EFFECTS OF TRICHODERMA HARZIANUM ON LETTUCE IN PROTECTED CULTIVATION

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ABSTRACT

Effects of Trichoderma harzianum on lettuce seedling development, plant quality characteristics at harvest and yield were investigated. Experiments were carried out in an unheated glasshouse where composted straw bales were used as the growing medium. A part of the experiment was carried out in a high tunnel where soil was the growing medium. Seeds were sown in peat and seedlings were grown in the presence of T. harzianum at dosages of 5, 10 and 15 g m^{-2} applied at sowing. Among the seedling characteristics studied only seedling fresh weight of cv. Yedikule was increased significantly. Effect of T. harzianum on lettuce yield was not statistically significant. In the glasshouse, the straw bales were composted with two different methods. In the method I, 134g N, 13g P, 25g K, and 76 g Ca, and in the method II, 54.3g N, 26g P, and 22.6g Ca were provided to the straw bales. The marketable yield of cv. Yedikule from the straw bales of method I was 503 g $plant^{-1}$ from 15 g m^{-2} whereas the control remained the lowest with 425 g $plant^{-1}$. For the same cultivar grown on the method II the highest marketable yield was obtained from 10 g m^{-2} with 303 g $plant^{-1}$ compared to the control (150 g $plant^{-1}$). The cv. Coolguard grown in the straw bales was tested only with the method I and the highest yield was from 15 g m^{-2} with 570 g $plant^{-1}$ compared to 551 g $plant^{-1}$ from the control. Dosage main effect regarding marketable yield in soil in the tunnel was not significant.

Key words: Trichoderma - Lactuca sativa - biological control agent - Organic/ecological horticulture

INTRODUCTION

Lettuce (Lactuca sativa L.) of the family Asteraceae (formerly Compositae) originates from wild Lactuca serriola and it was initially cultivated in the Mediterranean and the Near East [22]. The crop is grown widely around the World and its production reached ca. 22.2 Million Mt in 2005, and Turkey with a production of 375 000 Mt in the same year was one of the major producers [5]. The lettuce is a cool season leafy vegetable crop grown from September to April in the Mediterranean and mainly under plastic covered tunnels to the north west of Turkey.

Lettuce suffers from various fungal diseases in its entire growing period from germination of seeds to the mature plant stage. For example damping-off causes serious losses, and in general pests and diseases may be responsible for losses up to 50% [14]. Chemical fungicides are used most commonly against the pathogenic fungi however alternative methods of biological control are being tested increasingly. Trichoderma harzianum is one of the biological control agents used successfully against Sclerotinia and other pathogenic fungi in lettuce and other crops [17, 18, 30]. In addition to the control by T. harzianum of the pathogenic fungi through microbial interactions, it was demonstrated that T. harzianum and Trichoderma asperellum induced defense responses and systemic resistance as one of the mechanisms of biological control [28, 29]. It was also reported that T. harzianum can be used successfully as an alternative to methyl bromide [13].

Positive effects of Trichoderma spp. are not limited to the above, many species of Trichoderma promoted growth and development of seedlings of vegetable and non-vegetable crops, namely cabbage, cucumber, lettuce and cotton [6, 15, 23, 26, 28]. Furthermore, cucumber, bell pepper and strawberry yields were increased significantly following the application of Trichoderma spp. [1, 7, 12, 25]. However, onion and tomato yields were not increased by the same agent [2, 8, 24]. Yedidia et al. [28] suggested that growth promotion effect in the presence of T. harzianum in the rhizosphere was due to increased root surface area allowing the roots to explore larger volumes of soil therefore more nutrients were made available to the plants especially under nutrient-limited soil environments. In vitro studies have shown that micronutrients and insoluble phosphates became soluble and available, therefore useful to the roots interacting with T. harzianum in the root zone [4].

