

## USING *BACILLUS MEGATERIUM* VAR. *PHOSPHATICUM* AND CALCIUM SUPER PHOSPHATE FOR MANAGEMENT SESAME WILT DISEASE

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**ABSTRACT:** *Bacillus megaterium* var. *phosphaticum* (BMP) have been used to control sesame wilt disease caused by *Fusarium oxysporum* f.sp. *sesami* in the presence of different doses of calcium super phosphate (CSP) at two successive seasons (2014 and 2015). CSP was add with soil preparation at the rate of 1, 2, 3 and 4 gm/pot and 50, 100, 150 and 200 kg/fed under greenhouse and field conditions respectively.

BMP plus CSP at the highest concentration either in greenhouse (4 gm/pot) or field conditions (200 kg/fed) was the most effective treatment in reducing sesame wilt disease. Also BMP plus CSP at the rate of 200 kg/fed promote plants growth, increased minerals uptake, seed yield and oil concentration under field conditions.

**Key words:** *Bacillus megaterium* var. *phosphaticum*, calcium super phosphate, *Fusarium oxysporum* f.sp. *sesami* and Sesame.

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### INTRODUCTION

Sesame (*Sesamum indicum* L.) is one of the oldest oil seed crops known, and its use probably goes back to 2130 BC (Weiss, 1983). Almost 100% of the world's sesame area is found in the developing countries (Ashri, 1998). In Egypt, sesame is grown in many governorates i.e., Ismailia, El-Sharkia, El-Fayum and Sohag. It is considered as the first oil seed crop in Ismailia Governorate, where it used in popular Egyptian foods (Serry and Satour, 1981). Wherever sesame is grown it is liable to be attacked by at least eight economically important fungal diseases (Kolte, 1985) and by 65 species of insects at different stages of its growth (Ahuja & Bkhetia, 1995), causing considerable yield losses. *Fusarium oxysporum* f.sp. *sesami* reported for the first time by Armstrong and Armstrong (1950) in North America and consider the most serious destructive pathogen present in all sesame growing areas in the world including Egypt (Xiao *et al.*, 1992, Raghuwanshia *et al.*, 1995, EL-Shazly *et al.*, 1999 and Ammar *et al.*, 2004).

Phosphorus is one of the major essential macronutrients for biological growth and development (Ehrlich *et al.*, 1990). It is involved in several key plant functions, such as energy metabolism, photosynthesis, respiration, nitrogen fixation, enzyme regulation, nutrient movement within plant and DNA composition. Therefore, Phosphorus is important in cell division and development of new tissue (Hameeda *et al.*, 2008). Generally phosphorus found in soil in insoluble form; phosphate-solubilizing bacteria can help to make it available (Rodríguez and Fraga, 1999, Richardson *et al.*, 2009 and Fernández *et al.*, 2012)

*Bacillus megaterium* var. *phosphaticum* belong to plant growth-promoting rhizobacteria (PGPR) and known for its ability to solubilize rock Phosphate material (Schilling *et al.*, 1998). PGPR are root-colonizing bacteria with beneficial effects, which include plant growth promotion, biological disease control and induced systemic resistance (Kloepper and Schroth, 1981, Kloepper, 1993, Ramamoorthy *et al.*, 2001, Zehnder *et al.*, 2001, Akgül and M. Mirik 2008 and Viruel *et al.*, 2014). Induced

resistance by PGPR has also been studied in recent years using crop plants and different plant pathogens (Zhou *et al.*, 1992 and Ramamoorthy *et al.*, 2002). This group of bacteria includes Phosphate-Solubilizing Bacteria can convert insoluble phosphates into soluble forms through acidification, chelation, exchange reactions and production of organic acids (Rodríguez and Fraga, 1999). They are found in soil but usually they are not enough in population, therefore inoculation of plants by a target microorganism at higher concentration than that normally found in soil is necessary (Vessey, 2003).

This study aimed to control sesame wilt disease caused by *Fusarium oxysporum* f.sp. *sesami*, enhance plant growth, increase seed and oil crop by using *Bacillus megaterium* var. *phosphaticum* in the presence of different doses of phosphate fertilizer under greenhouse and field conditions.

## **MATERIALS AND METHODS**

### **The pathogen**

*Fusarium oxysporum* f.sp. *sesami* (Zap) Cast was isolated from sesame plants showing wilt symptoms at Mallawy, Menia Governorate. The isolate was identified according to Booth (1971 and 1977) and used to inoculate sterilized barley seeds medium for 3 weeks at 20°C according to Van der Meer *et al.* (1983) to use as inoculum.

### **The host**

Sesame seeds cv. Giza 32 were procured from Oil Crops Res. Dept., Field Crops Res. Inst., ARC, Giza, Egypt. Seeds of approximately similar size were surface sterilized in 5% sodium hypochlorite (Clorox) for 2 min then thoroughly washed in sterilized distilled water and left to air dried under aseptic conditions.

### ***Bacillus megaterium* var. *phosphaticum* (BMP)**

Phosphate dissolving bacteria *Bacillus megaterium* var. *phosphaticum* (BMP) kindly obtained from the culture collection of the Agr. Microbiol. Dept., NRC, Dokki, Giza, Egypt. The bacterial strain was propagated in 250 ml conical flasks, each contain 125 ml sterilized nutrient broth medium. The flasks were incubated on a rotary shaker (120 rpm) at 27±1°C for 5 days, the bacterial cells were washed three times by sterilized distilled water and adjusted to 1x10<sup>9</sup> cfu/ml according to Callan *et al.* (1990). Prepared seeds were thoroughly mixed with bacterial suspension for 5 min with 1% Arabic gum then left to dry for 2 hrs under aseptic conditions before sowing.

