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Full Length Research Paper

Effect of chitin on growth and chlorophyll content of two medicinal plants

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The effect of chitin soil amendment was studied in the characteristics of organic glasshouse cultivation of the lemon balm and tarragon plants. Chitin in the peat substrate did not affect the length and weight of the lemon balm plant, while at the peat-sand substrate it increased the corresponding sizes and the total chlorophyll content. In the peat-sand and chitin substrate the increase of the characteristics of the aboveground parts of tarragon was higher than in the peat and chitin substrate. Chitin affected the tarragon leaves resulting in the increase of the total chlorophyll content.

Key words: Chitin, growth, chlorophyll, *Melissa officinalis, Artemisia dracunculus,* peat, sand.

INTRODUCTION

In order to modify the physicochemical and biological properties of the soil in organic farming systems, cultural practices with soil amendments are very commonly used (Sikora, 1992; Spiegel et al., 1987). In terms of organic farming, chitin or substances rich in chitin have been used as an amendment to control fungal diseases and root-parasitic nematodes (Gooday, 1990). Chitin is included in the indicative list of substances approved by IFOAM for producing organic crops and processing of products (Gelinas and David, 2004, IFOAM, 2008). Chitin $(C_8H_{13}O_5N)_n$, an aminopolysaccharide, is the second most abundant polysaccharide after cellulose in nature and is a natural polymer, a basic structural polysaccharide. The chitin production in the maritime environment is extremely high (Evans 1993, Mian et al., 1982).

Actinomycetes are considered to be the dominant organisms involved in the soil decomposition of chitin. The soil may be hydrolyzed by the various chitinolytic enzymes that are produced by bacteria, actinomycetes, fungi and plants, and has crucial ecological significance since it is a very important source of nitrogen (Abdel-Fattah and Mohamedin 2000, El-Sayed et al., 2002). It has been reported (Dufour et al., 2003) that the addition of an organic substance rich in chitin to the soil reduced the populations of root knot nematodes. This is due to the development of microorganism populations (fungi, bacteria, actinomycetes) that feed on chitin. This increase in microorganisms causes the regeneration of saprophytic species of nematodes.

The soil treatment with chitin maintains its suppressiveness by preventing the development of certain pathogens. The cause of chitin's suppressive effect is the release of ammonium concentrations and the promotion of chitinolytic microorganisms, which present microbial activity (Rodriguez-Kabana et al., 1984; 1989; Spiegel et al., 1987).

The high soil availability of nitrogen N positively affects the plant growth. The decrease of the nematodes' population upon the amendment of chitin in the soil is related to changes in the bacteria colony inside the plant tissues, resulting in an immediate effect on the plants' functions (Hallmann et al., 1999).The plants have their own ways of benefiting from this situation in the root and soil interaction (Hallmann, 2003). When chitin enters the soil in large amounts, it is phytotoxic due to the release of ammonium concentrations (Evans, 1993; D' Addabbo, 1995; Brown et al., 1995; Spiegel et al., 1987). D'

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Addabbo (1995) mentions that when chitin concentration in the soil exceeds 1%, it has a phytotoxic effect.

The amendment with chitin alone (without antagonists) moderately increased the growth of pepper plants (Rajkumar et al., 2008). Applications of plant compounds such as chitin increased tomato fruit yield compared to plants grown in untreated soil (Giotis et al., 2009).

Arndt and Leuprecht (1994) showed better plant growth with chitin use, which they assumed was a result of the nitrogen release in the soil.

The addition of chitin in the soil at 1% (w/w) eliminated plant-parasitic nematodes in cotton planting, confirming long-term nematode suppressiveness induced by this organic amendment (Hallmann et al., 1999). Sarathchandra et al. (1996) mention that the shoot weight of the ryegrass (*Lolium perenne* L.) was greater in soil amended with chitin, most probably due to N mineralised from chitin. The N stimulates shoot growth more than root growth (Gulmon and Turner, 1978). Ladner et al. (2008) report that the total plant biomass fresh weight and the shoot fresh weight at a chitin concentration of 100 g in tomato plants was higher when compared to the control plant. They also report that root biomass fresh weight of the tomato plants indicated no significant differences compared to the plants growing in the control soil.

Medicinal and aromatic plants, considered a gift of nature, are being used against various infections and diseases in the world since past history. Over the past decades, medicinal plants gained in global importance. In addition, there is growing interest in organically produced herbs, and such herbs have to conform to organic certification standards. Lemon balm (*Melissa officinalis* L.) belongs to the family Lamiaceae and is known as an officinal herb of a long tradition and with a large variety of uses (Zargari, 1990; Ribeiro et al., 2001). Tarragon (*Artemisia dracunculus* L.) is a small, shrubby perennial herb of the Asteraceae family. It is cultivated for the use of its aromatic leaves in seasoning, salads, spices (Sayyah et al., 2004).

The object of the present experiment was to inverstigate whether the amendment of soil with chitin will affect the growth of lemon balm and tarragon plants, as well as their chlorophyll content.

