Activity and bioformulation of *Trichoderma harzianum* for management of tomato diseases caused by soilborne pathogens

S. Yucel[1], S. Karacancı[2] and T. Ay[1]

[1]Biological Control Research Institute, Ministry of Food, Agriculture and Livestock, Adana, Turkey

[2]Department of Bioengineering, Ege University Engineering Faculty, Izmir, Turkey

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Tomato is an important crop in Turkey with total production of 11.8 million tons (183000 ha).

The major factors restricting greenhouse tomato production are the soilborne pathogens and nematodes in the Eastern Mediterranean region of Turkey. Greenhouse farmers get good results when they apply pre-plant solarization and reduced dose fumigant properly.

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When farmers apply control methods after disease symptoms, they can't obtain adequate control, hence pesticide residues and environmental problems may occur.

Thus, other alternative disease management options were considered among promising biological control methods.

Seedling companies generally add biological fungicide which contains *Trichoderma harzianum rifai* KRL-AG2 (T-22) into growing media.

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This study was conducted to determine the effect of local *Trichoderma harzianum* isolates against damping off, wilt and root rot diseases caused by of soil borne pathogens .

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MATERIALS AND METHODS

Determination of biological efficiency of *T. harzianum* isolates against soil-borne diseases (*Fusarium oxysporum , R. solani, P. aphanidermatum*) in climate room.

Three *T. harzianum* local isolates (T1,T2,T4) which were isolated from rhizophere in previous studies were applied to growth medium (700 g m^{-3}), and tomato seeds (cv H-2274) were sown.

Pot experiments were conducted for three pathogenic species and mixture of pathogens according to randomized parcels design.

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The experimental design included 5 characters (three *T. harzianum* isolate, *T. harzianum* strain *T-22* (KRL-AG2) and non-treated *Trichoderma*-control) with 4 repetitions. Four tomato plants were planted in each pot.

Pots were placed in a climate room at 25±2°C temperature, 70±5% humidity and 12 hours light-12 hours dark.

Disease symptoms were evaluated as infected/healthy, and plants with disease symptoms were re-isolated.

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Formulation of *T. harzianum* isolates

1. Bioformulation of *Trichoderma* by spray dryer.

Zeolite (clinoptilolite), dry milk and malt dextrin were added into the solution as a supporting material containing spores of *T. harzianum* isolates grown in rice hull- malt sprout mixture (1:1).

Bioformulation was then prepared by processing the resulting formulation in spray drier (Sargın et al., 2013).

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2. Determination of shelf life of bioformulation

Bioformulation of *T. harzianum* isolates were stored at 5°C and 20°C and then were isolated into *Trichoderma* Selective Medium (TSM) in order to determine zeolit supported formulation of conidi g^{-1} (Elad and Chet, 1981).

Colony forming units were recorded via colony counter. Shelf life was determined every 30 days for 6 months. Sing et al., (2007)

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Determination of biological efficiency of *T. harzianum* isolates against soil-borne diseases (*F. oxysporum, F. solani, R. solani*) in greenhouse.

Three *T. harzianum* isolates were applied into growing media prior to (700g m^{-3}) sow tomato seeds (cv H-2274). Tomato seedlings were transplanted into greenhouse soil which was known to be infested by *F. oxysporum, F. solani* and *R. solani* on September 25, 2014 when they were one month old.

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Seedlings were planted in plots which were untreated (C) and solarized (S) for 6 weeks.

Twenty five tomato seedlings were planted in each plot (12 m^2).

The treatment in the experimental group of consisted of a randomized complete block design with four replications of five treatments (T1, T2, T4, T-22 and control).

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All of the plants in each parcel were uprooted approximately after 11 weeks (on July 21) and disease symptoms were examined. The data were collected from plants showing root tip discoloration, or the browning of the vascular system . The pathogens were isolated from infected plants.

Tomato fruits were harvested weekly for 6 times from February 20 to April 9 in 2015 and data were collected.

