

Integration of EM-X Biofertilization and Sowing Methods on Encouragement of Sunflower Productivity and Charcoal Rot Control under Reclaimed Soil Conditions

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ABSTRACT

Two field experiments were carried out at 6 October farm, El-Nubaria Province, the desert backyard of El-Behaira Governorate- Egypt during the two successive summer seasons (2010 and 2011) to study the effect of the integration between different sowing methods and different bio fertilization treatments on sunflower (*Helianthus annuus* L.) var. Giza-102 productivity and charcoal rot disease management caused by *Macrophomina phaseolina* (Tassi) Goid under the reclaimed soil conditions. Rides sowing methods were found to be more effective in reducing *M.phaseolina* population and charcoal rot, therefore enhance sunflower productivity compared to the other examined sowing methods. EM-X { EM1 + *Bacillus subtilis* + mycorrhiza + *Azotobacter* sp.} was found to be more effective in reducing *M.phaseolina* population and charcoal rot, therefore enhance sunflower productivity compared to the other examined biofertilization treatments including the conventional chemical fertilization as the control treatment. The integration between ridges sowing method with tillage and EM-X { EM1 + *B. subtilis* + mycorrhiza + *Azotobacter* sp.} was found to be the furthestmost advised agricultural practices under these conditions as land degradation neutrality (LDN) technology that enhances land cover and area unit productivity under these conditions.

Keywords: Effective microorganisms (EM-X), biological fertilization, *Helianthus annuus*, *Macrophomina phaseolina*, productivity, LDN.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is a high quality oilseed crop characterized with high production in different climatic and soil conditions including drought, it also grows in wide range of growing season (Weiss 2000). In Egypt, serious shortage in edible oil supplies are dominant due to the limited cultivation areas and the rapid increase of demands resulted from the steady population growth. This gap between supplies and demands in edible oils could be overcome through either by horizontal expansion (introduce the oil crops into the crop pattern of the newly reclaimed lands) or by vertical expansion (implementation of more efficient agricultural practices) as reported by Dawood, Mona *et al.*, 2012. Yet, the newly reclaimed lands are mostly exposed to combination of environmental stresses including many desertification factors such as drought, salinity, fertility depletion and heat stress, hence diversity of incidence of plant diseases.

Charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goid is a major plant disease, which assumed economically damaging proportions for more than 500 plant species (Purkayastha, *et al.*, 2006). This pathogen develops under warm and dry weather condition as seed and soil borne pathogen causes root or stem rot that caused early death of maturing plants (Sadashivaiah *et al.*, 1986).

Chemical pesticides pose serious health hazards to the applicator, well as kill various beneficial organisms as a result of the environmental pollution (Bouizgarne 2013). Increasing awareness of human kind toward the ecosystem and environment has made a marked shift from systemic materials to bio-products.

Agricultural practices can deal efficiently with many environmental problems, and thus, mitigate enormous challenges that face contemporary agriculture including plant diseases. It ensures the implementation of the Millennium Sustainable Development Goals. (Singh *et al.* 2011). Various agricultural practices, including the use of cover and rotational crops, composts, tillage systems, sowing method and others have been promoted as management options for combating desertification and

enhancing soil quality and health. All agricultural practices are known to directly or indirectly affect abiotic stress, populations of soil borne pathogens and the severity of their resultant root diseases. The integration of some practices, such as tillage, organic fertilization, crop rotation, and residue management, can also increase microbial activity in the rhizosphere thus enhances the crop growth and productivity (Raaijmakers *et al.* 2009). Tillage is a mechanical soil manipulation for seedbed preparation, affects the rate and trend of soil degradation. In addition to establishing the seed-soil contact, tillage is used to alleviate soil compaction and so improve infiltration capacity; to dispose of pathogen-infested crop residue; to incorporate fertilizer into the root zone, and to eradicate weeds. The kind of tillage and its frequency depend on the soil and its related constraints to crop production. Wide range tillage alleviates unfavorable soil conditions (FAO, 1995). It also increase drainage, and soil temperature that lead to reduce the severity and damage of root rot pathogens to many crops (Dang *et al.*, 2015; and Verrellet *et al.*, 2017).

Agricultural productivity is strongly related to microbial activity in the soil system (Chaparro *et al.* 2014). The use of beneficial microorganisms makes a positive contribution to environmentally safe agriculture (Figueiredo *et al.* 2012). It can be a potential tool for sustainable agriculture as well as a trend for the future. Its beneficial effects include biological control of diseases and pests of different plant species, promotion of plant growth, increases in crop yield, and quality improvement of in addition to enrich soil fertility (Figueiredo *et al.*, 2016). Moreover, improvement soil physical and chemical properties and favors the growth and efficiency of symbiotic microorganisms such as free and symbiotic nitrogen fixing rhizobia and arbuscular mycorrhizal (AM) fungi (Sharma *et al.*, 2014).

The use of beneficial and effective microorganisms (EM1) as microbial inoculants in agriculture is a promising new technology (Higa, 1994). It has been shown to be effective in improving soil health and quality, inevitably raising the yield and quality of crops. Currently, EM

technology has been applied in more than 90 countries and regions, including Japan, the United States, France, Austria, North Korea and Egypt. Employing EM composting fertilizer and EM-activated liquid has been shown to promote root growth and improve the germination potential and germination rate. EM1 is a mixed culture of beneficial and naturally occurring microorganisms, such as species of photosynthetic bacteria (*Rhodospseudomonas palustris* and *Rhodobacter sphaeroides*), lactobacillus (*Lactobacillus plantarum*, *L. casei*, and *Streptococcus lactis*), yeasts (*Saccharomyces* spp.), and Actinomycetes (*Streptomyces* spp.) While EM5 is a modification product from EM1 that created by adding vinegar and ethyl alcohol during the fermentation process in order to have multifunction product such as foliar fertilizer and insect repellent (Higa, 2000). Using EM improves crop growth and yield by increasing photosynthesis, producing bioactive substances such as hormones and enzymes, controlling soil diseases, and accelerating decomposition of lignin materials in the soil and generally improves soil physical and chemical properties and favors the growth and efficiency of symbiotic microorganisms such as nitrogen fixing rhizobia and arbuscular mycorrhizal (AM) fungi (Sharma *et al.*, 2014 and Figueiredo *et al.*, 2016).

This work aimed to study the effect of different sowing methods and different biofertilization treatments particularly (EM-X) in addition to their interaction on sunflower productivity and charcoal rot control under the Egyptian reclaimed soil conditions.

