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# Effect of Oil Based Formulation of *Trichoderma* spp. on Growth Parameters of Cucumber Seedlings

**B. Sreenayana and S. Nakkeeran***

*Department of Plant Pathology, TNAU, Coimbatore – 03, Tamilnadu, India*

**Corresponding author*

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**A B S T R A C T**

Cucumber vascular wilt pathogen, *Fusarium oxysporum* f. sp. *cucumerinum* is one of the most destructive organisms that hampers the cucumber production under protected cultivation and results in huge economic loss to farmers. *Trichoderma* spp., a potent biocontrol agent screened against *F. oxysporum* f. sp. *cucumerinum in vitro* resulted that *T. virens* TRI 37 effectively inhibited the mycelial growth of pathogen to about 68.0% compared to other isolates. An oil based formulation of effective *Trichoderma* spp. was developed with initial conidial concentration of $1 \times 10^{10}$. The formulation remained stable for more than 180 days with conidial concentration of $1 \times 10^{8}$. Efficacy of oil based formulation on growth parameters of cucumber seedlings in protray experiments revealed that oil based formulation of *T. virens* TRI 37 effectively increased the shoot length (28.74 cm, 14.54 cm), root length (14.64 cm, 19.14) and stem girth (1.76 cm, 1.72cm) in comparison to other isolates in vermicompost: sand: soil (1:1:1) and coir pith medium respectively.

## Introduction

Cucumber (*Cucumis sativus* L.) is one of the important vegetable crops, widely grown around the world. The leading producers of cucumber in world are China, Russia and Turkey, while India stands 27$^{th}$ position in cucumber production. In India, cucumber is cultivated in an area of 1,07,500 ha with annual production and productivity of 1657 MT and 15.49 t/ha respectively as of 2018-19, whereas in Tamil Nadu, cucumber is cultivated in an area of 949 ha with an average production of 11,051 t/ha and with an average productivity of 11.58 t/ha and as of 2018-19. In recent days though cucumber cultivation is gaining momentum, the productivity of the crop is hampered due to the outbreak of pests and diseases in the protected cultivation. The continuous production of cucumbers in monoculture resulted in the occurrence of *Fusarium* wilt incited by *Fusarium oxysporum*

f. sp. c*ucumerinum,* which causes serious impact on cucumber cultivation all over the world (Ahmed, 2001). Monoculturing of cucumber for three continuous seasons increase the occurrence of *Fusarium* wilt as high as 70% with 10–50% reduction in the yield and thus resulting in complete crop failure (Chen *et al.,* 2010; Booth 1971; Shen *et al.,* 2008; Zhang *et al.,* 2008) leading to a significant economic loss for farmers. The management of this disease includes the use of resistant cultivars, grafting, crop rotation and soil replacement (Yu, 2001). However, chemical control, often result in environmental and food quality problems (Minuto *et al.,* 2006; Omar *et al.,* 2006). As an alternative approach, the application of biocontrol agents to suppress soil borne pathogens has been widely used. The successful survival and colonization of beneficial antagonist in host plant are essential for the biocontrol of disease caused by *F. o.* f. sp. *cucumerinum*. *Trichoderma* spp. are non-pathogenic soil-borne (free-living) fungi that colonize the roots of many plants as opportunistic, avirulent plant symbionts (Harman *et al.,* 2004).

Success of any biocontrol agent depends on the type of formulation. Formulation also affect the shelf life of a product, ability of a biocontrol organism to multiply and survive in the environment, ability to control the disease, ease of preparation and application and cost of preparation and application. Biocontrol organisms have been formulated in variety of ways like wettable powders, dry flowable formulations, dusts, granules, liquids, gels, oil based formulations, pellets, freeze dried, spray dried and vaccum dried formulations for seed treatment, foliar application and soil application. Most of the biocontrol agent formulations are carrier based containing beneficial microorganisms in a viable state deliberated for seed or soil application (Sivasakthivelan and Saranraj, 2013). The liquid fermentation derived talc formulation of *T. harzianum* with the addition of glycerol in the production medium could protect the tomato plants from *Fusarium* wilt incidence by 44-50% (Sriram *et al.,* 2011).

The current study was carried out to understand the effect of oil based formulation of *Trichoderma* spp. on growth parameters of cucumber seedlings in portray experiments. The shelf life of the oil based formulation was also studied.

