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CONTROL OF CHAMOMILE POWDERY MILDEW DISEASE USING POTASSIEN, CITRIEN AND CHITOSAN

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ABSTRACT

The effect of three resistance inducers, *i.e.* chitosan, citrien and potassien were studied under both greenhouse (2009/2010) and field condition in 2010/2011 and 2011/2012 growing seasons to control powdery mildew disease caused by *Sphearotheca fuliginea* in chamomile (*Matricaria chamomilla*). Results showed that all used inducers had decreased disease severity compared to non-treated control. In this respect, chitosan showed the highest effect on decreasing disease severity followed by citrien. Meanwhile, potassien gave the lowest effect. The decrease in disease severity of powdery mildew was positively expressed by an increase in crop parameters, *i.e* numbers, inflorescences weights, fresh, dry weights of plants and the principal components of chamomile essential oil.

INTODUCTION

Chamomile (*Matricaria chamomilla* L.) is one of the most important medicinal and aromatic plants worldwide and in Egypt. Chamomile plant, belonging to family *Asteraceae (Compositae*), mainly cultivated for obtaining the dry fluorescence that containing essential oil which is used in several medicinal industries and preparations of cosmetics compounds. The essential oil of chamomile, recognized as a heavy viscous liquid and being as blue colored due to chamazulene, contains many active compounds such as bisabolol, bisabolol oxide A, bisabolol oxide B, Beta, trans farnesene, bisabolon oxide and chamazulene (Reda *et al*., 1999).

Powdery mildew is considered one of the most important diseases attacking chamomile plant in its different cultivated areas. This disease was detected in Egypt for the first time in 1970. Then, the causal pathogen was identified as the fungus *Sphaerotheca fuliginea* Schlect. Fr. poll **(**Hilal *et al.,* 1998).

Chamomile powdery mildew frequently occurs in the open field, estimated percentages of infection reaching 20-80%. Disease symptoms are shown up as powdery spots on leaves, stalks and small shoots on the lower parts of the plant, then the infection progressively increase to the upper parts of plant. These spots are characterized as white color in the beginning of infection, and then turn to gray color. In the severe infection, diseased plant seems as covered with layer form talk powder and this causes great damage for plant foliar. Since; this disease has negative effect on the quantity and quality of inflorescences yield regarded to the major purpose of chamomile plantation, all the possible measures should be considered to manage it.

The use of fungicides against plant diseases causes several problems such as carcinogenicity, development of fungicidal resistance and phytotoxicity as well as adverse effects of human health and environmental balance. Thus, it is urgent to apply alternative safe efficient methods against plant diseases. Induced disease resistance can be defined as the process of active resistance dependent on the host plant physical or chemical barriers activated by biotic or abiotic agents (walters et al., 2007). The resistance of plant may induce when using citric acid (El-Saidy and Abd El-Hai, 2011). Chitosan is a non-toxic compound and was reported to be resistance inducer against some fungal pathogens (Lafontaine and Benhamou, 1996 and El-Mougy et al., 2002). Hence, this work aims to evaluate the efficiency of three resistance inducers on management of chamomile powdery mildew under greenhouse and field conditions in Egypt.

MATERIALS AND METHODS

Survey of Chamomile powdery mildew at Fayoum Governorate:

Assessment of naturally infected chamomile plants with powdery mildew for diseases incidence and disease severity was conducted during the period from December, 2009 to April, 2010 at five districts of Fayoum governorate located at Fayoum, Sinnoures, Tamyea, Abshaway and Yousef El Seddik. The survey was done three times during December, 2009, February and April, 2010.

Artificial inoculation technique:

Chamomile plants were inoculated after two weeks from transplanting with the pathogen conidia. Inoculation was accomplished by shaking or gently dusting infected samples with powdery mildew conidia over the healthy plants. Inoculated plants were covered with polyethylene bags for 24 hours to maintain enough moisture necessary for infection and kept under greenhouse conditions, a set of uninoculated plants were kept as check treatment. Inoculated plants were examined daily and disease symptoms incidence and severity was calculated at 10 days after inoculation according to the method of Whiteny *et al* (1983).