Straw bales can be used as a growing medium in the production of lettuce in glasshouses. Before planting, straw bales are composted using various composting regimes [3]. Trichoderma harzianum is used also as an activator in compost making, and it is known to enhance the composting process. Taking into account mainly growth and yield promoting effects of T. harzianum the present experiment was carried out to find out if these effects are also available in the growing of lettuce, especially in straw bales.

METHODS

A total of two experiments, one in the glasshouse in straw bales and the other in a high plastic tunnel in soil, were carried out in the Department of Horticulture, Faculty of Agriculture, Namik Kemal University, Tekirdag, Turkey between November 2003 and April 2004. Trichoderma harzianum used was a commercially available product (TrichoFlow WP™, Agrimm Technologies Ltd., New Zealand) and contained 10^8 cfu per gram. Water suspension of T. harzianum was drenched in the root zone both in the soil and in the composted straw bales. In both the experiments, T. harzianum was applied twice, i.e., at initial sowing of the seeds and later at planting. The cultivars used were Coolguard (Iceberg type; May Seed Co.), Tarsus (Battavia type; Vilmorin Co.), Nevada (Battavia type; Anadolu Seed Co.) and Yedikule (Cos type; Sluis and Groot). The characteristics evaluated were marketable yield (g plant^{-1}), head diameter (cm) and length (cm), number of leaves per plant and head firmness. Firmness of lettuce heads were evaluated by hand and the heads were given marks of ≤50, 50, 75 and 100, the firmest being the 100. All data obtained were analyzed statistically using MSTAT software and least significant differences between the means were calculated for 5% [20].

Experiment on seedling development

Effects of Trichoderma harzianum on seedling development were investigated. Seeds of the cultivars Yedikule and Coolguard were sown in peat and T. harzianum was applied at 5, 10 and 15 g m^{-2} dosages, 10 g m^{-2} being the company recommended dosage. Control plants were also available. Data on seedling fresh weight, root length and other seedling characteristics were obtained after approximately a month, just before planting.

Experiment in straw bales in the glasshouse

Composted wheat straw bales were used as the main growing medium in the glasshouse experiment. Seedlings obtained at the end of the initial seedling experiment were planted in straw bales. Because straw bales were composted using two different methods two separate experiments were set up and seedlings were planted in each of the experiment. Seedlings of the cv. Yedikule were planted in straw bales composted according to both methods therefore two experiments were carried out for

Table 1. Effects of *Trichoderma harzianum* dosages (g m^{-2}) on seedling growth of lettuce cultivars Yedikule and Coolguard grown in peat

	Yedikule				Coolguard			
Characteristic	Control	5	10	15	Control	5	10	15
Seedling fresh weight (g)	1.00ab	0.51b	0.72b	1.61a	0.22	0.24	0.19	0.26
Root length (cm)	7.6	7.9	7.3	7.0	6.0	4.4	5.9	3.9
Number of leaves	3.8	4.1	4.0	4.3	3.0	3.3	2.6	3.3
Seedling length (cm)	14.0	11.6	11.3	13.3	9.9	7.7	9.1	8.1
Hypocotyl length (cm)	5.0	3.0	4.9	8.5	6.9	7.1	8.6	10.1
Root weight (g)	0.18	0.07	0.28	0.20	0.05	0.02	0.03	0.03
Stem diameter (mm)	1.69	1.50	1.72	2.17	1.26	1.31	1.0	1.21

Means followed by the same letters do not significantly differ ($P < 0.05$); cv. Yedikule: 5% LSD for seedling fresh weight: 0.75

this cultivar. The cv. Coolguard was planted in straw bales composted according to the Method I only. The following procedure was used in each of the experiment: seeds of the cultivars Coolguard and Yedikule were sown in peat (Plantaflor) on December 12, 2003 and January 2, 2004, respectively. Seedlings of the cultivars Coolguard and Yedikule were planted in the straw bales on January 23, 2004 and February 24, 2004, respectively. Spacing both in and between the rows at planting was 0.3 m. A completely randomized blocks design with four replicates was used. T. harzianum was applied in the plots in three dosages at 5, 10 and 15 g m^{-2} where 10 g m^{-2} being the company recommended dosage for the product. An untreated (control) plot in each block was left and only water without the product was applied. Data analyzed were obtained from plots containing 35 plants. The straw bales were watered weekly with 10 L per bale and weeding was carried out three times. Plant protection practices were not applied during the growing period. Dimensions of the wheat straw bales used were 80 cm x 40 cm x 50 cm.

