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IMPROVING BIOACTIVITIES OF SOME BACTERIAL BIOCONTROL AGENTS AGAINST CHICKPEA ROOT ROT AND WILT CAUSAL ORGANISMS USING SKIMMED MILK.

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ABSTRACT

Biocontrol activities of *Pseudomonas* spp. are widely accepted as biocontrol agents for many diseases. The main target of this research work was to find out a proper and effective formula for the bio-agents which enhance, survivability, rhizosphere competence and biological control of chickpea root rot and wilt. The results obtained revealed that the use of skimmed milk and whey as nutrients and protecants improved the survivability of *Pseudomonas fluorescens* and *P. putida* up to 180 days without any dramatic decline when stored at room temperature (25 $\pm$ 5 ^{0}C). The formulation enhanced the rhizosphere competence of *P.fluorescens* and *P. putida* which increased the population in the rhizosphere of chickpea up to 75 days from sowing. The formulated isolates were used as seed coating or soil treatment or combined seed and soil treatments for biocontrol of fusarial wilt and rhizoctonia root rot of chickpea. Results showed a significant reduction of the percentages of infected plants . Seed treatment followed by soil application gave the lowest percentages of infection compared to the control. The saprophytic growth of *Fusarium oxysporum* f. sp. *ciceri* and *Rhizoctonia solani* in the experimental soil was significantly reduced after the application of powder formulation of the biocontrol agents.

Keywords: Powder formulation, Skimmed milk, *Pseudomonas* spp., Chickpea, *Fusarium oxysporum* f. sp. *ciceri* and *Rhizoctonia solani* , rhizosphere competence.

INTRODUCTION

Root rot and wilt are common diseases on chickpea wherever it is grown in Egypt particularly in Upper Egypt. Different methods of disease control can be applied to minimize the incidence of both diseases. However, biological control is considered one of the most recommended methods of disease control without any hazard of using chemicals or fungicides which affect the human health (Cook and Baker, 1983) or affects the useful microorganisms in soil (Kloepper and Schroth ,1981). Fluorescent pseudomonas strains have bean reported to control plant diseases caused by soil borne pathogens (Weller, 1988) and are known to survive in both rhizosphere and phyllosphere (Beattie and Lindow, 1995).Because of the biocontrol agents can not resist the hard natural conditions, one of the most severe problems is the short life of the bioagents (Burges ,1998). Formulations play a vital role in helping to solve these problems and in making microorganisms effective in practice. Talc powder formulation was effective till 6 months of storage, while peat formulation was effective up to 60 days of storage(Vidhyasekaran and Muthamilan, 1995).Also, Fravel *et al.* ,(1985) and Russo et al. (1996), capsulated *P .cepacia* and *P.fluorescens* using alginate-clay matrix, the resulting product showed a very short shelf-life at room temperature .Pregelatinized water dispersible starches and flours are currently marketed as food additives and used to encapsulate *Pseudomonas*

sp. to improve its survivability under harsh environmental conditions, shelf-life, persistence and activities (Connick *et al.* 1998;Amer and Rania El-Shennawy, 2004).Commercial preparation of biological control agents must be stable, posses' adequate shelf-life for at least one year (Amer and Utkhede, 2000).

The present study was carried out to use skimmed milk and whey as nutrients and protectants to formulate *Pseudomonas fluorescens* and *Pseudomonas putida* in powder formulation : to asses their shelf-life and survivability during storage ; to evaluate their competence ability in chickpea rhizosphere ; to test their activity in biological control of *Fusarium* wilt and *Rhizoctonia* root rot of chickpea as models of soil borne plant pathogens and to test the effect of their application on saprophytic growth of the pathogens in soil under greenhouse conditions.

