EFFICACY OF THREE OF THE MOST EFFECTIVE FACULTATIVE OLIGOTROPHIC PUTATIVEN_2-FIXING BACTERIAL STRAINS IN CONTROLLING DAMPING-OFF DISEASE OF TWO CAUSABLE PATHOGENIC FUNGI IN TOMATO.

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ABSTRACT

Complete identification was made for three strains of the facultative putative N_2-fixing bacteria using the 16s DNA sequencing studies and by detailed conventional API kit and other possible genetic determination. The efficacy of *Bacillus subtilis* subsp. spizizenii strain NRRL B-23049T (S4), *Naxobacter varians* strain CCUG 35299 (S5) and *Bacillus megatherium* strain IAM 13418. (S6), their potency against two pathogenic fungi causing damping-off and their effect on N-content of tomato plant. Inoculated tomato plants with any of the three bacterial strains, each alone or together, in presence of 1/3 N-recommended dose, significantly increased N- content and yield production. The fungi infested plants (*Py. altimum* and *R. solani*) showed pronounced decreases in yield and N-content. Incorporation of the three putative N2-fixers antagonizer strains partially alleviated the adverse effect of any of the infested fungi. The presence of the three antagonizer strains together increased the defense mechanism principles of plants like PO, PPO and phenol content. In case of the presence of the three antagonizers and 1/3 N-dose, survived plants reached 100% while other treatments showed fluctuated estimates between 20 and 96.7% due to the treatment and the plant used.

Key words: Oligotrophs, Oligonitrotrophs, Oligocarbotrophs, Putative N_2-Fixers, Biocontrol Agents.

INTRODUCTION

Rhizobacteria, belonging to diverse genera, are root-associated bacteria. Rhizobacteria that exert beneficial effects on plant development are termed "Plant Growth-Promoting Rhizobacteria" PGPR (**Kloepper and Schroth, 1978**). Plant growth promoting is a multitask phenomenon. The bacteria achieve this by suppressing plant pathogens, production of plant-growth regulators, fixing atmospheric nitrogen, and solublize phosphate and micronutrients. The first successful filed trials with PGPR as bio-control agent, was conducted with cucumber and demonstrated that seed treatment followed by soil drench application resulted in a reduction of bacterial wilt disease symptoms **(Wei *et al.*, 1995)** and also the control of bacteria angular leaf spot and anthracnose **(Wei *et al.*, 1996). Hallmann *et al.*,(1997)** reviewed the published studies and reported that PGPR have been associated with the growth promoting of several crops, including tomato, lettuce, potato, corn, cucumber, rice, and cotton. PGPR could have great impact on plant yields. **de Freites and Germida, (1992)** conducted a field trial with PGPR as bio-stimulants and bio-fertilizers for winter wheat and found a significant increase in wheat yield and demonstrated the potential of PGPR as field inoculants. In 1985, Gustafson Inc. (Planco, Texas) introduced the first commercial PGPR biological control agent in U.S.A (**Zehnder *et al.*, 2001).**

Fong *et al.*, (2008) examined the spatial variations in upper ocean (0-10 m) nutrient inventories, N_2-fixing microorganisms diversity and abundance, and rates of N_2-fixation in an anticyclonic eddy near station ALOHA. Satellite-based sea surface altimetry and ocean color observation revealed an anticyclonic eddy with enhanced chlorophyll in the upper ocean in the vicinity of station ALOHA. Within the eddy, near-surface chlorophyll concentrations were 5-fold greater than the surrounding waters. Inventories of NO^-_3 and $PO_4^{(-3)}$ in the eddy were similar to the concentrations historically observed at station ALOHA, while silicic acid inventories were significantly depleted. Quantitative PCR determination of nif H gene copies revealed relatively high abundances of several N_2 fixing cyanobacteria, including *Trichodesmium spp.* *Crocosphaera watsonli* and *Richelia intracellularis.* These results suggested that mesoscale, physical variability can play an important role in modifying the abundances of N_2-fixating microorganisms and associated rates of N_2 fixation in open ocean ecosystems.