### **Calcium super phosphate (CSP)**

Calcium super phosphate (15.5%) was added as one part during soil preparation before seed sowing at the rate of 1, 2, 3 and 4 gm/pot and 50, 100, 150 and 200 kg/fed under greenhouse and field conditions respectively (the recommended dose rate of 200 kg/ fed.).

### **Fungicide**

Topsin M-70% W.P. (Thiophanate) Diethyl 4-4 (O-phenylene) bis 3-thiolllophanate, manufacture by Nippa Soda, Japan. Prepared seeds were mixed with Topsin M-70% at the rate of 3 gm/kg seeds for 5 minutes with 1% Arabic gum then left to dry for 2 hrs under aseptic conditions before sowing.

### **Greenhouse experiment**

Pot experiments were carried out in the greenhouse of Onion, Garlic and Oil Crops, Plant Path. Res. Inst., ARC during 2014 and 2015 seasons. 30 cm in diameter plastic pots filled with sterilized sand-clay soil (1:1 v/v) were infested with the prepared *F.oxysporum* f.sp. *sesami* at the rate of 2 % w/w, 7 days before transplanting. Five pots were used as replicates for each treatment and control (infested pots free treatment). Each pot was seeded with 6 seeds in 1<sup>st</sup> June and irrigated when needed. Also,

the recommended dose of potassium sulphate (48%) and ammonium sulphate (20.6%) at rate of remember follow.

### **Field experiment**

Field experiment were carried out in complete randomized plots at Mallawy Agric. Res. St., Menia Governorate during 2014 and 2015 successive seasons in a heavily infested soil. Four plots as replicates for each treatment and control (free treatment plots) was adopted. Plot was 12 m<sup>2</sup> (4\* 3 m) contained 4 rows (60 cm apart). Each row contained 35 hill was sown with 5 seeds in 1<sup>st</sup> June. Plants were thinned to one plant per hill after 20 days from sowing. The recommended cultural practices for sesame production were adopted throughout the growing seasons.

### **Treatments**

- 1- BMP + 50 kg CSP
- 2- BMP + 100 kg CSP
- 3- BMP + 150 kg CSP
- 4- BMP + 200 kg CSP
- 5- 200 kg CSP
- 6- BMP
- 7- Topsin M-70%
- 8- Control

### **Nitrogen, phosphorus and potassium assessment**

Plants shoot content of N, P and K were measured 90 days after sowing for each season under field conditions as described by Jackson (1967).

### **Wilt disease incidence, morphological characters, seed yield and oil content**

Percentage of wilted plants was recorded 90 days after sowing for each season under greenhouse and open field (Ziedan, 1998). Plant height, no. of branches/plant, no. of capsules/plant, seed yield and oil content (A.O.A.C., 1980) were determined at the end of each season (July month) under field conditions.

### **Statistical analysis:**

The data were statistically analyzed and significance among means was assessed by least significant difference (LSD) at 5% probability level using SAS ANOVA program V.9 (Anonymous, 2014).

### **Results**

The data present in Table (1) show that all treatments under investigation in the two successive seasons (2014 and 2015) significantly decreased the percentage of infection compared to control in greenhouse. Decrease in infection compared to control ranged between 44.88% to 22.40% with BMP plus 4 gm CSP/pot and BMP plus 1 gm CSP/pot treatments respectively. The treatment with BMP plus 4 gm CSP/pot gave best reduction in percentage of infection which it gave 38.32% compared with either control (81.64%) or Topsin M-70% (41.66%) as mean of two seasons. The combined treatments between BMP and CSP were higher effective more than single treatments either BMP or CSP in reducing percentage of infection. Also, the percentage of infected plants that treated by BMP only increased more than plants that treated by the recommended dose of CSP only. The percentage of infection decreased gradually with the increasing of CSP dose as fertilizer in the presence of BMP in combined treatments.

The data present in Table (2) under field conditions show that all treatments under investigation in the two successive seasons (2014 and 2015) significantly decreased the percentage of infection compared to control. The percent of decrease in infection compared to control ranged from 36.92% to 26.85% with BMP plus 200 kg CSP/fed and BMP plus 50 kg CSP/fed treatments respectively. The best treatment in reduction the percentage of infection was BMP plus 200 kg CSP/fed which gave 36.25% infection compared with 57.47% infection at control, while Topsin M-70% as check control gave 27.05% infection in two season mean values. The combined treatments

between BMP and CSP were higher effective more than single treatments with either BMP or CSP in reducing percentage of infection. Both of single treatments i.e., full dose of CSP or BMP treatment, gave the

same mean value of two seasons (40.35% infection). The percentage of infection decreased gradually with the increasing of CSP dose as fertilizer in the presence of BMP in combined treatments.

**Table (1): Effect of *Bacillus megaterium* var. *phosphaticum*, calcium super phosphate and Topsin M-70% treatments on percentage of infection by *Fusarium oxysporum* f.sp. *sesami* on Giza 32 sesame plants under greenhouse conditions during growing seasons 2014 and 2015.**

| Treatment         | 2014  | 2015  | Mean  | Decrease (%) |
|-------------------|-------|-------|-------|--------------|
| BMP* + 1 gm CSP** | 63.35 | 63.35 | 63.35 | 22.40        |
| BMP + 2 gm CSP    | 60.02 | 56.67 | 58.35 | 28.53        |
| BMP + 3 gm CSP    | 50.01 | 43.32 | 46.66 | 42.84        |
| BMP + 4 gm CSP    | 40.00 | 36.64 | 38.32 | 53.06        |
| 4gm CSP           | 43.33 | 46.66 | 45.00 | 44.88        |
| BMP               | 63.36 | 66.70 | 65.03 | 20.34        |
| Topsin M-70%      | 40.00 | 43.32 | 41.66 | 48.97        |
| Control           | 79.97 | 83.30 | 81.64 | -            |
| L.S.D. at 5%      | 11.53 | 10.76 | -     | -            |

