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The biological activity of bacterial vaccine of *Pseudomonas putida2* and *Pseudomonas fluorescens3* isolates to protect sesame crop (*Sesamum indicum*) from *Fusarium* fungi under field conditions

Hammad Nawaf Farhan[1], Basher H. Abdullah[2] and. Ashwaq T. Hameed[3]

[1]Professor at Biology Department, College of Education for Pure Science, Al-Anbar University, Iraq (E-mail: drhammad51@yahoo.com).
[2]Dr. at Agronomy Department, College of Agriculture, Al-Anbar University, Iraq.
[3]Lecturer at Biology Department, College of Science, Al-Anbar University, Iraq.

ABSTRACT

This research investigated the biological effects of *Pseudomonas putida2 and Pseudomonas fluorescens3* as biocides to inhibit *Fusarium* fungi growth and as biofertilizers to improve growth characters of sesame crop grown in contaminated soil with *Fusarium* under field conditions compared with Dithen. Results showed mixture of vaccine *Pseudomonas putida2* + *Pseudomonas fluorescence3* together was more effect on *Fusarium* growth and increased growth characters much higher than each isolate alone. Both isolates scored significant improving in morphological, physiological and productivity characters for sesame compared with control and Dithen treatments. But mixture of *P. putida2* + *P. fluorescens3* treatment together (Fusant) as a biocide and biofertilizers gave higher significant results in increasing chlorophyll content, percentage of N, P, K in total dry weight of shoot, branch no./plant, height of plant, leaf area per plant, leaf no./plant, pods no./plant, grains no./pod, total weight of 1000 grains, total yield of grains/plot, and percentage of oil in sesame grains. The values were 3.21 mg/gm, 4.18%, 0.44%, 3.87%, 45.8 branch/plant, 151.7 cm/plant, 59.7 cm^2/plant, 428.3 leaf/plant, 146.7 pod/plant, 69.1 grain/pod, 2.92 gm/1000 grain, 982.3 gm (grains)/plot and 56.2 % oil in sesame grains respectively. While control treatment scored: 0.85 mg/gm, 1.77%, 0.11%, 1.43%, 14.6 branch/plant, 53.3 cm/plant, 25.5 cm^2/plant, 162.7 leaf/plant, 44.0 pod/plant, 31.3 grain/pod, 0.94 gm/1000 grain, 112.4 gm (grains)/plot and 26.6 % oil in sesame grains respectively.

Keywords: Biological Control, Pseudomonas Bacteria, *Fusarium* Fungi, Sesame Crop.

INTRODUCTION

Sesame crop is one of the important and strategic oil crops in the world. It has an economical importance to produce plant oil. Unfortunately, In Iraq, there are a lot of problems facing sesame agriculture like wilting disease, root rot and damping off for seedlings, which happen by some fungus which transfer from soil to the plant to infect the crop. *Fusarium* is one of these fungi. Infection by *Fusarium* fungi causes: 1) high reduction in germination percentage because the high reduction in embryo activity of seeds by fungus; (2) seedlings death at the first stage of growth; (3) death of the whole plant at flowering stage; and finally (4) reduction in production.

It has been estimated that total losses as a consequence of plant diseases reached 25 % of the yield in western countries and almost 50% in developing countries, one third of this is due to fungal (Bowyer, 1999). *Pseudomonas sp*. can produce siderophores compounds which can inhibit growth of plant pathogenic fungi (Kumar & Dube, 1992; Loper & Henkel, 1999; Lottmann *et al*., 2000; Khan *et al*., 2006; Abdel-Salam *et al*., 2007). Other metabolites compounds with siderophores can produced by *Pseudomonas sp* isolates (Loper, 1988; Weller, 1988; Kumar & Dube, 1992; Duijff *et al*, 1993; Dowling & O,Gara, 1994; Loper & Henkel, 1999; Lottmann *et al*., 2000). Moreover, most of *Pseudomonas putida* and *Pseudomonas fluorescen*s and other species can produce phenozine antibiotic compound which inhibit growth of plant pathogenic fungi (Thomashow & Weller, 1988). For example, *Pseudomonas chlororaphis* pcl 1391 inhibited successfully growth of *Fusarium oxysporium* which caused root rot for tomato crop (Chin *et al*., 1998). *Pseudomonas* isolates can also produce some antibiotic compounds which can inhibit fungi growth (Schroth & Hancook, 1982). Many strains of *P. fluorescens* are known to enhance plant growth