In general, biological control offers an environmentally friendly approach to the management of plant disease. The microbiologists and plant pathologists try to gain a better knowledge of biocontrol agents to understand their mechanisms of control and to explore new biotechnological approaches. The study of the bacteria in soil surrounding the root of growing plants and their antagonistic behavier towards some pathogens is important not only for understanding their ecological role and the interaction with plants and plant pathogens but also for any biotechnological application **(Mahmoud *et al*., 2008)**. Microorganisms can be used directly for biological control of soil borne pathogens or indirectly for the productions of active substances (e.g. antibiotics, hydrolytic enzyme, osmoprotective substances….etc).

Consequantly, the intended objective of the present work is to test the most effective strains for other potancy against some plant pathogenic fungi causing damping-off disease to an economic crop (tomato).

Materials and Methods

1. Bacterial inoculants preparations (Antigonizers) (Biocontrol agents):

For preparation of bacterial strains inoculants (antagonizers), each strain was grown individually on sterilized nutrient broth medium in flasks with 1 liter capacity on rotary shaker after through shaking for 72 hours incubation period at 30 ^{o}C. The growing organisms were concentrated by centrifuging the medium and cell sidements were aseptically collected and diluted, by the same medium, to 250 ml only (1/4 liter). In case of using a mixture of the three antagonizers, equal volume of the three strains were mixed together instantaneously before use.

2. Fungal inoculums preparation (pathogenic agents):-

For preparations of fungal inoculums, used as pathogenic agents, the two selected strains, *P. altimum* and *R. solani*, were grown in sterile sand (50g), maize meal (1.5g) and water (10ml) medium in 300-ml Erlenmeyer flask for 3 weeks at 28 °C in the dark. Fungal inoculums were carefully incorporated into the sterile soil of different pots, prior cultivation of tomato seedling, at the rate of 0.5% (w/v). The none infested pots were treated in the same way with fungi-free sterile sand - maize meal medium **(Kataria *et al.*, 1997**).

3. Layout of the experiment:

1-1- Control (no treatment) except P and K at the recommended doses. (R. doses) 2-2- Soil + Complete dose of N, P and K.		
3-Soil + 1/3dose of N, + P, K at R. doses 4-Soil + 1/3dose of N, + P, K at R. doses 5-Soil + 51/3dose of N, + P, K at R. doses 6-Soil + 1/3dose of N, + P, K at R. doses	7-+ *S4* + *S5* + *S6* + mixture of S4, S5 and S6	
8-7- Soil + P and K at recommended doses	*9-+ Pythium altimum*	
10- 8- Soil + complete dose of N, P and K	+ + *Pythium altimum*	
9- Soil + 1/3dose of N + P and K at R. doses	+ *P. altimum*	+ *S4*
10- Soil +1/3dose of N + P and K at R. doses 11- Soil +1/3dose of N + P and K at R. doses 12- Soil +1/3dose of N + P and K at R. doses 13- Soil + P and K at recommended doses 14- Soil + complete dose of N, P and K 15- Soil +1/3dose of N + P and K at R. doses 16- Soil +1/3dose of N + P and K at R. doses 17- Soil +1/3dose of N + P and K at R. doses 18- Soil +1/3dose of N + P and K at R. doses	+ *P. altimum* + *P. altimum* + *P. altimum* + *R. solani* + *R. solani* + *R. solani* + *R. solani* + *R. solani* + *R. solani*	+ *S5.* + *S6* + mixture of S4, S5 and S6 + *S4* +*S5* +*S5* + mixture of S4, S5 and S6
Each treatment was replicated three time		
S4 = *Bacillus subtilis* subsp. Spizizenii S5 = *Naxobacter varians* S6 = *Bacillus megatherium*		

The air dried soil used in this experiment was crushed and sieved to pass 2 ml sieve. Mechanical analysis of this soil was 39 clay, 32 silt and 29% sand, with clay loam texture class. Soil paste had pH of 7.4, EC. 9.2 mS, total soluble salts of 0.58% and 60.55% water holding capacity. The soil was sterilized (autoclaving at 121 ^{o}C for 1 hour) before divided into two suitable parts in order to add the P and K fertilizers requirements for the crop previously mentioned (tomato).

Pots experiment with 14cm width was executed for planting and were firstly prepared by care washing with water repeatedly then surface sterilized with 70% ethyl alcohol. Sterilized soil was distributed and packed in plastic pots at 5 kg /pot. As to the potassium and phosphorus fertilizers supplementation for tomato plant, they were added at the recommended doses, 35kg superphosphate (15% P_2O_5) and 100 kg potassium sulphate 48% K_2O)/feddan. respectively.