## Materials and Methods

### Fungal strains

Fungal strains like *T. virens* (KU666466), *T. koningiopsis* (MF423101), *T. asperellum* (KX533985), *T. koningiopsis* (MF405092), *T. harzianum* (KX533990), *T. harzianum* (KX533990), *T. harzianum* (KX533989), *T. asperelloides* (MK981226) and *F. o.*f. sp. *cucumerinum* **(**KY495294) provided from the culture collection of Department of Plant Pathology, Tamil Nadu Agriculture University, Coimbatore and used in the following experiments.

### Screening of *Trichoderma* spp. against *F. oxysporum* f. sp. *cucumerinum*

Seven different isolates of *Trichoderma* spp. were screened against *F. o.* f. sp.*cucumerinum* F1. A mycelial disc of the pathogen (9 mm dia.) from the actively growing four day culture of the pathogen was placed at one end and a 9mm mycelial disc of the mycelium from the actively growing *Trichoderma* spp. was placed at the other end in Potato Dextrose Agar Medium (Potato – 250g, Dextrose – 20 g, Agar – 20 g and Distilled water – 1 litre). The plates were incubated at 28 ± 2$^{o}$C for seven days. The linear growth of the pathogen and antagonists were measured when the pathogen attained full growth in control plates.

Per cent inhibition of the test pathogen by the antagonistic isolates was evaluated by dual culture technique (Dennis and Webster, 1971). The radial growth of mycelium in cm of pathogen and antagonists were measured and per cent inhibition of test pathogen by the antagonistic strain was evaluated by dual culture technique (Dennis and Webster 1971). The radial growth of mycelium in mm of pathogen and antagonists were measured and per cent inhibition (PI) was calculated $PI = \frac{C-T}{C} \times 100$, where C is the growth of test pathogen (mm) in the absence of the antagonist strain; T is the growth of test pathogen (mm) in the presence of the antagonist strain. Total of three replications were maintained for each isolate and the experiment was repeated twice to confirm the results.

## Preparation of oil based formulation of *Trichoderma* spp.

Based on the *in vitro* assay, the best four isolates (*T. virens* (KU666466), *T. koningiopsis* (MF423101), *T. asperellum* (KX533985) and *T. asperelloides* (MK981226)) with high conidial production was used for subsequent studies. The emulsion contained the following ingredients *viz.,* 1% glycerol (as an osmoticant), 1% PVP, 0.5% $ZnSO_4$ (increases the shelf life), 1% Tween 20 (emulsifying agent), distilled water and coconut oil. The conidia of 4 effective *Trichoderma* spp. were mass multiplied in Potato Dextrose Agar medium for 5 days. After complete colonisation of the media, the conidia was collected by centrifuging at 6000 rpm for 5 minutes at 28±2°C. The aqueous phase was prepared by adding 1% conidia, 1% glycerol, 1% PVP and 0.5% $ZnSO_4$ in 80 ml sterile distilled water and the oil phase was prepared by adding 1% tween 20 in 20 ml of coconut oil. The oil phase was then added to aqueous phase and stirred well. The pH of the formulation was adjusted to 6.5-7.0**.** All the formulation had an initial conidial concentration of $1x10^8$ per ml. The prepared formulations were distributed in four different falcon tubes and stored at room temperature.

## Shelf life of oil based formulation of *Trichoderma* spp.

The shelf life of the oil based formulation was recorded at monthly interval. The shelf life was assessed for a period of 180 days. The population was estimated by serially diluting the sample upto $10^8$ and subsequent plating on to Trichoderma Selective Medium (TSM) at 28±2°C. After 3 days conidial count was recorded. Four replications were maintained for each isolate.

## Effect of oil based formulation of *Trichoderma* spp. on growth parameters of cucumber seedlings

The cucumber seeds cv. Green long were treated with oil based formulation of *Trichoderma* spp. ($1 \times 10^7$) as per the following treatment schedule. The treated seeds were sown in protrays containing sterilised vermicompost: soil: sand (1:1:1) and coirpith. The seedlings were inoculated with oil based formulation at 7th and 15th day.

Five replications were maintained for each treatment with 25 seedlings/replication. The plants were grown for about 35 days. The growth parameters such as root length, shoot length, stem girth and no. of leaves were recorded.

