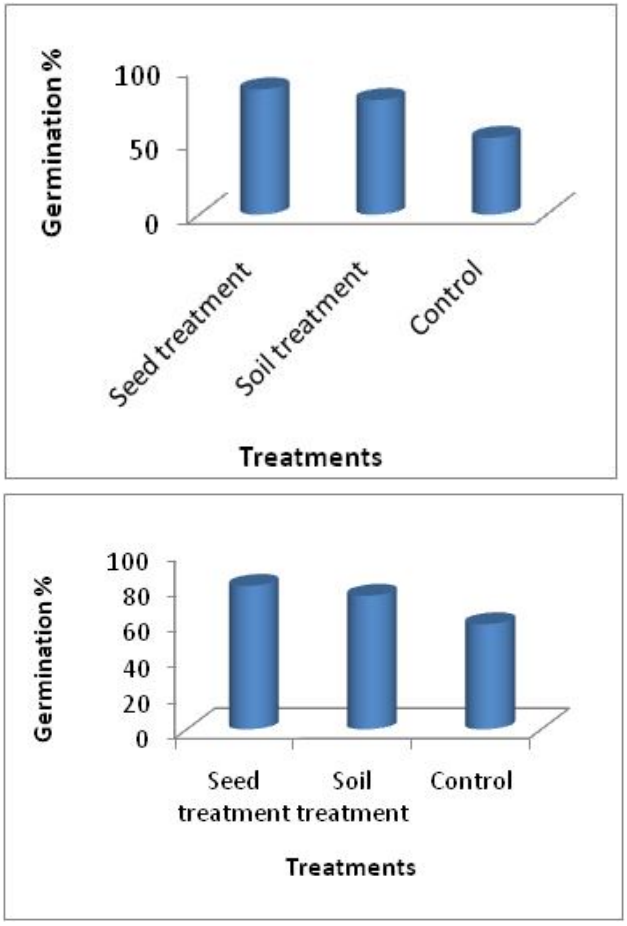


**Fig. 6 :A:** Effect of *Trichoderma* on germination percentage (%) of chilli infected with *R. solani;* **B:** Effect of *Trichoderma* on germination percentage (%) of chilli (un-inoculated)

## Effect of *Trichoderma* on seed germination of chilli

It is evident that highest germination was achieved in seed treatments with 84.8% and 80.8% in inoculated (Fig. 6 a) and non-inoculated (Fig. 6 b) soil respectively. However, both seed treatment and soil application had significant effects on germination while control had the least emergence with only 52% (inoculated) and 59.2% (non-inoculated). Also, treated seeds were seen to emerge two to three days earlier than untreated seeds. Similar results were reported by Asaduzzaman *et al*. (2010) and Joshi *et al*. (2010). They concluded that seed germination of chilli being greatly affected by seed treatments with *Trichoderma*. This indicates that *Trichoderma* has stimulatory effects and significantly enhances seed germination in plants.

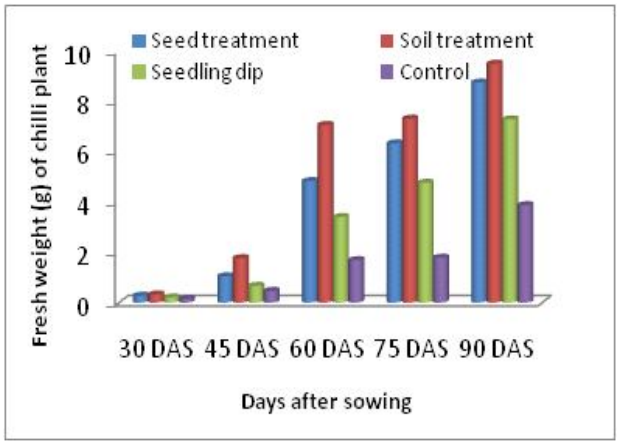


**Fig. 7 :** Effect of *Trichoderma* on fresh weight (g) of chilli plant

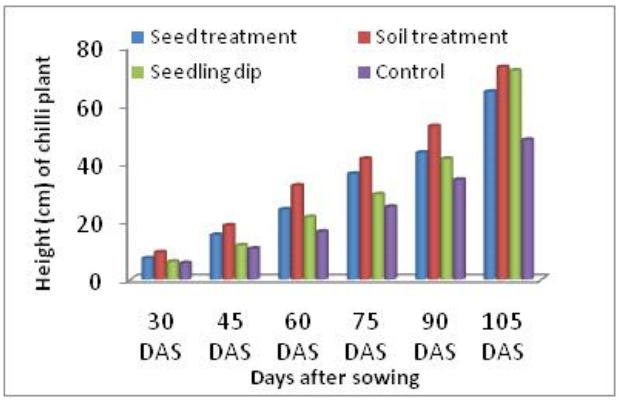


**Fig. 8 :** Effect of *Trichoderma* on height (cm) of chilli plant

## *Effect of Trichoderma on fresh weight of chilli plant*

The results of *Trichoderma* treatment on fresh weight of chilli plants are presented in Fig.7 and it shows that fresh weight of chilli plants was significantly increased in *Trichoderma*-treated plants as compared to control with soil application indicating to be significantly superior to seed and seedling treatments. Soil applications recorded maximum fresh weight of 9.51g followed by seed

