CONTROL OF CHOCOLATE SPOT DISEASE OF FABA BEAN BY GARLIC EXTRACT, COMPOST EXTRACT AND FUNGICIDES

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ABSTRACT: Crude extracts of garlic cloves (Allium sativum L.), compost extract and fungicides were tested for their effect on the infection of faba bean plants with chocolate spot disease caused by Botrytis faba. In vitro, these extracts reduced mycelial growth of Botrytis faba. The fungicides Galben-mancozeb 58% and Dithane M45 were more effective than garlic cloves and compost extract. Spraying faba bean plants with each of water extracts of garlic cloves, compost extract and fungicides under greenhouse conditions and field conditions reduced the infection with chocolate spot compared to the check plants. All tested fungicides were the more effective than extracts of garlic cloves and compost extract. Fungicides, Galbenmancozeb 58% and Dithane M45 were more effective in reducing the disease incidence. However, water extracts of garlic cloves and compost extract were less effective compared to fungicides. Extracts of garlic cloves or compost extract increased the number of weight of pods as well as weight of dry seed.

Keywords: Faba bean, chocolate spot, Botrytis faba, plant extracts, fungicides, compost extract.

INTRODUCTION

Chocolate spot disease of faba bean, caused by *Botrytis faba* Sard, is considered one of the most destructive diseases, especially in northern Egypt, (Mohamed, 1982). Effect of plant extracts on host parasite interaction was reported (El-Shami *et al.*, 1985). Garlic extract has been found to be insecticidal, antiprotozoal, antibacterial and antifungal (Barone and Tansey, 1977; Rippon, 1974 and Filiermans, 1973). Garlic leaf extract inhibits growth of some fungi (Mishra and Dixit, 1976). Khaled (1986) reported that juice of garlic green leaves inhibited growth of *Botrytis allii* more than onion green leaves. El-Sheikh (1992) found a toxicity effect of garlic juice of immature, mature and over mature Balady and Chinese cloves against *Botrytis allii*. Heweidy *et al.*, (1997) found a positive effect of extracts of garlic cloves and henna leaves in reducing mycelial growth of *B. faba*. El-Syed (2005) found that garlic extract was the most effective plant extracts, followed by onion and caraway. Amer *et al.*, (1994) found that the most effective fungicides

against chocolate spot of broad bean, both *in vitro* and *in vivo*, were iprodione and viclozolin and the greatest increase in their activity were achieved in combination with the adjuvant SCS2665 and Atlas G-5411. Heweidy (1998) obtained the lowest chocolate spot severity of two faba bean entries when the fungicides plant vax, copper Acrobat and Apron were individually applied 24 hrs before inoculation than that recorded if the chemical was used 24 hrs after inoculation process. Various reports indicated that Dithane M45 was the best fungicides significantly decreased chocolate spot severity on faba bean (El-Gindy, 2003, El-Gammal, 2005 and El-Syed, 2005). Chabousson (1985) and Trankner (1992) demonstrated that balanced nutrition with compost extract has been considered fundamental for maintaining plant health through its influence on plant resistance mechanisms.

Increasing interest in composting, as a waste management strategy, has led to increase research efforts directed toward utilization of various organic wastes in agriculture. One of the beneficial of compost extract-amended soils is the highly induced suppression of numbers of soil borne pathogens, such as *Pythium spp.* (Lewis et al., 1992; Pascual et al., 2000 and McKellar and Nelson, 2003), and *Fusaruim* spp. (Kavroulakis et al., 2005 and Borrero et al., 2006).

MATERIALS AND METHODS

A. Source of Botrytis faba Isolate:

Pathogenic isolate of *Botrytis faba*, the causal organism of chocolate spot disease, was kindly provided by Plant Pathology Dept., National Res. Center, Giza, Egypt. A spore suspension was prepared from 14 day old culture and adjusted to concentration of 2.5×10⁵ sopre/ml, which used for inoculation.

B. Laboratory experiments:

The effect of different concentrations of plant extracts, compost extract and fungicides on linear growth of *Botrytis faba* was tested. Garlic cloves with mechanical injury and diseases were discarded, cortexes were removed, and then the cloves were crushed in a worming blender with distilled water (50ml/250g) as described by El-Shami *et al.*, (1985).