MATERIALS AND METHODS

Bacterial antagonists, production and harvest:

The two antagonistic rhizosphere bacteria *Pseudomonas fluorescens* and *Pseudomonas putida*, both are effective as biocotrol agents (Weller,1988), were selected from the collection of the Department of Agricultural Botany, Faculty of Agriculture, Minufiya University, Shibin El-Kom, Egypt. Cultures were maintained on King's medium B (KMB) and developed as an antibiotic-resistant strains Vidhyasekaran and Muthamilan, (1995). The marked strains grew well on Kings medium B containing rifampicin (190 ug /ml) and streptomycin(30ug /ml),which inhibits all wild strains. To prepare inocula, the bacteria were grown at 30 ^{0}c on a rotary shaker (100 rpm) in 250ml Erlenmeyer flasks containing 100ml of KMB broth containing the selective antibiotics. When the cultures were fully turbid, the cells were collected by centrifugation and suspended in 260mM phosphate buffer (pH 6.5) and stored until use for formulations.

Preparation of powder formulations:

Rice flour, skimmed milk and whey based formulation was developed as follow : 320gm rice flour,500 gm vermiculite,10 gm skimmed milk,15 gm whey,10 ml vegetative oil and 10gm carboxymethyl cellulose as adhesive. All ingredients were mixed well under aseptic conditions .The PH was adjusted to 7.00 by adding calcium carbonate and the mixture was autoclaved for 30 min on each of two consecutive days. 400 ml milliliters of the bacterial suspension containing $8x10^{9}$ colony forming units (cfu) per milliliter was added to 1 kg of the sterilized mixture and mixed well under sterile conditions. The materials were packed in polythene bags, sealed, and incubated at room temperature (25 ^{0}C).

Survivability *of P. fluorescens* and *P. putida* in formulation:

Survivability of powder formulated bacterial biocontrol agents was assessed as colony forming units (CFU)for each bioagent.Samples were drown after intervals 0, 1, 2, 4, 6, 8 and 12 months after storage at room temperature. Dilution plate technique was used in which dilutions were prepared and 0.1 ml aliquots were plated on KMB.The independent samples

were analyzed with three replicates in a completely randomized design expriment.

Establishment of *P.fluorescens* and *P.putida* in the rhizosphere of chickpea:

Three parts of field soil and one part of well decomposed farm yard manure (FYM) were mixed together and filled in plastic pots of 25cm diameter .The antibiotic resistant strains of *P.fluorescens* (Pf5) and *P.putid* (Pp1) growing on KMB broth with selective antibiotics were used. Chickpea (Giza1) seeds were treated with the powder formulations of antibiotic resistant strains 10gm/kg seed following the method of Weller and Cook,(1986) shade dried overnight in laminar flow hood. The treated seeds were sown in pots containing unsterilized soil. Soil application of the powder formulations was carried out, on which 25gm of the powder formulation of each isolate was mixed individually with 2.5kg farm yard manure at the time of sowing. In another set of pots, seed treatment was followed by soil application of the powder formulation. Seeds and/or soils without the powder formulation treatment served as controls. Four seeds were sown for each pot and five pots were kept as one replicate, there are three replications and the pots were arranged in randomized block design. The rhizosphere populations of both biocontrol agents were assessed at intervals of 15 days. One plant from each pot was pulled out gently with intact roots and soil adhering to roots was removed. The root portions were cut and transferred to 100ml of sterile water in an Erlenmeyer flask. After thoroughly shaking the population of Pf5 and Pp1 in the suspensions was estimated by dilution plating on KMB agar containing 200ug cycloheximide /ml and the antibacterial antibiotics to witch the bacterium was made resistant. The plates incubated at 30c°, and fluorescent colonies were viewed under UV light and counted. Five Petri plates for each dilution and three replications were maintained. The root samples were weighed and the bacterial population was expressed per gram of root sample (dry weight).

Biological control assay of powder formulation of *P.fluorescens* and *P. putida* under greenhouse conditions:

Chickpea pathogens i.e. *Fusarium oxysporum* f.sp. *ciceri* the causal agent of fusarial wilt and *Rhizoctonia solani* the causal agent of rhizoctonia root rot of chickpea plants were freshly isolated from infected plants growing in Gemmeiza Research Station ,Agricultural Research Centre,Giza, Egypt. Pure cultures were maintained on potato dextrose agar medium.

The pathogen inoculums: The pathogens were grown on wheat bran-vermiculite (1:1w/w) mixture containing sucrose 10gm/kg in polyethylene bags for 10 days at 28c°.Ten grams of each pathogen inoculums containing actively growing mycelium were used as inoculums for each pot .Three parts of field soil and one part of farm yard manure were mixed together and filled in plastic pots of 25cm diameter.

