Available online at http://bajas.edu.iq
https://doi.org/10.37077/25200860.2019.204
College of Agriculture, University of Basrah

Basrah Journal of Agricultural Sciences

ISSN 1814 – 5868 Basrah J. Agric. Sci., 32 (2) 135-149, 2019 E-ISSN: 2520-0860

Evaluation of Biofungicide Formulation of *Trichoderma longibrachiatum* in Controlling of Tomato Seedling Damping-off Caused by *Rhizoctonia solani*

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Received 19 September 2019; Accepted 18 November 2019; Available online 31 December 2019

Abstract : This study was carried out in the laboratories and fields of Department of Plant Protection, College of Agriculture and aimed at isolating and identifying *Trichoderma* spp. from different agricultural soils in Basrah and evaluating of the efficacy of some polymeric materials in the formulation of *T. longibrachiatum* as biofungicides. The results showed that there was no direct effect of Polyvinyl acetate (PVA) on the growth of *Rhizoctonia solani* and *T. longibrachiatum* as inhibition percentage was 0%. While Polyurethane (PU) had low effect on growth of *T. longibrachiatum* as inhibition percent was 4.47%. The results of pot experiment showed that PVA reduced tomato seeds germination to50% compared to 66.6 and 70% in PU and control treatment respectively. The results of bio effect in the control of tomato seedlings disease caused by *R. solani* showed that *T. longibrachiatum* +PU (PUT) improve tomato seeds germination and enhanced plant growth as plant height reached 15.7cm compared with 13.5 and 12.9 in PVAT and control treatment. The results also showed that the polymeric material improved longevity of *T. longibrachiatum* spores for six months, but the spore viability began to decreased gradually during the storage period, reaching 6.03×10^6 and 2.54×10^6 cfu.g^{-1} with PVAT and PUT respectively after six months compared to the number of spores before storage of 5.09×10^6 and 1.77×10^6 cfu.g^{-1} respectively.

Keywords: *Trichoderma longibrachiatum*, Biofrmulation, Biocontrol, Damping-off.

Introduction

Trichoderma spp. is one of the essential biological agents used in the field of biological control, *T. harzianum* and *T. viride* have been introduced in controlling of plant pathogens (Sharma *et al*., 2014). Hjeljord & Tronsmo (1998) have indicated that *Trichoderma* can be used as seed treatment, seedling treatment or soil treatment to control soil borne fungi such as *R. solani*, *Fusarium* spp. and *Pythium* spp.

Trichoderma species play a crucial role in promoting plant growth according to different mechanisms (Harman *et al*., 2004). *Trichoderma* spp. has been examined and widely used to manage root and seedling diseases of many crops for long time. Recently *Trichoderma* spp. has been used to

reduce plant diseases on shoot part such as grey mould caused by *Botrytis cinerea* and powdery mildew on several crops (Sawant, 2014). Biofungicides are new promising alternative for chemical pesticides, for many reasons including high biodegradability, low probability of developing resistance and their suitability into integrated pest management applications (De La Crus *et al*., 2019).

Formulations of *Trichoderma* spp. have been widely used in plant disease management. The spores of the fungus *T. harzianum* were carried on talc (Bhat *et al*., 2009). Adzmi *et al*. (2012) used the microparticle method of *T. harzianum* spores in small granules by mixing them with Ca-alginate and Montmorillonite clay. Several materials, including cereals of plant crop such as wheat and barley, were used to propagate *T. viride* and were then carried on carriers such as starch and calcium carbonate (Al-Masaadi, 2014). The major aspects of successful biological control include the formulation and delivery system for bioagent that achieved efficient disease control, various material have been used in mass production of *Trichoderma* spp. such Sorghum, Millets grain, wheat brane, Talc, Kaolin and sodium algenate (Jeyarajan, 2006; Locatelli *et al*., 2017).

Polyvinyle acetate (PVA) is widely used polymer, and products of this polymer are good adhesives for paper, plastic, foil, leather and cloth as well as used in book binding and wood gluing (Ebnesajjad, 2011). In other study, Zhu & Zhuo (2001) used starch with polyvinyl alcohol in preparation of herbicide 2,4,5-T to showing slow release and promote longer life in the environment while minimizing its side effects.