BMP\* *Bacillus megaterium* var. *phosphaticum* and as seed dressing, CSP\*\* calcium super phosphate/pot

**Table (2): Effect of *Bacillus megaterium* var. *phosphaticum*, calcium super phosphate and Topsin M-70% treatments on percentage of infection by *Fusarium oxysporum* f.sp. *sesami* on Giza 32 sesame plants under natural infested soil at Mallawi Agric. Res. Sta., Menia Governorate during growing seasons 2014 and 2015.**

| Treatment         | 2014  | 2015  | Mean  | Decrease (%) |
|-------------------|-------|-------|-------|--------------|
| BMP* + 50 kgCSP** | 41.40 | 42.70 | 42.04 | 26.85        |
| BMP + 100 kgCSP   | 39.65 | 41.76 | 40.70 | 29.18        |
| BMP + 150 kgCSP   | 38.25 | 40.70 | 39.48 | 31.31        |
| BMP + 200 kgCSP   | 35.70 | 36.80 | 36.25 | 36.92        |
| 200 kgCSP         | 41.05 | 39.65 | 40.35 | 29.79        |
| BMP               | 40.00 | 41.05 | 40.53 | 29.48        |
| Topsin M-70%      | 26.60 | 27.50 | 27.05 | 52.93        |
| Control           | 56.06 | 58.88 | 57.47 | -            |
| L.S.D. at 5%      | 1.17  | 1.63  | -     | -            |

BMP\* *Bacillus megaterium* var. *phosphaticum* and as seed dressing, CSP\*\* calcium super phosphate/fed

## Using *Bacillus megaterium* var. *Phosphaticum* and *calcium super* .....

Generally, the data present in Tables (1) and (2) clearly show that BMP plus the highest dose of CSP treatment gave the best reduction in the percentage of infection either in greenhouse or field conditions.

According to the mean value of two seasons, the present data in Table (3) show that all treatments under investigation increased seed yield and oil content more than control and Topsin M-70% treatments, except BMP plus 50 kg CSP/fed treatment in seed yield which have no significant different with Topsin M-70% at two successive season. Seed yield increased by 69.05% and 18.10% with BMP plus 200 kg CSP/fed and BMP plus 50 kg CSP/fed treatments respectively, while Topsin M-70% gave 20.00% increase compared to control. From other hand, oil content increased under all treatments more than control and Topsin M-70%. Oil content increase ranged between 12.48% with 200 kg CSP/fed and 4.56% with BMP plus 50 kg CSP/fed, while Topsin M-70% increased oil content by 4.51% compared to control. Generally, BMP plus 200 kg CSP/fed treatment gave heights seed yield and oil content/fed which gave 4.44 arddab/fed and 55.79% oil followed by BMP plus 150 kg CSP/fed which gave 3.66 arddab/fed and 56.04% oil, while 200 kg CSP/fed as single treatment came in the third position with 3.44 arddab/fed and 57% oil compared with 2.63 arddab/fed and 133.29 unit oil/fed with control treatment.

The data present in Table (4) show that all treatments under investigation increased plant height, no. of branches/ plant and no. of capsules/ plant significantly more than control except BMP plus 50 kg CSP/fed treatment on plant height which have no

significant different with control. All treatments increased the tested parameters more than Topsin M-70% which decrease plant height (-1.30%) and gave the lowest percent of increasing in no. of branches/plant (9.74%) and no. of capsules/ plant (3.17%) compared with control. BMP plus 200 kg CSP/fed treatment was the best one in increase plant height and no. of branches/ plant which gave 190.69% and 85.39% increase respectively compared with control. While 200 kg CSP/fed treatment increased no. of capsules/ plant more than BMP plus 200 kg CSP/fed where they gave 51.77 % followed by 47.87% increase respectively compared with control.

According to mean values, the data present in Table (5) show that all treatments increased N, P and K content in plant more than control except BMP plus 100 kg CSP/fed treatment which decreased N content by -4.23% less than control. Also, all treatments increased plants content of N, P and K more than Topsin M-70% except BMP plus 25 kg CSP/fed and 100 kg CSP/fed treatments. BMP plus 200 kg CSP/fed treatment increased plants content of N and K more than any other treatments where it gave 21.14% and 18.09% increase more than control respectively. While both of BMP plus 200 kg CSP/fed and 200 kg CSP/fed treatments increased P content by the same percent (102.38%) compared to control.

Generally, the mentioned results pointed to BMP plus 200 kg CSP/fed as best treatment under investigation for controlling sesame wilt disease caused by *Fusarium oxysporum* f.sp. *sesami* and promote healthy growth and minerals uptake which reverses by increase on seed and oil yield.

Table (3): Effect of *Bacillus megaterium* var. *phosphaticum*, calcium super phosphate and Topsin M-70% treatments on seed yield and oil content on Giza 32 sesame plants under natural infested soil by *Fusarium oxysporum* f.sp. *sesami* at Mallawi Agric. Res. Sta., Menia Governorate during growing seasons 2014 and 2015.