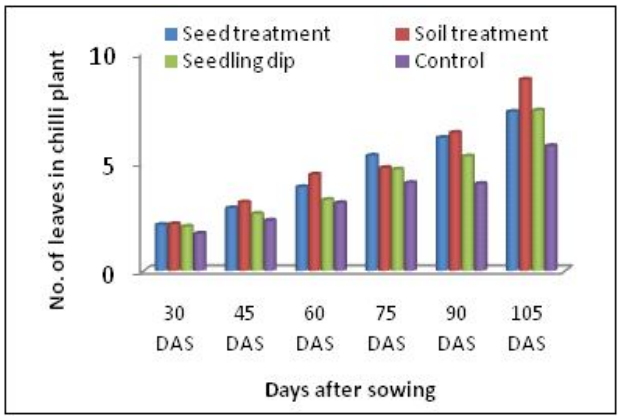


**Fig. 9 :** Effect of *Trichoderma* on number of leaves in chilli plant

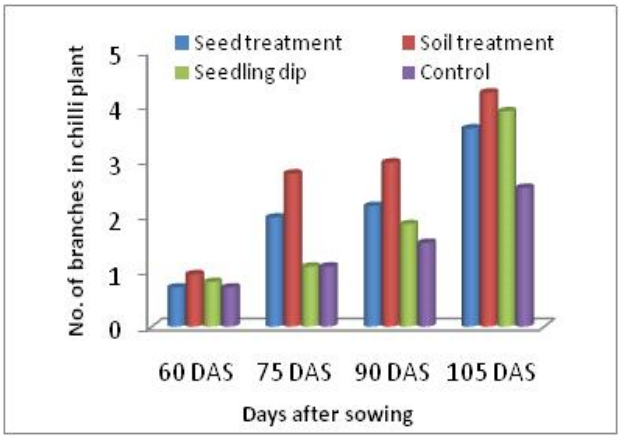


**Fig. 10 :** Effect of *Trichoderma* on number of branches in chilli plant

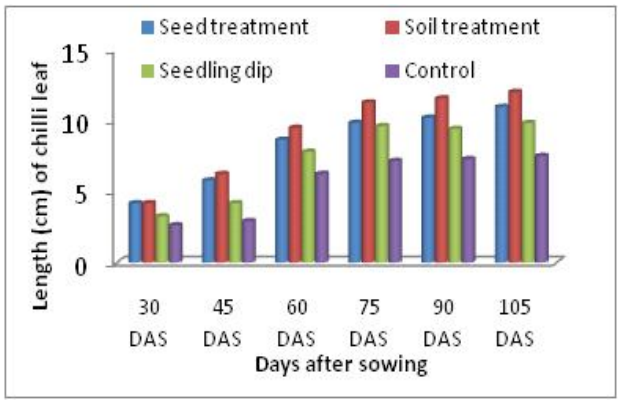


**Fig. 11 :** Effect of *Trichoderma* on length (cm) of chilli leaf

treatment (8.76g) at 90 DAS, while the minimum fresh weight recorded was 3.88g from control. Seedling dip treatment recorded the fresh weight of 7.29g which is significantly lesser than seed and soil treatments though higher than control. Khair*et al*. (2010) reported that *Trichoderma* significantly increased fresh weight of bean plants. Similar results were reported by Uddin *et al*. (2011) where higher fresh shoot and root weight as well as fresh seedling weight in egg plant and tomato plants

