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RESEARCH ARTICLE

Ability of Manufacturing of Bioformulations using *Trichoderma spp* for Biological Control of Some Plant Pathogenic Fungi

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Abstract

The aims of this study were to Ability of manufacturing of bioformulation using Trichoderma spp, Seven isolation of Trichoderma spp are evaluated as antagonist of five soil-borne fungal diseases, in dual culture, antagonistic activity of T. harzianum (Th3) was showed highest percentage inhibition compare with the other Trichoderma species, which caused 100, 79.16, 69.23, 63.88 and 58.33 % inhibition mycelial growth of Fusarium solani , Fusarium oxysporum f. sp. lycopersici, Fusarium oxysporum f. sp. melonis, Macrophomina phaseolina and Rhizoctonia solani respectively. Among five groats grains used as substrate for Th3, Corn substance has recorded the maximum colony forming unit (CFU) produce 27 x 10^8 g or ml followed by Millet, Barley, Wheat and Rice which record 20,66, 16, 10,33 and 8,66 respectively. Prepared nine different Trichoderma formulations and tested for their shelf life up to 150 day the result showed formulation which has higher shelf life and stress protection was ThT and ThS which record 20.33x108 and 19.66 x108 CFU/ ml respectively and followed by ThG, STCG, CTCG and LTCG which record 11.33 x108, 4.33 x108, 3.33 x108 and 3 x108 CFU/ ml while minimum CFU was by ST ,LT, and CT to 1.33 x108 CFU/ ml, 1 x108 CFU/ ml, and 0.66 x108 CFU/ ml respectively.

Keywords: *Bioformulation, Soil-borne fungal diseases, Antagonistic, Substance, Shelf life.*

Introduction

Soil-borne diseases cause considerable damage and losses major crop worldwide, Alternatives to the use of chemicals fungicide for disease control, may lead to toxic residues and contaminations environmental, which have severe negative effect to the human, animals and plants [1, 2]. Many researchers have shown the potential of *Trichoderma* spp. in control Soil-borne plant disease caused by *Macrophomina phaseolina*, *Rhizoctonia solani* [3] and *Fusarium* spp [4].

The genus *Trichoderma* spp one of the important groups found in many ecosystems and widely studied worldwide as a biocontrol agent, the antagonistic behavior which are based on the multiple mechanisms has led to agricultural applications, *Trichoderma* strains have either, indirectly by competing for nutrients and place, produce promoting plant growth and many antibiosis, or, directly mechanisms by mycoparasitism [5, 6] and [7, 8]. Today more than 60% of the produced bio-fungicides which used in biological control of plant disease based on formulation of *Trichoderma* spp they are safe for humans, and support the plants without adverse reactions [9, 11]. The success of a bio-formulation in managing plant diseases must be have highly effective against the target pathogens also on the ability survive long time under the conditions that occur in nature [12].Two types of bio-formulation are commonly used widely, solid and liquid bio-formulation, *Trichoderma* spp. produce two types of propagules, conidia wich is most commercial *Trichoderma*-based formulations and chlamydospores, which able to tolerance the hard condition and extended preservation time [13, 15]. This study aimed to evaluation effect of some species of *Trichoderma* spp on some Soil-borne plant diseases therefore, was to develop a cost-effective substrates to produce propagules and manufacture solid and liquid bio-formulation with evaluation the shelf life.

Materials and Methods

Trichoderma spp

In this study Seven isolation of *Trichoderma* spp., three isolation of *T. harzianum* (Th1,

Th2, and Th3), three isolation of *T. viride* (Tv1, Tv2, and Tv3) and *T. hamatum* (Tha) ,were obtained from the Department of Plant protection and, College of Agriculture , the University of Basra, Iraq.

Plant Pathogenic Fungi

The Plant pathogenic fungi *F. solani* (FS), *F. oxysporum f. sp. lycopersici* (FOL), *F. oxysporum f. sp. melonis* (FOM), *R. solani* (RS) *and M.phaseolina* (MP) were obtained from the Department of Plant protection and, College of Agriculture, the University of Basra, Iraq.

Evaluation of *Trichoderma* spp Isolates against Plant Pathogenic Fungi using Dual Culture Method

The seven different isolates of *Trichoderma* spp tested for antagonism against broad range of soil-borne plant diseases by using dual culture techniques as developed by Morton and Stroube, 1955. The mycelial bits of 5 mm diameter of *Trichoderma* spp pathogen were placed opposite to each other on petri plates containing sterilized PDA [16]. The plates were run in triplicates with one control set maintained without inoculating the *Trichoderma* spp, the plates were incubated at 25±20C. For one week. The growth of pathogen tested against all the isolates of *Trichoderma* spp. The data were recorded regularly on the growth of the pathogen and *Trichoderma* spp isolates; Antagonistic activity was measured as zone inhibition and growth reductions, the more activation strains used to prepare the bio formulations.

