# EVALUATION OF SOME PRODUCTS IN CONTROLLING CHOCOLATE SPOT OF FABA BEAN AND THEIR EFFECT ON THE GROWTH AND YIELD PARAMETERS

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***Abstract***

Plastic pots sown with seeds of faba bean variety Giza 429 susceptible to chocolate spot disease caused by Botrytis fabae were grown in a screenhouse . Two biofungucides (Bioarc and Biozeid), one green Algae (Ulva) and two chemical fertilizers (Alpha and Oris) were utilized in this research. Data obtained showed that disease severity was higher in the season of 2008/2009 than the season of 2009 / 2010. Bioarc and Biozeid significantly controlled the disease more than the other tested products.Bioarc and Ulva gave the highest increase in fresh weight, while Bioarc and Biozeid were superior in increasing the dry weight. Ulva and Alpha showed the higher plant height and also the number of branches of shoots. In addition, Alpha and Biozeid gave the highest weight of 100 seeds while Ulva and Alpha significantly increased the number of pods / plant. Also , Ulva was the only product increased seed number / pod. Determination of NPK level in faba bean shoot revealed that tested materials increased the level of the three elements to different degrees. Bioarc and Alpha significantly increased the highest level of P while the effect of the other materials was variable. Similar trends were observed in the two seasons of tria**ls.**

**Key words:** faba bean, chocolate spot, *Botrtis fabae*, biofungucides, and chemical fertilizers.

## INTRODUCTION

Faba bean (*Vicia faba* L.) is considered one of the most important legumenous crops due to its high nutritive value for both energy and phytoprotein contents. Cultivated area of faba bean in Egypt reached to 184.000 feddans in 2010 yielded 1.495.920 ardab {ardab=155 Kg} at rate of 8,13 ardab/feddan ( Food legume statics department , field crop research institute, ARC.)

More than 100 pathogens attack faba bean in the Mediterranean region (Hebblethwaite , 1983). *Botrytis fabae,* the principal causal pathogen of chocolate spot disease in Egypt caused severe injury in the northern regions of Egypt. In Egypt faba bean production is still limited due to the negative effects of chocolate spot disease on the growth and yield components.

This is attributed to the prevailing favorable climate factors such as relative humidity (El Helaly , 1938). Biological control of *B.fabae* by different bioagents was reported by Abd El Moiety *et al.*, (1990 ) and Omar *et al.*, (1987).

Abou-zeid *et al.*, (2003 ) used antagonistic microbes to reduce the use of chemical pesticides in IPM system. The current research aimed at studying some new products in order to reduce such deleterious impacts of chocolate spot disease on the morphological features and crop yield of faba bean.

## MATERIALS AND METHODS

Table 1 . The tested products , their active ingredients , types and rate of use per 1 liter of water.

| Tested products | Active ingredients | Type | Rate of use per/ L. |
|---|---|---|---|
| BioARC | *Bacillus megaterium* 6% (iv/w) | Biofungicide | 2.5g |
| Biozeid | *Trichoderma album* 2.5 % (w/w) | Biofungicide | 2.5g |
| Sea weeds | *Ulva* spp | Green algae | 2.5g |
| Alpha | Uric nitrogen (0.6%) + water – soluble phosphorus pentoxide ($P_2O_5$ ). (12%)+ water soluble boron (4.2%) | Chemical | 2 ml |
| Oris | Uric nitrogen (3%) + water soluble phosphorus pentoxide ($P_2O_5$). (15%)+ water soluble zinc (4.2)% | Chemical | 2 ml |

The former commercial products were tested under the screenhouse conditions of biological resource center standard (BRCS) Plant Pathology Research Institute (PPRI) , Agriculture Research Center (ARC) during the two successive seasons 2008 / 2009 and 2009/2010. Table (1) presents information about the used products.

**1- Isolation of *Botrytis fabae* and inoculum preparation.**

Faba bean leaves showing chocolate spot disease symptoms were collected during the growing season 2008/ 2009 from various fields located at Giza governorate. Leaves were cut to small pieces, washed with tap water then disinfested with 0.5% of sodium hypochlorite solution for 2 minutes then washed with sterilized distilled water several times and cultured on faba bean leaf extract agar (FBLA) (Leach and Moore,1966).

The recoverd isolates were sub- cultured on FBLA medium incubated at 20° C for 12 days to obtain dense sperulation.

Ten milliliters of sterilized distilled water were added on the sporulating cultures plates of *B.faba*.The conidia were released by scraping the growth with a glass rode. Two layers of sterile muslin were used to filter the spore suspensions to remove the mycelial residues and the spore suspension was adjusted to 2.5 x $10^5$ spore / ml using a haemocytometer (Abou-Zeid *et. al.,* 1985 ).