4. The greenhouse experiment:

Tomato seedlings (*Salanum lycopersicum L.cv. casel rock*) provided from ministry of agriculture at Al Azab branch, Fayoum Governorate, 30 days age at time of transplanting was implicated in this experiment. Before planting in soil, seedlings roots were repeatedly emersied in the desired bacterial inoculums and allowed to partial drying. One ml of the appropriate antagonizer culture was added instantinously on the surface of the soil near plant roots. Every pot was planted with four seedlings which, as possible, were morphologicaly uniform with nearly the same lengths. Nitrogen fertilizer application was done after 15 days of planting at the suitable dose as KNO_3 (21% N).

The pre damping-off recorded two days after transporting the seedlings to the soil. While the post damping-off was at 15 days. After 30 days of planting, plants were carefully removed from pots, washed with distilled water to get red of soil particles, and then dried with paper towel to eliminate any excess or dust. Morphological estimates were limited to the determination of whole plant dry weight, and percentage of survived plants from pre and post damping-off disease. All collected plants in all treatments were kept and prepared for proceeding analyses.

Chemical and enzymatical determinations were limited in total nitrogen, total phenols content and peroxidase (PO) and polyphenyloxidase (PPO) enzyme activities in vegetative parts of tomato plants.

Total nitrogen was determined according to the well known method described in **Bremner (1965).**

Estimation of total phenols, PO and PPO were carried out by the method described by **Ramamoorthy *et al.*, (2002).** Vegetative parts of the tested plant was carefully uprooted without causing any damage to the vegetative part. Three plants were sampled from each replication separately. Fresh samples were washed in running tap water and homogenized.

Results and discussions

The use of soil microorganisms with the aim of improving nutrients availability, specially nitrogen, for plants is an important practice and necessary for agriculture. During the past couple decades, the use of plant growth promoting rhizobacteria (PGPR) for sustainable agriculture has increased tremendously in different parts of the continent. Soil PGPR can affect growth by different direct and indirect mechanisms such as, increasing the mineral nutrients solubilization and nitrogen fixation, repression of soil borne pathogens by production of HCN, siderophores, antibiotics, and/or competition for nutrients, improving plant stress tolerance to drought, salinity, metal toxicity and production of phytohormons such as IAA. There are now several bacterial inoculants currently commercialized that seem to promote growth by various mechanisms: suppression of plant disease (bioprotectants), improved nutrients acquisition (biofertilizers), or phytohormons production (biostimulants). Bacteria in the genera *Bacillus*, *Stretomyces*, *Pseudomonas* and *Agrobacterium* are the biological control agents predominantly studied and increasingly marketed.

Biofertilizers are also available for increasing crop nutrient uptake of nitrogen from N_2-fixing bacteria, iron uptake from siderophores – producing bacteria, sulphur uptake from sulphur oxidizing bacteria and phosphorus uptake from phosphate solubilizating bacteria.

The most effective facultative oligotrophic bacterial strains obtained, from previous part, were examined and also chosen for some of the different characters mentioned above (IAA production, Salyselic acid production, zinc and phosphate solubilization, N_2-fixation, cellulase and chitinase production, oxidase, catalase activities and lactose fermentation), were used in present part of the study. It is worth to mention that the three strains used in this part as PGPR were isolated from the same soils at Fayoum Governorate and were completely identified as *Bacillus subtilis subsp. spizizenii* strain NRRL B-23049T, *Naxobacter varians* strain CCUG 35299 and *Bacillus megatherium* strain IAM 13418. The effect of these three strains on growth of tomato (*Salanum lycopersicum L.cv. casel rock*) when added separately or together as PGPR or when added to control damping–off of the previously mentioned crop, separately or in mixed inoculums. The two challenged pathogenic fungi used as infested agents named *Pythuim altimum* and *Rhizoctonia solani*. The two pathogenic fungi were tested alone or in presence of the antagonizing bacterial strains each alone or mixed together.