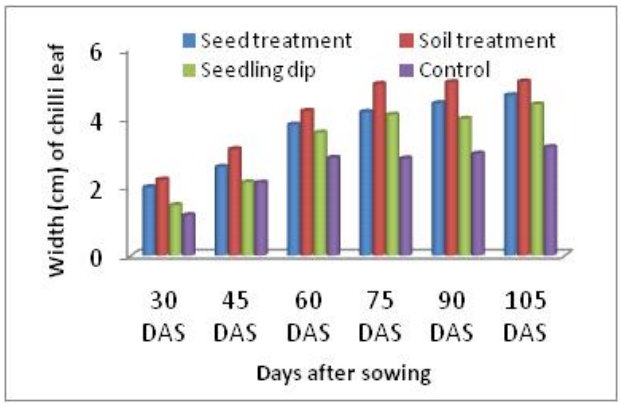


**Fig. 12 :** Effect of *Trichoderma* on width (cm) of chilli leaf

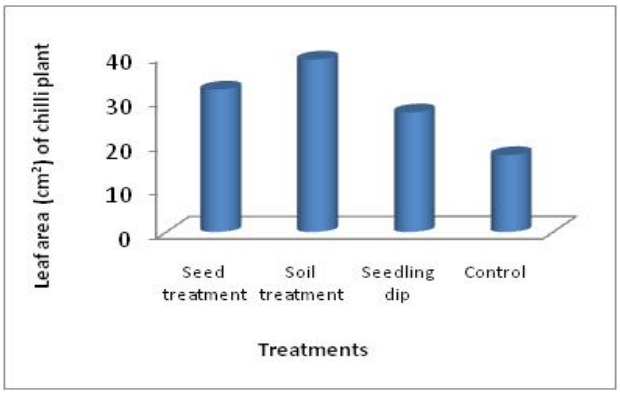


**Fig. 13 :** Effect of *Trichoderma* on leaf area ($cm^2$) of chilli plant

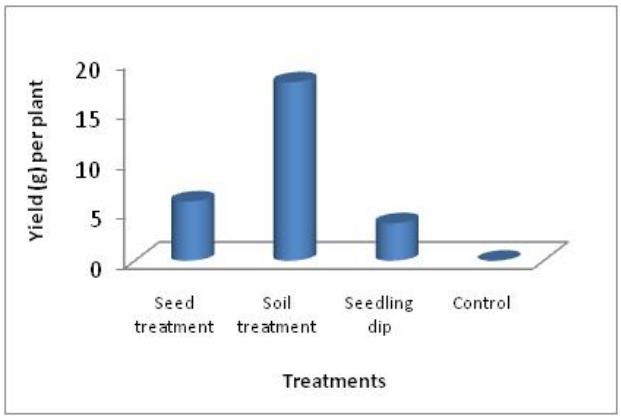


**Fig. 14 :** Effect of *Trichoderma* on yield of chilli plant

when treated with *Trichoderma*. Theseabove studies confirm the findings of the present study. This stimulatory effect of *Trichoderma* may be due to its interaction with plants in the root zones forming symbiotic association thereby increasing plant nutrient content in the soil by breaking down complex organic matter; and nutrient exchange (Howell, 2003; Harman, 2006).

### *Effect of Trichoderma on height of chilli plant*

In all the treatments, significant increase in plant height was seen(Fig.8).There is no significant

difference among the treatments except between soil application and seed treatment. The maximum plant height recorded was 73.06cm from soil treatment followed by 71.87cm from seedling dip which is statistically at par with seed treatment (64.61cm) at 105 DAS. However, the minimum height of chilli plants was recorded from control with 48.03cm at 105 DAS. It is, therefore, evident in this present study that the chilli plants treated with *Trichoderma* were significantly taller compared to untreated-plants. This may be due to the ability of *Trichoderma* to produce phytohormones such as Indole Acetic Acid and Gibberellic acid that contribute to greater plant growth as reported by Chawdappa*et al*. (2013). It is also known that plants treated with *Trichoderma* have better nutrient uptake as suggested by Saba *et al*. (2012). This confirms the findings of Joshi *et al*. (2010) and Subash *et al*. (2013) where treated chilli plants were taller than untreated plants. Khair*et al*. (2010) also reported the increase in plant height of beans treated with different strains of *Trichoderma* and no significant difference between the treatments. Several mechanisms may be involved such as production of plant hormones, vitamins, conversion of non-utilizable compounds into available forms for plant to take, increase in nutrient uptake and translocation of minerals and solubilisation of insoluble minerals like rock phosphate which all contributes to increased growth rate in *Trichoderma*-treated plants as suggested by various workers (Manoranjitham *et al*.,2001; Champawat and Sharma, 2003; Srivastava, 2004; Shabir and Rubina, 2010 and Uddin *et al*.,2011).

***Effect of Trichoderma on number of leaves in chilli plant***

Soil treatment recorded the maximum number of leaves (8.75) per plant at 105 DAS (Fig.9) as compared to control (5.71). Seedlingand seed treatments recorded 7.34 and 7.28 number of leaves respectively which are statistically at par. It is apparent that *Trichoderma*-treated chilli plants has increased number of leaves as compared to untreated thus confirming the findings of Subash *et al*. (2013). Similar increase in leaf number was reported by Khair *et al.* (2010) in beans and Roslee *et al*. (2012) in cauliflower plants treated with *Trichoderma*. Furthermore, Najam *et al*. (2014) suggested that the ability of *Trichoderma* to enhance leaf number may be due to its capability to act through several mechanisms and induce resistance to various abiotic stresses such as temperature, water deficit, salt and osmotic stress. Similar findings were made by Bae *et al*. (2009) who reported that *Trichoderma* can significantly increase the ability of cocoa plants to tolerate water scarcity and increase root growth as well which contributes to increased leafnumber.