Percentage of mycelial growth inhibition was calculated according to the formula: MGI% = (dc - dt) ×100/dc

Where, dc= fungal colony diameter in control sets, dt= fungal colony diameter in treatment sets.

Standardization of Suitable Substrate for the Growth of *T. harzianum*

Different five groats grains (Corn, Millet, Wheat, Barley and Rice) were used as substrate to to produce spores of *T. harzianum* (Th3), 50 g at 50% MHC were placed in 250-mL Erlenmeyer flasks, and autoclaved at 121.5°C (15 lb/inch2) for 15 min. cooled down to room temperature were transferred to, 5 discs (5mm) from actively mycelial tips of 7 days old *T. harzianum* (Th3) cultures were aseptically transferred to each substrate, The flasks were loosely sealed with lids and incubated at laboratory condition (28±2°C) for 12 days, the experiment were replicated three times, depended to the CFU to selected best substrate.

Preparation of the Bioformulation

Nine different *T. harzianum* (Th3) formulations (3 Wettable, 6 liquid) were prepared, (Table 1).

- Wettable formulations prepared by added sterile distil water to the substance seed culture medium (100 ml: 4g) and then screened with 100 mesh sieve, the suspension mixed with carriers based which includes Talc, Starch and Gypsum (1:2), and mixed each formulation with 2 % carboxyl methyl cellulose (CMC) and 0.2 % chitosan, after drayed under shade at room temperature.

- liquid formulation prepared by added substance seed culture medium to each of oil Sesame oil, Corn oil and Linseed oil (4g: 100 ml) and then screened with 100 mesh sieve, and divided in to two groups, one group mixed with 2 % carboxyl methyl cellulose (CMC) and 0.7 % glycerol, the other group let without Ingredients.

The Wettable formulations were kept in polyethylene bags and the liquid formulations kept in glass vials at room temperature, the population was analysed at every month depended to the CFU to selected best formulation.

Table 1: Preparation of the bio-formulations

Wettable formulations		
No.	**Method Preparation of the bioformulation**	**Code**
1	Suspension of Th3 + Talc 70g + (1:2) + carboxyl methyl cellulose (CMC) and 0.2 % chitosan.	ThT
2	Suspension of Th3 + Starch (1:2) + 1 % carboxyl methyl cellulose (CMC) and 0.2 % chitosan.	ThS
3	Suspension of Th3 + Gypsum (1:2) + 1 % carboxyl methyl cellulose (CMC) and 0.2 % chitosan.	ThG
Liquid formulation		
4	Sesame oil + substance seed culture medium Th3 (4g: 100 ml screened with 100 mesh sieve) + 2 % CMC and 0.7 % glycerol.	STCG
5	Corn oil + substance seed culture medium Th3 (4g: 100 ml screened with 100 mesh sieve) + 2 % CMC and 0.7 % glycerol.	CTCG

6	Linseed oil + substance seed culture medium Th3 (4g: 100 ml screened with 100 mesh sieve) + 2 % CMC and 0.7 % glycerol.	LTCG
7	Sesame oil + substance seed culture medium Th3 (4g: 100 ml screened with 100 mesh sieve) only.	ST
8	Corn oil + substance seed culture medium Th3 (4g: 100 ml screened with 100 mesh sieve) only.	CT
9	Linseed oil + substance seed culture medium Th3 (4g: 100 ml screened with 100 mesh sieve) only.	LT

Statistical Analyses

All the experiments were performed with 3 replications and analyses of variance and comparison means were done by the SPSS Statistics program version 24, Data were tested by least significant differences (LSD) ($p<0.05$).

Results

Inhibition Percentage of *Trichoderma* spp against Soil Borne Fungi Diseases

The result showed all seven *Trichoderma* species isolates antagonism to all soil-borne plant diseases (Table 2 and Figure 1) *T. harzianum* (Th3) isolate showed highest percentage inhibition with significantly different among the five pathogenic fungi compare with the other *Trichoderma* species, which caused 100, 79.16, 69.23, 63.88 and 58.33 inhibition of mycelial growth of *Fusarium solani* , *Fusarium oxysporum* f. sp. *lycopersici*, *Fusarium oxysporum* f. sp. *melonis*, *Macrophomina phaseolina* and *Rhizoctonia solani* respectively.