Six concentrations 5, 10, 25, 50, 75 and 100% of garlic extracts were prepared and used throughout the study. Discs (5mm in diameter), obtained from 6 days old culture of *B. faba* was placed in sterilized Petri dishes (9mm). Three ml of each tested concentration were added to 20 ml PDA medium in Petri dishes then; 5 mm of the fungal disc was placed in the center of each

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Petri dish. Three replicates were used for each tested concentration of each extract.

Laboratory study were directed to study toxicity of Benlate (50% Benomyl Methyl-1) Dithane M45 (Dithio carbamate 80%) and Gallban mancozeb were tested 58%. Growth and sporulation of causal pathogen *Botrytis faba* were determined on PDA plates containing fungicides at rate of 10, 25, 50, 100, 200 and 400 ppm. Linear growth of fungi was measured when fungal growth filled up the control plates.

In vitro assay of compost extract against Botrytis faba: compost extracted were mixed with tap water at the ratio 1:4 (v/v) and extracted at 24°C for 72hr while stirred thoroughly. After extraction, the mixtures were filtered through 3 layers of cheesed and the filtrates were sterilized by either filtration or by thermal treatment at 121°C for 30min (Zhang et al., 1998). Different dilutions of the compost extracted were prepared, i.e. 0, 10, 20, 30, 40 and 50% and tested.

Concentration of the tested compost extracted was poured in each sterilized Petri plates (9 cm Φ), then followed by adding PDA medium. The plates were inoculated individually with an equal disc (5 mm Φ) of *Botrytis faba* and inoculated at 20°C. The control plates were supplied with 5ml of sterilized distilled water. Three replicates were used for each treatment. Linear growth of developed colony of *B. faba* was measured when the fungus completely covered the surface of the plate in the control treatment.

C. Greenhouse experiments:

Effect of fungicides on chocolate spot severity:

Extract (garlic), fungicides i.e. Galben-mancozeb 58%, Benlet and Dithane M-45, compost extract were applied as foliar spray on 35-days old faba bean plants (C, V, Giza 402 and Giza 461). Three days later, all plants were sprayed with spore suspension *B. faba* (2.5×10⁵ spore/ml). All plants were covered with polyethylene bags for 48hr to maintain high relative humidity, and plants were kept in greenhouse under daily observation for 7 days. Three replicates were used and ten seeds were sown in each replicate. Disease severity was determined based on a 1-9 disease railing scale of Bernier *et al.*, (1983). (Table 1).

Disease Severity % =
$$\frac{n \times v}{gN} \times 100$$

Where:

- (n) = Number of plants in each category.
- (v) = Numerical values of symptoms category.
- (N)= Total number of plants.
- (g) = Maximum numerical value of symptom category.

Table (1): The infection type scale of faba bean chocolate spot disease (Bernier et al., 1983).

Disease rating	Host status	Description
1	Highly resistant	No disease symptoms, or very small, specks.
3	Resistant	Few small discrete lesions
5	Moderately resistant	Same coalesced lesions with some defoliation
7	Susceptible	Large coalesced sporulating lesions, 50 % defoliation, some dead plants
9	Highly susceptible	Extensive lesions on leaves, stems, and pods, sporulation, stem gridling, blackening and death of more than 80 % of plants.

Effect of different treatments on activity of Chitinase enzyme:

Chitinase activity was determined after 10 days of pathogen inoculation (13 days after treatment). Extraction of enzyme from faba bean leaves was done according to method of Tuzun et al., (1989).

Chitinase activity was determined by colorimetric of Boller and Mauch (1988). Colloidal Chitin was used as a substrate and dinitro salicylic acid as reagent to measure reducing sugars.

Chitinase activity was expressed as mMN-acetylglucosamine equivalent released/gram fresh weight tissue/60 minutes.

D. Field experiments:

The field experiments were conducted at Sers El-Layian Agric. Res. Stat., where chocolate spot disease is naturally occurred at the middle of November during two growing seasons (2006-2007) and (2007-2008). The experimental layout was divided to equal plots each one consisting of 4 rows (3.5m). The recommended agricultural practices were applied as usually recomended.

Five different treatments, i.e. plant extract (garlic cloves), 3 fungicides i.e. Benlate-50, Dithane M-45, and compost extract (1kg/20liter water) were tested for their efficiency in controlling faba bean chocolate spot disease of under field conditions.