Methods of composting the straw bales

Method I, modified from Papadapoulos [21]: The straw bales set in the glasshouse were watered daily with short intervals using 5 liters of water per bale for three days. The straw bales were provided with an NPK composed fertilizer of 15-15-15 (N, 15% min.; P_2O_5 15% min.; K_2O 15% min.) at 200 g and Calcium Ammonium Nitrate (CAN; 26% N) at 200 g, and watered daily in the following day 4 and 5. The straw bales were then watered briefly in the following day 6 to day 9. On the day 10, each straw bale was provided with 200 g CAN and watered briefly but frequently. In this method of composting, the amount of fertilizers above corresponded to mineral nutrients of 134g N, 13g P, 25g K, and 76 g Ca. The watering regime was continued also in the following days of 11 and 12. Then the straw bales were watered with 10 liter per straw bale from the day 13 to 22 until the temperature inside the straw bales was well below 35 ^{o}C, suitable for planting. Analysis of samples from the straw bales composted through the method I showed that the N concentration was 1.13%.

Method II, modified from Altintas and Varis [3]: The straw bales were briefly watered daily with short intervals in the initial seven days then in the day 8 each straw bale was provided with 130 g DAP (18% N, 46% P_2O_5) and 119 g CAN (26%). In this method of composting, the amount of fertilizers above corresponded to mineral nutrients of 54.3g N, 26g P, and 22.6g Ca. In the day 9 and 10 each of the straw bales were watered with 5 liters of water daily. In the following days the watering was continued and increased gradually until the day 18 that is when the temperature inside the straw bales was well below 35^{o}C. Analysis of samples from the straw bales composted through the method II showed that the N concentration was 0.72%.

Experiment in soil in high plastic tunnel

In experiment in the high plastic tunnel the cultivars Coolguard, Yedikule, and Tarsus were used. Seeds were sown in peat (Anadolu Peat Co., Turkey) on November 4, 2003 and seedlings were planted in soil on January 13, 2004. The plant spacing was 0.3 m both in and between the rows. Following planting, T. harzianum was applied at 1, 2, and 4 g m^{-2} to the plots set in the split plots design with three replicates. In the design, dosages and the cultivars were the main plots and sub-plots, respectively. Only water without the antagonistic fungus was applied to the root zones in the untreated (control) plots. A total of 13 plants were maintained in each plot. T. harzianum was applied twice, once at sowing, and once at planting in the soil, during the growing period. The experiment was ceased on April 22, 2004. The soil was a silty-clay loam containing 64 ppm P_2O_5 and 344 ppm K_2O with a pH of 7.5. The organic matter content of the soil was

Table 2. Effects of *Trichoderma harzianum* dosages (g m^{-2}) on yield (g $plant^{-1}$) and quality characteristics of lettuce cultivar Yedikule grown in the straw bales composted with the methods I and II (please see material and methods section for explanation of the methods)

Characteristic	Method I					Method II				
	Control	5	10	15	5% LSD	Control	5	10	15	5% LSD
Marketable yield (g)	425	492	476	503	n.s.	150	257	303	263	n.s.
Head diameter (cm)	8.1	8.1	8.1	8.7	n.s.	5.7b	5.2b	8.2a	7.8a	1.82
Head length (cm)	27.1	27.8	27.4	29.1	n.s.	17.8b	21.7ab	23.7a	22.9a	3.89
Number of leaves	65.2	61.5	65.3	64.4	n.s.	31.5b	50.5a	60.0a	48.8a	15.70
Head firmness	78.0	76.8	74.5	77.3	n.s.	40.8	32.5	55.8	55.5	n.s.