Table 2: Inhibition percentage of Trichoderma spp against soil borne fungi

	% Inhibition over control				
	Plant pathogenic fungi				
Antagonist	F. solani	*F. oxysporum* f. sp. *lycopersici*	*F. oxysporum* f. sp. *melonis*	*M.phaseolina*	*R.solani*
T.h1	59.37^{a}	30.76^{e}	41.66^{a}	38.88^{a}	27.22^{a}
T.h2	62.5^{ab}	46.15^{b}	50^{b}	47.22^{e}	27.77^{a}
T.h3	100^{d}	69.23^{d}	79.16^{c}	63.88^{d}	58.33^{c}
T.v1	71.87^{e}	46.15^{b}	41.25^{a}	41.66^{ab}	27.77^{a}
T.v2	53.12^{f}	42.3^{a}	51.66^{b}	41.66^{abc}	38.88^{b}
T.v3	62.5^{b}	43.84^{a}	58.33^{d}	42.77^{c}	38.88^{b}
T.ha	50^{j}	61.53^{c}	50^{b}	40.55^{ab}	27.77^{a}

Values are the average of three replicates, in each vertical column followed by same letter do not differ significantly, ($p<0.05$), analyzed using least significant difference

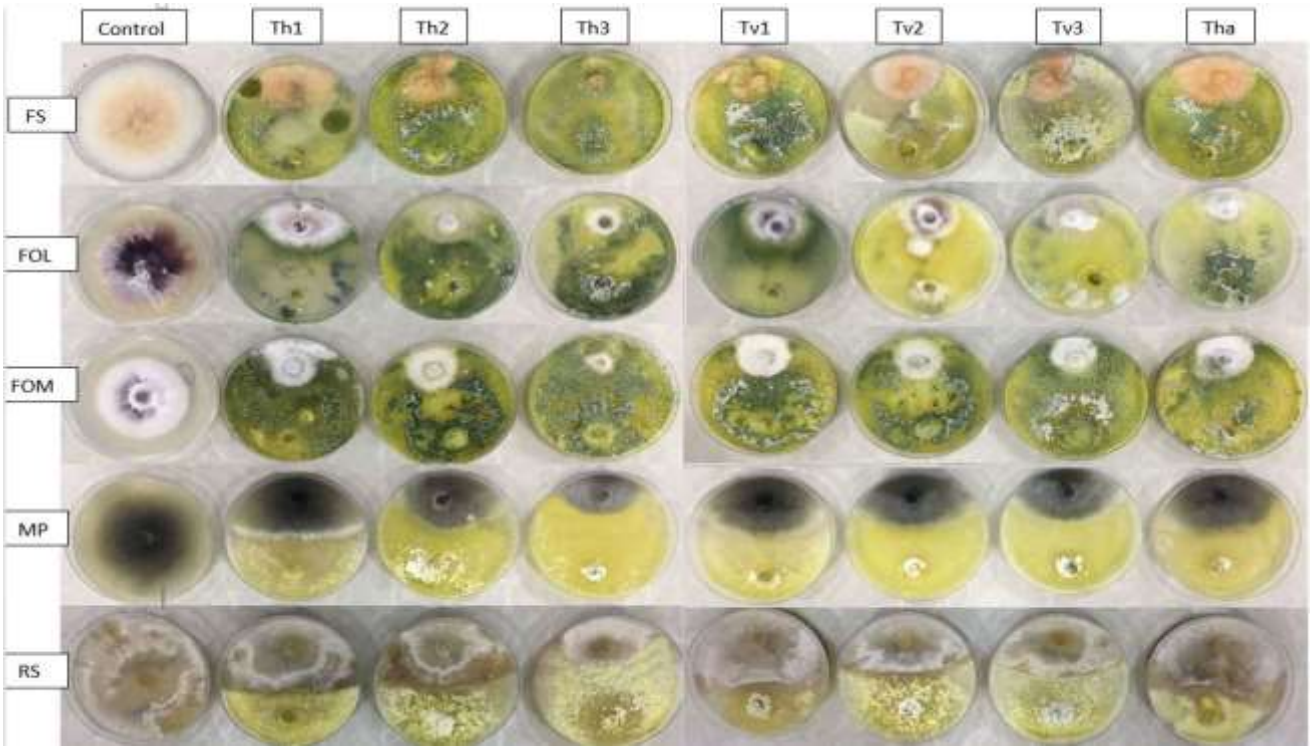

Figure 1: Efficacy of *Trichoderma* spp against soil-borne fungal diseases in dual culture method

Population of *T. harzianum* Th3 in Different Groats Grains Substrates

After 12 days of cultivation *T. harzianum* the results are presented in Figure 2. Among the five groats grains, Corn substrates has recorded the maximum colony forming unit (CFU) produce 27 x 10^{8} g or ml with significantly different among the others five substrates which followed by Millet, Barley, Wheat and Rice which record 20,66, 16, 10,33 and 8,66 respectively.

