2013 International Conference on Agriculture and Biotechnology
IPCBEE vol. 60 (2013) © (2013) IACSIT Press, Singapore
DOI: 10.7763/IPCBEE. 2013. V60.25

The Elicitor Response in Lettuce

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Abstract. In this study, it was investigated that the effect of treatments of *Trichoderma harzianum (T. harzianum)*, chitosan and methy jasmonate on total chlorophyll content, plant growth parameter and yield of lettuce. *T. harzianum*, chitosan and methyl jasmonate was applied to the lettuce (*Lactuca sativa*. cv. 'Arapsacı') grown in greenhouse conditions. Changes in vegetative growth, total chlorophyll content, leaf relative water content, plant weight, marketable yield, number of leaves per plant, and number of deformation leaves per plant were determined after treatments. Values obtained from average plant height, marketable yield and number of leaves per plant parameters in the plants subjected to *Thichoderma* treatment was higher than methyl jasmonate and chitosan treatments. It was determined that the *T. harzianum* treatments increased the marketable yield of lettuce 12.29%. Total chlorophyll content increased with *T. harzianum*, whereas no difference was found between methyl jasmonate and chitosan treatments. Data further suggest that *T. harzianum* has a promotional effect on plant growth mechanism of lettuce as well as the effect of bio-pesticides.

Keywords: Chitosan, growth parameter, *Lactuca sativa*, methyl jasmonate, *Trichoderma*

1. Introduction

The ability to provide adequate food and fibre is becoming increasingly strained and continued improvement in sustainable plant disease management is required to help meet these demands [1]-[2]. Plant diseases often substantially reduce quality and quantity of agricultural commodities [3]-[4]. The introduction of more effective biologically based products with a broader spectrum of biological effects or better activity against key target organisms has provided alternative management option. These biologically based products used for different purposes in plants. Usually the user area increase resistance against plant diseases. In addition to these areas, there are also effects on plant physiology.

Trichoderma harzianum is one of the biological control agents used successfully against *Sclerotinia* and other pathogenic fungi in lettuce and other crops [5]-[7]. Positive effects of *Trichoderma* spp. are not limited to the above, many species of *Trichoderma* promoted growth and development of seedlings of vegetable and nonvegetable crops, namely cabbage, lettuce and cotton [8]-[10]. Chitosan, a deacetylated derivative of chitin, has also been reported to enhance disease resistance in plants [11] and chitosan is a natural, low toxic and inexpensive compound that is biodegradable and environmentally friendly with various applications in agriculture; it is obtained by the deacetylation of chitin. In agriculture, chitosan has been used in seed, leaf, fruit and vegetable coatings, as fertilizer and in controlled agrochemical release, to increase plant productivity [12], to protect plants against microorganisms [13] and against oxidative stress and to stimulate plant growth [13]-[14]. In the latter studies, a positive effect of chitosan was observed on the growth of roots, shoots and leaves of various plant species. Similar results were determined within sweet pepper and radish [14]-[15]. Methyl jasmonate is a well-known defense elicitor that is often used to mimic the effects of wounding by herbivores [16]-[20]. Taking into account mainly growth and yield promoting effects of *T.*

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harzianum. chitosan and methyl jasmonate the present experiment was carried out to find out if these effects are also available in the growing of lettuce.

2. Materials and Methods

2.1. Plant Materials

Lettuce (*Lactuca sativa* cv.Arapsacı) was grown in pots containing soil and sand mixture (soil:sand; 75:25 v/v). Seeds of lettuce were germinated in September 2012 and later transplanted to the greenhouse. Growing conditions consisted of a day/night temperature regime of 22 ± 2/18 ± 2 ℃ and 16-hour photoperiod. Fertilizer with concentrations of 15-15-15 mg L^{-1} of N, P_2O_5 and K_2O, respectively, was uniformly applied within one month to the soil.

