

Original Research Article

<https://doi.org/10.20546/ijcmas.2017.607.308>Antagonistic Activity and Shelf Life Study of *Trichoderma harzianum* (Rifai)

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Trichoderma harzianum, a biological control agent seems to be suitable for management of plant pathogens. In dual culture, antagonistic activity of *T. harzianum* was effective against *Sclerotium rolfsii* showing 66.60 percent inhibition, *Fusarium solani* with 56.70 percent inhibition and 30.90 percent inhibition against *Fusarium oxysporum*. The formulation study states that it can be also promoted in liquid formulation which has higher shelf life and stress protection. Paraffin oil, soy bean oil, combination of paraffin oil and glycerol (1:1), paraffin oil and soybean oil (1:1), soya bean oil and glycerol(1:1), were used. Absence of colonies of *Trichoderma harzianum* was observed in soybean oil and glycerol (1:1) at 42nd day, whereas on 49th day *Trichoderma* showed nil growth in paraffin oil and glycerol (1:1). On 56th day paraffin oil and soybean oil showed nil growth of *Trichoderma*. Paraffin oil showed higher activity of *Trichoderma harzianum* with 20×10^7 cfu/ml followed by soybean oil with 2.1×10^6 cfu/ml at 56th day.

Introduction

Soil borne root infecting fungi are difficult to eradicate because they produce resting structure like sclerotia, chlamydospores or oospores for their survival for a longer period of time under adverse environmental conditions (Baker and Cooke, 1974). Use of fungicides for the control of soil borne diseases is costly and also produces environment and health hazards to men and adversely affects the beneficial microorganisms in soil (Dluzniewska, 2003). An alternative management should be given greater emphasis which is eco friendly, safe and effective than chemical methods. In this regard, use of *Trichoderma harzianum*, a

biological control method seems to be suitable for management of the disease (Howell *et al.*, 2000). *Trichoderma* is a biocontrol agent that has been widely used and reported effective against several fungi viz., *Pythium* (Naseby *et al.*, 2000), *Fusarium* (Lewis and Papavizas, 1987), *Rhizoctonia* (Sivan and Chet, 1986), and *Sclerotium* (Jegathambigai *et al.*, 2010). These pathogens cause diseases viz., damping off, wilt, root and collar rot and stem canker.

Trichoderma harzianum is present in all soils and other diverse habitats. They are better rhizosphere colonizers than plant pathogens,

hence compete with other organisms for food and shelter there by reducing the chances of colonization by pathogenic fungi. *Trichoderma* have antifungal, antinematode, plant growth promoting and plant defense inducing activities (Zaidi *et al.*, 2004). The commercial formulations of *Trichoderma* available in the market are talc based and there are also other formulations based on organic carriers such as neem cake, cow dung, tea waste, coffee husk (Bhai *et al.*, 1994) sorghum grains (Sarma *et al.*, 1998) etc. In talc based formulations, the viability of the spores are less and cfu decreases with increase in storage period.

Even though organic carrier based formulations maintain the viability of spores, they are always prone to spoilage by insects and other microbes in the long term and moreover these formulations are too bulky and difficult to transport in large quantities. In general, the major obstacle to the commercialization of such products is the development of a shelf-stable formulated product that retains biocontrol activity similar to that of the fresh product (Janisiewicz and Jeffers, 1997). Hence, study was initiated to test the spore viability of *Trichoderma harzianum* in different oils and its antagonistic activity.

Materials and Methods

Bioefficacy

The three soil borne pathogens, *Sclerotium rolfsii*, *Fusarium oxysporum* and *Fusarium solani* (local isolates) cultures and similarly *Trichoderma harzianum* (native isolate of DRYSRHU) culture maintained at Biocontrol Laboratory of Horticulture College and Research Institute, Anantharajupet, were used for the antagonistic studies. *Trichoderma harzianum* was screened for their antagonistic activity through dual culture method on

potato dextrose agar (PDA) plates, against these pathogens. In this method, culture discs of 5mm each, cut from the margins of actively growing cultures of antagonistic and pathogen, were placed at opposite points in petriplate. Control plates were maintained for *Trichoderma harzianum* and pathogen and three replications were maintained for each treatment and the petriplates were incubated at 28±1°C for observation. Radial growth of *Trichoderma harzianum* and pathogen was observed for each treatment up to 7 days of incubation and the percent growth inhibition (I) of pathogen was calculated using the formula (Vincent, 1947).

$$I(\%) = C-T/C \times 100$$

Where

I=Percent inhibition of pathogen by antagonistic.

C=Radial growth (cm) in control.

T=Radial growth (cm) in treatment.

Shelf life study in different oil formulations

Two grams of dry spore was aseptically transferred into pre-sterilized 100ml of liquid carrier and stirred for better spore suspension. Five different liquid carriers *viz.*, paraffin oil + glycerol (1:1) ratio (T1), paraffin oil + soybean oil (1:1)ratio (T2), soybean oil + glycerol (1:1) ratio (T3), paraffin oil (T4) and soybean oil(T5) were used for the study.

The oils were kept at room temperature and the product was serially diluted to obtain 10⁷ concentration and 1ml was poured in sterilized Petriplates and thereafter, potato dextrose agar medium was poured @ 20ml/plate. Plates were rotated horizontally for uniform distribution of inoculums and incubated at 28±1°C and colony forming units were recorded at every week interval (Sathiyaseelan *et al.*, 2009).