Seed treatment: chickpea seeds (Giza-1) were coated with the powder formulation of each biocontrol agent at 5gm/kg .Ten treated seeds were sown per pot already inoculated with the pathogen one week before sowing .Seeds dressed with the fungicide carbendazim (Bavistin) at the rate of 2.5gm/kg

seeds used for comparative treatment.Pots inoculated with pathogen only and sown with surface sterilized seeds served as control.

Soil treatment: The powder formulations of *P. fluorescens* and *P. putida* were added individually to pathogens inoculated pots at the rate of 5gm/pot before sowing .Ten surface sterilized seeds were sown per pot. Each treatment comprised four replicates. Combinations of seed treatment and soil treatment were kept, in which the powder formulation treated seeds were sown in soil treated with powder formulation as mentioned before with soil application. Observations on fusarial wilt and rhizoctonia root rot incidence were recorded as percentage of infection after 30, 60, and 90 days from sowing. The data were analyzed as analysis of variance.

Assay for saprophytic growth of *F.oxysporum* f.sp. *ciceris* and *R solani*.

The saprophytic growth of *F. oxysporum* f.sp. *ciceri* and *R.solani* .in soil was determined 45 days after sowing with a method in which autoclaved beet seeds was used as a trap for the pathogens with minor modification (Lewis and Papavizas,1987).In this method, autoclaved ,non-infested beet seeds were added to 200gm of sieved soil from each treatment(biocontrol experiment) as bait to trap the pathogens. This experiment was set up in a completely randomized block design with three replicates. After 3 days from incubation, seeds were retrieved and washed, ten seeds from each replication were surface sterilized and placed in plates containing 2% water agar. The data were recorded as percentage of beet seeds colonized by the pathogen, which gives an indication of saprophytic growth of the pathogens in the soil. Infested soil with pathogen served as control.

RESUTS AND DISCUSSION

In all carrier formulations, *P.fluorescens* (Pf5) and *P.putida* (Pp1) survived up to 180 days without any dramatic decline from the initial population. After this period there was a little decline in the population by the end of storage time. Shelf-life of *P.fluorescens* (Pf5) and *P.putida* (Pp1) in rice flour-skimmed milk powder formulation was assessed for one year at room temperature (Table 1) and Fig-1.From an initial population of 9.92 log cfu/g of *P.fluorescens* the population increased to 10.6 log cfu/g (106.60 % survival) after one month of storage and 9.79 log cfu/g (98.87% survival) after two months of storage. There was a slow decline in number of viable cells when onwards and even after 12 months of storage the powder formulation retained substantial number of viable cells 6.09 log cfu/g (61.50% survival) as compared to the initial population. .Though there was a slow decline in population after 4 months of storage, the powder formulation retained high population of *P. fluorescens* up to 6 months compared to initial population.

From an initial population of 10.09 log cfu/g of *P.putida* the population increased to 10.48 log cfu /g(103.86% survival) after one month of storage and 10.17 log cfu/g(100.63% survival) after two months of storage .There was a very slow decline in population numbers onwards and even after 12 months of storage, the powder formulation retained substantial population numbers of 7.68 log cfu /g (75.90% survival).Though there was a very slow decline in the population after 6 months of storage, the powder formulation

retained high population of *P.putida* up to one year of storage compared to initial population.

Table-1: Survival ability of *Pseudomonas fluorescens* and *P. putida* (log^{-1} cfu/gm) in skimmed milk –rice flour powder formulation in storage at room temperature.

Duration of storage (months)*	Biocontrol agents / log^{-1} colony forming units (cfu) per gm formulation			
	Pseudomonas fluorescens		*Pseudomonas putida*	
	log^{-1} cfu/gm	survival %**	log^{-1} cfu/gm	survival %**
O time	9.92 b	100,00 b	10.09 c	100.00 b
1	10.60 a	106.60 a	10.48 a	103.86 a
2	9.79 c	98.87 b	10.17 b	100.63 b
4	8.63 d	87.13 c	9.39 d	93.00 c
6	7.25 e	73.40 d	8.43 e	83.50 d
8	6.48 f	65.43 e	8.07 f	79.60 e
12	6.09 g	61.50 f	7.68 g	75.90 f
LSD 0.05%	0.002	2.274	0.06	1.02

*The formulation was stored up to 12 months; a period adequate for using fresh inoclum of the bioagents from year to year.