MATERIALS AND METHODS

Two field experiments were carried out at 6 October farm, El-Nubaria Province, the desert backyard of El-Behaira Governorate (experienced previous history of charcoal rot disease incidence)- Egypt during the two successive summer seasons (2010 and 2011) to investigate the effect of the integration between biofertilization treatments with EM-X and different sowing methods on sunflower (*Helianthus annuus* L.) var. Giza-102 productivity and charcoal rot control under the Egyptian reclaimed soil conditions.

The physical and chemical properties of the experimental soil was determined and included the following characters: sand 91.40%, silt 3.50%, clay 5.10%, pH 7.68, organic matter 0.18%. Ca CO₃ 1.00%, E.C. 0.50 mmhos/cm³. The available total N, P, K were 7.50, 2.80, 18.0 ppm, respectively at 0-60 cm depth as described by Chapman and Pratt (1978).

Except for the sowing method (rows with no tillage), the experimental soil was ploughed twice, ridged and divided into plots 4 meters long and 3.60 meter apart including 6 (ridges or rows) with 0.60 and 0.20 cm apart between (ridges or rows) and hills respectively and the total plot area was (14.4 m²). Accordingly, sowing methods treatments were as follows; {rows with no tillage (Flat)}, {rows with tillage (Furrow)}, and {ridges with tillage}.

During soil preparation 20 m³ of complete fermented animal dung was added as a base for the whole experiment. Unless the treatment advice adding only 50% or 100% of the complete doses of the

conventional chemical fertilization during the soil preparation i.e. 150 kg Calcium super phosphate/fed. (15.5% P₂O₅), and 45 kg N/fed. as ammonium sulphate (20.6% N) in three equal doses at sowing, after thinning and pre-configured buds flowering, in addition to 50 kg/fed. potassium sulphate (48% K₂O) was added after plant thinning.; the rest of the experiment received only the fertilization treatments which were as follows;

1. Conventional: full dose of the recommended dose of the chemical fertilization.
2. Biofert.1:EM₁ {4 Lit/ fed. with irrigation water (0.4%) + 2 Lit. /fed. (7.5%) foliar application.
3. Biofert.2:EM₁ {4 Lit/ fed. with irrigation water (0.4%) + 2 Lit. /fed. (7.5%) foliar application + 50% of the conventional chemical fertilization.
4. Biofert.3:EM₅ {4 Lit/ fed. with irrigation water (0.4%) + 2 Lit. /fed. (7.5%) foliar application.
5. Biofert.4: (EM₁+*Bacillus subtilis*) {4 Lit/ fed. with irrigation water (0.4%) + 2 Lit. /fed. foliar application. (7.5%).
6. Biofert.5:(EM₁+ Arbuscular Mycorrhiza) {4 Lit/ fed. with irrigation water (0.4%) + 2 Lit. /fed. foliar application (7.5%).
7. Biofert.6:(EM₁+ *Azotobacter chroococcum*) {4 Lit/ fed. with irrigation water (0.4%) + 2 Lit. /fed. foliar application (7.5%).
8. Biofert.7: Later will be known as EM-X; (EM₁+ *Bacillus subtilis*.+ *Azotobacter chroococcum*.+ Arbuscular Mycorrhiza). {4 Lit/ fed. with irrigation water (0.4%) + 2 Lit. /fed. foliar application (7.5%).

The effective microorganisms (EM₁) contains selected species of microorganisms including predominant populations of lactic acid bacteria and yeasts, and smaller numbers of photosynthetic bacteria, actinomycetes and other types of organisms such as mycorrhizae (Higa, 1999). Consequently, (EM₅) is a modification product from EM₁ that created by adding vinegar and ethyl alcohol during the fermentation process in order to have multifunction product such as foliar fertilizer and insect repellent (Higa, 2000). Later on, the new modification of EM-X was made by Abd El-Ati (under publication) as a new formula for EM₁ through different experiments for series of *Bacillus subtilis*.+ *Azotobacter chroococcum*.+ arbuscular mycorrhiza which were fermented in the classical EM₁ for 45 days before usage. The EM₁ was kindly obtained from EM project- EEAA- Ministry state of Environmental Affairs, while the liquid culture of *Bacillus subtilis*., *Azotobacter chroococcum*. and arbuscular mycorrhiza were kindly obtained from microbial research center (Cairo-MIRCEN), the unite biofertilizers, Faculty of Agriculture, Ain Shames University. The Biofertilizers application scheme was applied three times in equal doses following the same scheme of the nitrogen fertilizer as described by Abd El-Ati (2006).

Seeds of sunflower var. Giza- 102 were obtained from Agricultural Research Center, Giza, Egypt, were sown at 15 March in both seasons at seeding rates 5 kg/fed. (3-5 seeds per hill) and thinned at 15 days after sowing date.

Drip irrigation method was applied immediately after sowing, then once every week intervals, according to the agricultural practices in the region.

The experimental design that used in this experiment was split plot design in four replicates where the sowing methods occupied the main plots, the fertilization treatments were arranged in the sub plots.

Data Recorded:

I-Disease assessment:

At seedling stage: Damping-off was assessed as percentage of the pre- and post-emergence damping-off 15 and 45 days after planting.

At maturity stage: Charcoal-rot was assessed as disease incidence and disease severity before harvest. Disease incidence was evaluated as percentage of the disease plants 90 days after planting according to Morsy (2005).

Disease severity index was calculated as described by Bhattacharya *et al.*, (1985). The extent of infection by *M. phaseolina* was indicated by the presence of dark brown lesion and also by the presence of microsclerotia of the fungus on root systems. Healthy and infected plants were divided into four groups as follows: (1) Healthy plants = No root rot symptoms, (2) Slightly infected plants = Dark brown to black spots on collar as well as on primary roots, (3) Heavily infected plant = Weak and stunted plants with rotting of roots, (4) Plants dead = Dead and fallen plants. Lesions on the entire root system and the Disease Severity Index (D.S.I) were calculated as follows:

$$D.S.I. = \frac{0(H^n) + 1(S^n) + 2(H^m) + 3(D^n)}{\text{Total number of plants examined}}$$

Where:-

- (Hⁿ)= Number of healthy plants
- (Sⁿ) =Number of slightly infected plants
- (H^m)= Number of heavily infected plants
- (Dⁿ)= Number of dead plants

II- Determination of microbial and *Macrophomina* sp. populations:

Soil samples were obtained from each treatment to a depth 15cm. The population of microsclerotia of *Macrophomina* sp. was evaluated by the assaying of a single 10-g subsample from each sample, using the technique and semi selective medium previously described by Mihail and Alcorn (1982). Microbial populations were

evaluated by using suspension of 1g (dry weight equivalent) in 10 ml of sterile water was prepared. One ml of the soil suspension was then diluted serially (ten-fold) and used to estimation bacterial and fungal total counts by standard spread-plate dilution method as described by Seeley and Van Damark (1981).