promotion and reduce severity of various diseases and induced systematic resistance, biological control of pathogens (Ganeshan and Kumar, 2005). Fusants between *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* showed higher antagonistic efficiency reached three times more than the efficiency of *P. fluorescens* and two times more than efficiency of *P*. *aeruginosa* in controlling the plant pathogen *Fusarium oxysporium* (Abdel-Salam *et al*., 2007). There was a direct inhibition when *Pseudomonas fluorescens* used alone against *Pythium aphanidermatum,* with cowpea plants, and provided a reduction of the disease index from 3.44 to 1.06 (Nwaga *et al*., 2007). *Pseudomonas spp* seem to be the most successful Biocontrol agent against *Pythium ultimum* in a number of reports (Hadedorn *et al*., 1993 and Georgakopoulos *et al*., 2002).

The ability of *pseudomonas sp.* in stimulating germination and plant growth may be related to some compounds like plant hormones e.g. gibberellins, cytokines, Indole acetic acid (IAA) and polysaccharides (Deweger *et al*., 1987; Kloepper *et al*., 1992; Glick *et al*., 1997; and Abed *et al*., 2009). Indeed, fluorescent *Pseudomonas* belongs to plant growth promoting rhizobacteria (PGPR) (Ganeshan and Kumar, 2005). Using biofertilizers in the biological experiments increased the quantity and quality characters of plant when seeds treated with them (Burr *et al*., 1978; Hossain, 1987; Smith and Goodman, 1999; Hameed & Farhan, 2007 and Abed *et al*., 2009). Many strains of *Pseudomonas fluorescens*, significantly reduced the extent of both wheat coleoptiles growth retardation and wheat and barley seedling blight caused by *Fusarium culmorum* by 53 – 91 % (Khan *et al*., 2006). The effect of *Pseudomonas sp* vaccines on germination, morphological and physiological characters of crops was studied by many researchers. Weller and Cook (1986) found increasing in growth and productivity of wheat when seeds treated with *Pseudomonas fluorescens* against *Pythium* fungi. While Deluz *et al*., (1998) found significant increasing in germination and productivity of wheat when seeds treated with *Pseudomonas putida* against fungi growth compared with untreated seeds. Hameed & Farhan (2007) found significant higher values in growth and germination percentage of *Sorghum bicolor* crop compared with control treatment by using bacterial vaccines of *Pseudomonas aureofaiciens & Pseudomonas putida*. The two isolates also improved growth characters of plants cultured in soil contaminated with *Rhizoctonia solani* fungi and significantly inhibited the effect of plant pathogen fungi.

The effects of *Pseudomonas* in improving other growth characters like leaf area, leaf number per plant and chlorophyll content were studied by many workers. Benani *et al*., (1994) found that *Pseudomonas* increased leaf area per plant by 30 %. Hameed & Farhan (2007) found similar results, that *P. aureofaiciens* and *P. putida* increased leaf area per plant and chlorophyll content in sorghum plant significantly compared with control and fungi treatments. The ability of *Pseudomonas sp* in increasing chlorophyll content in leaves may be related to supplying the plants with some nutrient elements like N and P (Hameed & Farhan, 2007). Moreover, they showed significant increase in levels of nitrogen and phosphorus in total dry weight of shoot per plant for all plants which treated with bacterial vaccines compared with fungi treatments.

This research investigated the biological effects of some isolated bacteria from Iraqi soils like *Pseudomonas putida2 and Pseudomonas fluorescens3* as biocides to inhibit *Fusarium* fungi growth and as biofertilizers to increase and improve percentage of germination, productivity, morphological and physiological characters for sesame crop grown under field conditions.

MATERIALS AND METHODS

Experiment no.1: The Biological Activity of *Pseudomonas putida*2 and *Pseudomonas fluorescens*3 as biocide to inhibit *Fusarium* fungi growth comparison with Dithen and Radiomil Fungicide under incubator Conditions.