Means followed by the same letters do not significantly differ ($P < 0.05$)

1.35%. The lettuce plants were fertilized with a total of 4 g m^{-2} NH_4NO_3 (33% N). Besides the initial one at planting, the plants were watered four times during growing. The watering was started 15 days after planting and maintained with the intervals of 10 days.

RESULTS

Application of T. harzianum increased lettuce yield and some quality characteristics however the increases were not statistically significant. Results presented are divided into three sections, i.e., T. harzianum effects at seedling stage, at harvest in the composted straw bales in unheated glasshouse, and at harvest in soil in the high plastic tunnel.

Seedling stage

In the seedling stage only the seedling fresh weight of the cultivar Yedikule was significantly increased and it was 1.61 g from 15 g m^{-2} compared to that of the untreated (control) plots with 1.00 g (Table 1). The highest increase in the seedling fresh weight of cv. Coolguard was also from 15 g m^{-2}, compared to the control but the increase was not statistically significant. In cv. Yedikule, the highest figures for number of leaves, hypocotyl length and stem diameter were obtained from 15 g m^{-2}, however, the longest roots were from 5 g m^{-2}, and the highest root weight was from 10 g m^{-2}. Similar results were obtained from cv. Coolguard in that for some of the criteria T. harzianum treated plots resulted in superior data, for example seedling fresh weight was the highest at 15 g m^{-2} with 0.26 cm and the stem diameter was the largest at 5 g m^{-2} with 1.31 mm. Interestingly, however, in some other characteristics studied such as root length, seedling length, root weight the control plants were with the superior figures.

Results at harvest in straw bales in glasshouse

T. harzianum did not increase the marketable yield significantly in both cultivars grown in straw bales (Table 2, 3). In the cv. Coolguard only method I of composting was employed without significant increase. In the glasshouse experiment with cv. Yedikule grown in the straw bales composted according to method I (only "method I" hereafter) yield from 15 g m^{-2} was the highest with 503 g $plant^{-1}$ whereas the control remained at 425 g $plant^{-1}$, the difference being little. However, the same cultivar grown on the straw bales composted according to method II (only "method II" hereafter) yield was the highest at 10 g m^{-2} with 303 g $plant^{-1}$ in comparison to the lowest from the control with 150 g $plant^{-1}$, twice that of the control (Table 2). Head diameter was significantly high at 10 g m^{-2} from method II with 8.2 cm. Head length and the number of leaves in method II were also significantly increased, whereas head firmness was not significantly affected. In the method I however the effect of T. harzianum was not significant for all the characteristics studied.

With regards to the comparison of the figures from method I and II of cv. Yedikule, it is striking that almost all the

Table 3. Effects of *Trichoderma harzianum* dosages (g m^{-2}) on yield (g $plant^{-1}$) and quality characteristics of lettuce cultivar Coolguard grown in the straw bales composted using the method I (please see material and methods section for explanation of the method)

Characteristic	Control	5	10	15	5% LSD
Marketable yield (g)	551	517	529	570	n.s.
Head diameter (cm)	11.8	11.6	12.4	12.2	n.s.
Head length (cm)	27.0	26.1	27.0	27.6	n.s.
Number of leaves	35.8	35.7	33.8	34.2	n.s.
Head firmness	79.7	78.3	75.5	80.0	n.s.

data from method I were greater than the corresponding data on method II (Table 2). For example yield from the plots of the control treatment, 5, 10 and 15 g m^{-2} of the method I were 425, 492, 476 and 503 g $plant^{-1}$, respectively, and each one of which was greater than the corresponding yield data from method II with 150, 257, 303 and 263 g $plant^{-1}$, respectively. The same is applicable to the other characteristics analyzed. It seems therefore that the method of composting and the fertilizers added to the straw bales made the difference due probably to the fact that straw bales composted according to method I were comparatively rich in nutrients. With regards to cv. Coolguard of method I, T. harzianum effect was not significant in all the characteristics studied (Table 3). A parallel experiment using method II was not available for cv. Coolguard therefore data from a second experiment using method II was not available. If such an experiment had been available, it could be expected that figures from method II would have been inferior to figures of method I, due to less nutrient availability in straw bales composted according to method II.