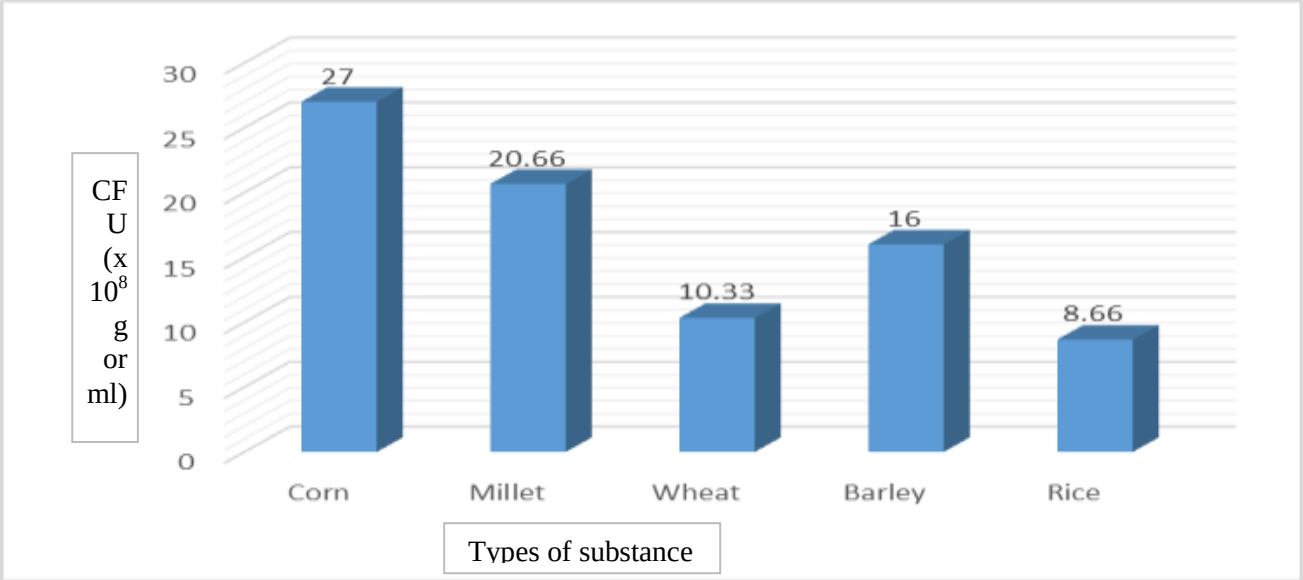

Figure 2: Population of T. *harzianum* Th3 in different groats grains substrates, LSD (p<0.05)

Shelf Life of the Bioformulations

The nine different *Trichoderma* formulations tested for their shelf life up to 150 day at room temperature and according to the results presented in Figure 3 in this period the maximum CFU was ThT and ThS which record 20.33x10^8 and 19.66 x10^8 CFU/ ml respectively with no significant difference and followed by ThG, STCG, CTCG and LTCG which record 11.33 x10^8, 4.33 x10^8, 3.33 x10^8 and 3 x10^8 CFU/ ml and the result showed decline of population level in formulation ST ,LT, and CT to 1.33 x10^8 CFU/ ml, 1 x10^8 CFU/ ml, and 0.66 x10^8 CFU/ ml respectively.