King B (KB) medium was prepared to activate bacteria growth (Cowan, 1977). While, Potato Dextrose Agar (PDA) was prepared to activate fungi growth (Agrios, 1988). Autoclave used to sterilize culture media under 121 C^0, pressure 15P/inch for 15 minutes. Oven used to sterilize all glasses under 180 C^0. Alcohol 70 % was used to sterilize benches, tables, others. Bacteria isolates were supplied from Biology Department, College of Sciences, Al-Anbar University. While, *Fusarium Sp* isolate was supplied previously from Department of Plant Protection, College of Agriculture, Baghdad University to Biology Department, College of Sciences, Al-Anbar University. Isolated bacteria were activated in liquid media prepared from each isolated bacteria in conical flask size 250 ml, contains 100 ml of KB liquid culture media, after contamination with bacteria each conical flask was incubated at 28 C^0 for 24 hours to activate

the two isolates bacteria, and then were kept in the fridge at 4 ^{0}C.

Small mycelium as a sample from *Fusarium* pathogenic fungi was cultured in sterilized Petri-dishes size 7 cm contain PDA culture media, then all dishes were incubated in the incubator at 25 C^0 to activate the growth of fungi for 24 hours. Fungi isolate was kept in the fridge at 4 ^{0}C.

Solid media culture was used in sterilized Petri dishes size 9 cm. Each Petri dish was contaminated completely with 0.1 ml of bacterial vaccine by using sterilized pipette (Micropipette). Then small disc from *Fusarium* fungi was taken by cork borer, each 4 mm diameter. Each disc was cultured in the center of each Petri dish to test the inhibition activity of each isolated bacteria. All dishes were incubated in the incubator at 25 C^0 to activate the growth of fungi for 24 hours. Then fungi isolate was kept in the fridge at 4 C^0.

0.5 gm from Dithen and Radiomil GMZ68 chemical fungicide were taken and mixed separately well in 1 liter of sterilized and distilled water. Then 200 ml from the mixture were put in sterilized conical flask size 500 ml contain KB nutrient media, and mixed homogenously. After preparing media, it was dropped and distributed in sterilized Petri dishes size 9cm. All treatments were contaminated with 0.4 cm in diameter from *Fusarium* according to the experimental design. Control treatment was left without contamination with bacterial vaccine or chemical fungicides (Al-Amery, 2003). All Petri dishes were kept in the incubator at 28 C^0 for 7days. Completely Randomized Design was used with three replicates in this experiment.

Pictures were taken for all treatments at the seventh day from the beginning of experiment (Fig. 1).

Inhibition percentage for fungi growth was calculated according to the following equation (Gamliel & Katan, 1993):

$$\text{Inhibition \%} = \frac{\text{Mean of fungi growth in control- mean of fungi growth in bacteria treatment}}{\text{Mean of fungi growth in control treatment}} \times 100$$

Experiment no. 2: The Biological Activity of Bacterial Vaccine of *Pseudomonas putida2* and *Pseudomonas fluorescens3* Isolates to Protect Sesame Crop (*Sesamum indicum*) from *Fusarium* Fungi under Field Conditions.

Preparation of Biocide: Preparation of bacterial vaccines was similar to that described in Experiment 1. Wheat bran powder was used as a carrier for bacterial vaccine, it was prepared and dried well. Then, it sieved by sieve size 250 micrometer. Carrier was sterilized in the Autoclave at 121 C^0 for 30 minutes (Amer & Utkhed, 2000). Under sterilized conditions inside Lab, Biocide was prepared for each isolate in concentration of 9 log 10^{15} cfu/1 ml in sterilized Petri dishes size 9 cm, each contains 10 ml of bacterial vaccine with 10 gm from wheat bran powder. All dishes were kept under incubator conditions at 30 C^0 for 3 days to dry them gently (Al-Amery, 2003). 1ml from each treatment was taken as a sample to test and count the total number of bacterial cells in 1ml under developed microscope as Clark's method (1965).

Dithen fungicide: Dithen fungicide was prepared as the following: 25 gm from Dithen chemical fungicide mixed well in 1 liter of sterilized and distilled water. After preparing solution, it was dropped and distributed in the experimental plot soil according to the experimental design (Al-Amery, 2003).