## Statistical analysis

All the experiments were analyzed independently. The treatment means were compared by Duncan's Multiple Range-Test (DMRT) (Gomez and Gomez, 1984). The package used for analysis was SPSS version 16.0.developed by IBM Corporation.

## Results and Discussion

### *In vitro* screening of *Trichoderma* spp. against *F. oxysporum* f. sp. *cucumerinum*

Seven different isolates of *Trichoderma* spp. were screened for *in vitro* antagonism against *F.oxysporum* f. sp. *cucumerinum* F1 by dual culture technique. The *in vitro* efficacy of antagonism of different *Trichoderma* isolates revealed that the growth of *F. oxysporum* f. sp. *cucumerinum* was suppressed maximum to an extent of 68.00 per cent over control by *T. virens* TRI 37 and was followed by *T. koningiopsis* TRI 41 and *T. asperellum* TRI 15 with 65.77 and 60.88 per cent inhibition respectively. *T. koningiopsis* TRI 44 had the lowest inhibition (44.88%) on the mycelial growth of *F. oxysporum* f. sp. *cucumerinum* F1. The four effective isolates was used for further studies (Table 1).

### Shelf life of oil based formulation

The population load of *T. virens* TRI 37 on zero day was 10.4 $x10^9$ cfu /ml. The final population after six months of storage was 3.1 $x10^8$ cfu /ml. The population load of *T. koningiopsis* TRI 41 on zero day was 9.9 $x10^9$ cfu /ml. The final population after six months of storage was 2.9 $x10^8$ cfu /ml. The population load of *T. asperellum* TRI 15 on zero day was 9.2 $x10^8$ cfu /ml. The final population after six months of storage was 2.9 $x10^7$ cfu /ml. Similarly, the population load of *T. asperelloides* TNAU Tad 1 on zero day was 8.8 $x10^8$ cfu /ml. The final population after six months of storage was 2.8 $x10^7$ cfu /ml (Table 2).

### Effect of oil based formulation of *Trichoderma* spp. on growth parameters of cucumber seedlings

The effect of *Trichoderma* sp. on root length, shoot length and stem girth in both medium (vermicompost: soil: sand and coir pith) were presented in table entitled on effect of *Trichoderma* sp. on growth parameters of cucumber seedlings (Table 3).

### Shoot length

Among the 10 treatments, shoot length was found to be more in the *T. virens* TRI 37 (28.74cm**)** treated plants followed by *T. asperelloides* TNAU Tad 1 (27.48cm) and *T. koningiopsis*

TRI 41 (26.38cm) seed treated plants grown in vermicompost: soil: sand (1:1:1) medium whereas shoot length was found to be more in the *T. asperelloides* TNAU Tad 1 (14.78 cm**)** treated plants followed by *T. virens* TRI 37 (14.54 cm) and *T. koningiopsis* TRI 41 (14.30 cm) seed treated plants grown in coir pith medium.

### Root length

Among the 10 treatments, root length was found to be more in the *T. virens* TRI 37 (14.64 cm**)** treated plants followed by *T. asperelloides* TNAU Tad 1 (14.58cm) and *T.koningiopsis* TRI 41 (14.58 cm) seed treated plants grown in vermicompost: soil: sand (1:1:1) medium whereas root length was found to be more in the *T. virens* TRI 37 (19.18 cm) treated plants followed by *T. asperelloides* TNAU Tad1 (19.08 cm**)** and *T. koningiopsis* TRI 41 (19.06 cm) seed treated plants grown in coirpith medium.

### Stem girth

Among the 10 treatments, stem girth was found to be more in the *T. virens* TRI 37 (1.76 cm**)** treated plants followed by *T. asperelloides* TNAU Tad 1 (1.66 cm) and *T. koningiopsis* TRI 41 (1.64 cm) seed treated plants grown in vermicompost: soil: sand (1:1:1) medium.