Greenhouse experiment:

Three different concentrations, *i.e.* 100, 75 and 50% were prepared from two commercial plant nutrients, *i.e.* Potassien (30% Phosphorus pentaoxide) and Citrien (15% active citric acid and 6% (Zn - Mn- Fe)) were obtained from the General Organization for Agriculture Equalization fund, ARC, Giza and one commercial product chitosan form of (Chitocare; containing chitosan 2.5% + N 1000 ppm + K 500 ppm + Zn 100 ppm +Cu 50 ppm + P 500 ppm + Mn 50 ppm + B 50 ppm) was obtained from Egypt Chemia Company Prepared concentrations were sprayed in advance to inoculation of 45 days old chamomile seedlings transplanted in 25cm diametr pots. Disease severity (%) was recorded 10 days after each spray and the average was recorded according to Whitney *et al* (1983). Randomized complete block design was applied to all treatments and statistical analysis for recorded data was done. Control treatment was sprayed with water only.

Field experiment:

Field experiments were carried out at Mineshat-Sabbry village, Etsa district at Fayoum Governorate using randomized complete block design during two successive growing seasons of 2009/2010 and 2010/2011. All the three chemical inducers were triple sprayed on chamomile plants grown under open field conditions, three weeks after transplanting, and repeated two times every two weeks. Control treatment sprayed with water only. Disease severity (%) was assessed according to Whitney *et al* (1983).

Oil extraction:

The essential oil of air dried samples of chamomile inflorescences (100 g) from sprayed plants with three resistance inducers was extracted by hydro-distillation for 3 h using a Clevenger type apparatus. The distillated oils were dried over anhydrous sodium sulphate and stored in tightly closed dark vials at 4^0 C until analysis.

Oil analysis by Gas Chromatograph (GC):

GC analysis was performed by using a Thermoquest Gas Chromatograph with a Flame Ionization Detector apparatus. The analysis was carried out using fused silica capillary DB-1 column (60 m x 25 mm i.d, film thickness 0.25µm). The operating conditions were as follows: Injector and detector temperatures were 250 and 300^0 C, respectively. Nitrogen was used as carrier gas at a flow rate of 1 mL min, oven temperature program, $60\text{-}250^0$C at the rate of 5^0 C min^{-1} and finally held isothermally for 10 min.

Chamomile growth and yield parameters

Effect on plant growth:

Morphological parameters include; plant height (cm), number of branches, fresh and dry weight (g)/plant, were recorded to study the effect of powdery mildew disease on the growth of chamomile plants sprayed with three resistance inducers grown under field conditions.

Effect on yield (inflorescence and oil components):

The effect different resistant inducer spray treatments on powdery mildew disease severity affected inflorescence yield of chamomile plants were assessed. Also, different parameters of inflorescence yield represented by the number of inflorescences/plant as well as fresh and dry weight of inflorescences/plant along with essential oil components were determined.

Disease incidence and severity assessment:

1- Percentage of disease incidence (DI %):

The percentage of powdery mildew disease incidence was calculated as follows:-

D I (%) = No. of infected plants / Total inspected plants X 100

2- Percentage of disease severity (DS %)

The percentage of disease severity was calculated using the disease scale levels from 0 to 4 after Whitney ***et al*., (1983)** as follows:

$$\text{Disease severity}(\%) = \frac{\sum(\text{rating No.} \times \text{No. of plants in each rating})}{\text{Total No. of plants} \times \text{highest rating}} \times 100$$

Where:
0 = No mildew colonies observed.
1 = 1 - 25% of plant area covered with mildew.
2 = 26 - 50% of plant area covered with mildew.
3 = 51 - 75% of plant area covered with mildew.
4 = 76 - 100% of plant area covered with mildew.

Statistical analysis:

Data obtained in these experiments were statistically analyzed using factorial design suggested by Snedecor and Cochran (1982). Least significant difference (LSD) at 5% probability was used to compare between treatment averages (Fisher, 1984).