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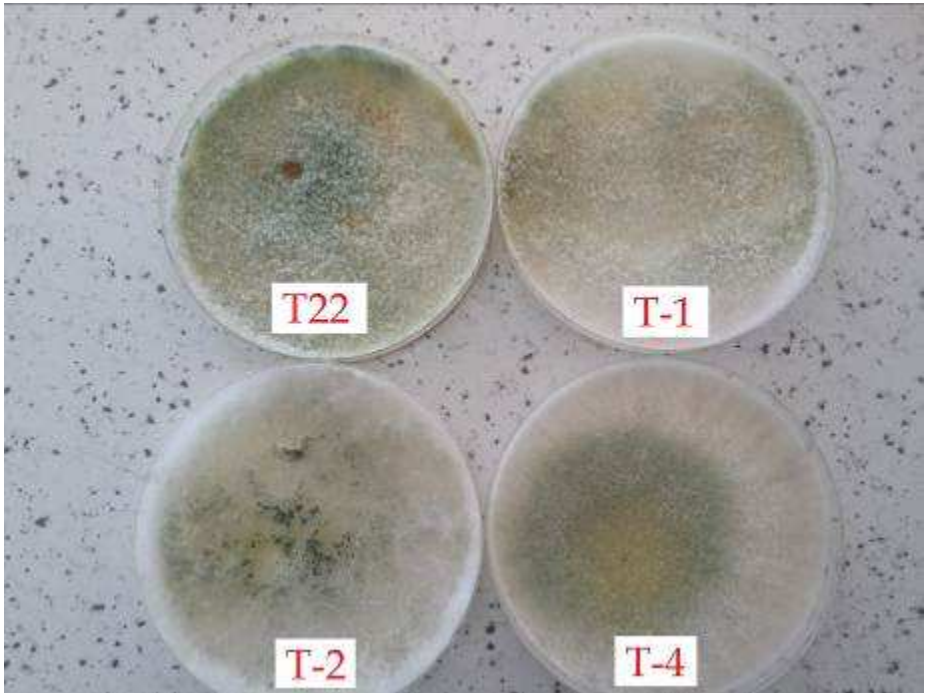
RESULTS



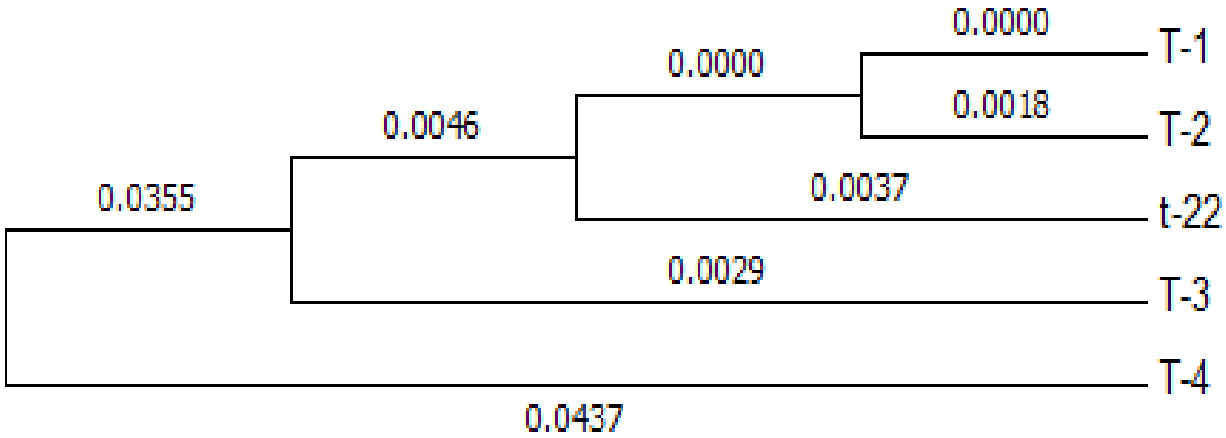


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Table 1. Effect (%) of *Trichoderma harzianum* (T1, T2, T4) bioformulation on root rot diseases of tomato in pots