2.2. Treatments

Trichoderma harzianum used was a commercially available product (TrichoFlow WPTM, Agrimm Technologies Ltd., New Zealand) and contained 108 cfu per gram. Water suspension of *T. harzianum* was drenched in the root zone. Two week old lettuce plants were sprayed homogenously with methyl jasmonate (Sigma-Aldrich, Germany)(above ground with deionized water containing 0.5 mM), chitosan (0.02%) (Sigma-Aldrich, Germany). The control plants were either sprayed with deionized water. The variables, total chlorophyll content in leaves and chlorophyll *a*/*b* ratio values were determined at harvest time. In all treatments, plant diameter (from the widest of plant), root diameter (from the widest of root) and plant height (from the root and end of leaf) of plants was determined. Leaves were separated, placed in bags and then in a drying oven at 80 ℃ for 2 days and weights determined. Content of relative water (RWC) at midday was determined with the formula: RWC = 100x[(FW- DW)/(TW- DW)] where FW = fresh weight, DW = dry weight, and TW = turgid weight. RWC was calculated with TW determined after fully hydrating fresh leaves in darkness at 4 ℃ for 18 hours [21]. Total chlorophyll and chlorophyll *a* and *b* were determined from the two youngest fully mature, symptomless leaves by extraction with 80% acetone and determining levels with a spectrophotometer (Shimadzu UV-120-01, Tokyo, Japan) at 645 and 663 nm, reported as mg/100 g FW [22]. Treatments were arranged in a completely randomized experimental design with 3 replicates, 5 plants in each replicate. Factor analysis of variance (ANOVA) was performed to partition the variance into main and interaction effects between variables. Means ± SE were calculated and differences tested for significance with LSD at the 5% level of significance. If the interaction was significant it was used to explain results.

3. Result and Discussion

The marketable yield and number of deformation leaves per plant are the most important indicators of lettuce. High marketable yield and low deformation leaves per plant in plants can be attributed to high productivity in lettuce. *T. harzianum* significantly increased yield both in leafy vegetable crops and fruit bearing vegetables such as cucumbers [23]-[24], [10]. In our study, an increase in marketable yield of lettuces treated with *T. harzianum* was observed. Average plant weight was similar among methyl jasmonate and chitosan treatments and visibly better than the control. Rabeendran et al. [10] also concluded that there were significant differences between plants subjected to *T. harzianum* and control plants with respect to marketable yield in lettuce. Foliar application of chitosan, improved average plant weight measures compared to untreated control plants but ANOVA results indicate that the effects of chitosan treatments were not significant (Table 1). This is contrary to the findings of Ghoname et al. [15] who found that mean weight of fruit and number of fruit of pepper grown in soil was increased significantly. Fresh weight of lettuce plants was increased significantly by the application of *T. harzianum* (Table 2). This result is similar in cucumber and cabbages [25]-[26]. The significant effect we observed on the leaf relative water content. Compared to the experimental controls, *T.harzianum* increased ($P < 0.05$) plant height and root diameter. There was no statistically significant difference between treated and control lettuces in plant diameter (Table 3). *T. harzianum* treatments caused an increase in root diameter. Similar results were also obtained by Björkman et al. [27]. Researchers investigated root development during growing in soil in greenhouse and determined an increase in sweet corn. In our experiments, response of plants to *T. harzianum* application varied in that while the increased plant height and root diameter at significant levels was recorded for some

organs of plants such an effect was not significant in others. Chlorophyll content was determined as high in *T.harzianum* treated plants, and low in methyl jasmonate and chitosan plants (Table 4). Farouk and Amany [28] carried out a study about chitosan in cowpea. The researchers determined increases in chlorophyll in water stress. Degradation of chlorophyll is a prominent phenomenon of senescence [29]. Reductions in the chlorophyll contents of plants became more severe with leaf aging and leaf yellowing. As can be seen in Table 4, significant differences were determined among the treatments with respect to the total leaf chlorophyll contents measured in the samples taken from harvested plant.

Table 1. Effects of biofunguside on yield in lettuce cv. Arapsacı

Treatments	Average plant weight (g plant^{-1})	Marketable yield (g plant^{-1})	Number of leaves per plant	Number of deformation leaves per plant
Control	345,01 ± 4.26 b	331,20 ± 1,26 b	75,00 ± 2,00 ab	3.00 ± 1,00 b
Trichoderma	384,09 ± 3,53 a	371,93 ± 1,95 a	79,00 ± 3,00 a	2,50 ± 0,50 b
Methy Jasmonate	353,26 ± 1,59 b	327,75 ± 1,80 b	69,65 ± 1,50 b	5,00 ± 0,01 a
Chitosan	355,15 ± 3,45 b	338,78 ± 1,16 b	70, 65 ± 2,00 b	3,33 ± 0,57 b
LSD	18,33	18.64	7,50	1,18

Table 2. Effects of elicitor on plant leaf parameter in lettuce cv. Arapsacı

Treatments	Leaf fresh weight (g)	Leaf dry weight (g)	Leaf Relative Water Content (%)
Control	15,37 ± 0,40 b	0,88 ± 0,028 a	83,66 ± 1,12 bc
T. harzianum	23,74 ± 1,98 a	0,98 ± 0,036 a	80,03 ± 1,55 c
Methy Jasmonate	16,18 ± 1,03 b	0,97 ± 0,130 a	90,43 ± 1,45 a
Chitosan	16,53 ± 1,25 b	0,93 ± 0,080 a	88,66 ± 2,43 ab
LSD	2,52	0,14	6,52