| Treatment details | |
|---|---|
| **T1** | Healthy Control |
| **T2** | Inoculated Control (*F. o.* f. sp. *cucumerinum* F1 inoculated soil) |
| **T3** | Seed treatment with of oil based formulation (OB) of *T. virens* TRI 37 |
| **T4** | Seed treatment with oil based formulation (OB) of *T. koningiopsis* TRI 41 |
| **T5** | Seed treatment with oil based formulation (OB) of *T. asperellum* TRI 15 |
| **T6** | Seed treatment with oil based formulation (OB) of *T. virens* TRI 37 in *F. o.* f.sp. *cucumerinum* F1(FOC) inoculated soil |
| **T7** | Seed treatment with oil based formulation (OB) of *T. koningiopsis* TRI 41 in *F. o.* f.sp. *cucumerinum* F1(FOC) inoculated soil |
| **T8** | Seed treatment with oil based formulation (OB) of *T. asperellum* TRI 15 in *F. o.* f.sp. *cucumerinum* F1 (FOC) inoculated soil |
| **T9** | Seed treatment with oil based formulation (OB) of *T. asperelloides* TNAU Tad 1 |
| **T10** | Seed treatment with oil based formulation (OB) of *T. asperelloides* TNAU Tad 1 in *F. o.* f.sp. *cucumerinum* F1(FOC) inoculated soil |

**Table.1** *In vitro* antagonistic activity of *Trichoderma* spp. against *F. o.* f. sp. *cucumerinum*

| Treatments | Growth of the pathogen* | Growth of the antagonist* | Percent inhibition over control** |
|---|---|---|---|
| *T. virens* TRI 37 | 2.88$^{a}$ (1.70) | 6.12$^{a}$ (2.48) | 68.00$^{a}$ (56.22) |
| *T. asperellum* TRI 15 | 3.52$^{b}$ (1.88) | 5.48$^{ab}$ (2.35) | 60.88$^{b}$ (51.93) |
| *T. koningiopsis* TRI 41 | 3.08$^{a}$ (1.75) | 5.92$^{b}$ (2.44) | 65.77$^{a}$ (54.86) |
| *T. koningiopsis* TRI 44 | 4.96$^{d}$ (2.23) | 4.04$^{c}$ (2.02) | 44.88$^{d}$ (47.28) |
| *T. harzianum* TRI 36 | 4.04$^{c}$ (2.01) | 4.96$^{b}$ (2.24) | 55.11$^{cd}$ (48.56) |
| *T. harzianum* TRI 35 | 3.68$^{bc}$ (1.91) | 5.32$^{ab}$ (2.32) | 59.11$^{bc}$ (50.89) |
| *T. asperelloides* TNAU-Tad1 | 3.62$^{bc}$ (1.90) | 5.38$^{ab}$ (2.33) | 59.77$^{bc}$ (51.29) |
| Untreated Control | 9.00$^{e}$ (3.08) | 0.00$^{d}$ (0.71) | - |

(Values are means of three replications, *Values in the parenthesis are square root transformed values, ** Values in the parenthesis are arcsine transformed values and followed by a common letter are not significantly different at 5% level by DMRT)

**Table.2** Shelf life of oil based formulation of *Trichoderma* spp.

| Treatments | Colony forming units (cfu/ml)* | | | | | | |
|---|---|---|---|---|---|---|---|
| | **0th day** | **30th day** | **60th day** | **90th day** | **120th day** | **150th day** | **180th day** |
| | **($10^8$cfu/ml)** | **($10^8$cfu/ml)** | **($10^8$cfu/ml)** | **($10^8$cfu/ml)** | **($10^7$cfu/ml)** | **($10^7$cfu/ml)** | **($10^7$cfu/ml)** |
| ***T. virens* TRI 37** | $104^a$ (2.02) | $81^a$ (1.91) | $75^a$ (1.96) | $72^a$ (1.86) | $42^a$ (1.51) | $37^a$ (1.42) | $31^a$ (1.49) |
| ***T. koningiopsis* TRI 41** | $99^b$ (1.99) | $80^a$ (1.90) | $72^b$ (1.85) | $63^b$ (1.79) | $39^b$ (1.59) | $36^a$ (1.41) | $29^b$ (1.46) |
| ***T. asperellum* TRI 15** | $92^c$ (1.96) | $78^b$ (1.89) | $68^c$ (1.83) | $58^c$ (1.76) | $37^c$ (1.43) | $35^a$ (1.40) | $29^b$ (1.46) |
| ***T. asperelloides* TNAU Tad 1** | $88^d$ (1.94) | $77^b$ (1.88) | $61^d$ (1.79) | $53^d$ (1.72) | $34^d$ (1.38) | $31^b$ (1.32) | $28^b$ (1.45) |

(Values are means of four replications, values in the parenthesis are log transformed values, values followed by a common letter are not significantly different at 5% level by DMRT)