1. Effect of the facultative oligotrophic putative N_2-fixers on growth and N- content of the experimented plant:

Fig. (1) and table (1), illustrate the effect of supplementing the plants (tomato) with mineral nitrogen fertilizer at recommended dose (C treatment) , compared with the negative control (B) which was deprived of any nitrogen fertilizer source, on nitrogen content and the plant dry weight obtained at the end of the experiment (30 days). Apart from the blank and the control treatment, the followed four treatments which received 1/3 recommended dose of nitrogen plus the putative nitrogen fixers strains individually or in mixed inoculants, there was, generally, no significant differences between values of total nitrogen contents and total dry weight of tomato plants except that treatment which received *B. subtilus* in preference to the nitrogen content only. The 1/3 dose nitrogen fertilizer in presence of the mixed inoculum of the three

putative N_2- fixers gave high significant values of nitrogen contents and dry weight of three tomato plants which were very near to that of the control (complete dose treatment C).
Generally, the supplementation of tomato plants with different bacterial strains (S_4, S_5 and S_6) increased, significantly, nitrogen contents and plant dry weight but were slightly less than what obtained in the mixed inoculum treatment (S mix).

Fig. (1): Effect of the Facultative oligotrophic Putative N_2- fixers on growth of Tomato plants

C = complete dose of nitrogen
B1= 1/3 dose of nitrogen + *S4*
B2=1/3 dose of nitrogen + *S5*
B3 = 1/3 dose of nitrogen + *S6*
B mix= 1/3 dose of nitrogen + mix of (S4, S5 and S6)

Table (1) Effect of Facultative oligotrophic putative N_2-fixers strains on N and total phenol contents, PO and PPO activities and dry weight of plants (3 plants) in presence or absence of challenged fungi in tomato. Plants.

No	treatment	PO nm min/g	PPO nm min/g	Total phenol mg/g	Dry weight	Total N %
1	B	٠.١١٣	٠.١٤٦	٠.٥٣٠	6.41	0.93
2	C	٠.١٣٠	٠.١٦٥	٠.٦١٣ ^{ac}	10.$^{10\ a}$	1.94 ^{a}
3	1/3 N + S_4	٠.١٧٢ ^{a}	٠.١٨٢ ^{a}	٠.٩٠٦ ^{ab}	8.89 ^{b}	1.79
٤	1/3 N + S_5	٠.١٩٣ ^{ab}	٠.١٩٥ ^{a}	١.٠٦١ ^{b}	8.52 ^{b}	1.82
٥	1/3 N + S_6	٠.٢١٠ ^{b}	٠.٢١٥ ^{b}	١.٠٨٢ ^{abc}	8.53 ^{b}	1.93 ^{a}
٦	1/3 N + S_{mix}	٠.٢٤٩ ^{c}	٠.٢٢٤ ^{bg}	١.١٠٩ ^{bd}	9.79 ^{a}	1.95 ^{a}
7	B + Py	٠.٢٦٤	٠.٢٧٤	١.١١٩	1.2	0.81
8	C + Py	٠.٢٨٨ ^{df}	٠.٢٩٦ ^{ch}	١.١٢١ ^{abc}	1.56	1.17
9	1/3 N + S_4 + Py	٠.٢٩٥ ^{cf}	٠.٣١٨ ^{d}	١.١٢٦ ^{e}	5.70 ^{c}	1.45 ^{b}
١٠	1/3 N + S_5 + Py	٠.٢٨٩ ^{d}	٠.٣٤٨ ^{e}	١.١٧٧ ^{e}	6.55 ^{d}	1.47
١١	1/3 N + S_6 + Py	٠.٣١٦ ^{e}	٠.٣٤٩ ^{e}	١.١٩٤ ^{e}	5.97 ^{cd}	1.51
١٢	1/3 N + S_{mix} + Py	٠.٣٦٢	٠.٣٨٥ ^{f}	١.٢٦٢ ^{e}	6.77 ^{d}	1.65
13	B +Rh	٠.٢٥٨	٠.٢٦٥	١.١١٣	1.32	0.87
14	C +Rh	٠.٢٧٤ ^{df}	٠.٢٧٨ ^{g}	١.١١٩ ^{bc}	2.82	0.99
15	1/3 N + S_4 +Rh	٠.٢٩٦ ^{de}	٠.٢٩٥ ^{cg}	١.١٢١ ^{de}	6.20 ^{cd}	1.41
١٦	1/3 N + S_5 +Rh	٠.٢٨٨ ^{d}	٠.٣١٢ ^{cdh}	١.٥٣ ^{e}	5.28 ^{c}	1.43
١٧	1/3 N + S_6 +Rh	٠.٣١٦ ^{e}	٠.٣٣٤ ^{de}	١.١٧٦ ^{e}	6.99 ^{d}	1.45 ^{b}
١٨	1/3 N + S_{mix} +Rh	٠.٣٣٢ ^{e}	٠.٣٨٥ ^{f}	١.٦٤٢ ^{e}	7.02 ^{d}	1.53
	LSD	٠.٠٣١	٠.٠٢٢	٠.٤١	1.13	0.021