RESULTS

1. Survey of chamomile powdery mildew

Data presented in **Table (1)** indicate that among 6 locations surveyed Etsa ranked the highest average of recorded for powdery mildew disease incidence percentage (80.21%) during the period from December, 2009 to April, 2010; followed by 78. 29% in Yousef El Seddik while the lowest recorded disease incidence percentage was 68.61% in Tamyea. Disease severity (DS %) highest average values were 64.18% and 61.43% at Yousef El-Seddik and Abshaway , respectively. Meanwhile, the lowest recorded average disease severity value was 51.08 % in Tamyea . The averages of disease incidence (DI %) and disease severity (DS %) were increased gradually and reached the maximum on April, 2010 as shown in Table 1.

Table (1): Survey of chamomile powdery mildew disease incidence and severity in the different growing areas at Fayoum Governorate during 2009/2010

Location	Disease assessment from December, 2009 to April, 2010							
	Diseases incidence %				Disease severity %*			
	Dec.	Feb.	Apr.	Average (%)	Dec.	Feb.	Apr.	Average (%)
El-Fayoum	41.56	81.00	92.23	71.59	28.74	63.91	70.43	54.36
Sinnoures	40.00	82.36	90.25	70.87	28.00	65.36	69.51	54.29
Tamyea	40.32	79.00	86.50	68.61	22.74	62.50	68.00	51.08
Abshaway	45.84	86.29	93.40	75.17	35.18	70.41	78.69	61.43
Yousef ElSeddik	49.38	88.34	97.16	78.29	38.56	74.92	79.06	64.18
Etsa	51.13	90.50	99.00	80.21	32.58	69.26	77.92	59.92
Mean	44.70	84.58	93.10	74.12	30.97	67.73	73.94	57.54
L.S.D. at 5%	*4.81*	*4.27*	*4.92*	-	*4.04*	*4.12*	*4.40*	-

*** according to the method of Whiteny *et al* (1983).**

2.1. Effect of commercial plant nutrients and chitosan on powdery mildew disease severity of chamomile under greenhouse condition.

Data in Table (2) showed the effect of the tested resistance inducers *i.e*. Chitosan, Citrien, Potassien at three different concentrations (100, 75 and 50%) on the percentage of disease severity of chamomile powdery mildew before inoculation with *S. fuliginea* under greenhouse conditions. All tested concentrations caused a significant decrease in disease severity compared to control. Increasing concentration of the tested inducers led to a decrease in disease severity. In addition, the highest decreasing effect on disease severity before inoculation was obtained when plants sprayed with Chitosan (17.70%) compared to control (48.18%) while, the lowest effect was recorded when plants treated with Potassien then followed by Citrien, that showed mean disease severity being 27.90 and 26.17%, respectively.

Table (2): Effect of Three different sprayed concentrations prepared from two commercial plant nutrients and chitosan on powdery mildew disease severity of chamomile under greenhouse condition

Treatments** (Resistance inducers)	**Concentration (%)***		
	100%	**75%**	**50%**
	Disease severity (%)		
Citrien	22.59	24.07	31.85
Potassien	24.81	26.29	32.59
Chitosan	14.37	17.33	21.40
- Control	52.13	45.52	46.88

Least significant difference (L.S.D.) at:		
	1%	5%
Treatments (T)	3.87	4.18
Concentration (T)	3.35	3.62
Interaction (T X C)	6.70	7.24

**** Three different sprayed concentrations; prepared from two commercial plant nutrients, *i.e.* Potassien (30% phosphorus pentaoxide) and Citrien (15% active citric acid and 6% (Zn - Mn- Fe) and chitosan (commercial product)**

Effect of commercial plant nutrients and chitosan spray on powdery mildew disease severity of chamomile under field conditions:

Table (3) showed the effect of three resistance inducers, *i.e*. Citrien, Potassien and chitosan on disease severity of chamomile powdery mildew during two successive seasons. Under field conditions, the used inducers caused significant decrease in the percentage of disease severity compared to the control. During the first season, chitosan was the most effective one in reducing powdery mildew followed by Citrien while Potassien was the lowest. The recorded percentages of disease severity were 28.79, 36.29%, and 41.50, respectively. Also, the results trend of the three resistance inducers was similarly recognized during the second season being 28.85, 32.82 and 38.69%, respectively, compared with control 60.86%. These results are confirmed with the results obtained under greenhouse conditions.