**Table.3** Effect of oil based formulation of *Trichoderma* spp. on growth parameters of cucumber seedlings

| Treatments | Growth Parameters | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Vermicompost : Soil : Sand (1:1:1) | | | | Coir pith | | | |
| | Root Length | Shoot Length | Stem girth | No. of leaves | Root Length | Shoot Length | Stem girth | No. of leaves |
| **Healthy Control** | 11.42$^{d}$ | 22.96$^{g}$ | 1.38$^{d}$ | 5.6$^{d}$ | 17.30$^{bc}$ | 12.96$^{b}$ | 1.56$^{bc}$ | 4.6$^{d}$ |
| **Inoculated Control (FOC inoculated soil)** | 12.46$^{cd}$ | 23.36$^{g}$ | 1.48$^{cd}$ | 5.8$^{cd}$ | 16.50$^{c}$ | 14.36$^{a}$ | 1.54$^{c}$ | 5.2$^{bcd}$ |
| **ST with OB formulation of *T. koningiopsis* TRI 41** | 14.58$^{a}$ | 26.38$^{cd}$ | 1.64$^{ab}$ | 6.6$^{ab}$ | 19.06$^{a}$ | 14.30$^{a}$ | 1.68$^{ab}$ | 5.2$^{bcd}$ |
| **ST with OB formulation of *T. virens* TRI 37** | 14.88$^{a}$ | 28.74$^{a}$ | 1.76$^{a}$ | 6.8$^{a}$ | 19.18$^{a}$ | 14.54$^{a}$ | 1.72$^{a}$ | 5.2$^{bcd}$ |
| **ST with OB formulation of *T. asperellum*** | 12.34$^{cd}$ | 25.34$^{ef}$ | 1.60$^{bc}$ | 6.0$^{bcd}$ | 17.38$^{bc}$ | 13.78$^{ab}$ | 1.62$^{ab}$ | 5.0$^{cd}$ |
| **ST with OB formulation of *T. koningiopsis* TRI 41in FOC inoculated soil** | 12.92$^{bc}$ | 25.92$^{de}$ | 1.62$^{abc}$ | 6.4$^{abc}$ | 17.30$^{bc}$ | 14.38$^{a}$ | 1.62$^{ab}$ | 5.8$^{abc}$ |
| **ST with OB formulation of *T. virens* TRI 37 in FOC inoculated soil** | 13.84$^{ab}$ | 26.94$^{bc}$ | 1.62$^{abc}$ | 6.6$^{ab}$ | 17.34$^{bc}$ | 14.04$^{a}$ | 1.64$^{ab}$ | 5.0$^{cd}$ |
| **ST with OB formulation of *T. asperellum* in FOC inoculated soil** | 12.82$^{bc}$ | 24.52$^{f}$ | 1.58$^{bc}$ | 6.0$^{bcd}$ | 18.14$^{ab}$ | 14.58$^{a}$ | 1.66$^{ab}$ | 6.2$^{ab}$ |
| **ST with OB formulation of *T. asperelloides*** | 14.64$^{a}$ | 27.48$^{b}$ | 1.66$^{ab}$ | 6.6$^{ab}$ | 19.08$^{a}$ | 14.78$^{a}$ | 1.66$^{ab}$ | 6.4$^{a}$ |
| **ST with OB formulation of *T. asperelloides* in FOC inoculated soil** | 13.82$^{ab}$ | 25.30$^{ef}$ | 1.62$^{abc}$ | 6.0$^{bcd}$ | 17.74$^{bc}$ | 14.28$^{a}$ | 1.58$^{bc}$ | 6.2$^{ab}$ |

(*ST – Seed Treatment, OB – Oil Based and FOC – *F. o.* f. sp. *cucumerinum* F1. Values are means of five replications with 25 seedlings per replication and values followed by a common letter are not significantly different at 5% level by DMRT)

Whereas stem girth was found to be more in the *T. virens* TRI 37 (1.72 cm) treated plants followed by *T. koningiopsis* TRI 41 (1.68 cm) and *T. asperelloides* TNAU Tad 1 (1.66 cm**)** seed treated plants grown in coirpith medium.