Table 3. Effects of elicitor on plant growth in lettuce cv. Arapsacı

Treatments	Plant diameter (cm)	Plant height (cm)	Root diameter (cm)
Control	21,50 ± 2,50 a	19,75 ± 2,50 b	1,45 ± 0,45 b
T. harzianum	25,00 ± 1,00 a	23,50 ± 2,00 a	2,00 ± 0,10 a
Methy Jasmonate	22,33 ± 1,80 a	21,00 ± 1,70 ab	1,50 ± 0,20 b
Chitosan	20,33 ± 1,85 a	18,33 ± 1,15 b	1,46 ± 0,50 b
LSD	7,34	3,65	0,43

Table 4. Effects of elicitor on total leaf chlorophyll, chlorophyll a and chlorophyll b in lettuce cv. Arapsacı

Treatments	Total leaf chlorophyll content (mg 100g^{-1}FW)^{a}	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Chlorophyll *a/b* ratio
Control	1,10 ± 0,11 ab	0,15 ± 0,01 b	0,92 ± 0,05 a	0,163
T. harzianum	1,55 ± 0,54 a	1,05 ± 0,38 a	0,51 ± 0,16 b	2,058
Methy Jasmonate	0,55 ± 0,06 c	0,36 ± 0,03 b	0,21 ± 0,02 c	1,714
Chitosan	0,60 ± 0,13 bc	0,39 ± 0,09 b	0,22 ± 0,03 c	1,772
LSD	0,37	0,16	0,54	

Lettuce (*Lactuca sativa* L.) is grown widely around the World and 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. *T. harzianum* is biological control agents used a lot of pathogenic fungi. The results obtained confirmed earlier suggestions that using *Trichoderma* as a biocontrol agent is an ideal solution for an integrated control programme combining biological and chemical methods [30]-[31]. The results of this study showed that *Trichoderma* for biocontrol of plant not only affects the quality of the positive, can be

used to improve the efficiency and quality. The results of this study obtained that the effect of *Trichoderma harzianum* on growth and quality lettuce plant characteristics, and yield were statistically significant.

4. Acknowledgments

This work was supported by the he Research Fund of the Uludag University (2010/14).

5. References

[1] L. Harrington. Sustainability in Perspective: Strengths and limitations of farming systems research in contributing to sustainable agriculture. *J. Sustain. Agric*. 1995, 5, 41–59.

[2] Y. L. Nene. Sustainable Agriculture: Future hope for developing countries. *Can. J. Plant Pathol.* 1996, 18, 133–140.

[3] L.M. Seitz, D. B. Sauer, H.E. Mohr, D.F. Aldis. Fungal growth and dry matter loss during bin storage of high-moisture corn. *Cereal Chem.* 1982, 59, 9–14.

[4] S.C. Alderman, D.D. Coats, F.J. Crowe. Impact of ergot on kentucky bluegrass grown for seed in northeastern *Oregon. Plant Dis*. 1996, 80, 853–855.

[5] Jones E.E., Stewart A., *Biological control of Sclerotinia minor in lettuce using Trichoderma species Proc*. 50th N.Z. Plant Protection Conf., 1997, pp.154-158.

[6] Lewis J.A., Lumsden R.D. Biocontrol of damping off of greenhouse grown crops caused by Rhizoctonia solani with a formulation of Trichoderma spp. *Crop Prot.*, 2001, 20: 49-56.

[7] J.M. Whipps. Microbial interactions and biological control in the rhizosphere. *J. Exp. Bot.* 2001, 52: 478-511.

[8] U. Bal, S. Altintas. Positive side effect from Trichoderma harzianum, the biological control agent: Increased yield in vegetable crops. *J.Environ. Protect.Ecol.* 2006, 7 (2): 383-387.

[9] L.E Hanson. 2000. Reduction of Verticillium wilt symptoms in cotton following seed treatment with Trichoderma virens. *The J. of Cotton Sci.* 2000, 4: 224-231.

[10] N. Rabeendran, D.J. Moot, E.E Jones, A. Stewart. Inconsistent growth promotion of cabbage and lettuce from Trichoderma isolates. *N.Z. Plant Prot.* 2000, 53: 143-146.

[11] N. Benhamou, J.W. Kloepper, S. Tuzun. Induction of systemic resistance to Fusarium wilt of tomato plants by seed treatment with chitosan. *Phytopathol.* 1994, 84, 1432–1444.

[12] N. New, S. Chandrkrachang, W.F. Stevens. *Application of chitosan in Myanmar's agriculture sector, in: Proceedings of the Sixth Asia Pacific Chitin and Chitosan Symposium,* May 23–26, 2004, The National Universityof Singapore.