Results and Discussion

Bioefficacy of *Trichoderma harzianum*

Antagonistic effect based on the dual culture studies indicated that *T. harzianum* significantly inhibited the mycelial growth of plant pathogens (Table 1). The inhibition percent was 56.7 with clear inhibition zones in *Fusarium solani*, whereas highest percent inhibition of 66.60 was observed with *Sclerotium rolfisii* at an incubation period of 7 days, but only 30.90 percent inhibition was

recorded with *Fusarium oxysporum* on 7th day. Susanto *et al.*, (2005) documented highest inhibition capacity of 97.8% in dual culture analysis when *Trichoderma harzianum* used as antagonistic fungi. The antagonistic activity of *T. viride* was effective against *Rhizoctonia solani* (54.91% inhibition) followed by *Macrophomina phaseolina* (39.39% inhibition), *Aspergillus flavus* (37.11% inhibition) and inhibition of 28.40% against *Fusarium carthami* as recorded by Sathiyaseelan *et al.*, (2009).

Table.1 Bio-efficacy of *Trichoderma harzianum* against fungal pathogens

Antagonist	Percent inhibition over control		
	<i>Sclerotium rolfisii</i>	<i>Fusarium oxysporum</i>	<i>Fusarium solani</i>
<i>Trichoderma harzianum</i> (DrYSRHU)	66.60	30.90	56.70

Table.2 Oil based formulation of *Trichoderma harzianum*

Interval	Shelf life of <i>Trichoderma harzianum</i> in oil formulations (cfu)				
	Paraffin oil + glycerol (1:1)	Paraffin oil + soybean oil (1:1)	Soybean oil + glycerol (1:1)	Paraffin oil	Soybean oil
0 day	4.9 x 10 ⁹	4.9 x 10 ⁹	4.9 x 10 ⁹	4.9 x 10 ⁹	4.9 x 10 ⁹
7 th day	6.4 x 10 ⁸	39.2 x 10 ⁸	23 x 10 ⁸	47 x 10 ⁸	40 x 10 ⁸
14 th day	5.8 x 10 ⁴	5.0 x 10 ⁷	9.5 x 10 ⁷	40 x 10 ⁸	34 x 10 ⁸
21 st day	6.5 x 10 ³	7.9 x 10 ⁴	7.6 x 10 ⁵	39 x 10 ⁸	27 x 10 ⁸
28 th day	4.0 x 10 ³	8.4 x 10 ³	4.3 x 10 ⁴	35 x 10 ⁸	15 x 10 ⁸
35 th day	2.0 x 10 ³	6.7 x 10 ²	2.1x 10 ²	30 x 10 ⁸	5.7 x 10 ⁸
42 nd day	1.0 x 10 ³	3.2 x 10 ²	Nil	28 x 10 ⁸	24 x 10 ⁷
49 th day	Nil	1.1 x 10 ²	Nil	25 x 10 ⁸	7.0 x 10 ⁷
56 th day	Nil	Nil	Nil	20 x 10 ⁷	2.1 x 10 ⁶

Oil based formulation of *Trichoderma harzianum*

Results indicated that on 0 day, the cfu count was 4.9x10⁹ in all oils whereas on 7th day, gradual increase in cfu was observed in all oils with highest cfu colonies in paraffin oil (47x10⁸ cfu/ml) followed by soybean oil (40x10⁸cfu/ml), paraffin oil + soybean oil (39.2x10⁸ cfu/ml), soybean oil + glycerol

(23x10⁸cfu/ml) and lowest cfu colonies in paraffin oil + glycerol (6.4x10⁸ cfu/ml). Only paraffin oil and soybean oils recorded highest cfu colonies with 40 x 10⁸ and 34 x 10⁸ cfu colonies, respectively on 14th day whereas on 28th day all oil based formulations showed decrease in cfu except in paraffin oil with 35 x 10⁸. Similar trend in observation with respect to cfu was recorded on 35th day i.e. 30 x 10⁸ cfu in paraffin oil followed by 5.7 x 10⁸

in soybean oil, 2.0×10^3 in paraffin oil + glycerol (1:1), 6.7×10^2 in paraffin oil + soybean oil (1:1) and 2.1×10^2 soybean oil + glycerol (1:1). Complete absence of *Trichoderma harzianum* colonies were noticed only in soybean oil + glycerol on 42nd day but in other oils, cfu were observed viz., 28×10^8 in paraffin oil, 24×10^7 in soybean oil, 1.0×10^3 in paraffin oil + glycerol (1:1) and 3.2×10^2 in paraffin oil + soybean oil (1:1) but on 49th day, colonies could be recorded only in paraffin oil, soybean oil, paraffin oil + soybean oil. In the last day of observation i.e. on 56th day, only in paraffin oil and soybean oil *T. harzianum* colonies i.e. 20×10^7 and 2.1×10^6 , respectively could be counted indicating that these oils could retain the spore viability for longer period compared to other oils and its combination used in the studies but higher colonies were noted in former oil only (Table 2).

The work carried out by Sathiyaseelan *et al.*, (2009) on shelf life supported our findings that *Trichoderma viride* in paraffin oil was better than other formulation with 28×10^8 cfu/ml followed by soya bean oil with 6×10^7 cfu/ml at 49th day as compared to absence of colonies were observed in soybean oil + glycerol (1:1), paraffin oil + glycerol (1:1) and paraffin oil + soybean oil (1:1) at 35th, 42nd and 49th day, respectively.

In conclusion the combination of *Trichoderma harzianum* in paraffin oil retains the spore viability of antagonistic fungi for longer period as compared to other oils.

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