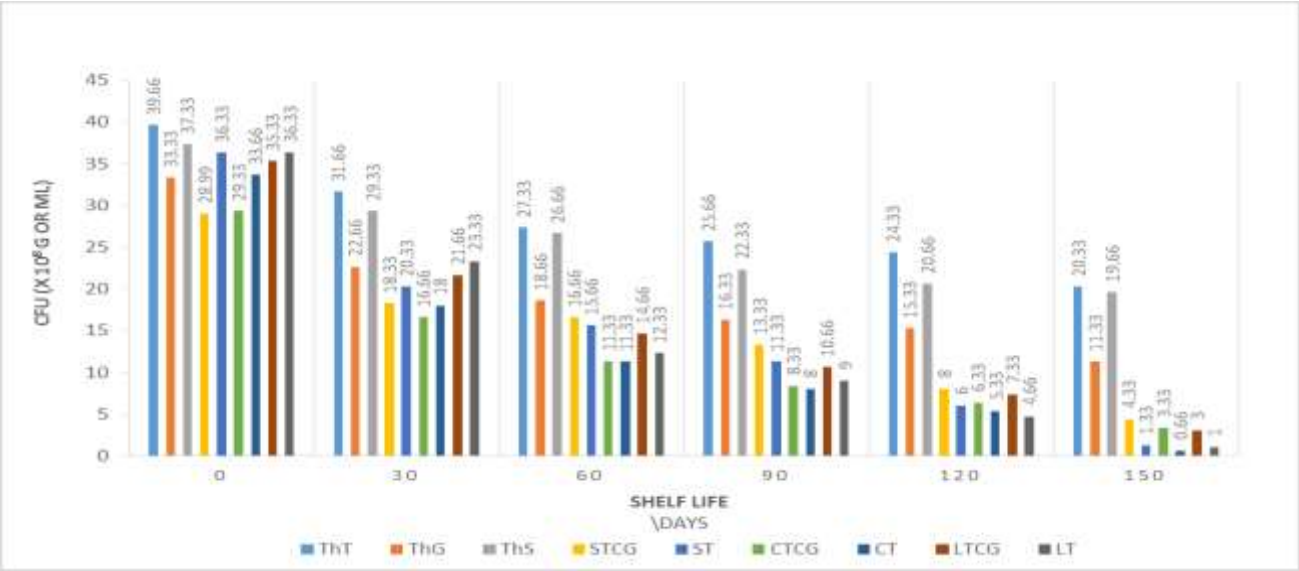

Figure 3: Shelf life of different formulations of *T. harzianum* (Th3) at room temperature, (p<0.05)

Discussion

Result of antagonistic effects of *Trichoderma* species showed all the seven isolation exerted an inhibitory activity against to control the plant diseases, *T. harzianum* (3) record highest percentage inhibition, *Trichoderma* strains have multiple mechanisms effect as biocontrol against fungal either directly and indirectly depended on the changes in gene expression which include mycoparasitism involves nutrient competition, hyper parasitism and antibiosis [17, 23].

Among the five groats grains based substrates tested for supporting the Population of *T. harzianum* (3), and after 12 days of cultivation the Corn substrates has recorded the maximum CFU (27 x 10^8 g), and this in agreement with the results of Panahian, et al [24].While Cavalcante, [25] remember that Rice, Corn bran and Wheat bran suitable substrate to produce *Trichoderma* spp conidia. Biotic factors especially C and N effect on conidiospore production in many species of *Trichoderma* [26] also structure and porosity of the

substrate important to make the nutrients availability and accessibility, by effect on water distribution and penetrate of the fungus [25, 27].Result of the formulations stored at room temperature after 150 day showed the maximum CFU record in ThT ($20.33x10^{8)}$ and ThS ($19.66\ x10^{8)}$ CFU/ ml), and this because nature of the organic and sources of carbon, many studies worked to development of the bioformulations for biocontrol plant disease, Al-Waily and Mshari [28] prepare bioformulation by use the starch and gypsum as a carrier material and reported shelf life up to 180 days at room temperature, while Reddy, *et al* [29]. Study states that it can prepare liquid formulation with the addition of glycerol which has even after 12 months of storage; Dinesh R. and Tewari, A. K. [30].Used different liquid and wettable formulation which stored for long time.

Shelf-life of the bioformulation depended on ability of the carrier materials to reduction of metabolic activity, the suitable Oxygen availability and control of moisture [31, 33]. The former provided higher production yields, greater UV-resistance and had.

Conclusion

Trichoderma species perfect agent to reduced growth of all the five soil borne pathogens of *F. solani* , *F. oxysporum* f. sp. *lycopersici*, *F. oxysporum* f. sp. *melonis* , *M. phaseolina* and *R. solani, Trichoderma* sp based biofungicides as an alternative to chemical fungicides which are ecological and environmental hazards, groats grains, Corn showed to be a suitable substrate for spore production of *T. harzianum,* Talc and starch suitable carrier material to prepare the bioformulation and which stored for long time [34, 35].

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