| Treatment         | Seed yield (araddab/fed) |      |      |              | Oil content % |       |       |              |
|-------------------|--------------------------|------|------|--------------|---------------|-------|-------|--------------|
|                   | 2014                     | 2015 | Mean | Increase (%) | 2014          | 2015  | Mean  | Increase (%) |
| BMP* + 50 kgCSP** | 3.00                     | 3.20 | 3.10 | 18.10        | 52.00         | 54.00 | 52.99 | 4.56         |
| BMP + 100 kgCSP   | 3.08                     | 3.65 | 3.36 | 28.10        | 53.88         | 54.13 | 54.00 | 6.56         |
| BMP + 150 kgCSP   | 3.50                     | 3.83 | 3.66 | 39.52        | 56.20         | 55.88 | 56.04 | 10.58        |
| BMP + 200 kgCSP   | 4.33                     | 4.55 | 4.44 | 69.05        | 56.58         | 55.00 | 55.79 | 10.09        |
| 200 kgCSP         | 3.25                     | 3.63 | 3.44 | 30.95        | 57.25         | 56.75 | 57.00 | 12.48        |
| BMP               | 3.28                     | 3.48 | 3.38 | 28.57        | 56.25         | 55.70 | 55.98 | 10.46        |
| Topsin M-70%      | 3.05                     | 3.25 | 3.15 | 20.00        | 53.63         | 52.30 | 52.96 | 4.51         |
| Control           | 2.58                     | 2.68 | 2.63 | -            | 50.50         | 50.85 | 50.68 | -            |
| L.S.D. at 5%      | 0.38                     | 0.25 | -    | -            | 1.53          | 1.41  | -     | -            |

BMP\* *Bacillus megaterium* var. *phosphaticum* and as seed dressing, CSP\*\* calcium super phosphate/fed

Table (4): Effect of *Bacillus megaterium* var. *phosphaticum*, calcium super phosphate and Topsin M-70% treatments on Plant height, no. of branches/plant and no. of capsules/plant oil content on Giza 32 sesame plants under natural infested soil by *Fusarium oxysporum* f.sp. *sesami* at Mallawi Agric. Res. Sta., Menia Governorate during growing seasons 2014 and 2015.

| Treatment         | Plant height (cm) |        |        |              | No of branches/plant |      |      |              | No of capsules/plant |        |        |              |
|-------------------|-------------------|--------|--------|--------------|----------------------|------|------|--------------|----------------------|--------|--------|--------------|
|                   | 2014              | 2015   | Mean   | Increase (%) | 2014                 | 2015 | Mean | Increase (%) | 2014                 | 2015   | Mean   | Increase (%) |
| BMP* + 50 kgCSP** | 175.00            | 179.80 | 177.38 | -0.07        | 4.50                 | 4.50 | 4.50 | 34.83        | 130.50               | 133.50 | 132.00 | 28.62        |
| BMP + 100 kgCSP   | 184.63            | 184.50 | 184.56 | 3.98         | 4.88                 | 5.08 | 4.98 | 49.06        | 135.75               | 137.75 | 136.75 | 33.25        |
| BMP + 150 kgCSP   | 184.00            | 186.13 | 185.06 | 4.26         | 5.63                 | 5.68 | 5.65 | 69.29        | 143.25               | 144.50 | 143.88 | 40.19        |
| BMP + 200 kgCSP   | 190.25            | 191.13 | 190.69 | 7.43         | 6.00                 | 6.38 | 6.19 | 85.39        | 150.25               | 153.25 | 151.75 | 47.87        |
| 200 kgCSP         | 185.00            | 190.00 | 187.50 | 5.63         | 5.50                 | 5.53 | 5.51 | 65.17        | 156.00               | 155.50 | 155.75 | 51.77        |
| BMP               | 181.50            | 183.88 | 182.69 | 2.92         | 5.13                 | 5.45 | 5.29 | 58.43        | 133.50               | 109.75 | 121.63 | 18.51        |
| Topsin M-70%      | 173.88            | 176.50 | 175.19 | -1.30        | 3.63                 | 3.70 | 3.66 | 9.74         | 106.00               | 105.75 | 105.88 | 3.17         |
| Control           | 175.63            | 179.38 | 177.50 | -            | 3.25                 | 3.43 | 3.34 | -            | 103.50               | 101.75 | 102.63 | -            |
| L.S.D. at 5%      | 1.44              | 3.05   | -      | -            | 0.56                 | 0.33 | -    | -            | 3.85                 | 2.90   | -      | -            |

BMP\* *Bacillus megaterium* var. *phosphaticum* and as seed dressing, CSP\*\* calcium super phosphate/fed

***Using Bacillus megaterium var. Phosphaticum and calcium super .....***

**Table (5): Effect of *Bacillus megaterium var. phosphaticum*, calcium super phosphate and Topsin M-70% treatments on Giza 32 sesame plants shoot content of N, P and K under natural infested soil by *Fusarium oxysporum* f.sp. *sesami* at Mallawi Agric. Res. Sta., Menia Governorate during growing seasons 2014 and 2015.**

| Treatment               | N (%) |      |      |              | P (%) |      |      |              | K (%) |      |      |              |
|-------------------------|-------|------|------|--------------|-------|------|------|--------------|-------|------|------|--------------|
|                         | 2014  | 2015 | Mean | Increase (%) | 2014  | 2015 | Mean | Increase (%) | 2014  | 2015 | Mean | Increase (%) |
| <b>BMP*+ 50 kgCSP**</b> | 0.90  | 0.90 | 0.87 | 1.17         | 0.20  | 0.10 | 0.14 | 32.14        | 1.20  | 1.30 | 1.28 | 8.83         |
| <b>BMP+100 kgCSP</b>    | 0.84  | 0.80 | 0.82 | -4.23        | 0.13  | 0.15 | 0.14 | 30.95        | 1.25  | 1.25 | 1.25 | 6.38         |
| <b>BMP + 150 kgCSP</b>  | 0.95  | 1.00 | 0.98 | 13.70        | 0.20  | 0.13 | 0.16 | 54.76        | 1.28  | 1.35 | 1.31 | 11.70        |
| <b>BMP + 200 kgCSP</b>  | 1.03  | 1.05 | 1.04 | 21.14        | 0.20  | 0.23 | 0.21 | 102.38       | 1.38  | 1.40 | 1.39 | 18.09        |
| <b>200 kgCSP</b>        | 1.04  | 1.03 | 1.03 | 20.26        | 0.23  | 0.20 | 0.21 | 102.38       | 1.23  | 1.30 | 1.26 | 7.45         |
| <b>BMP</b>              | 0.95  | 1.00 | 0.98 | 13.70        | 0.13  | 0.18 | 0.15 | 42.86        | 1.20  | 1.28 | 1.24 | 5.32         |
| <b>Topsin M-70%</b>     | 0.85  | 0.95 | 0.90 | 4.96         | 0.09  | 0.15 | 0.12 | 15.48        | 1.18  | 0.88 | 1.03 | -12.77       |
| <b>Control</b>          | 0.84  | 0.88 | 0.86 | -            | 0.09  | 0.13 | 0.11 | -            | 1.15  | 1.20 | 1.18 | -            |
| <b>L.S.D. at 5%</b>     | 0.08  | 0.11 | -    | -            | 0.09  | 0.08 | -    | -            | 0.08  | 0.30 | -    | -            |