***Effect of Trichoderma on number of branches in chilli plant***

According to the present study, the number of branches was also observed to be higher in treated-plants compared to untreated-plants (Fig.10). Soil treatment recorded the highest number of branches (4.25) followed by seedling treatment (3.91) and seed treatment (3.60) at 105 DAS, where the latter two are statistically at par. The minimum number of branches was recorded from control with 2.52 at 105 DAS which is significantly lower than that of all the treatments. This confirms the findings of Khair*et al*. (2010) who reported the significant increase in number of branches in all *Trichoderma* treatments. Better and fast growth in plants can result in enhanced development of various plant parts and higher growth leads to more branching. This may be due to the action of *Trichoderma* in growth enhanc-ements which also involves the decomposition of organic materials and solubi-lisation of insoluble compounds to make more minerals available to plants in forms which can be utilized by them (Manoranjitham *et al.*,2001 and Uddin *et al*., 2011).

***Effect of Trichoderma on length and width of chilli leaf***

The observations regarding the length and width of chilli leaf are presented in Figs. 11 and 12 respectively.The data reveal that the *Trichoderma*-treated plants have longer leaves than untreated-plants. The maximum length of leaf was recorded in soil treatment (12.03cm) followed by seed treatment (10.98cm) and seedling dip (9.84cm) at 105 DAS. The treatments show significantly different results and soil treatment is superior to all treatments. The minimum leaf length recorded was 7.52cm from control which is significantly lower compared to all the treatments under study.

It was also observed that the leaves in treated-plants are wider than those in untreated-plants. The maximum width recorded was 5.04cm from soil

treatment followed by 4.64 cm from seed treatment and 4.38cm from seedling dip treatment while the minimum of 3.14cm was recorded from control at 105 DAS. The size of the leaves depends on the ability of the plants to grow vigorously during vegetative stage till flowering stage. Also, early planting date or in this case, early emergence leads to better growth because the plant had enough time to complete the vegetative stage which resulted in bigger leaves. Since *Trichoderma* can act as a bio-fertilizer and increase nutrient uptake as reported by Saba *et al*. (2012), it ultimately promotes plant growth which includes vigorous and healthy plant parts as well.

### *Effect of Trichoderma on leaf area of chilli*

The observations regarding leaf area are presented in Fig.13. The maximum area of chilli leaf was recorded from soil treatment (38.98cm$^2$) at 105 DAS. The minimum leaf area recorded was 17.39cm$^2$ from control. All treatments gave significantly higher results as compared to control. Seed treatment recorded 32.198cm$^2$ while seedling dip treatment recorded 27.05 cm$^2$ of leaf area which are significantly lower than that of soil treatment. It is evident that in the present study, the plants produced bigger leaves with larger surface area upon *Trichoderma* treatment. This may be due to the vigorous growth of the plant, as discussed above, which may have been contributed by the interaction of plant roots with *Trichoderma* and its ability to promote root growth and better nutrient uptake as well as production of growth hormones and vitamins as discussed (Manoranjitham*et al*. 2001; Champawat and Sharma, 2003; Srivastava, 2004; Shabir and Rubina, 2010 and Uddin *et al*.,2011). *Trichoderma* was also reported to facilitate root colonization in host plants by production and regulation of hormonal signals (Saldajeno*et al*. 2014) such as auxin (Hoyos-Carjaval*et al*. 2009) which promotes root growth thereby promoting shoot growth and leaf growth.

### *Effect of Trichoderma on yield of chilli*

The yield of chilli fruit per plant was recorded collectively till 120 DAS. The observations on yield are presented in Fig.14.It is discernible from the illustration that the maximum fruit yield (with three harvests) was recorded from soil treatment with a total of 17.82g. From the other treatments harvesting could be done only once with a total fruit yield of 5.92g from seed treatment and 3.74g from seedling treatment. Plants in control did not produce fruits even at 120 DAS.

It can be concluded that *Trichoderma*-treated plants started fruiting earlier and hence produced higher yield than untreated plants. The early emergence led to faster vegetative growth which ultimately resulted in early maturity and fruiting of treated chilli plants. According to the present study, it is evident that *Trichoderma* plays a beneficial role which promote plant growth indicated by enhanced growth of vegetative plant parts (as discussed in previous sections) and thereby result in higher yield. This confirms the findings of Khair*et al*. (2010); Subash *et al*. (2013); Najam *et al*. (2014) and Sandham and Sinha (2016).

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