B = Blank = Soil + P,K Control= Soil + N, P, K
1/3N= 1/3 Recom. Dose Py = *Pythium altimum*
Rh= *Rhizoctonia solani* S_4 = *B. subtilis subsp. spizizenii*
S_5 = *Naxobacter varians* S_6= *B. megatherium*
$S_{mix} = (S_4 + S_5 + S_6)$

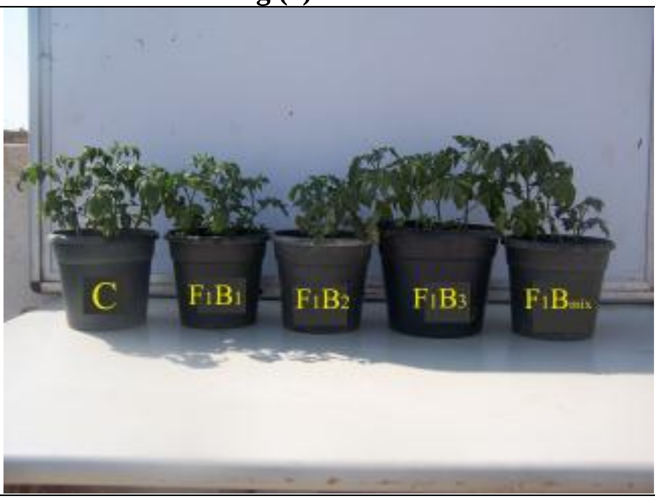
* Each value in the table is a mean of 3 reps.
* Means of the same colum followed by the same alphabetical letters in the table were not statistically significant (5%).

When the plants were challenged with the two pathogenic fungi (Fig (2) A, B) (*Py. altimum* (Py) and *R. solani* (Rh), significant decreases in nitrogen content and dry weight were observed in the blank (B+Py) control, due to the weak growth of the infested plants in these treatments. When the plants were supplemented with the putative N_2- fixers in presence of 1/3 nitrogen dose and the infested fungi, the adverse effect of the fungi was partially alleviated to some degrees and there were considerable increase in total nitrogen and total dry weight of the plant. Again, S_6 (*B. megatherium*) was slightly pronounced in that respect and gave to somewhat significant values than other treatments but still less than the mixed treatment (S mix). The infestation with the two fungi gave near and similar results indicating that supplementing the plants with 1/3 nitrogen fertilizer dose in presence of the putative oligotrophic N_2-fixers may alleviated the adverse effect of the pathogenic fungi on N-content and plant dry weight yield of the plant. **Albino *et al.,* 2006** obtained similar trends with some putative N_2-fixers belonging to the genera ***Bacillus, Methylobacterium, Paenibacillus, Pseudomonas,and Sphangomonas*** isolated from the rhizosphere of some plants. Similar trends were obtained in the same respect by **Bin *et al*., 2000, Asghar *et al.,* 2002, Gray and Amith 2005 and Figueiredo *et al*., 2008.**

2. Facultative oligotrophic putative N_2-fixers as biocontrol agents

The increased reflection on environmental concern over pesticide use has been of great importance in biological disease control and have further encouraged the exploitation of putative antagonistic microflora in disease management. Biological control is an environment-friendly strategy to reduce crop damage caused by plant pathogens. There are four advantages to microbial control: 1) appears to be ecologically safer than chemicals because control agents are not accumulated in the food chain, 2) Some biocontrol agents can provide persistent control 3) biocontrol agents have slight effects on ecological balance.