[13] S. Farouk, K.M. Ghoneem, A. Ali Abeer. Induction and expression of systematic resistance to downy mildew disease in cucumber plant by elicitors. *Egyptian J. Phytopathol.* 2008, 1-2: 95-111.

[14] S. Farouk, A.A. Mosa, A.A. Taha, M. Ibrahim Heba, A.M. EL-Gahmery. Protective effect of humic acid and chitosan on radish (*Raphanus sativus* L. var. *sativus*) plants subjected to cadmium stress. *J. Stress Physiol. Biochem.* 2011, 7 (2):99-116.

[15] A.A. Ghoname, M.A. EL-Nemr, A.M.R. Abdel-Mawgoud W.A. El-Tohamy. Enhancement of sweet pepper crop growth and production by application of biological, organic and nutritional solutions. *Research J. Agric. Biol. Sci.* 2010. 6(7): 349-355.

[16] H. Gundlach, M.J. Muller, T.M. Kutchan, M.H. Zenk. Jasmonic acid is a signal transducer in elicitor-induced plant-cell cultures. *Proceed. of the National Academy of Sci.* 1992, 89: 2389–2393.

[17] M. McConn, R.A. Creelman, E. Bell, J.E. Mullet, J. Browse. Jasmonate is essential for insect defense Arabidopsis. *Proceed. of the National Academy of Sci.* 1997, 94: 5473–5477.

[18] I.T. Baldwin, W. Hamilton. Jasmonate-induced responses of Nicotiana sylvestris results in fitness costs due to impaired competitive ability for nitrogen. *J. Chem. Ecology* 2000, 26: 915–952.

[19] N.M. Van Dam, M. Horn, M. Mares, I.T. Baldwin. Ontogeny constrains systemic protease inhibitor response in Nicotiana attenuata. *J. Chem. Ecology*. 2001, 27: 547–568.

[20] L. Pauwels, D. Inze, A. Goossens. Jasmonate-inducible gene: what does it mean? *Trends in Plant Sci.* 2009, 14: 87–91.

[21] N. Goicoechea, J. Aguirreolea, J.M. Garcia-Mina. Alleviation of verticillium wilt in pepper (*Capsicum annuum* L.) by using the organic amendment COAH of natural origin. *Sci. Hort*. 2004, 101:23–27.

[22] D. Arnon. Copper enzymes in isolated chloroplasts; polyphenol oxidases in *Beta vulgaris*. *Plant Physiol.*1949, 24: 1–15.

[23] S. Altintas, U. Bal. Application of *Trichoderma harzianum* increases yield in cucumber (*Cucumis sativus*) grown in an unheated glasshouse. *J. Appl. Horticulture.* 2005, 7: 25-28.

[24] P. Poldma, A. Albrecht, A. Merivee. Influence of fungus Trichoderma viride on the yield of cucumber in greenhouse conditions, in:Proc. *Conference on Scientific Aspects of Organic Farming'*. Jelgava, Latvia 21-22 March 2002. pp. 176-180.

[25] P. Poldma, K. Jaakson, A. Merivee, A. Albrecht. Trichoderma viride promotes growth of cucumber plants. In '*Proc. Int. Conf. on Development of Environmentally Friendly Protection in the Baltic Region'* Transactions of Estonian Agricultural University 209, pp. 162-164, Tartu, Estonia, Sept 28-29, 2000 (BIOSIS, UK).

[26] I. Yedidia, A.K. Srivastva, Y. Kapulnik, I. Chet. Effects of Trichoderma harzianum on microelement concentrations and increased growth of cucumber plants. *Plant and Soil*. 2001, 235: 235-242.

[27] T. Björkman, G.E. Harman, L. Blanchard. Root development in sweet-corn inoculated with the biocontrol fungus Trichoderma harzianum *Amer. Soc. Horticult. Sci. Meeting*, Montreal (Abstract) http://www.nysaes.cornell. edu/ hort/ faculty/ bjorkman /other/ abstracts /Corn95. html, 1995.

[28] S. Farouk, A. Ramadan Amany. Improving growth and yield of cowpea by foliar application of chitosan under water stress. *Egyptian Jour. Biol.* 2012, 14: 14-26.

[29] H. Thomas, J.L. Stoddart. Leaf senecence. *Ann. Rev. Plant Physiol*. 1980. 31: 83-111.

[30] U. Bal, S. Altintas. Effects of Trichoderma Harzianum on Lettuce in Protected Cultivation. *Journal of Central European Agric.* 2008, 9: 63-70.

[31] A.Ahmed, C.P. Sánchez, M.E. Candela. Evaluation of induction of systemic resistance in pepper plants (*Capsicum annuum*) to *Phytophthora capsici* using *Trichoderma harzianum* and its relation with capsidiol accumulation. *European J. of Plant Pathol.* 2000, 106: 817–824.