MATERIALS AND METHODS

Seeds of lemon balm (*M. officinalis* L.) and tarragon (*A. dracunculus* L.) were sown in plastic trays, which were filled with potting soil (Gramoflor - potting soil, GhbH and Co., EN 12580) and covered with vermiculite (Agra- Vermiculite). The plastic seed trays remained in an environmental growth chamber (temperature 20 ± 2°C, relative humidity 90 ± 5%) until the first real leaves appeared and were afterwards transferred to a greenhouse (temperature 18 ± 2°C, relative humidity 70 ± 5%) for 15 days. The seedlings were transplanted to 2-litres pots (2 seedling per pot) filled with the following substrates: a) peat (Kekila), b) river sand and c) chitin (Chitin Sigma C7170), in the below mentioning substrates: 1. peat, 2. peat and chitin (2 g/l), 3. Peat-sand (2:1 v/v), 4. Peat-sand and chitin (2:1v/v +2 g/l chitin). The plants remained in the greenhouse for 15 days and were then transferred to a glasshouse, where they were randomly placed. Within the glasshouse, for each of these four substrates, there were four replications of 3 pots each randomly distributed. The assessment of the characteristics of the plants was performed 30 days after their transfer in the glasshouse and was repeated every 10 days afterwards. The fresh and dry weight of the several parts of the plant (leaves, shoot, root) as well as the height of the leaves, the length of the leaves and the root, and the number of leaves per plant, as well as the chlorophyll a and b content of the leaves, were measured.

Determination of chlorophyll content

The concentration of chlorophyll on the leaves was determined using the Shinano et al. (1996) method. The chlorophyll amount on the leaves was measured every 10 days after the plants were transplanted to the glasshouse. Every time and for each treatment three leaves were randomly collected from both plants of each pot, they were wrapped in plastic bags and transferred immediately in the laboratory for chlorophyll content estimation in the extracts. Three leaf discs (0.9 cm diameter) were placed in a test tube containing 5 ml of dimethylsulphoxide (DMSO; Sigma Chemical Co.). The test tubes were incubated at 65°C for about 90 minutes (Hiscox and Israestam, 1978) until all the chlorophyll was extracted in the DMSO. The concentration of chlorophyll a (Chla) was measured at 665nm and chlorophyll b (Chlb) 648nm, using a spectrophotometer (Shimadzu UV-160 L). The content of the chlorophylls was calculated according to Lichtenthaler and Wellburn (1983). The equations were;

Chlorophyll a, Chla = $14,85xA_{665} – 5,14xA_{648}$ (mg Chl a/ml)
Chlorophyl b, Chlb = $25,4xA_{648} – 7,36xA_{665}$ (mg Chl b/ml)
Total chlorophyll concentration (a+b), Chl(a+b) = Chla + Chlb (mg Chl/ml).

Statistical analysis

All data were tested by the analysis of variance (ANOVA), using the software SPSS 12. Duncan's multiple range test was performed at P = 0.05 for each of the significant variables measured.

RESULTS

Effect of chitin on the characteristics of lemon balm and tarragon plants

Chitin in the peat substrate did not seem to affect the fresh and dry weight of the lemon balm plant. On the contrary, in the peat-sand substrate chitin significantly increased the fresh and dry weight of the plant by 58% and 72% respectively (Table 1). The presence of chitin in the two substrates did not affect the height of the lemon balm plants (Figure 1).

In the peat substrate, chitin did not seem to affect the length and weight of the leaves and the shoots of lemon balm plants. However, in the peat-sand substrate there was a significant increase in both their length and weight, due to the chitin presence. The leaf length of lemon balm plants increased by 22%, and the fresh and dry weight by 67 and 63% respectively in the presence of chitin, compared to the corresponding substrates that did not

Table 1. Effects of chitin (2 g/l) on characteristics of lemon balm (*Melissa officinalis* L.) and estragon (*Artemisia dracunculus* L.) cultivated in greenhouse with substrates of: peat, peat and chitin (2 g/l), peat and sand (v/v2:1), peat, sand (v/v 2:1) and chitin (2 g/l). Each column represents the mean value of four repetitions.