Fig (2) A



C = complete dose of nitrogen
F1B1 = *Pythium altimum* +1/3 dose of nitrogen + *S4*
F1B2 = *Pythium altimum* +1/3 dose of nitrogen + *S5*
F1B3 = *Pythium altimum* +1/3 dose of nitrogen + *S6*
F_1Bmix = *Pythium altimum* +1/3 dose of nitrogen + mix of (S4, S5 and S6)

B

C = complete dose of nitrogen
F2B1 = *Rhizoctonia solani* +1/3 dose of nitrogen + *S4*
F2B2 = *Rhizoctonia solani* +1/3 dose of nitrogen + *S5*
F2B3 = *Rhizoctonia solani* +1/3 dose of nitrogen + *S6*
F_2Bmix = *Rhizoctonia solani* +1/3dose of nitrogen + mix of (S4, S5 and S6)

4) They are compatible with other control agents (**Datta *et al.,* 2011**). The same auther added that among the various antagonists used for the management of plant diseases *Fluorescent pseudomonads* in control of welt disease cased by *Fusarium spp*. and root- rot of wheat, cucumber and tulip. The fruit rot and die back diseases of chilli was managed by *B. subtilus*.

Among fungal disease, damping-off caused by species of *Pythium* and *Rhizoctonia* is very common. They are usually soil- borne and consequently affect of the crop (tomato). Fungicides may offer a degree of protection against pathogens, but their adverse affect on beneficial soil microorganisms and environment cannot be ignored. Therefore, biocontrol agents appear to hold promise in disease management. These microbes induce resistance in different plant species against the infection of fungi, bacteria and viral pathogens. In this part of study the author report on the use of some PGPR strains isolated from soil, based on disease suppression, growth enhancement and the role in the defense mechanism of plants against pathogenic fungi. i.e, the role of phenol (P), peroxidase (PO) and polyphenyloxidase (PPO) induced in plants by the isolated bacterial strains from soil on management of damping-off caused by the challenged fungi *Pythium* and *Rhizoctonia*. As shown from the table, in case of treated tomato plants with the three putative N_2-fixers antagonizes bacterial strains and their mixtures (3, 4, 5 and 6 treatments), there were generally, significant increases in nitrogen content and dry weight of plants which may be due to the production of growth promoting substances by these strains or the increase in the defence mechanisms of the plants due to the inoculation with bacterial strains that led to increases in PO, PPO and total phenol content of plants. All bacterial strains treatments (3, 4, 5 and 6) produce these compounds in significant amounts when compared with the negative and positive controls i.e not received any of the bacterial strains. The deleterious effects results from the infestation of maize plants with the two pathogenic fungi resulted in little increases in values of the defense mechanism principles (PO, PPO and phenol content), and consequently gave relatively higher percentages of damping-off maize plants (treatments 7 and 8), when compared with the damping-off plants in treatments supplied with the antagonizing bacterial strains only (3, 4, 5, and 6). For example the PO, PPO and phenol contents of the B and C treatments (1 and 2), were 0.113 and 0.146 nm/g and 0.530 mg/g plant material, respectively, in tomato plant with no treatment except the P and k fertilizers (1). While in treatment (2) the figures were slightly significant and averaged 0.130, 0.165 and 0.613, respectively. This may indicate that, the application of N- fertilizer in presence of P and k fertilizers strenthed, to somewhat, the plant and then rise its defense mechanism. The picture was more delight when different bacterial strains were added in presence of 1/3 N-fertilize. The corresponding figures were relatively high in treatment No.6 (1/3N + mix) as the values reached 0.249, 0.224, and 1.109 which were highly significant when compared with the positive control (2). The other three treatments showed relatively less, but significant, values (3, 4 and 5 treatments).

The pre and post damping-off tomato plants were markedly related to the normal resistance of non infested plants which gave a very high percentages of survived plants 100% in the first five treatments (1 to 6). The 30 cultivated tomato plants were survived. The post damping-off recorded plants showed absence of any subdueded plants. When the preceding treatments (1 to 6) were replicated on tomato plants but in presence of challenged fungi, the picture was significantly different (7 to 12 treatments). The previously mentioned survived plants figures of tomato were decreased in all the six treatments. The lowest decreases were recorded in the twelve treatment (1/3N + S mix + py) as the percentage of damping-off plants were (100%), survived plants were 100%, while the higher percentages were recorded on seventh treatment (B + Py) reached (33.3%), survived plants were 66.7%. These figures, were coincide with the production of defense compounds accumulated in plant material. The PO, PPO and total phenol were 0.264, 0.274 nm min/g and 1.119 mg/g at the seventh treatment. While it were 0.362, 0.385 and 1.262 in the twelve treatment, respectively. This may be due to the increasing resistance of plants due to the presence of the mixed bacterial strains used as fungi antagonizers. The differences were statistically significant. The control treatment (C + Py) gave to somewhat similar values as to negative control (B + Py) and insignificant differences were observed. While similar decreases in subdueded tomato plants were recorded in the following treatments (from 9 to 12) as to the presence of the antagonizing bacterial strains which were correlated also by the PO, PPO and polyphenol content values.