BMP\* *Bacillus megaterium var. phosphaticum* and as seed dressing, CSP\*\* calcium super phosphate/fed

## Discussion

PGPR are root-colonizing bacteria able to exert beneficial effects, which include plant growth promotion, use as bio-fertilizers, biological disease control and induced systemic resistance (Kloepper, 1993, Ramamoorthy *et al.*, 2001 and Zehnder *et al.*, 2001). Phosphorus is one of the major essential macronutrients for biological growth and development (Ehrlich, 1990). The biggest reserves of phosphorus are rocks and other deposits, such as primary apatite and other primary minerals formed during the geological age and organic matter (Paul and Clark, 1988). Several reports have examined the ability of different bacterial species to solubilize insoluble phosphate compounds, such as tri-calcium phosphate, di-calcium phosphate, hydroxyl apatite and rock phosphate (Goldstein, 1986). There are considerable populations of phosphate solubilizing bacteria in soil and in plant rhizospheres (Sperberg, 1958 and Alexander, 1977). Considerable concentration of phosphate solubilizing bacteria is commonly found in

the rhizosphere in comparison with nonrhizosphere soil include both aerobic and anaerobic strains (Katznelson *et al.*, 1962 and Raghu and MacRae, 1966). *Bacillus megaterium var. phosphaticum* is known for its ability to solubilize rock phosphate material (Schilling *et al.*, 1998). In this study the combination between *Bacillus megaterium var. phosphaticum* with different doses of calcium super phosphate approved their capability to control sesame wilt disease and improve minerals uptake, plant growth, seed and oil crop.

Among PGPR, *Bacillus* spp. and *Azotobacter* spp., have been reported to be effective against a broad spectrum of plant pathogens, including fungi, bacteria and viruses in many plant species (Morsy *et al.*, 2009, Abd-El-Monaim, 2010 and Mogle and Mane, 2010). *Bacillus megaterium* has been shown the potential to inhibit or suppress a range of plant diseases, occurring on both the roots and aerial parts of plants (Islam and Nandi, 1985, Liu and Sinclair, 1992, Bertagnolli *et al.*, 1996, Chiou and Wu, 2001, Jock *et al.*, 2002, Jung and Kim, 2005

and Padgham and Sikora, 2007). *Bacillus megaterium* significantly reduced severity of pepper crown blight caused by *Phytophthora capsici* in field experiments (Akgül and Mirik, 2008), reduced growth of *Fusarium oxysporum* f.sp. *lycopersici* significantly and decreased tomato wilt incidence under greenhouse and field conditions (Abd-El-Monaim, et al., 2012). Kong et al. (2010) demonstrated that the marine bacterium *Bacillus megaterium* could be used as a bio-control agent against peanut kernels disease caused by *A. flavus* and significantly reduce the biosynthesis of aflatoxins and expression of aflR and aflS gene. Application of PGPR bacteria had stimulation effect on the population of rhizospher microorganisms and increased their numbers significantly (El-Mehrat et al., 2012 and Mahrous et al., 2015). Suppressing pathogens and producing biologically active compounds (Khalid et al., 2004). The effect of PGPR as bio-agents may be due to attacking and binding the pathogenic organisms by sugar linkage and begins to secrete extracellular protease and lipase (Zaghloul et al., 2007) and produce antibiotics products of secondary metabolites such as phenazine-1-carboxylic acid (PCA), 2, 4-Pyrrolinitrin and Oomycin (Ehteshamul-Haque and Ghaffar, 1993 and Knudsen et al., 1995). Several bio-control agents such as *Trichoderma* spp., *Bacillus subtilis* and *Bacillus megaterium* able to control root diseases of sesame in the field (Elewa et al., 2011, Ziedan et al., 2011 and 2012).

Application of phosphate solubilizing bacteria to soil or seed helps to improve solubilization of fixed soil phosphorus and to induce plant growth (Broadbent et al., 1977). *Bacillus megaterium* increased plant growth, quantitative and qualitative parameters of tomato fruits growing under field conditions in two successive seasons and increased fresh and dry weights of survival tomato plants growing in pots compared with control (Abd-El-Monaim, et al., 2012). Plant height, seed weight per plant and 1000-seed weight

increased in sesame plants as a result of use *Bacillus megaterium* (Mahrous et al., 2015). Two strains of *Bacillus megaterium* increased the pepper yield by 36.2 and 47.7% under *Phytophthora capsici* infection (Akgül and Mirik, 2008). Co-inoculation of *Bacillus megaterium* var. *phosphaticum* and *Bacillus mucilaginosus* strains synergistically solubilized rock P and K which were added into the soil and make them much more available for uptake by pepper and cucumber roots (Han and Lee, 2006). Growth enhancement by *Bacillus* spp. may be associated to its ability to produce hormone, especially IAA (Sheng and Huang 2001), and siderophore (Ito, 1993 and Hu and Boyer 1996). It is also known that availability of phosphate in soils is important for the uptake of N from soils and its utilization in plant (Kim et al., 2003). Higher available of phosphate and other nutrients due to the solubilization with inoculation by *Bacillus megaterium* var. *phosphaticum* might cause an enhancement of N uptake, resistance to stress, stabilize soil aggregates and improve soil structure and organic matter content (Al-Taweil et al., 2009). Root colonizing and plant growth promoting bacteria referred to affect plant growth by increasing nutrient cycling, suppressing pathogens and producing biologically active compounds (Khalid et al., 2004). Finally Retain more soil organic N and other nutrients in the plant-soil system, thus reducing the need for fertilizer N and P and enhancing release of the nutrients (Baset et al., 2010).