Two faba bean cultivars <u>i.e.</u> Giza 402 and Giza 461 faba bean, 45 days old, were sprayed with fungicides at the recommended doses and garlic extract concentration 100% and compost extract of concentration 50%. Spraying was repeated every 15 days, so that plants were sprayed 3 times during the growing season. Three replicates were used for each treatment. The same aforementioned methods were used without any treatments were used as control. Disease severity was recorded as previously mentioned. Effect of different treatments on crop growth parameters i.e. no. of branches pods/plant and weight of 100 seed was recorded.

RESULTS

I-The pathogen: **B.** fabae:

The pathogencity of <u>B</u>. <u>fabae</u> isolate was confirmed on faba bean leaves in obtained isolates from faba bean leaves in a preliminary test and was used throughout all greenhouse study.

II- 1. Effect of garlic extract on mycelial growth of B. faba:

Different concentrations of garlic extracts were tested against *B. faba* linear growth. Results shown in Table (2) indicate that increasing plant extracts concentrations significantly decreased the fungal linear growth. At 75 % concentration, mycelial growth was completely inhibited.

Table (2): Effect of different concentration of garlic cloves extracts on the mycelial growth of *B. faba* at 18°C *in vitro*.

Concentration,%	Linear growth (cm)	Growth reduction, %
5	8.25	8.3
10	6.77	24.8
25	4.90	45.5
50	2.66	71.1
75	0.00	100
100	0.00	100
Control	9.00	
	L.S.D. 0.05 = 0.23	•

2.Effect of fungicides on mycelial growth of *B. fabae*:

Different concentrations (0-400 ppm) of each of Benlate, Dithane M-45 and Gilben Mancozeb 58%, were tested against B. faba in Petri dishes. Results shown in Table (3) clearly indicate that Benlate was the most effective fungicide followed by Dithane -M45 and Gilben-Mancozeb 58%, in reducing fungal growth B. faba.

Table (3): Effect of different fungicides on mycelial growth of B. fabae, in vitro.

Fungicide Concentration (ppm)/ Linear growth (cm)										
	0	10	25	50	100	200	400			
Benlate	9.00	6.00	4.00	2.00	0.00	0.00	0.00			
Dithane M45	9.00	6.00	5.00	4.00	1.00	0.00	0.00			
Gillben-Mancozeb 58%	9.00	7.80	6.90	6.00	4.00	1.00	0.00			
L.S.D. for fungicides 0.05 = 0.77 & L.S.D. for concentration 0.05 = 0.77										

3. Effect of compost extract on mycelial growth of B. fabae, in vitro:

Data in Table (4) indicate that compost extract treatment reduced linear growth of Botrytis faba comparing to the control. Moreover, increasing the compost extract concentration increased gradually the reduction of linear growth.

Table (4): Effect of different compost extract on chocolate spot pathogen B. faba in vitro.

Compost extract (conc. %)	mycelial growth (cm)	Growth reduction, %							
0	9.00	-							
10	8.00	11.11							
20	7.50	16.66							
30	7.00	22.22							
40	40 6.00 33.33								
50 6.00 33.33									
L.S.D. 0.05 = 0.91									

III- Effect of different treatments on chocolate spot severity, under greenhouse conditions:

The results in Table (5) reveal that garlic extract, at different concentrations, reduced chocolate spot infection caused by *Botrytis faba* under controlled greenhouse conditions. Meanwhile, all tested fungicides significantly reduced chocolate spot infection compared with control. In this respect, Benlate was the best fungicide followed by Dithane M45 and Gilban-Mancozeb 58%. The compost extract treatment significantly minimized the chocolate spot symptoms than control. The results show also that, Giza CV. 402 was more susceptible to the disease than the other cultivar (Giza 461).

Table (5): Effect of garlic extract, fungicides and compost extract on severity of chocolate spot disease of faba bean under greenhouse conditions.