Fusarium fungi activation: *Fusarium* fungi was cultured in Potato Dextrose Media (PDM) in Petri dishes size 9 cm. Suspended solution of *Fusarium* fungi was prepared by adding 150 ml from distilled and sterilized water to one Petri dish which contains fungi only. Electrical mixer was used for this purpose. This fungi mixture was added to the plots soil in depth of 5 cm three days before planting (on 12th May, 2009) according to the experimental design (Al-Amery, 2003).

Preparing of Sesame seeds: Sesame seeds from local variety were sterilized by sodium hypo chloride 1% for 3 minutes, and washed gently by sterilized and distilled water then dried well (Beckker & Cook, 1988). Seeds were soaked in sucrose solution 1% then all seeds were covered with bacterial vaccine carrier at rate 50 gm/1 kg seeds in sterilized Petri dishes under sterilized conditions to prevent any contamination (Al-Rajab, 2005).

Planting: The land of the biological experiment was divided well by ridges into 18 plots (experimental units). Each plot was 1x1m. Width of each ridge was 25 cm to prevent contamination among the plots. Soil samples were taken to test the chemical and physical characters (Black, 1965 and Page *et al*, 1982), (Table 1). Randomized Complete Block Design (RCBD) was used with three replicates in this experiment under field conditions (Fig. 2). There was six treatments in each block as described in Table (3). There was two lines in each plot. Nitrogen and phosphorous fertilizers were added before planting as recommended to all plots. 15 sesame seeds with

bacterial vaccine were planted in depth 3 cm in each pit 3 days after contamination the soil with fungi vaccine (15th, May 2009). There was 14 pits in each plot. The distance between pits was 15 cm and between lines was 50 cm. Comparison treatment was left without contamination with fungi or bacterial vaccine or Dithen (No addition). Control treatment was left without contamination with bacterial vaccine or chemical fungicides, just with Fusarium fungi (Al-Amery, 2003). Germination percentage was recorded three weeks after planting. Plants in each pit were thinned to 2 plants. On 5th July 2009 (50 days after planting), leaf number per plant, branch number per plant and height of each plant were measured and calculated in each treatment. Fig. (2) shows general photo for the experiment site under field conditions, it was taken 21 days after planting. Table (1) shows some physical and chemical characters of the study soil before planting.

Item	Unit
pH	7.6
EC	3.4 dsms/m
Soil density	1.33gm/m^3
Ionic exchange capacity	26%
Silt	37%
Clay	27%
Sand	36%
Soil texture	Sandy loam
Organic matter	0.95
N	2300mg/kg
K	22ppm
P	11.8ppm

Measurements: 65 days after planting, many samples of leaves from each treatment were selected randomly and harvested to determine chlorophyll content (a+b) (Witham *et al*., 1971). The following characters were calculated and measured 100 days after planting: Total number of leaf per plant, Height of plants (cm), branches number per plant Leaf area per plant was measured as the following: LA = L x W x 0.76, when L = length of leaf (cm), W = wide of leaf cm) and 0.76 = constant, According to Liang *et al*, (1973). All plants in each plot were harvested individually and carefully 112 days after planting (on 7th September, 2009) to calculate the following: Pods number per plant, Grains number per pod per plant, total weight of 1000 grains, total yield of grains per plot. Percentage of nitrogen (N), phosphorus (P) and potassium (K) in total dry weight of shoot were tested 65 days after planting, according to Dubis *et al*., (1956), Black (1965) and Sawhney and Randlhir (2000). While percentage of oil in the grains was tested and recorded according to Harbone (1973) and Sorenson (1974).

RESULTS AND DISCUSSION:

Results showed a clear effect for the ability of the two isolated bacteria in inhibition to fungi growth under incubator conditions cultured in KB media (Table, 2). *Pseudomonas putida2 and Pseudomonas fluorescens3* gave high significant inhibition against fungi growth compared with control, Radiomil and Dithen treatments. In respect of *Pseudomonas putida2* treatment, fungi growth reached 4.8 mm in diameter. And for *Pseudomonas fluorescens3* treatment, the fungi growth reached 4.43 mm. No growth in the mixture treatment of the two isolates together, while the mean of fungi growth in control treatment reached 82.7 mm. In Dithen and Radiomil treatments the growth reached 29 mm and 33 mm respectively (Fig. 1).