## REFERENCES

- A.O.A.C. (1980). Official Methods of Analysis. Association of Official Agricultural Chemists. 13 teed A.O.A.C., Washington, D.c.
- Abd-El-Monaim, M.F. (2010). Integrated management of damping-off, root and/or stem rot diseases of chickpea with sowing date, host resistance and bioagents. Egypt. J. Phytopathol., 38: 45-61.



**Using *Bacillus megaterium* var. *Phosphaticum* and *calcium super* .....**

- Abd-El-Monaim, M.F., M.A. Abd-El-Gaid and M.A. El-Morsy (2012). Efficacy of rhizobacteria and humic acid for controlling Fusarium wilt disease and improvement of plant growth, quantitative and qualitative parameters in tomato. *J. Pl. Pathol.*, 1: 39-48.
- Ahuja, D.B., D.R.C. Bkhetia (1995). Bioecology and management of insect pests of sesame. A review. *Journal of Insect Science*, 8: 1–19.
- Akgül, D.S. and M. Mirik (2008). Biocontrol of *Phytophthora capsici* on pepper plants by *Bacillus megaterium* strains. *J. Pl. Patholo.*, 90(1): 29-34.
- Alexander, M. (1977). *Introduction to Soil Microbiology*. New York: Wiley and Sons.
- Al-Taweil, H.I., M.B. Osman, A.A. Hamid and W.W.M. Yussof (2009). Development of microbial inoculants and the impact of soil application on rice seedlings growth. *Am. J. Agric. Biol. Sci.* 4: 79-82.
- Ammar, S.E., M.S. El-Shazly, M.A. El-Ashry, M.A. Abd-El-Sattar and M.A.S. El-Bramawy (2004). Inheritance of resistance to Fusarium wilt disease in some sesame hybrids. *Egypt. J. Appl. Sci.* 19: 36-55.
- Anonymous, (2014). "Statistical Analysis System". *SAS User's Guide: Statistics*. SAS Institute Inc. Editors, Cary, NC, 27513, USA.
- Armstrong, J.K. and G.M. Armstrong (1950). A Fusarium wilt of sesame in United States. *Phytopathol. J.*, 40: 785.
- Ashri, A. (1998). Sesame breeding. In: JANICK J. (ed.): *Plant Breeding Reviews*. Vol. 16. John Wiley and Sons Inc., New York.
- Baset, M.M.A., Z.H. Shamsuddin, Z. Wahab and M. Marziah (2010). Effect of plant growth promoting rhizobacterial (PGPR) inoculation on growth and nitrogen incorporation of tissue cultured Musa plantlets under nitrogen free hydroponics condition. *Aust. J. Crop Sci.* 4: 85-90.
- Bertagnolli, B.L., F.K. DalSoglio and J.B. Sinclair (1996). Extracellular enzyme profiles of the fungal pathogen *Rhizoctonia solani* isolate 2B–12 and of two antagonists, *Bacillus megaterium* strain B153-2-2 and *Trichoderma harzianum* isolate Th008.1. Possible correlations with inhibition of growth and biocontrol. *Physiological and Molecular Pl. Patholo.* 48: 145-160.
- Booth, C. (1971). *The genus Fusarium*. Commonwealth Mycological Institute, Kew, Surrey, England 237pp.
- Booth, C. (1977). *Fusarium laboratory guide to the identification of the major species*. Commonwealth Mycol. Inst. NN. Appl. Biologists, Kew, Surrey, England, p: 1-58.
- Broadbent, P., K.F. Baker, N. Franks and J. Holland (1977). Effect of *Bacillus* spp. on increased growth of seedlings in steamed and in nontreated soil. *Phytopathol.* 67: 1027-1034.
- Callan, N. W., D. E. Mather and J. B. Miller (1990). Biopriming seed treatment for biological control of *Pythium ultimum* pre-emergence damping-off in sh2 sweet corn. *Pl. Dis.*, 74: 368-372.
- Chiou, A.L. and W.S. Wu, (2001). Isolation, identification and evaluation of bacterial antagonists against elliptica on lily. *Phytopathologische Zeitschrift*, 149: 319-324.
- Ehrlich, H.L. (1990). Mikrobiologische und biochemische Verfahrenstechnik. In: Einsele A, Finn RK, Samhaber W, (ed.) *Geomicrobiology*, 2nd ed. Weinheim: VCH Verlagsgesellschaft.
- Ehteshamul-Haque, S. and A. Ghaffar (1993). Use of rhizobia in the control of root rot diseases of sunflower, okra, soybean and mungbean. *J. Phytopathol.* 138: 157-163.
- Elewa, I.S., A.F. Sahab, M.H. Mostafa and E.H. Ziedan (2011). Direct effect of biocontrol agents on wilt and root-rot diseases of sesame. *Archives Phytopathol. Pl. Protec.*, 44(5): 493-504.
- El-Mehrat, H.J., A.A. Ragab, S.A. Fawaz and H.H. Abotaleb (2012). Enhancement of Guava fruits quality by using