Treatment	(Giza 402) conc./dis.sev.							(Giza 461) conc./dis.sev.					
	1	2	3	4	5	6	1	2	3	4	5	6	
*Garlic extract	15.55	13.33	9.99	6.66	5.55	5.00	12.22	9.99	6.00	3.33	1.11	0.00	
**Benlate	15.55	11.11	8.88	5.5	3.33	0.00	11.11	8.22	4.44	1.11	0.00	0.00	
**Dithan M45	15.55	12.22	9.9	6.6	4.44	2.22	11.11	9.00	5.55	2.22	1.11	0.00	
**Gilban Mancozeb	15.55	13.33	11.11	7.77	5.55	3.33	11.11	9.99	6.66	4.44	2.00	0.00	
***Compost extract	15.55	15.55	14.00	12.22	9.99	6.66	11.11	10.00	7.77	6.66	4.00	4.00	
L.S.D. at 0.05	6.82								7.2	1			

^{*} Conc. of garlic extract, (0,10,25,50,75 &100)

IV- Effect of different treatments on chitinase activity in fababean plants:

Data in Table (6) show the efficacy of plant extracts, fungicides and compost extract on chitinase activity of faba bean under greenhouse conditions. The obtained results show that all treatments of garlic extract, fungicides or compost extract significantly increased chitinase activity. The most effective treatment was Benlate 200 ppm which increased the activity 100%. Garlic at 75%, and compost extract (40%) increased the enzyme activity by more than 90.2%.

^{**} Conc. of fungicides, (0,25,50,75,100 &200) ppm respectively

^{***} Conc. of compost extract, (0, 10, 20,30,40,50 %)

Table (6): Chitinase plant inoculated with *B. faba* as affected by extract garlic cloves activity on faba bean, fungicides and compost extract under greenhouse conditions.

Treatment	Chitinase activity	Increase %								
Plant extract (garlic cloves)										
50%	1.9	40.6								
75%	2.50	90.2								
Benlate										
75ppm	2.00	51.3								
100ppm	2.2	66.4								
200ppm	2.7	100								
(Dithane M45)										
75ppm	1.5	30.5								
100ppm	2.0	50.9								
200ppm	2.4	88.0								
Compost extract										
20%	1.6	35.2								
40%	1.9	45.6								
Control	1.1									
L.S.D. at 0.0	L.S.D. at 0.05 non significant									

V- Field experimental:

a- Effect of different treatments on chocolate spot infection:

Data in Table (7) show the efficacy of garlic extract, fungicides and compost extract as foliar treatments on chocolate spot disease, under field conditions. The obtained results in two growing seasons (2006-2007), (2007-2008) revealed that the tested materials reduced significantly disease severity of *Botrytis faba* compared to untreated control. In this respect, Benlate was the most effective treatment followed by Dithane M45. However, both garlic extract and compost extract significantly decreased the infection compared with control. Data also show that response of Giza 461 showed the best results, although it seemed to be less susceptible than Giza 402.

Table (7): Effect of spraying faba bean plants with garlic extracts, fungicides and compost extract on incidence of chocolate spot disease of faba bean during two seasons (2006-2008).

Treatment	Season/Disease severity											
			2006	-2007					200	7-2008		
Spray	1 spray 2 sprays				3 sprays 1 spray			2 sp	rays	3 sprays		
Cultivar	G402	G 461	G 402	G 461	G 402	G 461	G 402	G 461	G 402	G 461	G 402	G 461
Garlic extract	11.11	9.99	8.00	7.77	5.55	4.44	10.00	8.00	7.77	5.55	4.44	2.22
Benlate	1.94	0.83	0.55	0.00	0.00	0.00	2.2	0.55	1.11	0.55	0.55	0.00
Dithane M45	2.50	1.67	1.9	0.83	1.6	0.00	2.2	1.11	0.83	0.00	0.00	0.00
Compost extract	12.22	10.00	8.88	7.00	6.66	5.00	11.39	9.99	7.77	5.55	3.33	2.22
Control	22.77	14.72	20.55	12.22	15.55	11.11	20.77	15.88	18.44	12.72	11.11	10.00
L.S.D at 0.05	1.82	3.9	2.9	3.11	2.9	2.13	3.19	3.25	1.71	3.40	4.10	3.1

b- Effects on some plant growth characters:

Results present in Tables (8 a&b) indicate the number branches of faba bean plants were significantly increased than that the control in response to either the plant extracts (garlic cloves), the chemical control or compost extract application. The best mean (4.75 branches plant) was achieved at (2006-2007) season when Benlate and Dithane M45 were applied. The average numbers of control plant branches were 3.00 and 3.25, respectively for (2006-2007) and (2007-2008) seasons.