**2- Efficiency of the tested materials against chocolate sport disease on faba bean.**

The trial was carried out in the two successive growing seasons under the screen house conditions as mentioned above.

Five seeds of the susceptible cultivar of faba bean Giza 429 were cultivated in black polyethylene pots (35 cm diameter). Four pots replicated 3 times were used for each treatment.

The plants were sprayed four times during the two seasons of the experiments with the recommended dose of the tested materials (Table1) when they were 25 days old. After 24 hrs all plants were inoculated with spore suspension of *B.fabae* (2.5 x $10^5$ spore /ml )

The same number of pots were inoculated with the the same spore suspension only and left as check control.Transparent plastic bags were used to cover the plants in order to kept the relative humidity for 48 hrs.

All pots received the recommended dose of super phosphate (15.5 % $P_2 O_5$) at the rate of 200 kg / feddan before cultivation. While potassium sulphate (48% $K_2SO_4$) was added at the rate of 50kg/feddan 15 and 50 days after sowing. Ammonium sulphate (20.5%N) was utilized as a source of mineral N fertilization at the rate of 20 kg N/ feddan. Irrigation was done as needed. Disease severity was recorded after 2 , 4 , 7 and 14 days according to Abou–Zeid (1978 ).

Modified scale by Abou–Zeid (1978) was used to calculate disease severity applying the following equation:

$$\text{Disease severity \%} = \frac{n \times V \times 100}{9N}$$

**Where** :

n = plant number in every grade.

V = grade number.

N = total number of examined plants.

9 = maximum disease grade.

**3- Effect of the tested materials on the morphological characters and yield components.**

Plant samples of faba bean were taken at 90 days from planting to estimate the growth parameters such as: Plant height, number of branches, fresh and dry weight of plant shoot. In addition, number of pods / plant, seed / pod and weight of 100 seeds was recorded at harvest.

**4- Effect of tested materials on NPK content of plant shoot.**

Shoot samples were taken from 70 days old plant, treated with the tested materials as mentioned before. They were dried at 70° C then ground to fine powder. Two grams was wet digested with sulphuric and perchloric acids mixture according to Jackson (1967 ). N and P were estimated according to Chapman and Pratt (1965 ) while K was determined according to Hesse (1971).

Data obtained were statistically analyzed by (ANOVA) according to Snedecor and Cochran (1980) using M-stat program.

## RESULTS AND DISCUSSION

**1- Efficiency of the tested materials against chocolate spot disease on faba bean.**

Data obtained cleared that all the tested materials significantly reduced disease severity of chocolate spot disease on faba bean compared with control treatment. (Table 2 ) .

Table 2 . Effect of the tested materials on disease severety of chocolate spot on faba bean (Giza 429) during the two successive growing seasons 2008 / 2009 and 2009 /2010.

| Growing seasons / Tested materials | Disease severity % | |
|---|---|---|
| | 2008/2009 | 2009/2010 |
| BioARC | 13.15 | 11.21 |
| BioZeid | 17.35 | 16.42 |
| Ulva | 20.10 | 18.15 |
| Alpha | 25.16 | 23.15 |
| Oris | 27.40 | 25.05 |
| Control | 72.25 | 67.80 |
| L.S.D. | 1.90 | 2.10 |

These significant differences between the tested materials and control may be attributed to the effect of the antagonistic secretions of the two tested biofungicides (Bioarc and Biozeid) against *B. fabae*. The superiority of the two tested biofungicides Bioarc and Biozeid in minimizing disease severity of chocolate spot disease on faba bean is attributed to the effective mechanisms of their active ingredients.

It is known that *Trichoderma* spp inhibits the fungal growth by 3 mechanisms : competition (for space and nutrients ),parasitism (deriving nutrients from the host) and antibiosis ( producing antibiotics or inhibitory metabolites), Harman, (2006).Also,Lorito *et. al.*,(1994) mentioned that the mycoparasitism and competition and / or fungicidal action was referred to the capacity of *Trichoderma* to produce antibiotics or hydrolytic enzymes. Mahmoud *et al*.(2004 ) stated that certain *Bacillus* spp. were effective as bioagents by inhibiting the mycelial growth of plant pathogenic fungi and reducing dry weight as well as sporulation. Additionally, Sailaja *et al*. (1998) found that *Bacillus subtilis* induced resistance in plant to disease by stimulating the phytoalexins production and increasing the activity of lytic enzymes. Also, the same trend was observed in relation to the green algae Ulva. While the effect of the two chemical fertilizers may be refered to improving the health status of the plant which may increase its tolerance, in addition the possible role of the two microelements in trigerring certain metabolic pathways for groth regulators biosynthesis such as IAA .