- biofertilizer. *Ann. Agric. Sci. Moushtohor*, 50(2): 185-192.
- EL-Shazly, M.S., O.A. Abdul Wahid, M.A. El-Ashry, S.M. Ammar and M.A.S. El-Bramawy (1999). Evaluation of resistance to Fusarium wilt disease in sesame germplasm. *Int. J. Pest Manag.* 45: 207- 210.
- Fernández, L., B. Agaras, P. Zalba, L.G. Wall and C. Valverde (2012). *Pseudomonas* spp. isolates with high phosphate-mobilizing potential and root colonization properties from agricultural bulk soils under no till management. *Biol. Fert. Soils.* 48: 763-773.
- Goldstein, A.H. (1986). Bacterial solubilization of mineral phosphates: historical perspective and future prospects. *Am. J. Altern. Agri.*, 1: 51-7.
- Hameeda, B., G. Harini, O.P. Rupela, S.P. Wani and G. Reddy (2008). Growth promotion of maize by phosphate-solubilizing bacteria isolated from composts and macrofauna. *Microbiol. Res.* 163: 234-242.
- Han, H.S. and K.D.S. Lee (2006). Effect of co-inoculation with phosphate and potassium solubilizing bacteria on mineral uptake and growth of pepper and cucumber. *Pl. Soil Environ.*, 52(3): 130-136.
- Hu, X. and G.L. Boyer (1996). Siderophore-mediated aluminum uptake by *Bacillus megaterium* ATCC 19213. *Appl. Environ. Microbiol.*, 62: 4044-4048.
- Islam, K.Z. and B. Nandi (1985). Inhibition of some fungal pathogens of host phylloplane by *Bacillus megaterium*. *Zeitschrift Fur Pflanzenkrankheiten Und Pflanzenschutz-Journal of Pl. Dis. Protec.*, 92: 233-240.
- Ito, T. (1993). Enzymatic determination of itoic acid, a *Bacillus subtilis* siderophore, and 2,3-dihydroxybenzoic acid. *Appl. Environ. Microbiol.*, 59: 2343-2345.
- Jackson, M.L. (1967). *Soil Chemical Analysis* Prentice-Hall of India Private limited. New Delhi. pp. 144-197.
- Jock, S., B. Volksch, L. Mansvelt and K. Geider (2002). Characterization of Bacillus strains from apple and pear trees in South Africa antagonistic to *Erwinia amylovora*. *Fems Microbiology Letters*, 211: 247-252.
- Jung, H.K. and S.D. Kim (2005). An antifungal antibiotic purified from *Bacillus megaterium* KL39, a biocontrol agent of red pepper Phytophthora blight disease. *J. Microbiol. Biotechnol.*, 15: 1001-1010.
- Katznelson, H., E.A. Peterson and J.W. Rovatt (1962). Phosphate dissolving microorganisms on seed and in the root zone of plants. *Can. J. Bot.*, 40:1181-1186.
- Khalid, A., M. Arshad and Z.A. Zahir (2004). Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. *J. App. Microbiol.*, 96: 473-480.
- Kim, T., W. Jung, B. Lee, T. Yoneyama, H. Kim and K. Kim (2003). Effects on N uptake and remobilization during regrowth of Italian ryegrass (*Lolium multiflorum*). *Environ. Exp. Bot.*, 50: 233-242.
- Kloepper, J.W. (1993). Plant growth-promoting rhizobacteria as biological agents. In: Metting F.B. Jr., (ed.). *Soil Microbial Ecology Applications in Agricultural and Environmental Management*, pp. 255-274. Marcel Dekker, New York, NY, USA.
- Kloepper, J.W. and M.N. Schroth (1981). Relationship of in vitro antibiosis of plant growth promoting rhizobacteria to plant growth and the displacement of root microflora. *Phytopathol.*, 71: 1020-1024.
- Knudsen, I.M.B., J. Hockenhull and D.F. Jensen (1995). Biocontrol of seedling diseases of barley and wheat caused by *Fusarium culmorum* and *Bipolaris sorokiniana*: Effects of selected fungal antagonists on growth and yield components. *Pl. Pathol.*, 44: 467-477.
- Kolte, S.J. (1985). *Diseases of Annual Edible Oil Seed Crops*. Vol. II: Rapeseed,