Results also show that the number of faba bean pods /plant revealed a significant higher effect of fungicides, with garlic extracts and compost extract on treated plants compared untreated ones. Pods weight of all tested faba bean cultuvars was significantly increased than control, where Dithane M45 and Benlate had the best effect. Also, seed weight of both tested faba bean cultivar was significantly increased than control. Hundred seeds weight of Giza 461 cultivar was increased significantly comparing to those of Giza 402.

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Dithane M 45 L.S.D. 0.05 Compost extract Treatment Garlic extract Control Benlate No. of branc hes 0.64 2.00 2.50 3.50 3.75 3.00 1.45 19.7 32.0 Pods No. 26.7 33.0 26.5 Pods weight (g) Giza 402 3.73 58.2 76.0 88.3 94.0 78.2 Seeds weight (g) 3.86 7.55 50.2 53.7 79.7 83.2 100 seed weight (g) 40.25 3.04 41.0 69.0 75.5 59.0 branches No. of 0.65 3.00 3.50 3.25 4.50 4.50 1.13 24.7 37.5 Pods No. 27.7 36.2 30.7 Giza 461 Pods weight (g) 2.48 91.2 58.5 70.0 94.5 85.7 Seeds weight (g) 2.34 52.0 55.0 85.5 83.0 77.7 100 seed weight (g) 3.13 44.0 73.5 68.0 42.0 76.7

Table (8 a): Effect of treating faba bean seed with garlic extracts, fungicides and compost extract on some field component/plant under field conditions (2006-2007).

Dithane M 45 Compost extract Treatment Control Garlic extract Benlate L.S.D. 0.05 No. of branches 3.00 0.63 1.00 2.75 3.75 4.00 Pods No. 0.93 22.5 29.7 34.7 35.0 27.7 Pods weight (g) 2.28 58.7 90.0 82.0 Giza 402 80.7 96.7 Seeds weight (g) 1.97 53.2 73.7 83.5 90.0 76.5 100 seed weight (g) 1.45 41.5 57.2 59.0 72.0 75.0 No. of branches 0.73 3.25 3.76 3.50 4.75 4.25 Pods No. 0.81 25.7 31.2 36.5 38.0 32.0 Giza 46' Pods weight (g) 1.62 60.586.7 98.0 95.590.0 Seeds weight (g) 1.76 91.2 55.0 79.7 88.5 85.0 100 seed weight (g) 3.96 44.0 55.2 88.0 75.2 70.0

Table (8 b): Effect of treating faba bean seed with garlic extracts, fungicides and compost extract on some field component/plant under field conditions (2007-2008).

DISCUSSION

Botrytis faba Sard is the causal pathogen of chocolate spot disease of faba bean in Egypt. This disease is considered one of the most destructive diseases of this popular field crop, especially in Northern Egypt (Mohamed, 1982). The results of the present study clearly indicated that garlic extract, fungicide, and compost extract reduced growth of B. fabae, in vitro. It has been demonstrated that the tested plant extract were effective in reducing plant diseases (Filiermans, 1973; Rippon, 1974; Barona and Tansey, 1977 and El-Shami et al., 1985), juice of garlic green leaves inhibited the fungal growth. El-Sheikh, (1992), Mohamed, (1997), and El-Syed (2005) found that garlic extract was the most effective plant extract in vitro. All, tested fungicides at all tested concentrations have affected significantly growth of B. faba, where Benlate was the most effective fungicide followed by Dithane-M45 and Gillbane-Mancozeb 58%, respectively. Amer et al., (1994) and Heweidy (1998), found such effects of fungicides on chocolate spot of faba bean both in vitro and in vivo, when used 24hrs before inoculation than that recorded if the chemical was used 24hrs after inoculation. Benlate, Dithane M45 and copper oxychoride completely stopped the growth of B. faba in vitro. Benlate showed the best effect (El-Gindy, 2003). Application of the fungicides, at three times intervals resulted significantly in less chocolate spot disease symptoms as compared with control. In both growing seasons, Benlate and Dithane M45 showed the best results. These results are in agreement with those of El-Gammal, (2005) and El-Syed, (2005). Spraying faba plants with compost extract at 50% reduced significantly disease severity of chocolate spot compared with untreated control. However, increasing the concentration of compest resulted in a great reduction in disease severity when compared with the control treatment, after 45 days from application. Chabousson, (1985), and Trankner, (1992) demonstrated, that balanced nutrition with compost extract has bean considered fundamental for maintaining plant health through its influence on plant resistance mechanisms. One of the beneficial properties of compost extract application is the highly induced suppression of numbers of soil-borne pathogens, such as Pythium spp. and Fusaruim oxysporum (Lewis et al., 1992, Pascual et al., 2000; McKellar and Nelson, 2003), Rhizoctonia Solani and Fusarium spp. (Kauroulakis et al., 2005 and Borrero et al., 2006). Generally, garlic clove extract and compost extract could be considered as alternatives for control of chocolate spot disease of faba bean. These results are promising and integration of such treatments with fungicide application should be considered for further research.