		Peat	Peat+Chitin	Peat+Sand	Peat+Sand+Chitin
Melissa officinalis L					
Plant	Height (cm)	$46,18^{a}\pm1,66$	$39,76^{b}\pm1,66$	$38,70^{b}\pm1,66$	$42,93^{ab}\pm1,66$
	Fresh weight (g)	$9,93^{a}\pm0,80$	$8,90^{a}\pm0,80$	$6,00^{b}\pm0,80$	$9,50^{a}\pm0,80$
	Dry weight (g)	$1,75^{a}\pm0,15$	$1,45^{ab}\pm0,15$	$1,09^{b}\pm0,15$	$1,88^{ac}\pm0,15$
Leaf	Length (cm)	$4,57^{a}\pm0,08$	$5,41^{b}\pm0,10$	$4,05^{c}\pm0,09$	$4,94^{d}\pm0,08$
	Fresh weight (g)	$4,79^{a}\pm0,39$	$4,17^{a}\pm0,39$	$2,56^{b}\pm0,39$	$4,27^{a}\pm0,39$
	Dry weight (g)	$0,87^{a}\pm0,08$	$0,71^{ab}\pm0,08$	$0,54^{bc}\pm0,08$	$0,89^{abd}\pm0,08$
Root	Length (cm)	$32,06^{a}\pm1,74$	$28,08^{a}\pm1,74$	$28,17^{a}\pm1,74$	$28,97^{a}\pm1,74$
	Fresh weight (g)	$4,13^{a}\pm0,38$	$3,82^{ab}\pm0,38$	$2,85^{b}\pm0,38$	$4,14^{a}\pm0,38$
	Dry weight (g)	$0,68^{a}\pm0,06$	$0,54^{ab}\pm0,06$	$0,43^{b}\pm0,06$	$0,74^{ac}\pm0,06$
Stem	Length (cm)	$6,01^{a}\pm0,56$	$6,51^{ab}\pm0,58$	$4,54^{ac}\pm0,67$	$6,48^{ab}\pm0,54$
	Fresh weight (g)	$1,04^{a}\pm0,15$	$0,99^{a}\pm0,15$	$0,63^{ab}\pm0,18$	$1,09^{ac}\pm0,15$
	Dry weight (g)	$0,20^{a}\pm0,03$	$0,22^{ab}\pm0,03$	$0,13^{bc}\pm0,03$	$0,26^{abd}\pm0,03$
Artemisia dracunculus L					
Plant	Height (cm)	$27,63^{a}\pm1,29$	$31,65^{b}\pm1,29$	$27,54^{a}\pm1,29$	$32,09^{b}\pm1,29$
	Fresh weight (g)	$1,43^{a}\pm0,21$	$2,50^{b}\pm0,21$	$1,21^{a}\pm0,21$	$2,18^{b}\pm0,21$
	Dry weight (g)	$0,29^{a}\pm0,04$	$0,49^{b}\pm0,04$	$0,22^{a}\pm0,04$	$0,42^{b}\pm0,04$
Leaf	Length (cm)	$4,12^{a}\pm0,13$	$5,03^{b}\pm0,10$	$3,53^{c}\pm0,13$	$4,70^{d}\pm0,11$
	Fresh weight (g)	$0,66^{a}\pm0,11$	$1,10^{b}\pm0,11$	$0,43^{a}\pm0,11$	$0,99^{b}\pm0,11$
	Dry weight (g)	$0,11^{a}\pm0,02$	$0,20^{b}\pm0,02$	$0,07^{ac}\pm0,02$	$0,14^{ad}\pm0,02$
Root	Length (cm)	$13,30^{a}\pm1,04$	$14,58^{a}\pm1,04$	$16,05^{a}\pm1,04$	$15,69^{a}\pm1,04$
	Fresh weight (g)	$0,63^{a}\pm0,09$	$0,94^{b}\pm0,09$	$0,56^{a}\pm0,09$	$0,80^{ab}\pm0,09$
	Dry weight (g)	$0,13^{a}\pm0,02$	$0,18^{b}\pm0,02$	$0,11^{ac}\pm0,02$	$0,16^{abc}\pm0,02$
Stem	Length (cm)	$8,53^{a}\pm0,84$	$13,71^{b}\pm0,84$	$9,01^{a}\pm0,84$	$12,79^{b}\pm0,84$
	Fresh weight (g)	$0,29^{a}\pm0,18$	$0,42^{a}\pm0,18$	$0,74^{a}\pm0,18$	$0,34^{a}\pm0,18$
	Dry weight (g)	$0,08^{a}\pm0,02$	$0,11^{ab}\pm0,02$	$0,04^{ac}\pm0,02$	$0,08^{abc}\pm0,02$

The values that are followed by the same letter don't differ statistically between them, in a significance level of 5% ($P<0.05$).

contain any. In addition, the length and weight of the shoots increased significantly in the peat-sand substrate. In the presence of chitin, the shoots' length increased by 43%, the fresh weight by 74% and the dry weight by 102% (Table 1). The weight and the height of the tarragon plants were significantly increased in both substrates that contained chitin (Figure 2).

In the peat with chitin substrate the fresh and dry weight of the plants increased by 75% and 71% and in the peat-sand substrate by 80 and 93% respectively. The percentage of mean plant height increase was lower than the corresponding percent weight increase of the plants in the presence of chitin. In the peat and chitin substrate, the plant height was 15% increased and in the peat-sand substrate 17%, compared to the corresponding substrates that did not contain chitin (Table 1). Chitin significantly increased the weight and length of the tarragon leaves as well. In the peat substrate, the fresh and dry weight of the tarragon leaves increased by 68% and 85%, whereas in the peat-sand substrate, the fresh and