The metrological factors such as temperatures and relative humidity may be the predisposetion of faba bean plants and favor to the mycelial growth and sporulation of the causal pathogen of chocolate spot disease on faba bean.

**2- Effect of the tested materials on the growth parameters of faba bean under screenhouse conditions.**

Date obtained in Table (3) indicate that all tested materials exhibited positive effect on the morphological characters of faba bean plants compared with the control treatment.

Results revealed that values of the estimated parameters were more higher during the season of 2008 / 2009 than 2009 / 2010 probably due to variation in the environmental condition.

Data showed that the higher fresh weight was obtained with Bioarc and the green algae ( Ulva ), respectively. In addition, the higher dry weight was obtained when plants were treated with Bioarc and Biozeid followed by the green algae Ulva. Also,taller plants were obtained when the green algae (Ulva) and the chemical fertilizer ( Alpha ) were used. Higher branch number on the plant was obtained with the two former materials , respectively. Oris ( chemical fertilizer )gave the least values for all examined parameters.

Table 3 . Effect of the tested materials on the growth parameters of faba bean under greenhouse conditions.

| Tested materials | 2008/2009 | | | | 2009 /2010 | | | |
|---|---|---|---|---|---|---|---|---|
| | FW/g | DW/G | PH/CM | BN/P | FW/G | DW/G | PH/CM | BN/P |
| Bioarc | 111.75 | 51.30 | 82.52 | 8.60 | 100.40 | 46.25 | 79.15 | 6.30 |
| Biozeid | 82.15 | 45.25 | 85.65 | 8.25 | 75.15 | 40.15 | 80.13 | 6.75 |
| Ulva | 91.35 | 40.91 | 93.25 | 10.15 | 84.33 | 37.22 | 85.33 | 8.20 |
| Alpha | 73.20 | 37.60 | 87.15 | 9.95 | 58.15 | 35.90 | 83.40 | 7.80 |
| Oris | 72.10 | 35.15 | 81.90 | 7.30 | 55.30 | 31.10 | 79.10 | 6.00 |
| Control | 63.5 | 30.71 | 76.10 | 5.15 | 55.75 | 27.90 | 69.50 | 4.25 |
| L.S.D. | 3.45 | 3.20 | 2.36 | 1.93 | 3.95 | 3.40 | 2.42 | 2.20 |

Fw/g = fresh weight (g ) Dw/g = dry weight ( g )

PH/cm = plant height (CM) BN /p = branches number / plant

**3- Effect of the tested materials on yield parameters of faba bean under screenhouse conditions.**

The efficiency of the five tested materials for improving yield parameter was evaluated during the two experimental seasons 2008/2009 and 2009/2010 in the screenhouse . Data shown in Table (4) indicate that all the tested materials affect the yield parameters to different degrees compared with the control treatment. While the chemical fertilizer Alpha followed by the biofungicide Biozeid increased the weight of 100 seeds / g (70.32 and 69.20, respectively), the green algae ( Ulva ) followed by the chemical fertilizer (Alpha ) increased the number of pods / plants. At the same time , the green algae (Ulva ) was the only material that increased the number of seeds/pod compared with control treatment and among the other tested materials with no significant differences. The former data was obtained during the growing season 2008 / 2009. The same trend was observed in the season of 2009 / 2010.

Table 4 . Effect of the tested materials on the yield parameters of faba bean under screenhouse conditions.

| Growing seasons | 2008/2009 | | | 2009/2010 | | |
|---|---|---|---|---|---|---|
| Yield parameters Treatments | 100Sw/g | PN/p | SN/p | 100sw/g | PN/p | SN/p |
| Bioarc | 65.15 | 36.10 | 4.0 | 64.35 | 33.40 | 4.0 |
| Biozied | 69.20 | 43.33 | 4.0 | 66.75 | 37.22 | 4.0 |
| Ulva | 67.05 | 68.15 | 5.0 | 66.30 | 56.25 | 5.0 |
| Alpha | 70.32 | 53.35 | 4.0 | 68.05 | 47.83 | 4.0 |
| Oris | 58.10 | 36.41 | 4.0 | 53.90 | 33.75 | 4.0 |
| Control | 57.10 | 34.15 | 4.00 | 58.15 | 32.25 | 4.00 |
| L.S.D. | 2.51 | 3.42 | N.S | 2.62 | 3.35 | N.S |

100 SW/g = 100 seeds weight /g PN/p = pod number / plant SN/p= seeds number / pod

The obtained data during the two experimental seasons indicated that all growth and yield parameters were significantly increased with all tested materials compared with control treatments. These enhancements may be referred to the effect of the tested materials on some components of plant cells such as their contents of same nutrients and growth regulators such as cytokinin, auxin and gibberellin. Each of these components plays an important and essential role in plant growth beside its vital function.