**Survival (%)during storage was calculated compared to initial population (log^{-1} cfu/gm)as starting time.

***Means followed by the same letter are not significantly different according to Duncan's multiple range test (P=0.05).

The results obtained showed that the rice flour-skimmed milk powder formulation supported the growth and survival of *P.fluorescens* and *P.putida* and improved their shelf-life for 12 months with effective population and retained more than 6 log cfu/g for *P.fluorescens* and more than 7 log cfu/g for *P .putida* even after 12 months of storage. Shelf-life may also vary to certain extent with isolates. Plant-growth promoting rhizobacteria (PGPR) have been reported to survive in certain dry formulation (Suslow,1980) populations of PGPR didn't decline in tale mixture with 20% xanthan gum after storage for 2 months at 4 ^{0}C (Kloepper and Schroth ,1981).Vidhyasekaran and Muthamilan(1995) found that *P. fluorescens* can survive well in talc or peat-based formulations for more than 8 months. Amer (2007),encapsulated *P.fluorescens* in biopolymer gel matrix ,the bacteria survived poorly during storage and declined after 6 months of storage. *P.putida* population declined after 45 days of storage at different carriers (Amer and Utkhede,2000) .In pregelatinized corn flour powder formulation, the survival of *P. fluorescens* was prolonged up to one year with 59%survival,also survived in semolina talc powder up to 4 months of storage and declined after 6 months (Amer and Rania EL-Shennawy,2004). *P. fluorescens* and *P.putida* a non-spore former survived well in rice flour-skimmed milk for one year of storage and this could be due to the effect of nutritional value of skimmed milk and whey as well as the ingredients acting as protecting to the cells during formulation process and during storage

Establishment of *P.fluorescens* and *P.putida* in chickpea rhizospher:

When rice flour –skimmed milk powder formulations of antibiotic resistant *P. fluorescens* and *P. putida* were applied to seeds under green house

conditions the bacteria could be detected in the rhizosphere (Table-2).The populations increased gradually with time and after 15 days from sowing was 16.53 and 13.6 x10^6 cfu/gm for *P.fluorescens* and *P .putida* ,respectively and reached to the maximum population after 60 days which recorded 28.93 and 23.2 x10^6 cfu/gm for *P.fluorescens* and *P.putida* respectively, and declined to 15.23 and 12.1 x10^6 cfu/gm after 90 days. Soil application of the formulations, the rhizosphere bacterial populations of both isolates were increased compared to seed application in all experimental time. The rhizosphere population was 19.36 and 19.16 x10^6 cfu /gm for *P. fluorescens* and *P. putida*, respectively after 15 days and increased to 29.23 and 28.8x10^6 cfu/gm for both isolates after 60 days and remained in high population number even after 90 days from sowing which recorded 27.13 and 24.2x10^6 cfu/gm .