Polyurethane (PU), which is used in a wide range of areas including surface coating, fibre industry, flexible sponges, home furniture industry and automotive industry, has the characteristics of being low or almost zero thermal conductivity and has no harmful effect on plants as well as its high degradable by a number of fungi and soil bacteria (Russell *et al*., 2011).

Considering the increase need to develop *Trichoderma* formulations in a way that can be used easily by farmers. This study aim to develop a formulation of *T. longibrachiatum* FBY1 with high cell viability and biological activity under prolonged condition of storage and evaluate the efficiency to control Tomato seedling damping-off caused by *R. solani*

Materials & Methods:

Isolation of *Trichoderma* spp.

Soil samples and roots of different healthy plants, Which were free from any disease signs were collected such plants iclude okra, sunflower, and cowpea. The roots were cut into small pieces 1 cm and then sterilized superficially with NaOCl solution. (At a concentration of 10% from commercial product) for three minutes and dried on sterile filter paper and then washed with sterile distilled water also for three minutes and dried. Four pieces of each plant root were transferred on Petri dishes containing the potato dextrose agar (PDA) medium, and the experiment was carried out with three replicates per plant. Soil samples were suspended by mixing the soil with distilled water at a ratio of 1: 9 (weight/volume) and made a series of dilution, 1 ml of dilution 10^{-4} for each PDA plates. The plates were incubated at 25 ± 2°C. When the colonies appeared, the isolation of *Trichoderma* spp. were purified.

Molecular identification of the fungus *Trichoderma* spp.

Trichoderma isolates were grown on the PDA, DNA was extracted according to Kerenyi *et al*. (1999). The DNA fragments of *Trichoderma* spp. isolates were amplified with using the primers shown in table (1). After confirming the amplification of the PCR product by electrophoresis, 20 µl of the amplification product per isolation was sent to Macrogen for the purpose of determining sequences of nitrogen bases in the genes used and then matching them with the NCBI.

Table (1) Sequences of primers used in PCR technology.

Sequence name	Sequences of prefixes
Its1	5TCTGTAGGTGAACCTGCGG3
Its4	5TCCTCCGCTTATTGATATGC3

Isolation of *R. solani* causing tomato seedlings damping-off disease

R. solani was isolated from tomato seedling infected with damping-off disease as explained in paragraph 1.

Effect of *Trichoderma* spp. on the growth of *R. solani*

Daul-culture Technique was used to test the antifungal efficiency of *Trichoderma* spp. The level of antagonism was evaluated according to Bell *et al. (*1982).

Preparation of *T. longibrachiatum* in the dry formulation

T. longibrachiatum was grown on the seeds of millet *Panicum miliaceum* L. The spores were harvested by washing the fungal spores with 50 ml sterile distilled water containing 0.02 ml of Tween 80% per 50 g of millet seeds coated with *T. longibrachiatum*. The fungus was filtered in a sterile flask using two pieces of sterile gauze. The suspension was then centrifuged, placed in sterile 10 ml plastic tubes and centrifuged at 4000 rpm for 10 minutes (Locatelli *et al*., 2017). The suspension was counted using Haemocytometer. the average number of spores was 6.53×10^{10} .ml-1

The concentrated suspension was mixed with talc with ratio 1:2, Neeedle 25×0.7 mm was used to inoculated talc with spore suspension and mixed thoroughly to prevent coagulation, and then dry in the oven at 35 ± 2 °C for 24 hours. After drying the concentrated suspension on the talc, pass the sterile preparation through a metal clip with a hole size of one mm to obtain a homogeneous volume of the dry formulation. The dry formulation (concentrated dried suspension on talc) was used in the production of the polymeric formulation.

The pathogenic fungus *R. solani* was also prepared on millet seeds in the same way as *T. longibrachiatum*.

The effect of polymeric materials on germination of tomato seeds and seedling growth

The following materials were used in the production of bioformulations of *T. longibrachiatum.*

1- Poly vinyl acetate (PVA) Deli Company/ China.