Table (3): Effect of commercial plant nutrients and chitosan spray on disease severity of chamomile powdery mildew during two successive growing seasons of field experiment

Treatments** **(Resistance inducers)**	**Growing season**	
	2010/2011	**2011/2012**
	Disease severity (%)	
Citrien	36.29	32.82
Potassien	41.50	38.69
Chitosan	28.79	28.85
- Control	60.86	64.33
(L.S.D.) at 0.05	*7.62*	*8.89*

**** sprayed with effective concentration prepared from two commercial plant nutrients, *i.e.* Potassien (30% phosphorus pentaoxide) and Citrien (15% active citric acid and 6% (Zn - Mn- Fe) and chitosan (commercial product) .**

Effect of commercial plant nutrients and chitosan spray on some growth parameters of chamomile during two successive growing seasons of field experiment.

Data presented in Table(4) indicated that all treatments spray led to significant increase in plant highest, number of branches and fresh and dry weights of chamomile plants compared to control treatment. In the first season, the greatest effect (61.83 cm) was recorded for Citrien followed by 58.05cm for Chitosan, respectively, as compared to control (48.33cm.). The lowest effect on plant height was recorded (52.50 cm) on plants treated with Potassien. The greatest average numbers of branches were 15.23 and 14.35 obtained when plants were treated with Citrien and Chitosan; respectively as compared to the control (10.83). The lowest mean number of branches was 12.65 recorded after plant treatment with Potassien. In addition, the best recorded values of fresh and dry weights of plant were 664.04g and 136.26 g , respectively for the plants treated with Citrien. The corresponding figures recorded for control treatment were 526.39g and 120.31g; respectively.

2.4. Effect of commercial plant nutrients and chitosan spray on yield components of chamomile during two successive growing seasons of field experiment.

Data shown in Table (5) revealed that all sprayed treatments caused significant increase in chamomile inflorescences yield including inflorescence number and inflorescence fresh and dry weights compared with control treatment. In the first season, the highest number of inflorescences recorded were 566.20 and 548.57, for plants treated with Chitosan and Citrien; respectively. The lowest number recorded was 508.13 when plants were treated with Potassien. On the other hand, the highest fresh and dry weights of inflorescences were 178.81 g and 37.55 g; respectively when plants were treated with chitosan compared to the control that recorded 128.75 and 27.11g, respectively. The lowest fresh and dry weights of inflorescences for plants treated with Potassien were 159.24g and 30.60 g. On the other hand, the highest recorded fresh and dry weights of inflorescences treated with chitosan were 166.22g and 34.47g, compared with similar recorded values 124.41 and 27.41g of control treatments; respectively. The lowest recorded fresh and dry weights of inflorescences were 146.65g and 27.80 g for plants treated with Potassien.

2.5. Effect of commercial plant nutrients and chitosan sprays on essential oil components of chamomile.

Data presented in **Table (6)** and **Fig.(1)** showed that chamomile essential oils contain seven components. Two components namely; Bisabalol oxide A and Bisabalol oxide B were the main components occurred in highest percentages. However, the highest recorded value of Bisabalol oxide A was 47,878 in plants treated with Chitosan followed by 44,992 and 44,014 for treatments treated with Potasin and Citrien, respectively compared with 44,267 for control treatment. On the other hand, Bisabalol oxide B recoded its highest percentage in treatment sprayed with Citrien followed by Chitosan treatment.