Bruce (1990) and Deotale *et. al.* (1998) found that gibberellins caused increase in cell division and cell enlargement. Also Castro and Vello (1983) stated that gibberellins increased internodes number / plant and length of plant internodes.

**4- Effect of the tested materials on NPK content of faba bean shoot :**

All tested materials showed no significant change in NPK content but Bioarc and Alpha led to a significant increase of P content in faba bean shoot comparing with control treatment in the two growing seasons table ( 5 ).

Table 5 . Effect of the tested materials on NPK content in faba bean shoot.

| Tested materials | 2008/2009 | | | 2009/2010 | | |
|---|---|---|---|---|---|---|
| | N% | P% | K% | N% | P% | K% |
| Bioarc | 2.75 | 0.38 | 1.62 | 2.73 | 0.37 | 1.58 |
| Biozeid | 2.73 | 0.32 | 1.52 | 2.71 | 0.30 | 1.50 |
| Ulva | 3.14 | 0.31 | 1.48 | 3.15 | 0.29 | 1.46 |
| Alpha | 3.21 | 0.36 | 1.61 | 3.26 | 0.36 | 1.57 |
| Oris | 3.11 | 0.33 | 1.84 | 3.08 | 0.31 | 1.80 |
| Control | 2.40 | 0.30 | 1.45 | 2.53 | 0.28 | 1.39 |
| L.S.D. | 1.01 | 0.04 | 0.21 | 1.05 | 0.05 | 0.24 |

Data in Table (5) show that NPK levels were higher in faba bean shoots during the season of 2008 / 2009 than the growing season 2009 / 2010.

In both seasons the higher level of N was obtained by the chemical fertilizer Alpha followed by the green algae ( Ulva ).

On the other hand, the biofungicide Bioarc and the chemical fertilizer Alpha showed higher level of P in faba bean shoots in the two experimental seasons. The chemical fertilizer Oris and the biofungicide Bioarc gave the higher level of K. Bioarc increased the levels of NPK more than Biozeid in the shoots of faba bean during the two seasons of trials, however, to a non significant degree.

Increasing levels of N, P and K after using the tested materials may be attributed to the effect of tested materials on metabolites accumulation in the plant cells and consequently increase the growth and yield parameters observed during the experimental seasons.

Finally, based on this study, the use of Bioarc and the other tested materials as foliar spray in control chocolate spot is recommended as a safer approach to the environment and public health, and maximizing the growth and yield of faba bean plants.

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# تقييم بعض المنتجات فى مكافحة مرض التبقع البنى وأثرها على النمو والمحصول فى الفول البلدى

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تم تقييم بعض المنتجات والمركبات الحيوية ودراسة تأثيرها على مرض التبقع البنى فى الفول البلدى والمتسبب عن الفطر *Botrytis fabae* وعلى تحسين نمو وإنتاجية الفول البلدى باستخدام صنف حساس ( جيزة 429 ) خلال موسمى 2009/2008 – 210/2009 تحت ظروف الصوبة بوحدة تعريف الكائنات الدقيقة– معهد بحوث أمراض النباتات– مركز البحوث الزراعية– جيزة.

وقد أوضحت الدراسة التى أجريت على إستخدام اثنين من المركبات الحيوية (بيوزيد– بيوأرك) ( Bioarc and Biozeid)، واحد الطحالب الخضراء (Ulva)، و اثنين من المخصبات (Alpha و Oris) أن شدة الإصابة بالمرض كانت أعلى في موسم 2009/2008 عنه فى موسم 2010/2009.

وقد قللت بوضوح المعاملة بالبيوزيد والبيوأرك الإصابة بالمرض عن المنتجات الأخرى المختبرة. وقد أعطت المعاملة بالبيوأرك والألفا زيادة كبيرة فى الوزن الطازج بينما أدت المعاملة بالبيوأرك والبيوزيد إلى الزيادة فى الوزن الجاف.

وقد شوهدت زيادة واضحة فى طول النبات وعدد الأفرع والتفريعات عند المعاملة بمركبات الـ Ulva مع Alpha. بالإضافة إلى أن إستخدام مركبات الألفا والبيوزيد أعطت أعلى زيادة لوزن 100 بذرة بينما المعاملة بالألفا والأولفا زادت بوضوح من عدد القرون / نبات . بالإضافة إلى أن المعاملة بالأولفا فقط قد زادت من عدد البذور/ قرن.

تقدير المعدلات السمادية ( NPK) فى أفرع الفول البلدى أشارت إلى أن كل المعاملات أدت إلى زيادة العناصر الثلاثة بدرجات مختلفة. وقد زادت مركبات الألفا والبيوأرك من مستوى الفوسفور زيادة معنوية. وقد لوحظ هذا الاتجاه خلال الموسمين.