Results at harvest in soil in tunnel

All the quality characteristics and yield of lettuce grown in soil in the tunnel were not significantly affected by the application of T. harzianum that is the dosage main effect was not statistically significant. However, cultivar and dosage interaction was significant for head length (Table 4). In this experiment dosages applied were lower than the ones applied in the glasshouse experiment. The highest dosage main effect on the marketable yield was from 2 g m^{-2} with 165 g $plant^{-1}$ in comparison to the control treatment with 141 g $plant^{-1}$. While 2 g m^{-2} was with the highest yield in the cultivars Tarsus and Nevada with 160 g and 187 g $plant^{-1}$, respectively, 4 g m^{-2} produced the heaviest lettuce heads with 156 g $plant^{-1}$ from cv. Coolguard. The head diameter was the highest from 2 g m^{-2} in the cultivars Coolguard and Nevada with 7.5 cm and 6.5 cm, respectively, whereas in the cv. Tarsus the control treatment produced the largest diameter with 6.7 cm.

DISCUSSION

T. harzianum treatment was significantly effective in eliciting increased growth response only in one of the seedling characteristics studied but yield was not significantly increased. The significant effect we observed on the fresh weight of cv. Yedikule was in line with earlier reports. Fresh weight, shoot length, dry weight and leaf area of cucumber seedlings as well as seedling weight of cabbages were increased significantly by the application of T. harzianum and T. viride [23, 27, 28]. Root development in the present work was not significantly increased which is contrary to the findings of Björkman et al. [9] who determined in sweet corn grown in soil in glasshouse that root dry weight was significantly increased. In our experiments, response of seedlings to Trichoderma application varied in that while the increased growth response at significant levels was recorded for some organs of seedlings such an effect was not significant in others. Also, the response of the cultivars tested presently varied.

The effect of T. harzianum on yield was not significant either. This is contrary to the findings of Rabeendran et al. [26] who found that yield of lettuce grown in soil was increased significantly. T. harzianum significantly increased yield both in leafy vegetable crops and fruit bearing vegetables such as cucumbers [1, 25, 26] and ornamental peppers [19]. The increased yield in cucumber reported by Poldma et al. [25] was obtained only in the last year of a four year experiment. Rabeendran et al. [26] reported that cabbage seedling development was enhanced in only one of the two experiments carried out under identical conditions. Additionally, the effect on seedling characteristics was significant after 28 days from sowing, but after 42 days.

Rabeendran et al. [26] suggested that the enhanced growth response was more pronounced under sub-optimal conditions and obtained significant yield increase in lettuce from the field experiment, soils of which, due to fungal infestation was considered sub-optimal. One of the lettuce experiments we presented here was also

Table 4. Effects of *Trichoderma harzianum* dosages (g m^{-2}) on yield and quality characteristics of lettuce grown in soil in high plastic tunnel

Dosage	Cultivar	Marketable yield (g $plant^{-1}$)	Head diameter (cm)	Head length (cm)	Number of leaves	Head firmness
Control	Coolguard	131	6.0	14.0b	30.5	82.0
Control	Tarsus	149	6.7	14.6b	35.9	86.7
Control	Nevada	143	5.2	14.4b	35.0	76.9
Control	DME*	141	5.9	14.3	33.8	81.9
1 g m^{-2}	Coolguard	134	6.8	13.9b	31.9	94.4
1 g m^{-2}	Tarsus	116	4.9	13.4b	34.0	75.6
1 g m^{-2}	Nevada	158	6.1	16.8a	37.1	78.7
1 g m^{-2}	DME	136	5.9	14.7	34.3	82.9
2 g m^{-2}	Coolguard	149	7.5	13.3b	29.6	72.2
2 g m^{-2}	Tarsus	160	5.6	14.1b	37.3	78.9
2 g m^{-2}	Nevada	187	6.5	16.4a	38.2	92.2
2 g m^{-2}	DME	165	6.5	14.6	35.0	81.1
4 g m^{-2}	Coolguard	156	6.2	14.4b	32.4	86.1
4 g m^{-2}	Tarsus	126	4.6	14.3b	35.4	80.0
4 g m^{-2}	Nevada	160	5.5	14.1b	33.8	86.3
4 g m^{-2}	DME	147	5.4	14.3	33.9	84.1