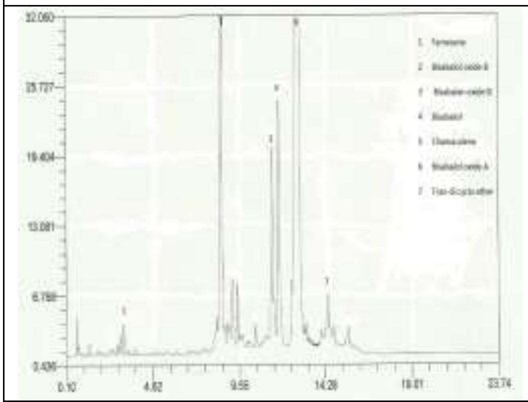
T 4.5

Table (6) : Effect of commercial plant nutrients and chitosan sprays on essential oil components of chamomile

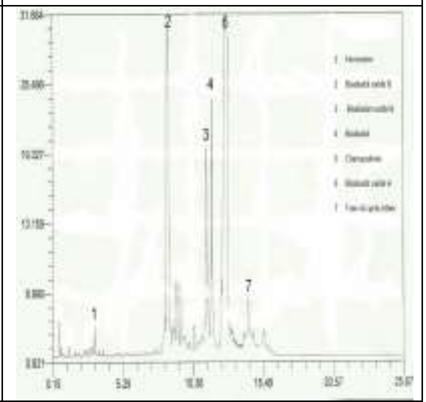
Essential oil Components	Treatment sprays			
	Control	Chitosan	Potassien	Citrien
Farnesene	1.361	0.595	1.575	1.619
Bisabalol oxide B	20.169	24.161	20.334	27.220
Bisabalan oxide B	8.659	7.221	8.730	5.270
Bisabalol	7.589	8.747	7.651	7.073
Chamazulene	-	-	-	2.699
Bisabalol oxide A	44.267	47.878	44.992	44.014
Tran-di cyclo ether	5.929	1.198	5.978	4.412
U.K.	12.026	10.200	10.740	7.693

Analysis of essential oil of chamomile plants treated with:

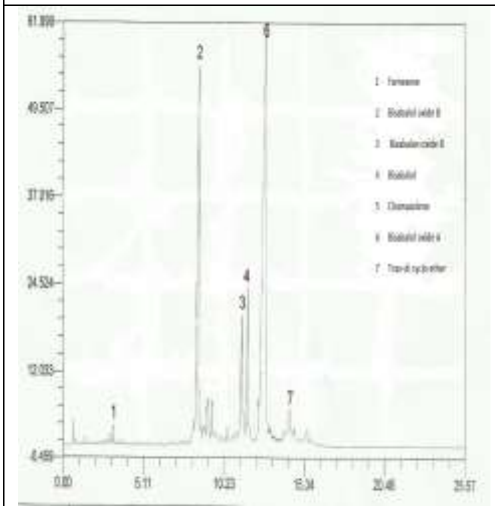
Control



Chitosan



Potassien



Citrien

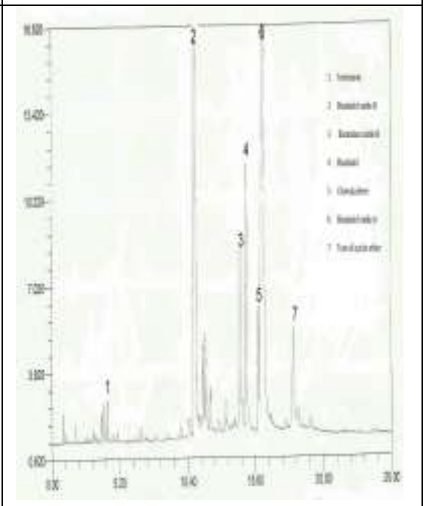


Fig (1): GLC chromatogram of chamomile essential oil distilled from inflorescences treated with commercial plant nutrients and chitosan sprays compared with control.

Discussion

Chamomile is one of the most important medicinal herbs native to southern and eastern Europe. It is also grown in Germany, Hungary, France, Russia, Yugoslavia, and Brazil. Also, chamomile has been used in herbal remedies for thousands of years, as known in ancient Egypt, Greece and Roman. It is used mainly as an anti-inflammatory and antiseptic as well as antispasmodic.