2- Polyurethane (PU): prepared by mixing equal 1: 1 ratio of Diphenyl dimethyl isocyanate (MDI) with Polyester polyol.

The effect of polymeric materials in germination of tomato seeds on Water Agar medium.

Polymers were added by 1% to WA and after autoclaving at 121 °C and pressure of 1.5 kg .cm^{-1}The medium was poured with polymeric materials into Petri dishes and left to be solid and then planted with tomato seeds, steriled with sodium hypochlorate 10%. Each Petri dish contained 15 seeds. The experiment was carried out with three replicates for each treatment. The control treatment included tomato seeds planted in dishes containing only WA medium.

The effect of polymeric materials on germination of tomato seeds (pot experiment)

The soil mixed with Peatmoss in ratio of 1:3 then sterilized for half an hour twice for two days and the polymeric material was added to sterile soil by 1%. The soil was placed in plastic pot and moistened. After three days planted with tomato seeds with ten seeds per pot. The germination percentage was calculated after 21 days of planting using the equation:

$$\text{Germination rate \%} = \frac{\text{Number of germinating seeds}}{\text{Total seeds number}} \times 100$$

The effect of polymeric materials on the growth of *R. solani* and *T. longibrachiatum*.

The polymeric materials used in this study were added by 1% to the PDA and sterilized in the autoclave. After sterilization, the media was poured in Petri dishes. Each dish was inoculated with a 0.5 cm diameter disc taken from the edge of the five-day fungus colony. The dishes were incubated at 25 ±2 ºC. The diameter growth was determined by using the average of two perpendicular diameters after the fungal growth in the control treatment reached the edge of the dish. The experiment included three dishes for each polymeric material and three dishes without any polymeric material as control treatment as follows:

1- PVA × *R. solani*.

2- PU × *R. solani*.

3- *R. solani* (control).

4- PVA × *T. longibrachiatum*.

5- PU × *T. longibrachiatum*.

6- *T. longibrachiatum* (control).

After the growth was reached the edge of the dish, the percentage of inhibition was calculated according to the equation in Shaban & Al-Malah (1993).

$$\textbf{Inhibition percentage\%} = \frac{\textbf{average of diameter of colony fungus in control} - \textbf{average of diameter of colony fungus in control treatment}}{\textbf{average of diameter of colony fungus in control}} \times 100$$

Formulation of *T. longibrachatium* by polymeric material

Formulation PVAT

PVA Sterilized and added to *T. longibrachiatum* spores whitch dried on talc. The formula was dried under 40°C for 12 hours (Santos *et al*., 2015) and stored at laboratory temperature for further use.

Formulation PUT

Ten grams of *T. longibrachiatum* with talc was added to 15 ml of Polyester polyol, and mixed thoroughly to obtain a homogeneous mixture. 15 ml of MDI was added to the mixture vortex for prober time. The mixture left to dry in room temperature for 15 min. The mixture was passed through sieves to granules size less than 5 mm and stored for further use.

Evaluation of the efficiency of bioformulation in control of tomato seedlings damping-off disease caused by *R. solani*

This experiment was conducted in a greenhouse and used plastic pots 17 × 18 cm capacity 3kg soil. The soil was sterilized with commercial formalin. and the soil was inoculated with the pathogen *R. solani* inoculum (grown on millet seeds) ratio 1% w / w. After three days of inoculation, the soil treated with formulations of *T. longibrachiatum*, added three concentrated for each formula 0.5,1 and 2 g.kg soil^{-1}. Pots was planted with sterile tomato seeds, and the experiment was carried out at the rate of four replicates for each treatment, The treatment were as follows:

1- PVAT + *R. solani*

2- PUT + *R. solani*

3 - pathogen *R. solani* (control)

The percentage of germination of tomato seeds after three weeks of planting was calculated. The plant height, wet and dry weight of the shoots and roots were calculated after six weeks of planting.

Evaluation of polymeric material in longevity of *T. longibrachiatum* at room temperatures.