*: Dosage Main Effect
Means followed by the same letters do not significantly differ ($P < 0.05$); 0.05 LSD for Head length: 1.61

in soil in tunnel where soil sterilization was not realized therefore the soil was with a natural population of fungi at the beginning of the experiment. However, contrary to the above postulation, Trichoderma treatment did not enhance yield significantly. In the glasshouse experiment in the straw bales the two methods of composting represented optimal and suboptimal conditions with regards to N concentrations available in the straw bales. While % N in the straw bales composted through method I was 1.13%, it was 0.72% in the straw bales from method II. The yield increase through the method I by the application of 15 g m^{-2} was 1.18 times (503g / 425 g) that of the control whereas the increase through the method II by the application of 10 g m^{-2} was 2.02 times (303 g / 150 g) that of the control treatment (Table 2). In line with the postulation of Rabeendran et al. [26], this calculation shows that T. harzianum was more effective on the plants grown on straw bales containing lower N level, i.e., the sub-optimal level.

Type and species of Trichoderma may be an important factor in its effectiveness both in the control of phytopathogenic fungi and in the induction of enhanced plant growth and yield. More than one species of Trichoderma can enhance growth, development and yield on different plants: T. harzianum and T. viride in cucumber and bell peppers [7, 23, 28], T. virens in cotton [15], T. longipile, T. tomentosum and T. virens in cabbage [26]. It seems, however, that in this regard, some of the Trichoderma species may interact better with certain plant species. In other words so called “affinity” of some of the Trichoderma species is high only with some plant species, that is root exudates of some plants may induce or inhibit mycelial growth of certain Trichoderma species only. Pea and maize root exudates strongly induced the mycelial growth of T. longibrachiatum, T. harzianum and T. viride. However, the increasing effect on the mycelial growth by the lettuce root exudates was only slight for the above mentioned Trichoderma species. The mycelial growth was completely inhibited by the exudates of onion and cabbage for the Trichoderma species above. Mycelial growth of T. koningii, of the slow mycelial growth type, was not significantly affected by any of the exudates from all the plants tested [11]. The present authors determined that T. harzianum did not increase onion yield and plant characteristics significantly [2]. This may have been due to the strong inhibition of the mycelial growth by the onion root exudates [11]. While root exudates of onion caused the inhibition, the lettuce root exudates favored T. harzianum only slightly. Our results confirm that this slight positive effect may have reflected also slightly or greater but not significantly into the plant growth and yield promotion capability of T. harzianum in lettuce. Type of soil and soil x plant interaction were also effective in the success of Trichoderma species [10]. Also, in line with Celar [11], on the interaction of Trichoderma spp. and plant species, Rootshield, the commercial product containing T. harzianum 1295-22, a very aggressive strain, developed through protoplast fusion [9, 16], did not increase yield and development of onion roots significantly [24].

In conclusion, the effect of Trichoderma harzianum on most of the seedling characteristics, plant characteristics at harvest, and yield were not statistically significant. This further confirmed the earlier report on the interaction between root exudates and Trichoderma species where root exudates of lettuce only slightly enhanced mycelial growth of Trichoderma harzianum. It seems that this slight effect translated into slight but not statistically significant increases in the characteristics studied here. However, with the manipulation of dosage and frequency of Trichoderma application significant increases may be obtained in the characteristics studied.

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