III- At harvest:

A random sample of ten plants were taken from each plot at harvest time of both seasons to determine the following: plant height (cm), stem diameter (cm), head diameter (cm), 100 seed weight "seed index" (g), seed weight/head (g) , biological yield (kg/fed.), seed yield (kg/fed.), straw yield (kg/fed.) and the oil yield (kg/fed.) which was estimated by ground dry mature seeds into very fine powder to determine oil percentage using Soxhlet apparatus and diethylether according to A.O.A.C. (1990), then the oil yield was estimated by multiplying seed oil percentage by seed yield (kg/fed.).

Statistical Analysis:

Pooled data were subjected to the combined statistical analysis after passing the homogeneity test using M-STAT C, (Russell, 1991), while Duncan's multiple range test was used to verify the significant differences between treatments means as described by Duncan, (1955).

RESULTS

1. Effect of sowing methods:

On damping off and charcoal rot diseases:

Data in figs (1&2) underline that, the proper sowing method is one of the agricultural practices that reduce both damping off and charcoal rot diseases in sunflower. Sowing on ridges was the most effective sowing method to reduce both pre and post emergence damping off compared to row (furrow) and {row with no tillage (flat)} sowing methods (Fig 1). Consequently, data in Fig.2 illustrate that, both sowing on ridges and on rows reduced significantly incidence and severity of charcoal rot compared to (row with no tillage) sowing method. It also could be concluded that the disease incidence and severity was less in ridges than rows sowing method (Fig 2).

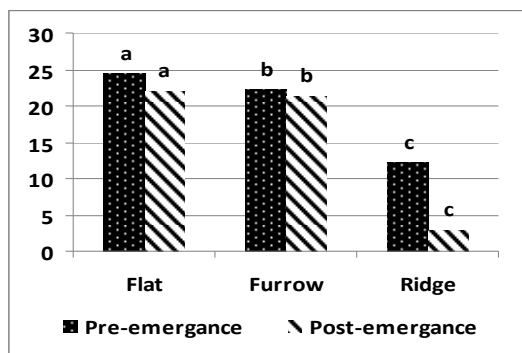


Fig. 1. Effect of sowing methods on damping off disease.

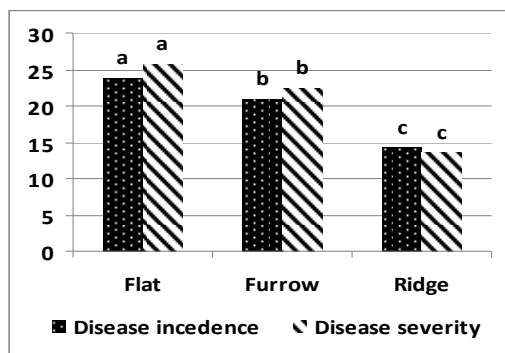


Fig. 2. Effect of sowing methods on charcoal rot disease.

On microbial (bacteria &fungi) and *Macrophomina* sp. Populations:

Data in Fig.3 emphasis that, the proper sowing method as one of the agricultural practices did not suppress

Macrophomina phasolina population density. It was clear that, no significant dereference between the three examined sowing methods i.e. (rows with no tillage), rows and ridges on *Macrophomina* sp. populations density. However,

results showed that, sowing methods as an agriculture practice play an important role in encouraging the soil microbial populations. The bacterial and fungal total counts showed high significant increase with ridges sowing method compared to rows and (rows with no tillage), respectively Fig. 3.

On sunflower var. Giza 102 yield & its components:

Data in Table. 1 highlight the effect of different sowing methods on sunflower var. Giza 102 yield and its components under the dominancy of sunflower damping off and charcoal rot diseases. The observations emphasis that under these conditions of abiotic stresses using ridges sowing method as one of the agricultural practices was more appreciated in increasing significantly all the studied characters of sunflower yield and its components; i.e. plant height (cm), stem diameter (cm), head diameter (cm), 100 seed weight "seed index" (g), seed weight/head (g), biological yield (kg/fed.), seed yield (kg/fed.), straw yield (kg/fed.) and the oil yield (kg/fed.) compared to (sowing in

rows with tillage) and (sowing in rows with no tillage), respectively.

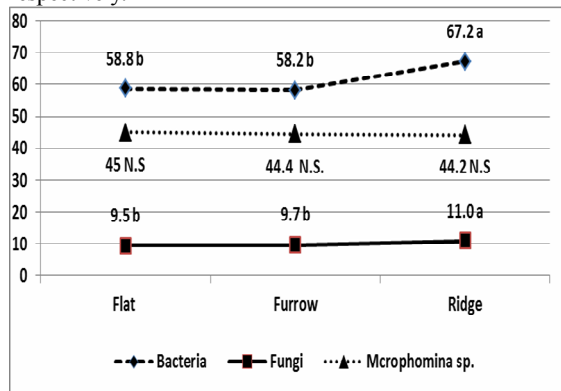


Fig. 3. Effect of sowing methods on microbial counts and *Macrophomina sp.* populations.

Table 1. Effect of different sowing methods on sunflower var. Giza 102 yield & its components grown under Nubaria province conditions (combined analysis of 2010 and 2011 summer seasons).

Sowing Method	Studied Characters								
	Plant Height (cm)	Stem Dia-meter (cm)	Head Dia-meter (cm)	Seed weight /head (g)	100 Seed weight (g)	Bio-logical Yield (kg/fed.)	Seed Yield (kg/fed.)	Straw Yield (kg/fed.)	Oil Yield (kg/fed.)
No tillage	143.2 C	16.2 C	11.1 C	30.1C C	2.82C	4315.3C	271.0C	4044.1C	77.7C
Rows	143.8 B	18.6 B	14.1 B	40.1B B	3.15B	7166.5B	368.5B	6798 B	115.8B
Ridges	157.0 A	21.5 A	16.4 A	46.7A A	3.56A AAAA	8902.8A	469.1A	4833.7A	174.1A

- No tillage = Rows with no tillage.
- Means having similar letters at same column has no significant differences at $P \geq 0.05$.