The formulations of *T. longibrachiatum* by using polymeric material was prepared as described in paragraph 7, then stored in a sterile plastic bottle at 25± °C. The evaluation of the shelf life of the bioagent of *T. longibrachiatum* was assessed by measuring the viability of the spores after several periods of storage of the preparations, which lasted from one to six months.

The viability of the spores was calculated according to dilution plate method. 1 mL of dilution 10^{-6} was taken for each formula and placed in Petri dishes containing PDA medium. The plates were moved in gently to ensure the distribution of the particles of the formula and incubated on 25 ± 2 °C for 24 hours. The experiment was carried out with three replicates per formula. CFU was calculated according to equation in Clark (1965):

The number of live reproductive units.g^{-1} = Average number of colonies × inverted dilution used.

Statistical analysis

All laboratory experiments were carried out with complete randomized design C.R.D. Pot experiments were carried out according to the design of the randomized complete block design R.C.B.D. The mean was compared with the least significant difference L.S.D. below the probability level of 1% for laboratory experiments and 5% for field experiments. Data were analyzed using the Genstat discovery edition 3 statistical software.

Results & Discussion:

Isolation and identification of *Trichoderma* spp.

Three isolates of *Trichoderma* were obtained from Al-Mudaina, Safwan and Al-Hartha area fig. (1). Molecular identification confirmed two isolates of *Trichoderma* had similarity of 99% with *T.longibrachatium* and one isolate had 99% similarity with *T. harzianum*. The sequences of nitrogen bases have been deposited at NCBI with Gen bank accession number LC499793.1, LC499794.1 and LC499795.1 respectively (Table 2).

T. longibrachiatum FBY1 was selected for the production of the bioformulation based on some of the good properties it possesses such high growth speed, production of high number of spores on substrate and high antagonistic activity against *R. solani*. This result was consisted Harman *et al*. (2004).

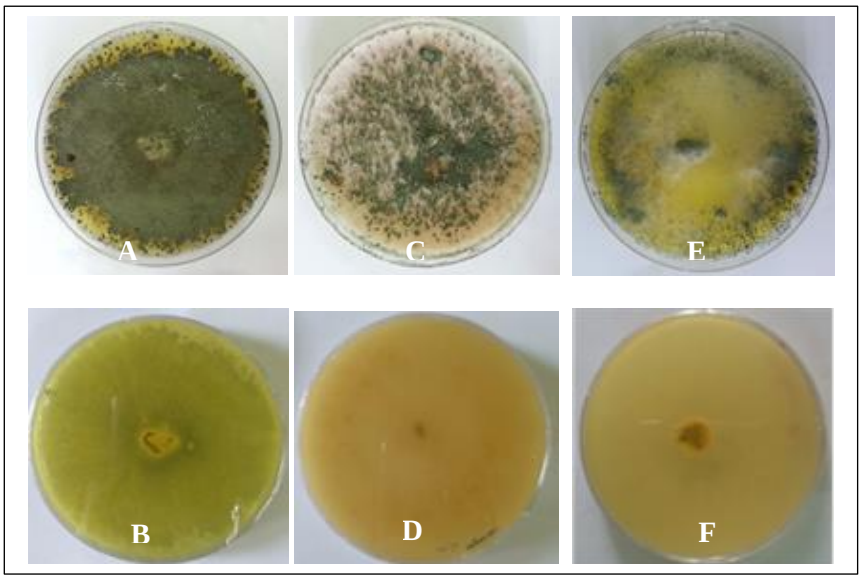

Fig. (1): Colony of *Trichoderma* spp. isolates on PDA: A: *T. longibrachiatum* FBY1, B-*longibrachiatum* FBY1(reverse), C: *T. longibrachiatum* FBY2, D: *T. longibrachiatum* FBY2(reverse), E: *T. harzianum* FBY3, F: *T. harzianum* FBY3(reverse).