Table 2*:* Comparison between the Biological activity of Pseudomonas sp. Bacteria with Dithen and Radiomil fungicide on diameter growth of Fusarium fungi which cultured in KB media under incubator conditions at 28 C^0 for seven days.

Treatments	Fusarium growth (mm)	% inhibition
Pseudomonas putida 2 + Fusarium	4.80	94.2
Pseudomonas fluorescens 3+ Fusarium	4.43	94.6
P. putida2 + P. *fluorescens 3*+ Fusarium	0.0	100.0
Dithen Fungicide + Fusarium	29.0	64.9
Radiomil + Fusarium	33.0	60.1
Control (Fusarium only)	82.7	-
LSD at 5%	11.2	-

Pseudomonas putida2 scored inhibition in fungi growth by 94.2%, while *Pseudomonas fluorescens3* scored inhibition in fungi growth by 94.6 %. Mixture of *P. putida2* + *P. fluorescens*3 treatment scored inhibition in fungi growth by 100 %. Dithen and Radiomil treatments scored inhibition by 64.9 % and 60.1 % respectively compared with control treatment. *Pseudomonas putida2 & Pseudomonas fluorescens3* were significantly higher effect as Biocides on fungi growth more than Dithen and Radiomil fungicide treatment. However, Dithen treatment was more effective than Radiomil treatment in this study. This suppression in fungi growth, may be related to the siderophores and other metabolites compounds

which produced by *Pseudomonas* isolates (Duijff *et al*, 1993; Dowling & O,Gara, 1994). Most of *Pseudomonas putida* and *Pseudomonas fluorescen*s and other species can produce phenozine antibiotic compound which inhibit growth of plant pathogenic fungi (Thomashow & Weller, 1988). This is in agreement with many workers in this subject (Wood & Pierson, 1996; Loper & Henkles, 1999; Ganeshan & Kumar, 2005). Moreover, For example, *Pseudomonas chlororaphis* pcl 1391 inhibited successfully growth of *Fusarium oxysporium* which caused root rot for tomato crop (Chin *et al*., 1998). This agrees with James & Gutterson (1986) who used *Pseudomonas fluorescens* to inhibit *Pythium ultimum* fungi which caused damping of on cotton crop.

Table (4) and Figs. (2 & 3) show a clear effect for the two isolates on increasing branches number per plant, height of plant and leaf area per plant of sesame crop. Values of the above characters for *Fusarium* treatment (control) were 14.6 branch/plant, 53.3 cm/plant and 25.5 cm^2/plant respectively. While when we added bacterial vaccine of *Pseudomonas putida2* to the seeds as a biocide to fungi treatments, values increased to 32.9 branch/plant, 105.7 cm/plant and 41.2 cm^2/plant respectively. Addition bacteria vaccine of *Pseudomonas fluorescens3* to the seeds in fungi treatments increased values to 34.6 branch/plant, 106.7 cm/plant and 43.2 cm^2/plant respectively. Mixture of the two isolates together gave significant increases: 45.8 branch/plant, 151.7 cm/plant and 59.7 cm^2/plant respectively. Similar results were observed in respect of leaf number per plant (Table, 5). *P. fluorescens3* treatment gave much higher values in increasing morphological characters against *Fusarium* fungi compared with *P. putida2* treatment and control treatments (Fig. 3). This may be related to the variation in siderophores compounds which produced by them, and to its tolerance to field conditions. The high inhibition in growth characters in all fungi treatments may be related to the toxic compounds which produced from plant pathogenic fungi to inhibit activity of seed embryo. This agrees with Hameed & Farhan (2007) who found that *P. aureofaiciens and P. putida* inhibited successfully growth of *Rhizoctonia solani* fungi on sorghum plant and with Chin *et al* (1998) who found that *P. chlororaphis pcl 1391* inhibited successfully growth of *Fusarium* on tomato crop, and with Becker & Cook (1988) with wheat crop.