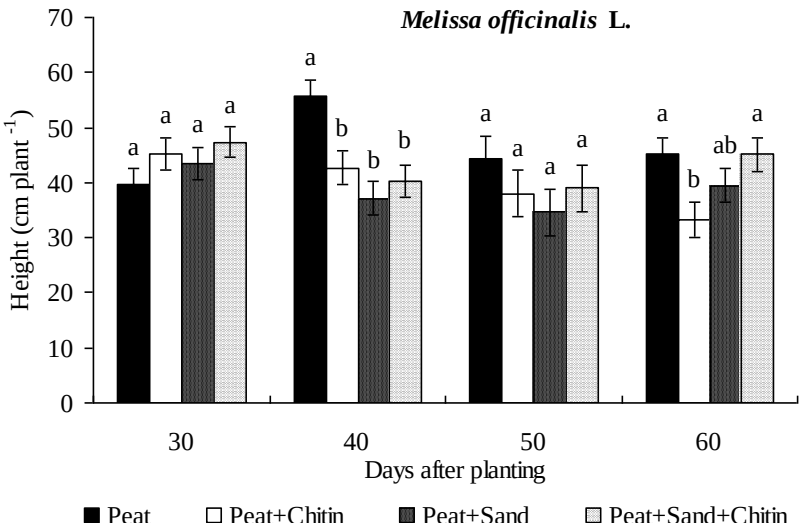

Figure 1. Effects of chitin (2 g/l) on the plant height of lemon balm (*Melissa officinalis* L.) cultivated in greenhouse with substrates of: peat, peat and chitin (2 g/l), peat and sand (v/v2:1), peat, sand (v/v 2:1) and chitin (2 g/l). Each column represents the mean value of four repetitions.
The values that are followed by the same letter donot differ statistically between them, in a significance level of 5% ($P < 0.05$).

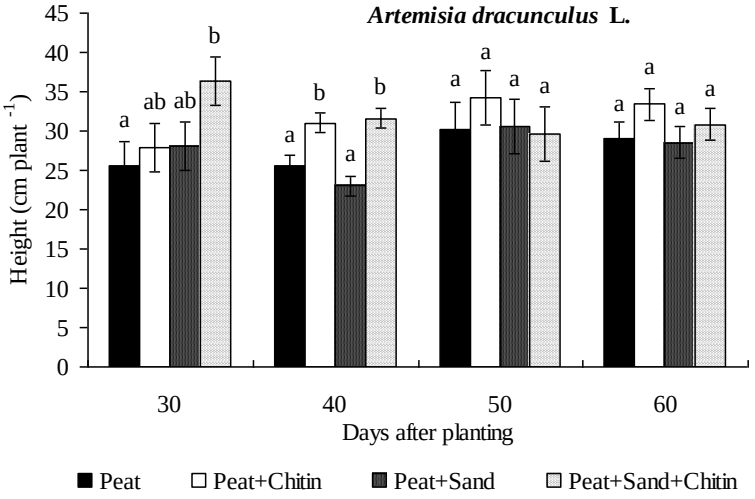

Figure 2. Effects of chitin (2 g/l) on the plant height of estragon (*Artemisia dracunculus* L.) cultivated in greenhouse with substrates of: peat, peat and chitin (2 g/l), peat and sand (v/v2:1), peat, sand (v/v 2:1) and chitin (2 g/l). Each column represents the mean value of four repetitions.
The values that are followed by the same letter donot differ statistically between them, in a significance level of 5% ($P < 0.05$).

dry weight increased by 131% and 106%, which is significantly higher that the corresponding ones without chitin. In the peat substrate, the leaf growth was 22% and in the sand and peat substrate it was 33% more compared to the corresponding ones that did not contain chitin (Table 1). On the other hand, chitin (2 g/l) did not affect the number of the leaves, but it increased significantly the length of the tarragon shoot. In the peat substrate the shoot length grew by 61% and in the peat-sand substrate by 42% more than in the corresponding substrates that did not contain chitin (Table 1). In the peat-sand with chitin substrate, the increase of the characteristics of the aboveground parts of the tarragon plants was higher than the corresponding ones in the peat and chitin alone

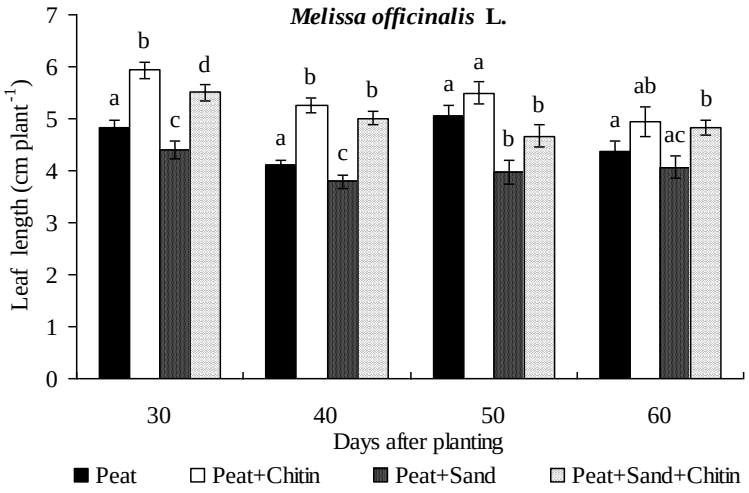

Figure 3. Effects of chitin (2 g/l) on the leaf length of lemon balm (*Melissa officinalis* L.) cultivated in greenhouse with substrates of: peat, peat and chitin (2 g/l), peat and sand (v/v2:1), peat, sand (v/v 2:1) and chitin (2 g/l). Each column represents the mean value of four repetitions.
The values that are followed by the same letter donot differ statistically between them, in a significance level of 5% (P < 0.05).