The causal organism of chamomile powdery mildew was identified as *Sphaerotheca fuliginea* (Schlecht.Fr.) Poll; according to the observed disease symptoms and morphological characteristics of conidial stage. Our results were in consistence with those by (Kapoor, 1967; Prasada *et al.,* 1968; EL-Zarka, 1970; EL- Kazzaz, 1981; Joshi and Saksena, 1983; Kenneth and Palti, 1984; Cheah *et al.,* 1996; Hilal *et al.,* 1998 and Bharat, 2003).

Survey conducted at six districts of El Fayoum governorate, indicated that chamomile powdery mildew was more severe in Yousef El-Seddik district followed by Abshaway. The lowest disease severity was recorded in Tamyea district. The recorded differences on the disease widespread may attributed to the differences in climatic conditions among these districts which encourage the increase of the powdery mildew severity on chamomile plants.

Chitosan, Citrien and Potassien were tested individually, as resistance inducer in controlling chamomile powdery mildew, at three different concentrations before and after inoculation with the pathogen. The results showed that all inducers decreased disease severity as compared to the check treatment. Chitosan followed by Citrien were highly effective treatments against disease control compared to the check treatments while Potassien had the lowest effect in this regard. These positive effects of chitosan, Citrien and Potassien in disease control of powdery mildew were reported on other crops (Hadwiger *et al,* 1984; El Ghaouth *et al.,* l992; Fajardo *et al.,* 1995; Reuveni et al.,1994 and 1996; Reuveni and Reuveni, 1998; Orober *et al*., 1999; Ehret *et al*., 2002; Mosa, 2002; Dik *et al*., 2003; Bautista *et at*., 2006 and Saleh *et al*., 2007).

Chitosan, a given name to a deacetylated form of chitin, is a natural biodegradable compound derived from crustaceous shells such as crabs and shrimps, whose main attributes correspond to its polycationic nature. Chitosan has been proven to control numerous pre - and post - harvest diseases on various horticultural commodities.

In addition to its direct effect on microbial activity, other studies strongly suggested that chitosan inducing a series of defense reactions correlated with enzymatic activities. Chitosan has been shown to increase the production of glucan-hydrolases, phenolic compounds and synthesis of specific phytoalexins with antifungal activity, and also reduces macerating enzymes such as polygalacturonases, pectin methyl esterase etc Ben-Shalom *et al*. (2003). Furthermore, chitosan induces structural barriers for example inducing the synthesis of lignin-like material. In some horticultural and ornamental commodities, chitosan increased harvested yield due to its ability to form a semi permeable coating Borkowski and Kowalczyk, (1999), chitosan extends the shelf life of treated fruit and vegetables by minimizing the rate of respiration and reducing water loss Borkowski *et al*. (2004). As a nontoxic biodegradable material, as well as an elicitor, chitosan has the potential to become a new class of plant protectant, assisting towards the goal of sustainable agriculture. These results are similar to those obtained by many researchers (Hadwiger *et al.,* 1984; El-Ghaouth *et al.,* l992; Fajardo *et al.,* 1995; Bautista *et al*., 2006; and Moret *et al*., 2009). In the present study it has been determined that the induced resistance compounds could be used as an effective and safe technique for controlling the disease in addition to avoid environmental pollution. Meanwhile, further studies on the alternatives control means using inducer resistance may be extended in the future. Furthermore, because of the new strategy of plant protection will consider keeping the environmental conditions free from the harmful pollution a priority of further studies are needed to encourage the use of alternatives chemicals for disease control.

Moreover, field experiments confirmed that reduction in disease severity due to the effects of the tested inducers as expressed on significant increase in plant height, number of branches and fresh and dry weight of the plants compared to the control treatment. Furthermore, resistance inducers increased the main components of chamomile inflorescences essential oil (Borkowski and Kowalczyk, 1999; Borkowski *et al.* (2004); Gharib, (2007) and Abdel - Aziz, 2010. Further trials with the nonspecific disease resistance compounds may be adviced.