2.Effect of bio fertilization treatments:

On damping off and charcoal rot diseases:

Data in Fig. 4 and 5 indicate that, there was significant impact of biofertilization treatments i.e. Bio1 (EM₁); Biofert.2 (EM₁+50% of conventional chemical fertilization); Biofert.3 (EM₅); Biofert.4 (EM₁+*Bacillus subtilis*); Biofert.5 (EM₁+ mycorrhiza) Biofert.6 (EM₁+*Azotobacter sp.*) and Biofert.7 (EM₁+ *B. subtilis* + mycorrhiza + *Azotobacter sp.*) for suppression both damping off and charcoal rot diseases, compared to the conventional chemical fertilization as the control treatment. Data in Fig. 4 illustrate that Biofert.7 treatment had the most significant effective to suppress both pre & post

emergence damping-off followed by Biofert.3, Biofert.4, Biofert.5, Biofert.6, Biofert.2 and Biofert.1 respectively, compared to conventional chemical fertilization as the control treatment. Meanwhile, Data in Fig. 5 show that, there are significant variations between the biofertilizer treatments for reducing charcoal rot compared with conventional as a control treatment. Biofert.3 and Biofert.7 (EM-X) treatments showed the minimum disease incidence and severity followed by {Biofert.4& Biofert.5 (with no significant differences)}, Biofert.6 and {Biofert.1& Biofert.2 (with no significant differences)} respectively, compared to conventional as a control treatment (Fig 5).

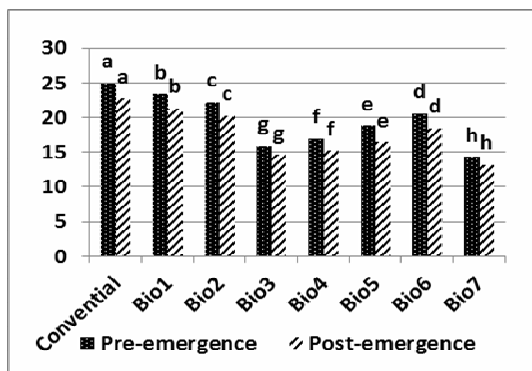


Fig. 4. Effect of biofertilization treatments on damping off disease.

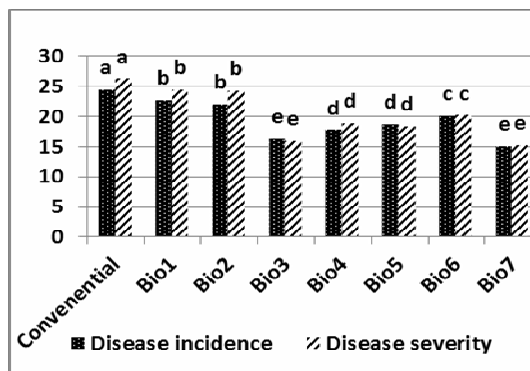


Fig. 5. Effect of biofertilization treatments on charcoal rot disease

On microbial (bacteria & fungi) and *Macrophomina* sp. populations

Data in Fig. 6 present that, the variation effectiveness of biofertilizers i.e. Biofert.1 (EM₁); Biofert.2 (EM₁ + 50% conv. chem. fert.); Biofert.3 (EM₅); Biofert.4 (EM₁+*Bacillus subtilis*) ;Bio5 (EM₁+mycorrhiza) Biofert.6 (EM₁+*Azotobacter* sp.) and Biofert.7 (EM₁ + *B. subtilis* + mycorrhiza + *Azotobacter* sp.) on *Macrophomina* sp. populations and soil microbial total counts compared with conventional as a control treatment. Data showed that, the lowest significant populations of *Macrophomina* sp. was recorded by Biofert.3 & Biofert.7 (EM-X) followed by Biofert.4 and Biofert.6 & Biofert.5 treatments respectively, compared with conventional as a control treatment. There was no significant reduction of the pathogen populations by Biofert.1 or Biofert.2 compared to conventional treatment (Fig. 6). Meanwhile, the biofertilization treatments encouraged significantly the soil microbial total counts compared to the conventional as a control treatment. The most significant increase in total counts of both soil bacteria and fungi was showed by Biofert.3, followed by Biofert.7 (EM-X), Biofert.6 ,Biofert.5 and Biofert.4 respectively, while, Biofert.2 and Biofert.1 treatments had the lowest significant effect when compared with conventional as a control treatment Fig. 6.

On sunflower var. Giza 102 yield & its components:

Data in Table 2 indicate the effect of different biofertilization treatments on sunflower var. Giza 102 yield and its components i.e. plant height (cm), stem diameter (cm), head diameter (cm), 100 seed weight "seed index" (g), seed weight/head (g), biological yield (kg/fed.), seed yield (kg/fed.), straw yield (kg/fed.) and the oil yield (kg/fed.) compared to the conventional chemical fertilization as the control treatment. Observations illustrated that under the dominancy of the biotic stress of

soil born diseases the application of the biofertilizers was more appreciated to increase sunflower yield and its attributes compared to the conventional chemical fertilization. The highest observations were obtained from Biofert.7 (EM-X) followed by Biofert.3,Biofert.6. Biofert.5, Biofert.4, Biofert.2, Biofert.1 then the conventional chemical fertilization as the control treatment, respectively.This was true except for head diameter (cm) where the highest observations were obtained from Biofert.7 (EM-X) followed by Biofert.3, then but with no significant differences Biofert.6, Biofert.5 and Biofert.4, as well as no significant differences between Biofert.2, Biofert.1 and the conventional chemical fertilization. Also, for oil yield (kg/fed.) the superior observations were obtained from Biofert.7 (EM-X) but with no significant differences between the other biofertilizers and compared to the conventional chemical fertilization as the control treatment.

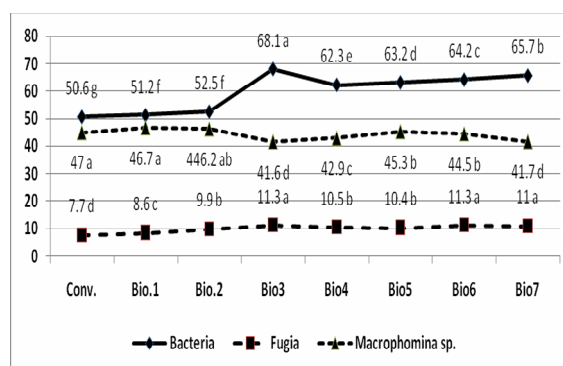


Fig. 6. Effect of biofertilization treatments on soil microbial counts and *Macrophomina* sp. populations.

Table 2. Effect of different fertilization treatments on sunflower var. Giza 102 yield & its components grown under Nubaria province conditions (combined analysis of 2010 and 2011 summer seasons).