Pods number per plant, grains number per pod per plant, total weight of 1000 grains and total yield of grains per plot for *Fusarium* treatment, were 44 pods/plant, 31.3 grains/pod, 0.94 gm/1000 grain and 112.4 gm/plot respectively, while when we added mixture of *Pseudomonas fluorescens3* and *Pseudomonas putida2* to the sesame seeds as a biocide against fungi treatments, the values increased significantly to 146.7 pods/plant, 69.1 grains/pod, 2.92 gm/1000 grain and 982.3 gm/plot respectively. Similar results were observed with vaccine of *Pseudomonas putida2* and *Pseudomonas fluorescens3* treatments when they used separately (Tables, 5 & 6). This may be related to the ability of *Pseudomonas sp* to produce promoter compounds (Deweger *et al*., 1987; Glick *et al*., 1997 and Abed *et al*., 2009) which can stimulate the growth and productivity of plants and inhibit growth of fungi Successfully.

Table (3) shows significant effect for *Pseudomonas sp* as biocides in increasing chlorophyll content and levels of N, P and K in total dry weight of shoot (gm) per plant compared with control and fungi treatments. *Fusarium* treatment gave 0.85 mg/gm, 1.52 %, 0.07 % and 0.98 % respectively. While mixture of the two isolates together gave high significant results: 3.21 mg/gm, 4.18 %, 0.44 % and 3.87 % respectively, this agrees with Abdel-Salam *et al*., (2007). Similar results were observed with vaccine of *Pseudomonas putida2* and *Pseudomonas fluorescens3* treatments when they used separately. Treatment of the mixture of the two isolates increased significantly percentage of oil in the sesame grains from 26.6 % in control treatment to 56.2 % , and no addition treatment scored 46.3 % (Table, 6). This agrees with Pierson and Weller (1994) in improving the growth of wheat. It is clear from results of this experiment, bacterial vaccine of the two isolates of *Pseudomonas* increased significantly the physiological characters per plant compared with control and other fungi treatments. *Pseudomonas* may protected sesame seeds from the toxic effect of fungi in contaminated soils with fungi by siderophores compounds which produced by *Pseudomonas* (Newman *et al*., 2001). Some compounds like siderophores and phenozine antibiotic produced from *Pseudomonas* species can inhibit growth of plant pathogenic fungi (Thomashow & Weller, 1988; Kumar & Dube, 1992; Dowling & O'Gara, 1994; Wood & Person, 1996; Duijff *et al*., 1999). The ability of *Pseudomonas* in increasing chlorophyll content in leaves may be related to the role of *Pseudomonas* in supplying the plants with some nutrient elements like N and P (Al-Rajab, 2005 and Hameed & Farhan, 2007

Table 3: Effects of *Pseudomonas putida2* and *pseudomonas fluorescens3* on chlorophyll content and percentage of N, P, K in dry weight of shoot of sesame crop planted in soil contaminated with Fusarium fungi under normal conditions.

Treatments	Chlorophyll a+b (mg/gm)	% N	% P	% K
Pseudomonas putida 2 + Fusarium	2.29	3.82	0.35	2.23
Pseudomonas fluorescens 3+ Fusarium	2.17	3.06	0.23	3.11
P. putida2 + P. *fluorescens 3*+ Fusarium	3.21	4.18	0.44	3.87
Dithen Fungicide + Fusarium	1.78	2.70	0.27	3.06
No addition	1.86	3.03	0.18	3.31
Control (Fusarium only)	0.85	1.77	0.11	1.34
LSD at 5%	0.81	1.52	0.07	0.98

Table 4: Effects of *Pseudomonas putida2* and *pseudomonas fluorescens3* on branch no./plant, height of plant (cm) and leaf area/plant (cm^2) of sesame crop planted in soil contaminated with Fusarium fungi under normal conditions.

Treatments	Branch no./plant	Height of plant (cm)	Leaf area/plant (cm^2)
Pseudomonas putida 2 + Fusarium	32.9	105.7	41.2
Pseudomonas fluorescens 3+ Fusarium	34.6	106.7	43.2
P. putida2 + P. *fluorescens 3*+ Fusarium	45.8	151.7	59.7
Dithen Fungicide + Fusarium	23.6	104.0	41.2
No addition	27.4	105.3	40.5
Control (Fusarium only)	14.6	53.3	25.5
LSD at 5%	5.8	13.7	10.7

Table 5: Effects of *Pseudomonas putida2* and *pseudomonas fluorescens3* on leaf number per plant, Pods number per plant and Grains no./pod of sesame crop planted in soil contaminated with Fusarium fungi under normal conditions.