*Trichoderma* spp. were considered as the potential biocontrol agents for the control of phytopathogenic fungi, oomycetes, and even nematodes (Monte 2001). Similarly in the present investigation *T. virens* TRI 37 inhibited the mycelia growth of the pathogen upto 68.00% followed by *T. koningiopsis* TRI 41 and *T. asperellum* TRI 15. The results were similar with the findings of Saravanakumar *et al.,* (2016). They screened 100 isolates of *Trichoderma* spp. and reported that *T. asperellum* strain CCTCC-RW0014 was effective against *F. o.* f. sp. *cucumerinum*. The *in vitro* studies confirmed the maximum antifungal activity of *T. virens* TRI37 against cucumber wilt pathogen (Vasumathi *et al.,* 2016). Similarly, Lopes *et al.,* (2012) screened 21 isolates of *Trichoderma* spp. and reported that *T. asperellum* inhibited *S. sclerotiorum* to an extent of 50%. These findings confirm the effect of *Trichoderma* spp. in inhibiting the mycelial growth of *F. o.* f. sp. *cucumerinum in vitro.*

In protray experiments, *T. koningiopsis* TRI 41 treated seedlings had more root length, shoot length, stem girth and no. of leaves compared to other isolates. When soil was amended with *T. harzianum* propagules, a 30% increase in seedling emergence was observed up to 8 days after sowing. On day 28, these plants exhibited a 95 and 75% increase in root area and cumulative root length, respectively, and a significant increase in dry weight (80%), shoot length (45%) and leaf area (80%). Similarly, an increase of 90% and 30% in P and Fe concentration respectively, was observed in *T. harzianum* inoculated plants of cucumber (Yedidia *et al.,* 2001). Similarly, T*richoderma harzianum i*solate T969 increased the vigor and their nutrient uptake of tomato plants. Seed germination rate was not affected by *Trichoderma* application, but shoot height, shoot diameter, shoot fresh and dry weight and root fresh and dry weight in tomato seedlings were increased when sown in *T. harzianum* T969 fortified soil and when compared to the control (Azarmi *et al.,* 2011). These results indicate the positive effect of *Trichoderma* spp. on growth parameters of cucumber plants.

The success of any biocontrol agent depends on the type of formulation. In the present study, oil based formulation of *Trichoderma* spp. such as *T. koningiopsis* TRI 41, *T. asperellum* TRI 15 and *T. asperelloides* TNAU Tad 1 was developed. The population load of *T. koningiopsis* TRI 41 on zero day was 10.4 $x10^8$ cfu /ml. The final population after six months of storage was 3.1 $x10^7$ cfu /ml. This is confirmed with the findings of Mbarga *et al.,* (2014), where they developed an oil based formulation of *T. asperellum* was for the control of cocao black pod disease caused by *Phytophthora megakarya* with a shelf life of about 22.5 weeks and conidial concentration of about $2.7 \times 10^7$ conidia/ml. This oil based formulation contained different additives such as vegetable oil (soybean oil - 74%), emulsifying agent (Tensiofix NTM-15%), Structural agent (Tensiofix 869-5%), carbon cource (Glucose-4%) and *T. asperellum* conidia (2%). The formulation showed complete inhibition of *P.megakarya* on sprayed detached pods and there was enhanced rate and duration of protection on sprayed cocao pods in field with 50% of pods protected for 3.2 weeks after spraying in field. This formulation was developed with an intension to supply for small cocao producers. Similarly, an invert emulsion (water in oil) of *T. harzianum* Rifai has been developed for the control of post-harvest diseases of fresh fruits

caused by *Botrytis cinerea, Rhizopus stolonifer* and *Pencillium expansum.* The invert emulsion consisted of sterile deionized water (45.25%), glycerine (4%), water soluble wax (0.75%), tween 20 (2.5%) and a mixture of coconut oil (19%) and soybean oil (28.5%) with a conidial concentration of about $4.6 \times 10^8$ conidia/ml of emulsion. *T. harzianum* Rifai containing conidia reduced the mean lesion diameter of *R. stolonifer* on apple, pear, peach and strawberry, *B.cinerea* on grape, pear, strawberry and kiwi fruit and *P. expansum* on grape, pear and kiwi fruit compared to control. The mean duration of minimum protection period was upto 59 days and percent reduction of disease was about 89% on unwounded apple fruit against infection with *R. stolonifer* (Batta, 2006).

In conclusion, the production of oil based formulation of *Trichoderma* spp. from locally available oil and emulsifiers resulted in increased vigor and growth parameters in protray experiments with increased shelf life. However, the efficacy of formulation under protected cultivation has to be evaluated.

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