**Using *Bacillus megaterium* var. *Phosphaticum* and *calcium super* .....**

- Mustard, Safflower and Sesame Diseases. CRC Press Inc., Boca Raton.
- Kong, Q., S. Shan, Q. Liu, X. Wang and F. Yu (2010). Biocontrol of *Aspergillus flavus* on peanut kernels by use of a strain of marine *Bacillus megaterium*. Inter. J. Food Microbiol., 139: 31-35.
- Liu, Z.L. and J.B. Sinclair (1992). Population dynamics of *Bacillus megaterium* strain B153-2-2 in the rhizosphere of soybean. Phytopathol., 82: 1297-1301.
- Mahrous, N.M., N.M. Abu-Hagaza, H.H. Abotaleb and Salwa. M.K. Fakhry (2015). Enhancement of growth and yield productivity of sesame plants by application of some biological treatments. American-Eurasian J. Agric. and Environ. Sci., 15(5): 903-912.
- Mogle, U.P. and R.Y. Mane (2010). Antagonistic effect of bio-fertilizers against seed born mycoflora of tomato (*Lycopersicon esculentum*). Res. J. Agri. Sci., 1: 255-258.
- Morsy, M. Ebtsam, K.A. Abdel-Kawi and M.N.A. Khalil (2009). Efficiency of *Trichoderma viride* and *Bacillus subtilis* as biocontrol agents against *Fusarium solani* on tomato plants. Egypt. J. Phytopathol., 37: 47-57.
- Padgham, J.L. and R.A. Sikora (2007). Biological control potential and modes of action of *Bacillus megaterium* against *Meloidogyne graminicola* on rice. Crop Protection 26: 971-977.
- Paul, E.A. and F.E. Clark. (1988). Soil microbiology and biochemistry. San Diego, CA: Academic Press, 1988.
- Raghu, K. and I.C. MacRae (1966). Occurrence of phosphate-dissolving microorganisms in the rhizosphere of rice plants and in submerged soils. J. Appl. Bacteriol. 29: 582-586.
- Raghuwanshia, K.S., N.N. Kune, C.D. Deokar, D.M. Veer and R.W. Bharud (1995). Seed-Borne infection of *Fusarium oxysporum* f.sp. *sesami* in different sesame cultivars. Sesame and Safflower Newsletter, 7: 24 -25.
- Ramamoorthy, V., T. Raguchander and R. Samiyappan (2002). Enhancing resistance of tomato and hot pepper to pythium diseases by seed treatment with fluorescent Pseudomonads. European J. Pl. Pathol. 108: 429-441.
- Ramamoorthy, V., R. Viswanathan, T. Raguchander, V. Prakasan and R. Samiyappan (2001). Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases. Crop Protection 20: 1-11.
- Richardson, A.E., P.J. Hocking, R.J. Simpson and T.S. George (2009). Plant mechanisms to optimize access to soil phosphorus. Crop Pasture Sci. 60: 124-143.
- Rodríguez, H. and R. Fraga (1999). Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol. Adv. 17: 319-339.
- Schilling, G., A. Gransee, A. Deubel, G. Lezovic and S. Ruppel (1998). Phosphorus availability, root exudates, and microbial activity in the rhizosphere. Z. Pfl. Ernähr. Bodenkde, 161: 465-478.
- Serry, M. and M. Satour (1981). Major disease of sesame and sources of resistance in Egypt. FAO Pl. Produc. Protec. Paper 29: 71-72.
- Sheng, X.F. and W.Y. Huang (2001). Physiological characteristics of strain NBT of silicate bacterium. Acta Pedol. Sci., 38: 569-574.
- Sperberg, J.I. (1958). The incidence of apatite-solubilizing organisms in the rhizosphere and soil. Aust. J. Agric. Res., 9:778a.
- Van der Meer, Q.P., J.L. Van Bennekom and A.C. Van der Giessen (1983). Screening for resistance to white rot caused by *Sclerotium cepivorum* Berk. in onions (*Allium cepa* L.) and leek (*Allium porrum* L.). Euphytica, 32: 697-701.
- Vessey, K. (2003). Plant growth promoting rhizobacteria as biofertilizers. Pl. Soil., 255: 571-586.

- Viruel, E., L.E. Erazzú, L. Martínez Calsina, M.A. Ferrero, M.E. Lucca and F. Siñeriz (2014). Inoculation of maize with phosphate solubilizing bacteria: effect on plant growth and yield. *J. Soil Sci. Pl. Nutri.*, 14(4): 819-831.
- Weiss, E.A. (1983). *Oil seed crops*. Longman, London, New York.
- Xiao, T.H., X.Y. Feng and X.R. Zhang (1992). Evaluation of introduced sesame germplasm. *Crop Genetic Resources* 4: 38-39.
- Zaghloul, R.A., Hanafy, A., Neweigy N.A. Ehsan and Neamat A. Khalifa (2007). Application of biofertilization and biological control for tomato production. 12th Con. of Microbiology, Cairo, Egypt, 198-212.
- Zehnder, G.W., J.F. Murphy, E.J. Sikora and J.W. Kloepper (2001). Application of rhizobacteria for induced resistance. *Euro. J. Pl. Pathol.*, 107: 39-50.
- Zhou, T., L. Rankin and T.C. Paulitz (1992). Induced resistance in the biological control of *Pythium aphanidermatum* by *Pseudomonas* spp. on European cucumber. *Phytopathol.*, 82: 1080.
- Ziedan, E.H.E. (1998). Integrated control of wilt and root-rot diseases of sesame in A.R.E. Ph.D. thesis, Faculty of Agric. Ain Shams Univ., 169 pp.
- Ziedan, E.H., I.S. Elewa, M.H. Mostafa and A.F. Sahab (2011). Application of mycorrhizae for controlling root diseases of sesame. *Pl. Protec. Res. J.*, 51(4): 355-367.
- Ziedan, E.H., M.H. Mostafa and I.S. Elewa (2012). Effect of bacterial inocula on *Fusarium oxysporum* f.sp. sesami and their pathological potential on sesame. *J. Agric. Technol.*, 8(2): 699-709.

## أستخدام *Bacillus megaterium* var. *phosphaticum* هزهاذ غهفئة

على ذلك زيهاك في هذا لب لنص ذبول السمس

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### الملخص العربى

تم أستخدم عذلة بكتيريا *Bacillus megaterium* var. *phosphaticum* لمكافحة مرض ذبول السمس المتسبب عن فطر *Fusarium oxysporum* f.sp. *Sesami* فى وجود معدلات مختلفة من سمد سوبر فوسفات الكالسيوم فى موسمين متتاليين (2014 و 2015). تمت أضافة السمد الفوسفاتى بمعدل 1، 2، 3، 4 جم/أصيص و 50، 100، 150، 200 كجم/فدان تحت ظروف الصوبة و الحقل على الترتيب. كانت المعاملة بالعذلة البكتيرية (*Bacillus megaterium* var. *phosphaticum*) فى وجود سمد سوبر فوسفات الكالسيوم الأحادى بمعدل 4 جم/ أصيص بالصوبة أو 200 كجم/فدان بالحقل أفضل المعاملات من حيث أختزال نسبة الأصابة بالمرض. كذلك أدت نفس المعاملة بالحقل الى تشجيع نمو النباتات، زيادة أمتصاص الأملاح، زيادة محصول البذرة و تركيز الزيت.