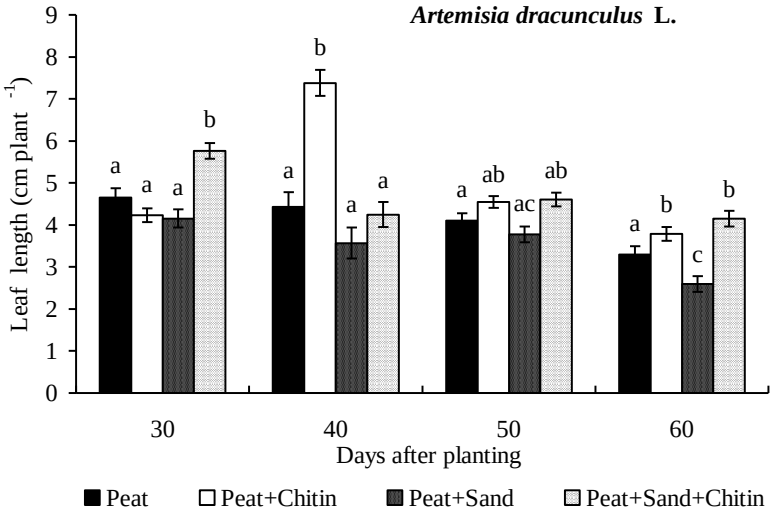

Figure 4. Effects of chitin (2 g/l) on the leaf length of estragon (*Artemisia dracunculus* L.) cultivated in greenhouse with substrates of: peat, peat and chitin (2 g/l), peat and sand (v/v 2:1), peat, sand (v/v 2:1) and chitin (2 g/l). Each column represents the mean value of four repetitions.
The values that are followed by the same letter donot differ statistically between them, in a significance level of 5% (P < 0.05).

substrates.

The change with time in plant height of the lemon balmplants for the period from the transplanting of the seedlings to the glasshouse until the end of the cultivation period is shown in Figure 1 and the change with time for the tarragon leaves in Figure 2. In the substrates with chitin amendment there is a growing tendency for the lemon balm leaf length, which is statistically significant compared to the corresponding leaf length in the substrates that did not contain any (Figure 3).

Chitin increased the length of the tarragon leaves in the peat-sand substrates from the seedling transplant to the glasshouse and until the end of the growing period (Figure 4).

In the lemon balm plants, the chitin in the peat substrate did not seem to affect either the fresh or dry weight of the

lemon balm root, while in the peat-sand substrate it significantly increased its weight. Also it significantly increased the tarragon root weight in the two plant substrates within chitin. It did not affect the length of the lemon balm or the tarragon root (Table 1).

Effect of chitin on the chlorophyll content

Chitin amendment did not seem to affect the lemon balm plants' total chlorophyll content (chlorophyll a + chlorophyll b) in the peat substrate, while it significantly increased it in the peat-sand substrate. The total chlorophyll content increased by an average of 49% compared to the corresponding substrates without chitin. This increase of the total chlorophyll content in the presence of chitin in this substrate is due to chlorophylls a and b; the increase of the chlorophyll a content in the leaves was at an average of 47% and the chlorophyll b content 60% (Figure 5).

The tarragon leaves showed a significant increase of the total chlorophyll content (chlorophyll a + chlorophyll b) in both substrates that contained chitin. The total chlorophyll content in the tarragon leaves in the peat and chitin substrate was 1.06 (mg g^{-1} fresh weight), an average 20% more, and in the peat-sand was 1.00 (mg g^{-1} FW), an average 24% more compared to the corresponding substrates without chitin, where the values were 0.89 and 0.80 (mg g^{-1} FW) respectively. This increase of the total chlorophyll content in the presence of chitin in the two substrates is mainly due to the higher chlorophyll a content. In the peat substrate the increase of the leaf chlorophyll a content was a mean of 20% and in the peat-sand substrate 26% (Figure 6).

During the plant growth in the glasshouse, the chitin amendment in the peat-sand substrate increased primarily the chlorophyll a content and secondly the chlorophyll b content, thus the total chlorophyll content (mg g^{-1} FW) in the lemon balm leaves increased (Table 2).

In addition, chitin significantly affected the tarragon leaves during the plant growth period by increasing the total chlorophyll content (mg g^{-1} FW). Indeed, this increase of the total chlorophyll content in the peat and chitin substrate reached up to 74% and in the peat-sand substrate 57%. The same trend is observed in both chlorophylls during the plant growth period in the glasshouse, but the increase differential was higher for chlorophyll a content than for chlorophyll b. The increase of chlorophyll a content in the peat substrate reached up to 70% and of chlorophyll b 35% in the plant leaves, while in the peat-sand substrate it was 58 and 38% respectively (Table 2).

DISCUSSION

Chitin has a direct effect on the plant functions (Hallmann et al., 1999). The plants have their own ways of benefiting from this situation in the soil-root interaction (Hallmann, 2003). In this study the growth responses of lemon balm and tarragon plant parts to chitin in two soil types were different when amending chitin in the soil and are consistent with Spiegel (1986), who reports that cultivating plants react differently to the addition of chitin. For example, tomatoes and beans react in a more sensitive way than corn (Spiegel 1986). The chitin in the peat substrate did not affect the leaf weight and length, the shoots and the entire lemon balm plant, but in the peat-sand substrate it increased them compared to the respective substrates that did not contain chitin.