The preceding experiment was repeated, with the only difference that challenged fungi was *Rhizoctonia solan*i instead *of Pythium altmum* and also the two previously described plant (tomato) was used. The picture was, nearly, the same and as shown in table and figures similar results were obtained as to the percentages of survived plants and the induction of the resistance principles mentioned before (PO, PPO and total phenol). The data of the second fungi used are illustrated in the table and figure represented from 13 to 18 treatments.

As early as **1998, Gasoni *et al.*,** reported that radish seed treatment with *B. cereus* in peat/virmiculate/clay formulation effectively controlled *Rhizoctonia* damping-off disease. Also *B. subtilus* strain isolated from the rhizosphere of fruit plants was used by **Jayalakshmi *et al.*, (1998),** to manage the fruit rot and die back diseases of Chilli plants. The same trend was also found by **Ryder *et al*., 1998**, where they reported that *B. cereus* and *B. subtilus* constantly reduced the severity of the take all disease of wheat grown in sodic and acid soil.

Explaining the defense mechanisms against pathogenic fungi **Chen *et al.*, 2000,** stated that root and crown rot cucumber caused by *Pythium* can be suppressed by *P. corrugate* strains and the peroxidase (PO) and polyphenyl oxidase (PPO) activities were increased in roots 2- 5 days after bacterization with *P. corrugate*. While **Ramamoorthy *et al.*, 2002**, stated that *P. fluorescence* was found to protect tomato plants from wilt disease caused by *F. oxysporium*. Induction of defense protein and chemicals against challenge inoculation with the fungi in tomato plants was pronounced. Phenolics were found to accumulate in bacterized tomato root tissues challenged with the fungi at one day after pathogen challenge. Activities of peroxidase (PO) and polyphenyloxidase (PPO) increased in bacterized root challenged with pathogen. The bacterial antagonist was found by **Tendulkar *et al.*, 2007** exhibiting highest antifungal activity against the rice plant fungus *M. grisea* which isolated from soil and identified as *B. licheniforms* BC 98. Besides *M. grisea*, the isolate also inhibited the growth of other phytopathogens such as *curvularia luneta* and *Rhizoctonia bataticola*. The active material was identified as peptide surfactin. The antagonist inhibits germination *of M. grisea*, a potent rice phytopatogens, and therefore appears to be a potential candidate for control rice blast disease. More recently, **Datta *et al.*, 2011**, stated that plant growth promoting rhizobacteria can enhance the growth and the productivity by exerting beneficial effects (direct or indirect). Fifteen strains were isolated from Chilli rhizosphere. Plant growth and yield attributes significantly increased when the 15 isolates were applied to the local Chilli cultivar. Two strains were *Bacillus spp.* and one *Streptomyces sp*. Remarkable increase in growth such as total number of fruits, plant weight and yield was recorded in plants with combined inoculation. A total of 186 bacterial strains isolated from various soil sources and plant species from Eastrn Anatolia region in Turkey were evaluated for their ability to suppress gray mold *(Botrytis cinerea pers*. Ex fr**)** occurred on strawberry cv. fern were studied by **Donmez *et al.*, 2011**. 36 strains were found effective to inhibit development of the fungi under in vitro conditions, and 13 of them, have greater inhibition zone, were selected as biocontrol agent. These antagonistic strains were identified as *B. lentimorbus, B. megatherium, B. pumilis, B. subtilus, Enterobacter intirmedius, Kurthia sibirica, Paenibacillus polymyxa* and *pantoea agglomerans*.. *Bacillus* were found more effective to prevent mycelial development on strawberry fruits in comparison to the control. It was shown that antagonistic bacterial strains inhibited *B. cinerea* and that they have a potential use in sustainable strawberry production.