Inhibition of *Trichoderma* spp. on the growth of *R. solani* and *T. longibrachiatum* FBY1

The results showed high antagonist ability of *T. longibrachiatum* isolates against *R. solani* as it reached score 1 according to

Bell *et al.* (1982) scales (Fig. 2). While *T. harzianum* reported 3 score of antagonism. Previous study indicated that *T. longibrachiatum* had antagonistic ability against many plant pathogenic fungi such as *R. solani*, *Macrophomina phaseolina* and *Pyricularia oryzae* (Al-Qaisi & Alwan, 2016).

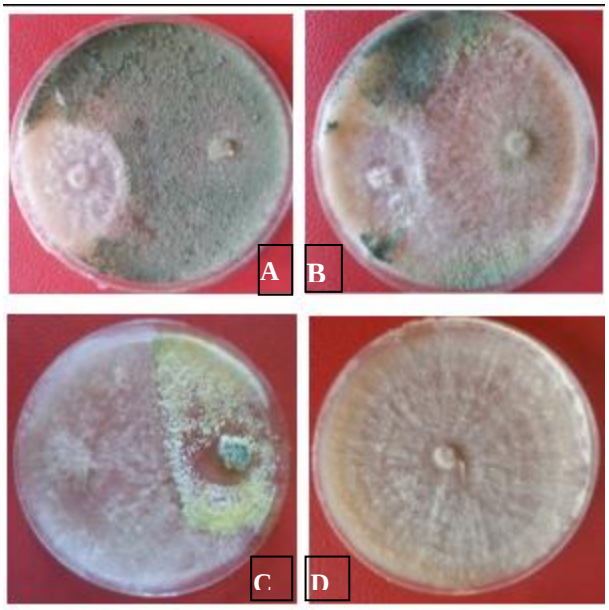

Fig. (2): Efficacy of *Trichoderma* spp. isolates in inhibition pathogen *R. solani*. *A: T. longibrachiatum* FBY1 × *R. solani*, B: *T. longibrachiatum* FBY2 × *R. solani*, C: *T. harzianum* FBY3× *R. solani* and D: *R. solani* (control).

Table (2): Isolates registered at NCBI.

Isolate No.	Isolate name	Accession No.	Isolation area
1	*T. longibrachiatum* FBY1	LC499793.1	Al-Mudaina
2	*T. longibrachiatum* FBY2	LC499794.1	Safwan
3	*T. harzianum* FBY3	LC499795.1	Al-Hartha

The efficacy of *Trichoderma* spp. in inhibition of plant pathogenic could be attributed to different mechanisms such as mycoparasitism and competition with the pathogens for space and nutrients as well as due to the production of antibiotics such as Gliotoxin and Viriden and lysis enzymes such as B-1,3-gluconase, Chitinase and Protease (Harman *et al*., 2004; Gajera *et al*., 2012; Vinale *et al*., 2012; Puyam, 2016). Activity of some *Trichoderma* spp. such *T. koningii* and *T. hamatum* against plant pathogenic fungi could be attributed to its ability to produce certain toxic compound such as Pyrones. (Ghisalberti *et al*., 1990).

Preparation of *T. longibrachiatum* FBY1 spores in dry formula

A dry formulation of *T. longibrachiatum* (Fig. 3) was obtained by mixing the spores suspension with talc. The spores concentration per gram of dry formula was 2.86×10^{10} CFU. The results were agreed with Kumar *et al*. (2014) in the ability of talc and charcoal to preserve the viability of *T. viride* grown on PD broth and added to talc at 30, 40 and 50 ml $.100^{-1}$ g talc and charcoal for 120 days.

Fig. (3) Dry talc formula of spores of *T. longibrachiatum* FBY1spores.

Effect of polymeric materials on seeds germination and growth of tomato seedlings.

The results in table (2) showed that was no significant effect of polymeric materials used for the production of the biological formulation on the germination of tomato seeds on the WA medium. The germination percentage was 93 and 91% using PVA and PU polymers compared to 89% in control treatment (Fig. 4). Most important to mention is there was no previous studies had examined the effect of PVA and PU on seed germination.

In the test of the effect of polymeric materials in the germination of the tomato seeds in pots experiment has shown in table (3), there were no significant differences in the effect of PU on the percentage of seed germination of tomato. The germination percentage was 66.5% compared with control treatment which was 70.0%. While PVA treatment caused a significant reduction in the percentage of germination of tomato seeds, which reached 50.0% compared with control treatment. The results of the PVA treatment were not consistent with the previous WA experiment.