Bio Fertiliza-tion treatments	Studied Characters								
	Plant Height (cm)	Stem Dia-meter (cm)	Head Dia-meter (cm)	Seed weight /head (g)	100 Seed weight (g)	Bio-logical Yield (kg/fed.)	Seed Yield (kg/fed.)	Straw Yield (kg/fed.)	Oil Yield (kg/fed.)
Conv.	139.3 H	17.4 H	12.4 D	33.2 H	3.01 H	5530 H	322.2 H	5207.7 H	128.6 AB
Biofert.1	140.5 G	17.8 G	12.6 D	36.3 G	3.05 G	5966.4 G	332.3 G	5634 G	102.8 B
Biofert.2	142.0 F	18.2 F	12.7 D	37.6 F	3.09 F	6281.9 F	343.8 F	5938 F	107.6 B
Biofert.3	149.8 B	19.7 B	15.1 B	41.7 B	3.02 B	7640.2 B	406.8 B	7233.6 B	135.0 AB
Biofert.4	143.6 E	18.4 E	14.0 C	38.9 E	3.14 E	6641.8 E	360.8 E	6281.1 E	114.6 AB
Biofert.5	145.3 D	18.8 D	13.8 C	40.0 D	3.20 D	6923.1 D	376.3 D	6546.9 D	120.7 AB
Biofert.6	147.2 C	19.2 C	13.9 C	40.9 C	3.23 C	7381 C	390.7 C	6990.2 C	126.9 AB
Biofert.7	152.4 A	20.6 A	16.2 A	43.2 A	3.37 A	7994.4 A	423.4 A	7570.7 A	144.0 A

• Conv. = Conventional, Biofert.1= EM₁, Biofert.2 = (EM₁+ 50% of conventional fertilization), Biofert.3= EM₅, Biofert.4= (EM₁+*Bacillus subtilis*), Biofert.5 = (EM₁+ Arbuscular Mycorrhiza) ,Biofert. 6= (EM₁ + *Azotobacter chroococcum*), Biofert.7 = (EM₁+ *Bacillus subtilis*.+*Azotobacter chroococcum*.+ Arbuscular Mycorrhiza).

• Means having similar letters at same column has no significant differences at P≥ 0.05.

3. Effect of the interaction between sowing methods and biofertilization treatments:

on damping off and charcoal rot diseases:

Data in table (4) indicate that, all the different interactions between sowing methods (rows with no tillage, rows andridges) and different biofertilization treatments {conventional, biofert.1, biofert.2, biofert.3, biofert.4, biofert.5, biofert.6, and biofert.7 (EM-X)}, reduced

significantly damping off and charcoal rot diseases in sunflower compared to the control treatment (row with no tillage × conventional fertilization). At seedling stage; data showed that ,the interaction treatments {ridge ×biofert.7 (EM-X)} had the superior significant suppression of pre & post emergence damping off, while the minimum suppression were obtained from (row with no tillage × conventional fertilizers) as the control treatment. At

maturity stage; observations revealed that, the superior significant suppression of charcoal rot incidence was obtained from the interaction {ridge × biofert.7 (EM-X)} followed by {ridge × Biofert.3}.

On microbial (bacteria & fungi) and *Macrophomina* sp. populations

Data in Table 5 illustrate that the integration between different sowing methods (rows with no tillage, rows and ridges) and different biofertilization treatments conventional, biofert.1, biofert.2, biofert.3, biofert.4, biofert.5, biofert.6, and biofert.7 (EM-X) had significant effects on the microbial total counts of both bacteria and fungi in addition to the total counts of *Macrophomina phasolinae* populations in sunflower rhizosphere (colonies x 10³ /g soil). Regarding the bacterial total count the superior observation was obtained from the integration between (ridge×biofert.3) and (row×biofert.3) with no significant differences while the minimum observation was obtained from (conventional fertilization × row with no tillage). Regarding the fungi total count the heist observations were obtained from (with no significant differences in between) from (biofert.7× ridge), (biofert.6× ridge) and (biofert.3× ridge), respectively, while the minimum total fungal count was obtained from (conventional fertilization × row with no tillage). In addition, In respect to *Macrophomina phasolinae* populations in sunflower rhizosphere highest reduction was obtained from (biofert.7× row) and (biofert.7× ridge) respectively, but with no significant differences, while the lowest reduction was obtained from (conventional fertilization× row with no tillage), (conventional fertilization× rows) and then (conventional fertilization× ridges) respectively with no significant differences as well.

Table 5. Effect of the interaction between different sowing methods and soil biofertilizer treatments on microbial total counts and *Macrophomina phasolinae* populations in sunflower rhizosphere (colonies x10³ /g soil) .

Bio-fertilizers	Row with no tillage			Row			Ridge		
	Microbial counts		<i>Macro-phomina</i> sp.	Microbial counts		<i>Macro-phomina</i> sp.	Microbial counts		<i>Macro-phomina</i> sp.
	B	F		B	F		B	F	
Conv.	48.6Ec	7.4Db	47.5Aa	47.5Eb	7.0Db	46.9Ab	55.9Ea	8.8Ca	46.6Ab
Biofert. 1	49.8Db	8.5Cb	47.0Aa	48.0Dc	7.8Dc	46.5Ab	55.8Ea	9.4Ca	46.7Ab
Biofert. 2	50.4Db	9.0Bc	46.9Aa	48.9Dc	9.6Cb	46.0Aab	58.6Da	11Ba	45.8ABab
Biofert. 3	67.6Ab	10.5Ac	42.0Da	68.2Aa	11.5Ab	41.9Dab	68.7Aa	12.0Aa	41.0Db
Biofert. 4	62.2Cb	9.9Bc	43.5Ca	61.4Cc	10.5Bb	42.8Db	63.8Ca	11.2Ba	42.4Cb
Biofert. 5	63.4Cb	9.7Bc	45.7Ba	62.5Cc	10.5Bb	45.1Bc	64.6Ca	11.0Ba	45.0Bb
Biofert. 6	63.7BCb	10.8Ab	44.9BCa	63.5Bb	10.7Bb	44.2Cb	65.9BCa	12.4Aa	44.5Bb
Biofert. 7	64.5Bc	10.4Ab	42.3Da	65.9Ab	10.0Bb	41.6Db	67.4Ba	12.5Aa	41.2Db

- Conv. = Conventional, Biofert.1= EM₁, Biofert.2 = (EM₁+ 50% of conventional fertilization), Biofert.3= EM_s, Biofert.4= (EM₁+*Bacillus subtilis*), Biofert.5 = (EM₁+ Arbuscular Mycorrhiza) ,Biofert. 6= (EM₁ + *Azotobacter chroococcum*), Biofert.7 = (EM₁+ *Bacillus subtilis*.+*Azotobacter chroococcum*.+ Arbuscular Mycorrhiza).
- B = Total bacteria F= Total Fungi.
- Means having similar latters at same column and similar small letters at the same row has no significant differences at P≥ 0.05.