On the contrary, the presence of chitin in the tarragon plants positively affected the aboveground parts in both substrates. In the peat and chitin substrate the leaf weight and length, the shoot length, as well as its weight and the plant height increased more than the equivalent in the peat substrate without chitin. In the peat-sand and chitin substrate the leaf weight and length, the shoot length, as well as the tarragon plant height increased. This study shows that the growth responses as a result of the chitin amendment in the substrates were higher, mainly in the tarragon plants. The chitin in the two substrates had a positive effect on the aboveground parts but did not affect the root of the tarragon. In the peat-sand substrate with chitin, the growth of the tarragon's aboveground parts was higher than the respective one in the peat and chitin substrate. The plant growth in the sand substrate seems to be the outcome of better soil physical properties (pore size distribution, particle size, porosity, water-holding capacity) and of microorganism populations (fungi, bacteria, actinomycetes) development that are fed on chitin and favoured plant growth conditions. Ben-Yephet et al. (2005) and Oka et al. (2007) also mention that the amendments were more effective in sandy soil than in soil from the organic farm, which contained more organic matter.

The result of this study shows that the increasing chitinolytic microbial activities in the soil which improve with chitin presence might be a major factor in the increase of plant length and weight and leaf chlorophyll content, because chitin addition to the soil is effective as a fertilizer amendment (El-Sayed et al., 2002). The relations between the soil environment, the chitin addition and the increase features of the plants are obviously complex and hard to assess. Chitin degradation by microorganisms plays an important role in the soil fertility and thus represents a significant source of energy and reflects the development of an adaptive microflora (Rodriguez-Kabana et al., 1983; Iglesias et al. 1994). Overall, the presence of chitin in the substrates tends to increase the height and the chlorophyll content of the lemon balm and tarragon plants.