Table (3): Effect of polymeric materials on tomato seeds germination.

Treatment	Germination %	
	Water Agar	Pots
PVA	93	50.0
PU	91	66.5
control	89	70.0

L.S.D 1%= 2.01 **L.S.D 5%= 4.36**

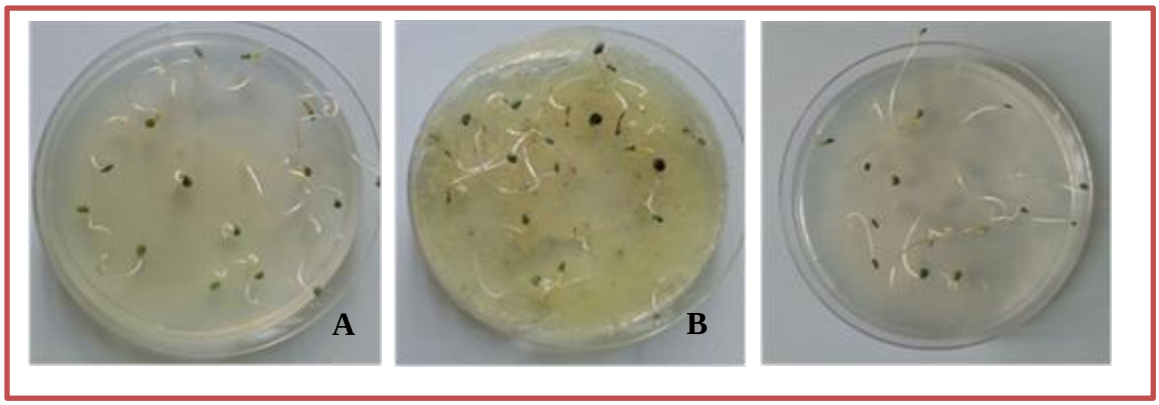

Fig. (4): Effect of polymeric materials on tomato seeds germination percentage on WA. A: PVA, B: PU, C: Control.

Moreover, the negative effect of PVA treatment may be due to the high viscosity of the polymeric material which adheres the soil granules and binds them together, which reduce the soil porosity.

The effect of polymeric materials on the growth of pathogen *R. solani* and *T. longibrachiatum*

The results of this experiment (Table 4) showed that there was no effect of both polymers on the growth of *R. solani*. The inhibition percentage was 0% for all treatments, and no changes were observed in the growth speed and shape of the fungus colony (Fig. 5). This indicates that these compounds are non-toxic. On the other hand the results showed no impact of PVA on the growth of *T. longibrachiatum* of the polymeric material on the growth of *T. longibracgiatum* , the inhibition percentage was 0%, while the PU inhibited the growth of the fungus at a low percentage which was 4.47% (Fig. 6). The differences in the growth of fungi may be due to the difference in the enzymatic abilities it possesses, such as lysis enzymes which break down the polymer materials. This results consistent with results of Mahajan & Gupta (2015) who pointed out that *F. solani*, *Curvolaria* sp. and *T. viride* possess the ability to break down polyurethane.

Table (4): Effect of polymeric materials on the growth of *R. solani* and *T. longibracgiatum*

Fungus	Treatment	average of diameter of colony (cm)	Inhibition %
R. solani	PVA	8.50	0
	PU	8.50	0
T. longibracgiatum	PVA	8.50	0
	PU	8.12	4.47

Fig. (5): Effect of polymeric materials on the growth of *R. solani*. A: PVA, B: PU and C: Control.