On sunflower var. Giza 102 yield & its components:

Results in Table. 6 illustrate that the integration between different sowing methods (rows with no tillage, rows and ridges) and different biofertilization treatments { conventional,biofert.1, biofert.2, biofert.3, biofert.4, biofert.5, biofert.6, and biofert.7 (EM-X)} had significant effects on improving sunflower var. Giza 102 yield and its components under the dominancy of the biotic stress of charcoal-rot. The observations demonstrated that under the dominancy of the severe soil borne disease such like charcoal-rot the integration between different agricultural practices such as the proper sowing method and the proper biofertilization treatment; where the conventional chemical

Table 4. Effect of the interaction between sowing methods and biofertilization treatments on charcoal rot.

Sowing methods	Bio Fertilizers	At seedling stage		At mature stage	
		Pre-emergence damping off	Post-emergence damping off	Disease incidence	Disease Severity
Row with no tillage (Flat)	Conv.	28.4 a	26.3 a	27.6 a	29.8 a
	Biofert. 1	27.1 b	25.2 b	26.5 a	28.3 b
	Biofert. 2	26.4 b	24.3 c	25.3 b	28.5 b
	Biofert. 3	21.6 f	19.5 f	21.2 f	22.3 g
	Biofert. 4	21.7 f	19.6 f	22.3 e	25.4 e
	Biofert. 5	23.6 e	20.0 f	23.1 d	24.1 f
	Biofert. 6	25.5 c	23.4 d	24.0 c	25.5 e
Biofert. 7	20.7 g	18.6 g	20.3 g	21.8 g	
Row (Furrow)	Conv.	26.5 b	25.4 b	25.6 b	27.5 c
	Biofert. 1	25.3 c	24.4 c	24.3 c	26.3 d
	Biofert. 2	24.5 d	23.5 d	23.2 d	26.0 d
	Biofert. 3	18.7 i	18.8 g	16.8 i	18.7 i
	Biofert. 4	20.5 g	19.6 f	18.6 h	20.5 h
	Biofert. 5	22.3 f	20.4 f	20.5 g	20.7 h
	Biofert. 6	23.4 e	21.5 e	21.7 e	22.5 g
Biofert. 7	16.5 k	17.5 h	15.4 j	17.0 j	
Ridge	Conv.	19.7h	16.5 i	20.0 g	21.7 g
	Biofert. 1	17.5 j	14.2 j	17.2 i	18.6 i
	Biofert. 2	15.6 l	12.7 k	17.7 i	18.5 i
	Biofert. 3	7.3 p	5.4 o	10.4 m	8.6 m
	Biofert. 4	8.5 o	6.5 p l	12.5 l	10.7 l
	Biofert. 5	10.6 n	8.7 n	12.7 l	10.3 l
	Biofert. 6	12.7 m	10.3	14.5k	12.5 k
Biofert. 7	5.7 q	3.5 q	9.5 m	6.7 n	

- Conv. = Conventional, Biofert.1= EM₁, Biofert.2 = (EM₁+ 50% of conventional fertilization), Biofert.3= EM_s, Biofert.4= (EM₁+*Bacillus subtilis*), Biofert.5 = (EM₁+ Arbuscular Mycorrhiza) ,Biofert. 6= (EM₁ + *Azotobacter chroococcum*), Biofert.7 = (EM₁+ *Bacillus subtilis*.+*Azotobacter chroococcum*.+ Arbuscular Mycorrhiza).
- Means having similar latters at same column has no significant differences at P≥ 0.05.

fertilization is prohibited, is a must in order to achieve an appreciated growth and yield under this conditions. The highest observations i.e. plant height (cm), stem diameter (cm), head diameter (cm), 100 seed weight "seed index" (g), seed weight/head (g), biological yield (kg/fed.), seed yield (kg/fed.), straw yield (kg/fed.) and the oil yield (kg/fed.) were obtained from the interaction treatment (biofert.7 × ridges) followed by (biofert.3 × ridges) respectively, while there were significant variations between the other interaction treatments, this was true in regard to all the studied characters except for 100 seed weight "seed index" (g), and the oil yield (kg/fed.) where the differences between most of the interaction treatments

were insignificant except the superior treatment ; the interaction (rows with no tillage × conventional fertilization) as control.

Table 6. Effect of the interaction between different sowing methods and soil amendment treatments on sunflower var. Giza 102 growth and yield & its components grown under Nubaria province conditions (combined analysis of 2010 and 2011 summer seasons).

Sowing Methods	Fertilization Treatments	Studied Characters								
		Plant Height (cm)	Stem Dia-meter (cm)	Head Dia-meter (cm)	Seed weight /head(g)	100 Seed weight (g)	Bio-logical Yield (kg/fed.)	Seed Yield (kg/fed.)	Straw Yield (kg/fed.)	Oil Yield (kg/fed.)
Rows with no tillage (Flat)	Conv.	127.8 T	14.8 S	9.7 N	19.8 W	2.67 R	2286.7 V	215.7 U	2070.7 V	62.4 k
	Biofert.1	129.6 S	15.4 R	NS	27.6 V	NS	3151.7 U	229.8 T	2921.7 U	NS
	Biofert.2	131.8 R	NS	NS	29.1 U	NS	3573.7 T	245.7 S	3327.7 T	NS
	Biofert.3	NS	17.1 N	NS	34.0 Q	NS	5574.0 P	NS	5264 P	NS
	Biofert.4	NS	NS	NS	30.1 T	NS	4171.3 S	270.7 R	3900.7 S	NS
	Biofert.5	135.9 P	16.2 P	NS	31.7 S	NS	4602.3 R	284.3 Q	4318.3 R	NS
	Biofert.6	NS	16.7 O	NS	33.1 R	NS	5328 Q	297.2 P	5030.7 Q	NS
Biofert.7	NS	NS	NS	35.2 P	NS	5834.7 O	NS	5519.3 O	NS	
Rows (Furrow)	Conv.	NS	NS	NS	35.7 O	NS	6156.3 N	322.7 N	5833.7 N	NS
	Biofert.1	NS	17.9 L	NS	36.8 N	NS	6455.7 M	NS	6122.7 M	NS
	Biofert.2	NS	18.3 K	NS	38.4 M	NS	6790.7 L	NS	6453 L	NS
	Biofert.3	146.9 I	NS	NS	42.6 J	NS	7810.3 H	407.5 I	7003 H	NS
	Biofert.4	NS	NS	NS	40.8 L	NS	7128.7 K	356.1 L	6772.7 K	NS
	Biofert.5	NS	NS	NS	NS	NS	7369.3 J	376.4 K	6793 J	NS
	Biofert.6	NS	19.0 I	NS	NS	3.21 H	7617.7 I	396.8 J	7220.7 I	NS
Biofert.7	NS	NS	NS	43.4 I	NS	8003 G	417.7 H	7585 G	NS	
Ridge	Conv.	NS	NS	NS	43.9 H	NS	NS	NS	NS	NS
	Biofert.1	NS	20.2 F	NS	44.4 G	NS	8292 F	NS	7857.7 F	NS
	Biofert.2	NS	NS	NS	45.2 F	NS	8481.3 E	447.9 F	8033.3 E	NS
	Biofert.3	164.0 B	22.8 B	NS	48.4 B	3.77 B	9536.3 B	502.9 B	9033.7 B	NS
	Biofert.4	153.7 E	NS	NS	45.7 E	3.5 D	NS	455.6 E	NS	NS
	Biofert.5	155.9 D	21.4 D	NS	46.9 D	NS	8797.7 D	468.2 D	8326.3 D	NS
	Biofert.6	159.6 C	21.9 C	NS	47.7 C	NS	9197.3 C	478.1 C	8718.3 C	NS
Biofert.7	169.5 A	24.8 A	19.8 A	50.9 A	3.87 A	10145.7 A	537.7 A	9607.7 A	201.5 A	