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مقاومة مرض البياض الدقيقى على البابونج باستخدام البوتاسين والسترين والشيتوزان

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تم دراسة تأثير ثلاثة من المواد المستحثة للمقاومة وهى الشيتوزان والسترين والبوتاسين تحت ظروف الصوبة (2010/2009) وتحت ظروف الحقل فى موسمى (2011/2010 و2012/2011) لمقاومة مرض البياض الدقيقى على نباتات البابونج.

أظهرت النتائج المتحصل عليها أن المواد المستحثة للمقاومة أدت الى خفض الاصابة مقارنة بالنباتات غير المعاملة بالاضافة لذلك فان الشيتوزان كان الأكثر كفاءة فى خفض شدة الاصابة يليه السترين بينما كان التأثير الاقل لمركب البوتاسين.جدير بالذكر أن خفض شدة الاصابة بالمرض كان مصحوبا بزيادة فى قياسات النباتات المختبرة مثل عدد ووزن النورات الناتجة وكذا طول وعدد أفرع النبات والوزن الطازج والجاف للنباتات. كما أن المعاملة بالمواد المستحثة للمقاومة أدت الى زيادة فى المكونات الرئيسية للزيت الطيار مقارنة بالغير معاملة.

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Table (4): Effect of commercial plant nutrients and chitosan sprays on growth parameters of chamomile during 2010/11 and 2011/12 growing seasons field experiment

Treatment *	Averages of growth parameters of chamomile											
	Plant height (cm)			Branches NO.			Plant fresh weight (g)			Plant dry weight (g)		
	2010/11	2011/12	Mean	2010/11	2011/12	Mean	2010/11	2011/12	Mean	2007/08	2008/09	Mean
Citrien	61.83	58.00	59.92	15.23	13.20	14.22	664.04	628.54	646.29	136.26	137.03	136.65
Potassien	52.50	47.67	50.09	12.65	10.82	11.74	559.11	582.29	570.70	121.25	117.23	119.24
Chitosan	58.05	52.55	55.30	14.35	11.02	12.69	651.38	624.88	638.13	136.59	130.86	133.73
Control	48.33	42.75	45.54	10.83	8.10	9.47	526.39	510.23	518.31	120.31	107.44	113.78
Mean	55.18	50.24	52.71	13.27	10.79	12.03	600.23	586.49	593.36	128.60	123.14	125.87
L.S.D. at 5%	4.67	3.8	-	4.04	2.5	-	7.42	4.09	-	8.5	8.2	-

- **sprayed with effective concentration prepared from two commercial plant nutrients, *i.e.* Potassien (30% phosphorus pentaoxide) and Citrien (15% active citric acid and 6% (Zn - Mn- Fe) and chitosan (commercial product)**

Table (5) : Effect of commercial plant nutrients and chitosan spray on chamomile inflorescence yield, plan fresh and dry weights during two successive growing seasons of field experiment

Treatment *	Averages of some yield components								
	Inflorescences No.			Fresh weight (g)/ plant			Dry weight (g)/ plant		
	2010/11	2011/12	Mean	2010/11	2011/12	Mean	2007/08	2008/09	Mean
Citrien	548.57	524.20	536.39	173.58	157.81	165.70	34.83	32.23	33.53
Potassien	508.13	478.43	493.28	159.24	146.65	152.95	30.60	27.80	29.20
Chitosan	566.20	434.50	500.35	178.81	166.22	172.52	37.55	34.47	36.01
Control	425.07	402.80	413.94	128.75	124.41	126.58	27.11	27.41	27.26
Mean	511.99	459.98	485.99	160.10	148.77	154.43	32.52	30.48	31.50
L.S.D. at 5%	16.85	10.6	-	9.0	11.6	-	10.7	1.5	-

*** sprayed with effective concentration prepared from two commercial plant nutrients, *i.e.* Potassien (30% phosphorus pentaoxide) and Citrien (15% active citric acid and 6% (Zn - Mn- Fe) and chitosan (commercial product).**