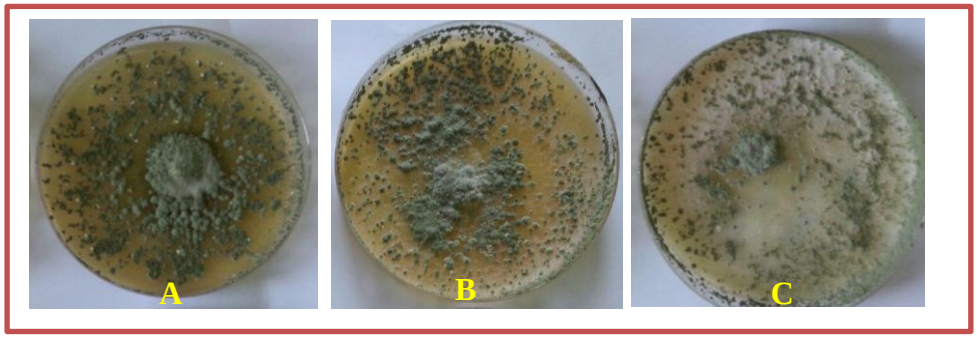

Fig. (6): Effect of polymeric materials on the growth of *T. longibrachiatum*. A: PVA, B: PU and C: Control.

The effect of bioformulation of *T. longibrachiatum* on tomato seedlings damping-off caused by *R. solani*

The results in the table (5) showed that *R. solani* has a significant impact on reducing the percentage of germination of tomato seeds which decreased from 70% to 52.5%. These findings are consistent with several studies in which *R. solani* was identified as one of the most important fungi caused seeds decay and damping-off diseases. (Saleh & Bedan, 2000; Latorre, 2004).

On the other hand adding of PUT and PVAT increased tomato seeds germination up to 72.5% compared with 52.5% in control treatment. The efficacy of PUT and PVAT formulation in reducing the negative effect of *R. solani* in tomato seed germination is due to *T. longibrachiatum*, which is active ingredient of these compound rather than the polymeric material itself.

Several studies have reported the efficacy of *Trichoderma* species in reducing the negative effect of several pathogens. This result was in agreement with Saleh & Bedan (2000) referred to that *T. harzianum* increased the percentage of tomato seed germination to 60.06% compared to 53.72% in control treatment. However, the results also agreed with Jovicic-Petrovic *et al*. (2017), who indicated that *T. longibrachiatum* had high efficacy in antibiosis to *Pythium aphanidermatum* led to reducing the infection of seedling damping-off in

cucumbers by 50 %. The treatment of PUT (0.5 g / kg) was 16.7 cm compared with the control treatments in inoculated and non-inoculated soils with *R. solani* at the height of 12.9 and 11.5 cm, respectively. The results of table (5) showed significant differences in plant highest. The treatment of PUT reported 16.8cm compared with control treatment inoculated and non-inoculated by *R. solani*, which gave 12.9 and 11.5 cm, respectively. The treatment of PUT (0.5 g .kg^{-1}) was 16.8 cm compared with the control treatments in inoculated and non- inoculated soils with *R. solani* at the height of 12.9 and 11.5 cm, respectively.

Table (5): Effect of bio formulations in the percentage of seed germination and plant height in soil infested with *R. solani*.

Treatment	Biofrmulation concentrate (g.kg $soil^{-1}$)	Germination percentage	Average of plant height (cm)
PVAT+ *R. solani*	0.5	65.0	14.3
	1	60.0	15.1
	2	50.0	15.0
	Average	58.3	14.8
PUT+ *R. solani*	0.5	67.5	16.7
	1	72.5	14.7
	2	82.5	15.3
	Average	74.1	15.6
R. solani	1% W/W	52.5	12.9
Control	Without pathogen	70.0	11.5

L.S.D. 5% Germination percentage= 1.84

L.S.D. 5% plant height= 2.73

The results in table (6) showed that there was no effect of *R. solani* in reducing the growth parameters of Tomato plant such as wet and dry weight of shoots and roots system, which was 1.5, 0.91 and 0.26 g in the control treatment of soil inoculated with the pathogen compared with the control treatment in soils not inoculated with pathogen which was 1.45, 0.66 and 0.22 g, respectively. This may be probably due to few number of plants in the pots, which have a few competition for water and nutrients and reflected positively on the growth indicators. The results of bioformulations showed that there were significant differences in the percentages of the wet and dry weight of the shoots and roots system in the soil infected with the *R. solani*. The results of PUT (0.5 g .kg^{-1}soil) gave 2.88 and 0.43 g for the higher and weight for both soft and dry weight of the shoots system respectively, compared with the control treatment inoculated by the pathogen which gave 1.50 g and 0.26 g respectively.