Treatments	Leaf no./plant	Pods no./plant	Grains no./pod
Pseudomonas putida 2 + Fusarium	297.3	101.3	51.2
Pseudomonas fluorescens 3+ Fusarium	374.7	106.0	52.8
P. putida2 + P. *fluorescens 3*+ Fusarium	428.3	146.7	69.1
Dithen Fungicide + Fusarium	269.3	88.3	47.0
No addition	272.3	94.0	49.3
Control (Fusarium only)	162.7	44.0	31.3
LSD at 5%	77.82	17.3	8.0

Table 6: Effects of *Pseudomonas putida2* and *pseudomonas fluorescens3* on Weight of 1000 grain (gm), Total yield of grains per plot (gm) and percentage of Oil in grains of sesame crop planted in soil contaminated with Fusarium fungi under normal conditions.

Treatments	Weight of 1000 grain (gm)	Total yield of grains per plot (gm)	% Oil in grains
Pseudomonas putida 2 + Fusarium	2.24	362.1	50.6
Pseudomonas fluorescens 3+ Fusarium	2.46	442.2	51.3
P. putida2 + P. *fluorescens 3*+ Fusarium	2.92	982.3	56.2
Dithen Fungicide + Fusarium	1.81	303.5	41.9
No addition	2.29	352.4	46.3
Control (Fusarium only)	0.94	112.4	26.6
LSD at 5%	0.38	49.0	10.5

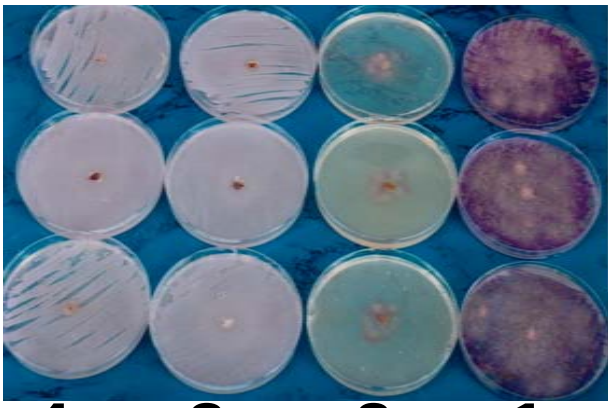

Fig.1 Shows the effects of pseudomonas
1. Control treatment (Fusarium).
2. Dithen + Fusarium treatment
3. P. Putida 2 + Fusarium treatment
4. P.fluorescens 3 + Fusarium treatment

The clear increase in percentage of N, P, K and oil by *Pseudomonas* treatments may be related to the ability of *Pseudomonas sp* to produce promoter compounds like indole acetic acid (IAA), cytokinins and polysaccharides (Deweger *et al*., 1987 and Glick *et al*., 1997, Abed *et al*., 2009), which can stimulate the growth of plants. Dithen treatment was applied once to the experiment soil at planting time but did not give clear effect as expected, this may be related to continuous irrigation water which may diluted the concentration of Dithen chemical against fungi. It is clear from the primary results of this experiment, bacterial Wheat bran powder as a carrier succeeded in this experiment under normal conditions to keep the bacterial cells in active way during the period of experiment.

CONCLUSIONS

It is difficult to sterilize soil in large area by chemicals and so difficult to kill fungi inside the plant. Use the pesticide chemicals in Agriculture cause contamination problems to the Ecological Resources. Biological control is one from many methods to protect crops and keep the Ecological Resources in the safe side. These two isolates have a dual effect as biocides (Biocontrol) and biofertilizers. There was a clear effect in improving the quantity and quality characters of sesame crop under field conditions. *Pseudomonas sp* is not specific against one group of fungi. This effect encourages using bacterial vaccines as Biocides in the applied field to protect plants from fungi diseases in wide range.

Fig. 2 General photo for the whole experiment was taken 21 days after planting.

Fig. 3 shows the differences between the six treatments 100 days after planting. T1=Pp2, T2=Pf3, T3=Pp2+Pf3, T4=Dithen, T5=No addition, pT6= Fusarium only

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