Obtained results clearly demonstrate the rhizocompetance and plant growth enhancing of the strains. Somewhat similar trends were obtained by **Ardokani *et al.*, 2011**, when using two isolates of *P. fluoresens* isolated from rhizosphere of cotton roots. Results indicated that the increase in crop produced reached in some treatments to 3.37 fold the control one, promoting seedling height, root length, seedling dry weight and root dry weight. *Stenacarpella mayds* and *St. macrspora*, the causable agents of white ear rot corn which is one of the most destructive in crop worldwide was studied by **Sagahón *et al.*, 2011**. These fungi are important mycotoxin producers that cause different pathologies in farmed animals and represent an important risk for humans. The same authors isolated 160 strains from soils of corn crops of which 10 showed antifungal activity against these fungi which were identified as *B. subtilus*, *Pseudonomas* spp., *P. fluoresense* and *Pantogea agglomerans* by sequencing of 16SrRNA gene and phylogenetic analysis.

Table (2) Effect of facultative oligotrophic antagonizer strains each alone or in mixture on survived percentage plants.

NO.	Treat	Tomato
1	B	100
2	C	100
3	1/3 N + S_4	100
4	1/3 N + S_5	100
5	1/3 N + S_6	100
6	1/3 N + S_{mix}	100
7	B + Py	66.7
8	C + Py	70.0
٩	1/3 N + S_4 + Py	96.7
١٠	1/3 N + S_5 + Py	٩٣.٤
١١	1/3 N + S_6 + Py	١٠٠
١٢	1/3 N + S_{mix} + Py	١٠٠
13	B + Rh	70
14	C +Rh	70
15	1/3 N + S4 +Rh	90
١٦	1/3 N + S5 +Rh	96.7
١٧	1/3 N + S6 +Rh	96.7
18	1/3 N + Smix +Rh	96.7

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نشاط وكفائة بعض السلالات المعزولة في مقاومة بعض الامراض الفطرية التي تصيب الطماطم وكذلك زيادة مقاومة هذه النباتات للاصابة بالامراض الفطرية

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تم دراسة كفاءة كل من *Bacillus subtilus, Naxobacter* sp. and *B. megatherium*. كل علي حده او مع بعضهما في مقاومة مرض الذبول في الطماطم في تجربة أصص وقد أستخدم نوعين من الفطريات المسببة لمرض الذبول *Rhizoctonia solani , Pythium altimum*, مضافة علي حده أو في وجود البكتريا المضادة لها السابق ذكرها.

كان لإضافة البكتريا المستخدمة مع ثلث كمية السماد المعدني النتروجيني الموصي بها. تأثير مباشر علي نمو النبات لكن كانت كمية الازوت في النبات لا تختلف عن الكنترول الذي لم يضاف له اي ميكروب علي حدها ما عدا معاملة *(B. megathrium)* فقد كان لها تميز نسبي في كمية الازوت بالنبات. أما أضافة الثلاثة سلالات معا فقد كان لها تاثير مميز علي كمية الازوت بالنبات وكذلك وزن المحصول الناتج والذي كان قريبا بدرجة كبيرة من الكنترول المحتوي علي NPK الموصي بها في الحقل سواء في محصول الذرة أو الطماطم.

أدي أجراء العدوى للنباتات بواسطة الفطريات المذكورة الي نقص في النسبة المئوية للازوت بالنباتات وكذلك المحصول الناتج النهائي في حالة الكنترول. وعند أمداد النباتات بالبكتريا المثبتة للازوت الجوي في المعاملات المسمدة ب ٣/١ الجرعة الطبيعية من الازوت أمكن التعلب جزئيا علي التاثير السئ للفطر علي محتوي الازوت والمحصول. وكان ميكروب ال *B. megathrium* أفضلها في ذلك.

وجد من النتائج أن وجود الثلاثة أنواع من البكتريا قد يكون سببا في زيادة مقاومة النباتات للامراض الفطرية نتيجة زيادة تكوين المركبات المساعدة في مقاومة النبات لامراض الذبول مثل مركبات PO, PPO, phenol حيث ان تراكمها كان أكثر في المعاملات التي أضيفت لها البكتريا المختلفة. وكانت المعاملة المحتوية علي الثلاثة سلالات بكتريا أكثر وضوحا.

وجد أن أضافة السماد الازوتي ٣/١ الجرعة في وجود P,K قد جعل النباتات أكثر قوة في المقاومة وخاصة عند وجود الثلاث ميكروبات (بكتريا) مع بعض كمجموعة واحدة حيث كانت نسبة النباتات الناجية التي لم يحدث لها ذبول ١٠٠% مع كلا الفطريين الممرضين.