The results indicated a positive effect of the formulations used in the experiment, which led to an increase in the weights of the treated plants compared to the control treatments for the inoculated and non-inoculated soils by *R. solani*. This effect is due to the efficiency of *T. longibrachiatum* used as an active ingredient in the

producing of bio formulations. The results were similar to those indicated by Saleh & Bedan (2000) and Abboud & Abboud (2010) in the efficiency of the biological control agent *T. harzianum* formulated on millet seeds. It achieved a significant reduction of seedling death on tomato caused by *R. solani* and a considerable increase in plant growth rate in greenhouse and field experiments. *Trichoderma* spp. Biological control of plant pathogenic fungi by *Trichoderma* spp. can be achieved by different mechanism such competition, production of antibiotics, mycoparasitism and induce systemic resistance (Ragina, 2015; Abbas *et al*., 2017; Fayyadh & Abbas, 2018).

Table (6): Effect of Biological formulations on tomato plant weights (g) in soil inoculated with *R. solani* after six weeks of planting.

Treatment	Biofrmulation concentrate	wet weight(g)		Dry weight(g)	
		shoot	Root	shoot	Root
PVAT+R.S	0.5	2.50	0.70	0.35	0.04
	1	2.64	0.72	0.38	0.06
	2	2.53	0.71	0.34	0.08
	Average	2.55	0.71	0.35	0.06
PUT+R.S	0.5	2.88	0.83	0.43	0.06
	1	2.04	0.58	0.33	0.06
	2	2.40	0.67	0.35	0.05
	Average	2.44	0.69	0.37	0.06
R.S	1%W/W	1.50	0.91	0.26	0.04
Control	Without pathogen	1.45	0.66	0.22	0.05
L.S.D.5%		0.70	0.16	0.09	0.02

Effect of polymeric material on longevity of *T. longibrachiatum*

The results of the experiment in table (7) indicated that the polymer materials used preserved the fungus alive for six months as the number of colony forming units, after storage period reached to $5.09x10^6$ and 1.77×10^6 after six month for each of PVAT and PUT respectively. Moreover, the results indicated that polymeric materials and talc could keep the spores active at room temperature. These results were agreed with Al-Mesaadi (2014) to keep the spores of *T. viride* active when formulated on carriers such as starch and calcium carbonate. He reported there were no significant differences between the two carriers during the 180 days of storage period and the rate of spores concentration was 17.57×10^7 and $16.43 \times 10^7 CFU.ml^{-1}$ respectively. The reason for the holding of the bio activity during the storage period is due to the efficiency of some polymeric materials in the encapsulation of the fungus spores and reduce the impact of external influences of heat and moisture, That's what Oancea *et al.* (2016) referred. The results also agreed with Locatelli *et al.* (2017) in capability of sodium genes mixed with some other polymers used in the coating of *Trichoderma* sp. In maintaining the vitality of the fungus at a rate of more than 10^6 CFU for

Table (7): Effect of polymeric material on the longevity of *T. longibrachiatum*.

Period/month	Bioformulation	
	PVAT	PUT
Zero time	6.03	2.54
1	5.78	3.08
2	3.66	1.14
3	4.39	1.23
4	5.16	2.47
5	5.60	1.97
6	5.09	1.77
Average	5.10	2.02
L.S.D. 1%	1.40	0.93

a period of up to 14 months at room temperature (28 °C).

Conclusions:

Results showed that possibility of using the polymeric material In production of a bioformulation, using the biological agent *T. longibrachiatum*. A reduction of infection of damping-off disease on Tomato caused by *R. solani*. The results indicated that polymeric materials and talc preserved the propagative active unit at room temperature (25±5°C) for six month.

Acknowledgement:

The authors is very much grateful to Department of Plant Protection, College of Agriculture, University of Basrah for their financial support.

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