• Conv. = Conventional, Biofert.1= EM₁, Biofert.2 = (EM₁+ 50% of conventional fertilization), Biofert.3= EM_s, Biofert.4= (EM₁+*Bacillus subtilis*), Biofert.5 = (EM₁+ Arbuscular Mycorrhiza) ,Biofert. 6= (EM₁ + *Azotobacter chroococcum*), Biofert.7 = (EM₁+ *Bacillus subtilis*.+*Azotobacter chroococcum*.+ Arbuscular Mycorrhiza).

• NS = not significant at P ≥ 0.05.

• Means having similar letters at same column has no significant differences at P ≥ 0.05.

DISCUSSION

The aspirational goal of a land degradation neutral world, to be realized by reducing the rate of land degradation and increasing the rate of restoration of degraded land, was agreed at the Rio+20 Conference in 2012. This land degradation was occurred through different biotic and abiotic stresses some are natural and the others are manmade, yet soil borne diseases is one of those biotic stresses that has direct impact on loss of land cover and decrease productivity of area unit hence desertification (Grainger, 2015). He added; in order to achieve land degradation neutrality in an infested area with soil borne diseases, we should protect new lands to be infested, restore the infested lands to be productive, and engage people to let them know the most proper agriculture practice in order to achieve the land degradation neutral world by 2030.

Tillage is one of those agricultural practices that determined to be a critical management practice to improve soil properties and to suppress soil-borne diseases and decrease population density of serious soil borne pathogens such as *Macrophomina phasolina* (Wrather and Kendig 1998). This may be due to that tillage reduces populations of weeds and volunteer crop plants that harbor pathogens between crops. It also buries plant pathogens from the

upper layers of the soil into deeper ones where they cause less or no disease (Dang *et al.*, 2015). Practices involved in the preparation of seed beds can greatly modify physical properties of soils such as moisture characteristics, bulk density, aeration and temperature profiles which in turn influence the incidence of disease. Forming the soil into hills, ridges or raised beds provides better drainage and irrigation, and healthy soil with high microbial diversity does play a role by being antagonistic to soil pathogens thus increase plant growth and productivity (Dang *et al.*, 2015).

As indicated in the results; the soil population density of *M. phasolina* was greater in the soil with no tillage (flat) than with tillage either row (furrow) or ridges but with nosignificant differences. However, the ridge sowing method increased bacterial and fungal total counts significantly more than row and row with no tillage sowing methods. Mbuthia *et al.*, (2015) indicated that, zero tillage without residues retention resulted in very low populations of micro-flora, while conventional tillage with residue removal resulted in the predominance of total fungi, bacteria, actinomycetes and fluorescent pseudomonas. Tillage was found to be enhance the propagation of the plant growth-promoting bacteria (PGPB) and other rhizospheric beneficial microorganisms , such as

biological nitrogen fixation and phosphate solubilization that can be assessed as plant growth promotion traits (Anikwe *et al.*, 2016).

Soil health is an important factor that affects plant growth promoting bacteria (PGPB) efficiency due to several characteristics such as soil type, nutrient pool, soil moisture, microbial diversity, and soil disturbances caused by management practices such as tillage, which all together play an important role in improving the plant growth and productivity (Anikwe *et al.*, 2016). This can easily describes the superior results obtained by using ridges then rows sowing methods respectively, compared to (rows with no tillage) as the control treatment.

Biofertilizers which applied as seed or soil inoculants is a terrific solution for soil fertility depletion particularly in harsh environments where biotic or abiotic stresses prevailed and chemical fertilization seems to be a great gamble (Singh *et al.*, 2011). It keep the soil environment rich in all kinds of micro- and macro-nutrients via nitrogen fixation, phosphate and potassium solubilisation or mineralization, release of plant growth regulating substances, produce of antibiotics and biodegradation of organic matter in the soil, biocontrol of pathogens and insect pests, which operation can significantly be useful in maintaining the sustainability of various crop productions, therefore improve plant growth and productivity (Sinha *et al.*, 2014). Similarly, Plant growth promoting rhizobacteria (PGPR) has positive impacts on plant growth and productivity; it act as phytostimulators, biofertilizers, thus enhance crop growth and yield through nutrient uptake and plant growth regulators. It also acts as biocontrol agents by production of antibiotics, triggering induced local or systemic resistance (Bouizgarne 2013). The PGPR or co-inoculants of PGPR and arbuscular mycorrhizal fungi (AMF) can advance the nutrient use efficiency of fertilizers seven times compared to the chemical fertilizers (Adesemoye and Kloepper, 2009).

In general, 60% to 90% of the total applied chemical fertilizer is lost, while only 10% to 40% remained in the soil to be taken up by plants, besides the enormous pollution that was introduced to the virgin environments through the chemical fertilization to introduce pollution as one of the most severe desertification factors to the harsh environment. In contrary, microbial inoculants have paramount significance in integrated nutrient management systems to sustain agricultural productivity and healthy environment, therefore mobilize the Millennium Sustainable Development Goals (SDG No. 15); concerning life on earth (Adesemoye *et al.*, 2009).