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مقاومة مرض التبقع البنى فى الفول البلدى باستخدام مستخلص فصوص الثوم والكمبوست والمبيدات الفطرية

رانيا زكى الشناوى ، سحر عباس السيد معهد بحوث أمراض النباتات – مركز البحوث الزراعية – الجيزة – مصر

الملخص العربي

يعتبر مرض التبقع البنى فى الفول أهم أمراض الفول البلدى فى مصر وخاصة فى مناطق شمال الدلتا. تم دراسة تأثير بعض البدائل كالمستخلصات النباتية والكمبوست مقارنة بالمبيدات لمكافحة المرض حيث تم استخدام مستخلص فصوص الثوم والكمبوست والمبيدات الفطرية (بنليت، دياثين م ٥٤، جالين مانكوزيب بتركيزات مختلفة لدراسة تأثيرةا على الفطر الممرض والمرض تحت ظروف المعمل والصوبة والحقل.

وقد تم دراسة تأثير بعض المعاملات عليها وتم التوصل الى النتائج الآتيةا:

- ١- في المعمل: أدت جميع المعاملات إلي اختزال النمو الميسليومي لفطر البوتريتس فابا وقد أدت المعاملات بجميع المبيدات المستخدمة ومستخلص الثوم إلي اختزال ملحوظ لنمو الفطر بينما أدت المعاملة بالكمبوست إلي اختزال بسيط في نمو فطر البوتريتس فابا نتيجة تعقيم المستخلص.
- ٧- تجارب الصوب: أدت جميع المعاملات الكمبوست بتركيز ٤٠% والثوم بتركيز ٥٧% إلي انخفاض نسبة حدوث مرض التبقع البني في الفول البلدي أما عن تأثير استخدام المبيدات فقد أدت إلي انخفاض ملحوظ في شدة الإصابة وكانت أكثر المبيدات فاعلية هو البنليت يليه دياثين أم ٥٥ ثم جالين مانكوزيب وقد أدت جميع المعاملات إلي زيادة نشاط أنزيم الشيتينيز في النباتات المعاملة وكانت أفضل النتائج المتحصل عليها عند تركيز البنليت ٢٠٠ جزء في المليون يلية تأثير مستخلص الثوم عند تركيز ٥٧% يليه الكمبوست.

- ٣- في تجارب الحقل أظهرت المبيدات تفوقاً ملحوظاً في خفض نسبة حدوث مرض التبقع البني بسنبة تصل إلي ٩٠% خلال موسمى الدراسة ٢٠٠١-٢٠٠٧، ٢٠٠٧-٢٠٠٨.
- ٤- وجد أن البنايت والدياثين أم ٥٠ كانوا من أكثر المعاملات تأثيراً في خفض شدة الإصابة بالمرض إذا ما قورنت بالكنترول.
- ٥- كان تأثير مستخلص الثوم والكمبوست أقل فعالية في خفض شدة المرض مقارنة بالمبيدات المستخدمة إذا ما قورنت بالكنترول. ويصفة عامة فان جميع المعاملات قد أدت الى خفض شدة الاصابة بدرجة معنوية اذا ماقورنت بالكنترول الغير معامل على كلا صنفى الفول تحت الدراسة جيزة ٢٠١، جيزة ٢٦١.
- ٦- أدت هذه المعاملات إلي زيادة في عدد القرون ووزن القرون ووزن البذور الجافة ووزن المائة بذرة مقاربة بالكنترول.