T.harzianum isolates	*P. aphanidermatum* (P)		*F. oxysporum*(F)		*R. solani*(R)		P+F+R	
	DI[1]	Effect	DI	Effect	DI	Effect	DI	Effect
T1	23.4a	58.9a[2]	20.3a	56.3a	18.7a	62.6a	28.1b	56.2a
T2	18.7a	67.8a	17.2a	63.7a	**15.6 a**	67.9a	20.3a	68.4a
T4	21.9a	61.4a	23.4a	49.6a	**23.4 a**	52.4a	25.0ab	60.2a
T-22	20.3a	64.8a	18.7a	58.7a	17.2a	66.2a	23.4ab	63.1a
Control	57.8b	-	**46.9b**	-	50.0b	-	**64.5c**	-

[1] DI= Disease incidence

[2] Means followed by the same letter in the same column do not statistically differ following the LSD's multiple comparison tests (p<0.05)


28 7 2015


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Table 2. Viability spore counts of bioformulation using *T. harzianum* isolates (x 10^5)cfu g^{-1}

Temp. °C	Isolates	Starting	Months					
			1.	2.	3.	4.	5.	6.
+5°C	T1	200	43	7	1.23	0.17	0.1	**0.1**
	T2	**170**	23	15.3	13.6	1.7	1.2	**0.8**
	T4	**200**	53	48	15.3	5.6	4.5	0.4
	T-22	**3000**	1126	600	120	95	65	**50**
+20°C	T1	200	16	0,8	0.8	0.1	0	0
	T2	170	20	2.6	0.2	0.1	0	0
	T4	200	36	11.6	0.3	0.1	0	0
	T-22	3000	963	160	26.7	8	8	2


K
T-22
T-4
T-1
T 2


KEKLIK
KEKLIK


K
T-2

Table 3. Effect of T. harzianum (T1, T2, T4) bioformulation on root rot diseases of tomato in greenhouse

Trichoderma isolates	Soil solarization		Control	
	DI[1]	Effect(%)	DI	Effect
T1	16.00ab	27.08 b[2]	20.00b	39.76 b
T2	7.00b	**67.92 a**	13.00b	**59.96 a**
T4	10.00b	54.17 a	17.00ab	47.54 b
T-22	9.00b	**62.92 a**	14.00a	**57.46 a**
Control	22.00b	-	33.00c	-

[1] DI= Disease incidence
[2] Letters next to numbers indicate different groups determined by LSD's multiple comparison tests ($p<0.05$)

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Tomato yield taken from February to April resulted to be 70 tons ha^{-1} in control plots while it was 90-95 tons ha-1 in the sites where a combination of *Trichoderma harzianum* with soil solarization was used.

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DISCUSSION

- We observed that local isolates and the commercial strain of *T. harzianum* were showing the same effect in greenhouse soil naturally infested by soilborne pathogens (*F. oxysporum, F. solani, R. solani*).

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- We noticed *Trichoderma* applications in solarized soil provided better results than in non-solarized soil.

- Similar results have been reported in previous studies about *Trichoderma* applications. Sharma (2008) observed such development as plant vigour, root colonization and disease control in some of the biologically-active Trichoderma isolates in tomato and cauliflower. The researcher also observed that *Trichoderma* performed better under solarized soils than non-solarized soils.

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- Initial concentration of the formulation prepared by using local *T. harzianum* ($1.7\text{-}2x10^7$) was lower than those of the commercial formulation ($3x10^8$).
- The reason of this difference could be the damage of *T. harzianum* spores due to spray drier used in this study. It is thought that the experiments could be repeated by using advanced spray drier device.

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- On the other hand, it is reported that the shelf life of dried spores of *T. harzianum* can be extended considerably when stored in sealed containers at low moisture contents (4-5%) and under refrigeration (5–7°C).
- It is also found that the loss and decrease of viability were faster at high temperatures (20°C)(Pedreschi and Aguilera , 1997) .

Similar results were taken in this study, as well.

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- The use of T2 and T4 bio-formulation with solarization in greenhouse increased the yield and reduced root rot symptoms in tomato.

- In addition, no significant difference between the effects of local and commercial formulation of *T. harzianum* on damping off and root rot diseases in tomato was observed in this study.

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CONCLUSIONS

- Application of solarization has become widespread in Mediterranean region of Turkey.
- Enhancement of IPM applications in protected vegetable cultivation site should be taken into consideration.
- The use of local *Trichoderma harzianum* biological fungicide combined with solarization could decrease the costs of application.
- Further studies will be conducted in order to increase the spore content of the formulation.

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