Azotobacter, Azospirillum, Rhizobium, cyanobacteria, phosphorus and potassium solubilising microorganisms and mycorrhizae are some of the PGPRs that were found to increase in the soil under no tillage or minimum tillage treatment (Aziz *et al.* 2012). Azotobacter plays an important role in the nitrogen cycle in nature as it possesses a variety of metabolic functions. Besides playing role in nitrogen fixation, Azotobacter has the capacity to produce vitamins such as thiamine and riboflavin, and plant hormones viz., indole acetic acid (IAA), gibberellins (GA) and cytokinins (CK). *A. chroococcum* improves the plant growth by enhancing seed germination and

advancing the root architecture by inhibiting pathogenic microorganisms around the root systems of crop plants (Bhardwaj *et al.*, 2014). Soil application with *Bacillus subtilis* decreased the incidence of damping-off and root rot, increased the number of survived peanut plants in *M. phaseolina* and/or *R. solani* infested soil in comparison with the control (Abd-El-Khair *et al.* 2016). Mechanisms involved in *Bacillus* sp. eliciting plant growth promotion include auxin production, increased uptake availability of phosphorus biocontrol abilities and induction of systemic resistance (Bouizgarne 2013).

According to Tokeshi *et al.* (1998) beneficial microorganisms (EM) were found to be suppressive to the soil-borne plant pathogen *Sclerotinia sclerotiorum*. Control of fungal pathogens may be attributed to the activity of lactic acid bacteria in the beneficial microorganisms mixture that produce lactic acid, a strong sterilizing compound (Higa 2000). EM technology itself imbedded a wide variety of products. As EM1 consists of lactic acid bacteria, yeasts, photosynthetic bacteria, actinomycetes and other types of organisms such as mycorrhizae which are mutually compatible with one another and coexist in based molasses liquid culture, while EM5 exceeds with ethyl alcohol and sugar cane vinegar (Higa, 1991), so they seemed to be more applicable under the study conditions. Referring to EM1 & EM5 biochemical structure; EM5 was superior to extract the biochemical materials from the decomposed plants as a mentioned before for its continuity of bio-solvents such as ethyl alcohol and esters which naturally formed from vinegar and ethanol by the EM existed microorganisms. This can answer the query about the superior results obtained from EM5 application more than EM1 under both greenhouse and field conditions. Both EM1 and EM5 contained some alcoholic sugars such as manitol, which formed during the fermentation process from ethanol and glucose that already existed in the both bio-products (Higa, 2000).

As the alcoholic sugars have a smaller linear shape molecule that was capable to enter the plant stomatal system easier and faster than any other molecule shape (Edwards *et al.*, 1998), thus provides its physical features to the biochemicals when associated together. These can easily describes the superior results in the reduction obtained in *Macrophomina* sp. populations and soil microbial total counts, thus plant growth and productivity compared with the conventional fertilization. It could be concluded that the rhizobacterial effects can occur via local antagonism to soil-borne pathogens or by induction of plant systemic resistance against pathogens. In addition, several substances produced by antagonistic rhizobacteria may be related to both pathogen control and promotion of plant growth and productivity, such as siderophores and antibiotics that was clear by different biofertilization application (Figueiredo *et al.*, 2016), particularly when {EM1 + *B. subtilis* + mycorrhiza + *Azotobacter* sp.} were integrated together under the name (EM-X).

Consequently, when take into consideration all the integration of the direct and indirect impacts of sowing method {rows, ridges and (rows with no tillage)} and the impacts of biofertilization treatments {biofert.1, biofert.2, biofert.3, biofert.4, biofert.5, biofert.6, and biofert.7 (EM-X)} compared to the control treatment (conventional

chemical fertilization). These impacts can simply explicate the superior results obtained from the integration between ridges sowing method and { EM₁ + *B. subtilis* + mycorrhiza + *Azotobacter* sp.} as (EM-X), compared to the other fertilization treatments particularly the { rows with no tillage ×conventional chemical fertilization} as the control treatment.

CONCLUSION

Charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goid is a major seed and soil borne pathogen that causes root or stem rot and sometimes causes early death of maturing sunflower plants, thus decrease the plant growth and productivity, and thus introduce land cover loss as one of the desertification indicators to the affected areas.

Under this biotic stress, the integration between the proper agricultural practices such as tillage, sowing method and biofertilization where the conventional fertilization seems to be a great risk for its pollution consequences on the stressed environment, in addition to increase plant water stress as a result of damaging both the vascular and root systems.

Therefore, the integration between ridges sowing method with tillage and { EM₁ + *B. subtilis* + mycorrhiza + *Azotobacter* sp.} named as (EM-X), compared to the other sowing methods and other fertilization treatments was the furthestmost advised agricultural practices under these conditions as land degradation neutrality (LDN) practical technology that enhance land cover and area unit productivity under these conditions.

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تأثير تكامل التسميد الحيوي بالـ EM-X وطرق الزراعة علي تشجيع إنتاجية عباد الشمس ومقاومة مرض العفن الفحمي تحت ظروف الأراضي المستصلحة.

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¹ قسم الإنتاج النباتي - مركز بحوث الصحراء - المطرية- القاهرة- مصر.

² قسم وقاية النبات - مركز بحوث الصحراء - المطرية- القاهرة- مصر.

أجريت تجربتان حقلتان في مزرعة 6 أكتوبر بمنطقة النوباريه بالظهير الصحراوي لمحافظة البحيره بجمهورية مصر العربية خلال الموسمين الصيفيين المتتابعين (2010-2011)، وذلك لدراسة تأثير التكمال بين طرق زراعه مختلفه ومعاملات مختلفه من التسميد الحيوي على كلا من إنتاجية محصول عباد الشمس وإدارة مرض العفن الفحمي الناتج عن المسبب المرضي ميكروفومينا فاسولينا تحت ظروف الأراضي المستصلحة. أوضحت النتائج ان طرق الزراعه على خطوط كانت الأكثر فاعليه لخفض تعداد الميكروفومينا فاسولينا ومرض العفن الفحمي وبالتالي أدت إلى تحسين إنتاجية محصول عباد الشمس بالمقارنه بطرق الزراعه الأخرى تحت الدراسه. أثبتت الدراسه أيضا أن المركب الحيوي EM-X والمشمتمل على (*Bacillus subtilis* + *Azotobacter sp* + mycorrhiza) (EM_1) الأكثر فاعليه لخفض تعداد الميكروفومينا فاسولينا ومرض العفن الفحمي وبالتالي أدت المعامله به إلى تحسين إنتاجية محصول عباد الشمس بالمقارنه بمعاملات التسميد الحيوي الأخرى والمشمتمل على التسميد الكميائي الموصى به كمقارنه. كما أظهرت النتائج أن التكمال بين طريقة الزراعه على خطوط ومركب EM-X من أفضل العمليات الزراعيه التي تدخل ضمن تقنيات تحيد آثار تدهور الأراضي والتي تعمل على تحسين حالة الغطاء النباتي وزيادة إنتاجيه وحده المساحه تحت تلك الظروف.