Conclusion

The lemon balm and tarragon aromatic plants exhibit

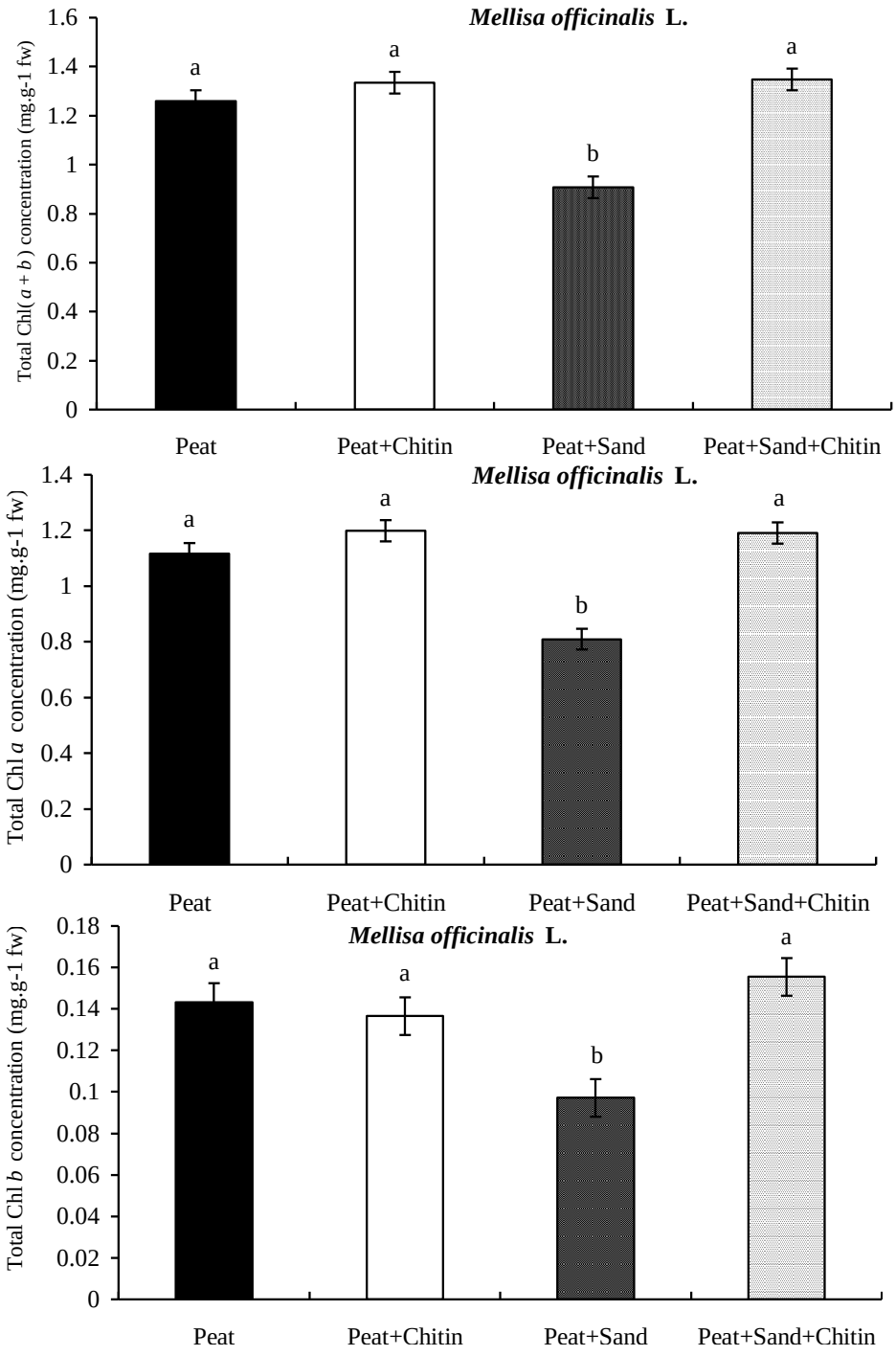

Figure 5. Effects of chitin (2 g/l) on chlorophyll a+b, a, and b content (mg.g-1 fresh weight) of lemon balm leaves (*Melissa officinalis* L.) cultivated in greenhouse with substrates of peat, peat and chitin (2 g/l), peat and sand (v/v 2:1), peat and sand (v/v 2:1) and chitin (2 g/l). Each column represents the mean value of four repetitions.
The values that are followed by the same letter don't differ statistically between them, in a significance level of 5% ($P < 0.05$).

different reactions to the amendment of chitin in two different soil types.

In the lemon balm plant, the chitin presence in the *peat substrate* did not affect the weight, length or total chlorophyll content of the leaves, the weight and length of the shoots or of the entire plant, but in the *peat-sand*

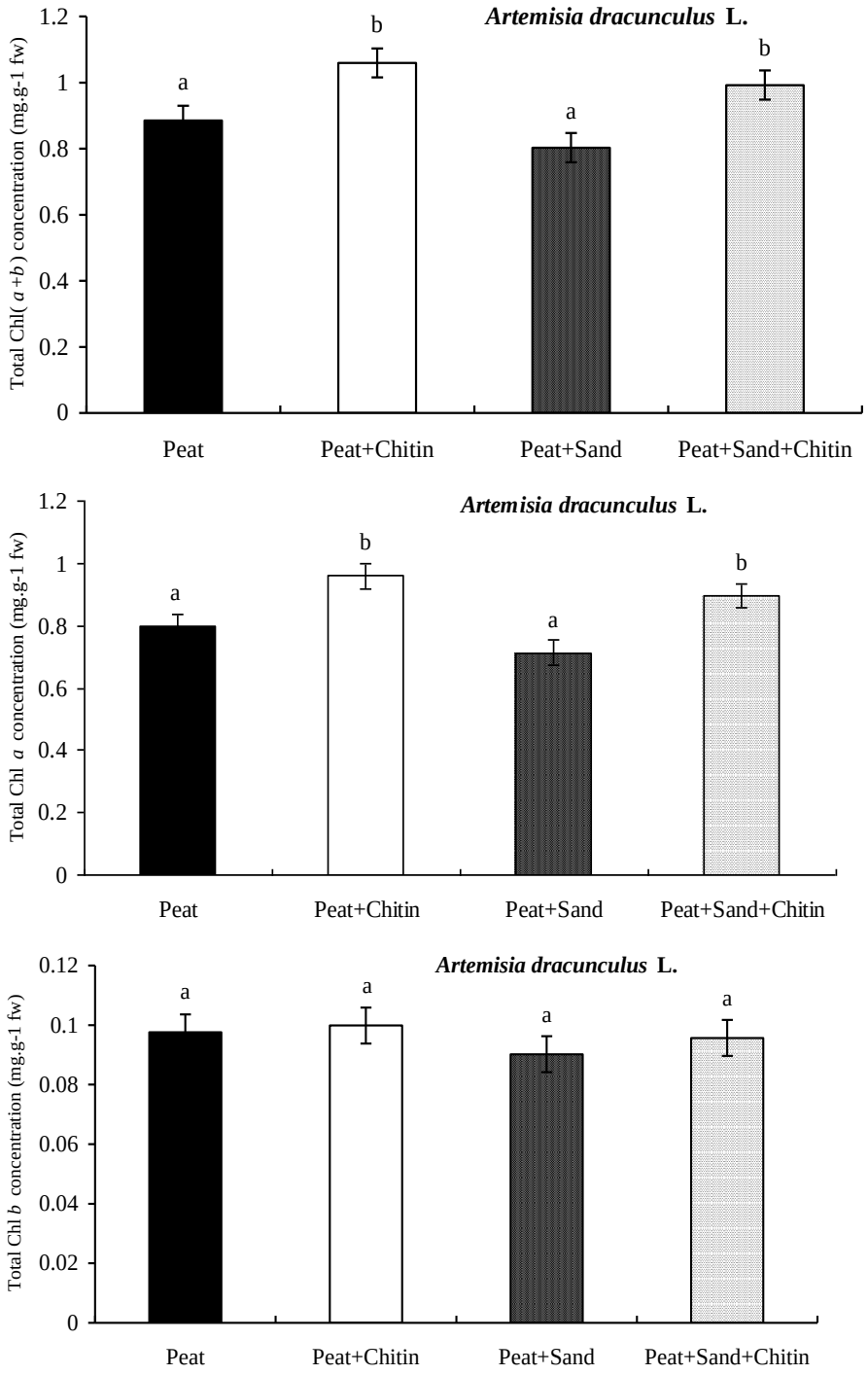

Figure 6. Effects of chitin (2g/l) on chlorophyll a+b, a, and b content (mg.g-1 fresh weight) of estragon (*Artemisia dracunculus* L.) cultivated in greenhouse with substrates of peat, peat and chitin (2g/l), peat and sand (v/v2:1), peat and sand (v/v 2:1) and chitin(2g/l). Each column represents the mean value of four repetitions.
The values that are followed by the same letter don't differ statistically between them, in a significance level of 5% ($P<0.05$).

substrate it increased the measured parameters compared to the respective substrates without chitin.

In the tarragon plants, chitin amendment had a positive effect on the characteristics of the aboveground parts in both substrates. In the sandy peat substrate with chitin, the growth of the aboveground parts' features was higher

Table 2: Effects of chitin (2 g/l) on the chlorophyll a+b, a, and b content (mg.g^{-1} fresh weight) of lemon balm leaves (*Melissa officinalis* L.) and estragon leaves (*Artemisia dracunculus* L.) cultivated in greenhouse with substrates of: peat, peat and chitin (2 g/l), peat and sand (v/v 2:1), peat and sand (v/v 2:1) and chitin (2 g/l). Each column represents the mean value of four repetitions.

(mg.g^{-1}fw)	Days	Peat	Peat+Chitin	Peat+Sand	Peat+Sand+Chitin
			***Melissa officinalis* L.**		
Chl(a+b)	30	1,98^{a}±0,07	1,70^{b}±0,07	1,27^{c}±0,07	1,70^{b}±0,07
	40	1,09^{a}±0,08	1,32^{ab}±0,08	0,95^{ac}±0,08	1,38^{ab}±0,08
	50	1,32^{a}±0,12	1,73^{b}±0,12	0,85^{c}±0,12	1,61^{ab}±0,12
	60	0,65^{a}±0,08	0,59^{a}±0,08	0,56^{a}±0,08	0,70^{a}±0,08
Chla	30	1,73^{a}±0,06	1,50^{b}±0,06	1,11^{c}±0,06	1,49^{b}±0,06
	40	1,03^{a}±0,07	1,25^{ab}±0,07	0,90^{a}c±0,07	1,30^{ab}±0,07
	50	1,11^{a}±0,10	1,48^{b}±0,10	0,70^{c}±0,10	1,32^{ab}±0,10
	60	0,59^{a}±0,06	0,56^{a}±0,06	0,53^{a}±0,06	0,65^{a}±0,06
Chlb	30	0,25^{a}±0,01	0,20^{b}±0,01	0,16^{c}±0,01	0,21^{b}±0,01
	40	0,06^{a}±0,02	0,07^{a}±0,02	0,05^{a}±0,02	0,07^{a}±0,02
	50	0,21^{a}±0,02	0,25^{ab}±0,02	0,15^{ac}±0,02	0,29^{ab}±0,02
	60	0,05^{a}±0,02	0,02^{a}±0,02	0,03^{a}±0,02	0,04^{a}±0,02
			***Artemisia dracunculus* L.**		
Chl(a+b)	30	1,47^{a}±0,09	1,35^{a}±0,09	0,99^{b}±0,09	1,21^{b}±0,09
	40	0,69^{a}±0,04	0,89^{b}±0,04	0,60^{a}±0,04	0,94^{b}±0,04
	50	0,59^{a}±0,11	1,02^{b}±0,11	0,88^{ab}±0,11	0,95^{ab}±0,11
	60	0,80^{a}±0,10	0,98^{a}±0,10	0,74^{a}±0,10	0,86^{a}±0,10
Chla	30	1,30^{a}±0,08	1,23^{a}±0,08	0,88^{b}±0,08	1,11^{ab}±0,08
	40	0,63^{a}±0,04	0,82^{b}±0,04	0,56^{a}±0,04	0,89^{b}±0,04
	50	0,52^{a}±0,09	0,89^{b}±0,09	0,74^{ab}±0,09	0,80^{ab}±0,09
	60	0,74^{a}±0,09	0,89^{a}±0,09	0,67^{a}±0,09	0,80^{a}±0,09
Chlb	30	0,16^{a}±0,01	0,12^{ab}±0,01	0,10^{ab}±0,01	0,11^{ab}±0,01
	40	0,06^{a}±0,01	0,07^{a}±0,01	0,04^{a}±0,01	0,06^{a}±0,01
	50	0,11^{a}±0,01	0,13^{a}±0,01	0,14^{a}±0,01	0,15^{a}±0,01
	60	0,06^{a}±0,01	0,08^{a}±0,01	0,08^{a}±0,01	0,07^{a}±0,01

The values that are followed by the same letter donot differ statistically between them, in a significance level of 5% (P<0.05).

than the respective in the peat and chitin substrate.

The relations between the soil environment, the chitin amendment and the plants' growth features are evidently complex and render their assessment a difficult task.

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