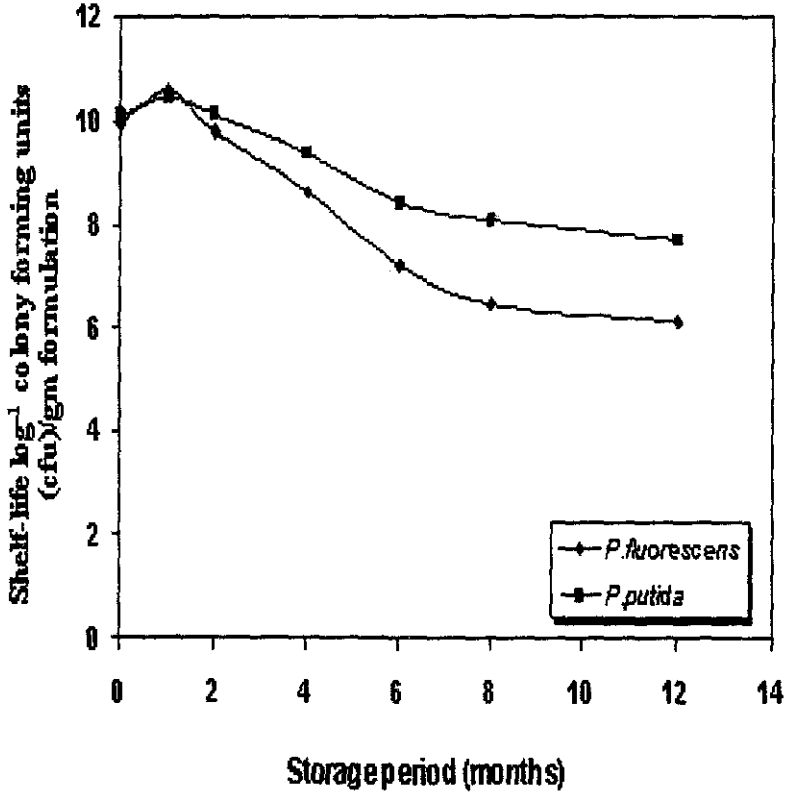

Fig (1): Shelf-life of *Pseudomonas fluorescens* and *P. putida* (log^{-1} Cfu/gm) in skimmed milk –rice flour powder formulation during storage at room temperature for one year.

Table-2: Population of marked isolates of *Pseudomonas fluorescens* and *P. putida* in chickpea rhizosphere applied as powder formulation with different application methods.

Antagonist	Treatment	Bio-agent population x10^6 cfu/gm after sowing					
		15 days	30 days	45 days	60 days	75 days	90 days
P.fluorescens	Seed	16.53 c	17.70 c	21.46 b	28.93 b	28.00 b	15.23 d
	Soil	19.36 a b	20.50 b	25.40 a	29.23 b	37.10 a	27.13 a b
	Seed +soil	20.16 a	21.30 a	26.00 a	31.20 a	38.36 a	27.90 a
P.putida	seed	13.60 d	15.00 d	20.17 b	23.20 c	21.13 e	12.10 e
	soil	19.16 b	19.96 b	21.16 b	28.83 b	24.20 d	24.20 c
	Seed +soil	19.60 a b	20.36 b	25.06 a	31.53 a	26.26 c	26.27 b
Control (free from bacteria)	0.0	0.00 e	0.00 e	0.00 c	0.00 d	0.00 f	0.00 f
LSD 0.05		0.94	0.89	1.39	1.38	1.27	0.90

Means followed by the same letter are not significantly different according to Duncan s multiple range test (P=0.05).

When the soil application followed seed treatment, the rhizosphere population of both isolates was higher throughout the experimental period of 90 days than seed treatment and it was 20.16 and 19.6 x10^6 cfu/gm after 15 days, 31.2 and 31.53 x10^6 cfu/gm after 60 days, 27.9 and 26.27x10^6 cfu/gm after 90 days from sowing for *P. fluorescens* and *P. putida*, respectively. The maximum population was recorded after 75 days with soil and , seed plus soil application. The root colonizing ability of *P. fluorescens* and *P.putida* , in general, was higher when applied as soil or seed and soil treatments throughout the experimental period of 90 days than seed treatment, and this indicates its ability as good root colonizer. It is possible to predict the rhizosphere competence of *Pseudomonas* bacteria based on their root colonizing ability (Lugtenberg *et al.*, 2001) science the root colonizing ability of a bacterium will determine its rhizosphere competence. The most widely studied bacteria by far in relation to biocontrol are *Pseudomonas* spp. Which are amongst the most effective root colonizing bacteria (Whipps, 2001).

Biological control assay of powder formulation of *P.fluorescens* and *P. putida* under greenhouse conditions:

The effect of seed, soil or seed and soil treatments with rice flour-skimmed milk powder formulation of *P.fluorescens* and *P. putida* on root rot(*Rhizoctonia solani*) and wilt (*F.oxysporum* f. sp.*ciceri*) incidence was tested under greenhouse conditions. Significant differences were obtained in controlling *R.solani* root rot as indicated in (Table 3).After 30 days from sowing, the lowest root rot incidence (4.0%) was in the fungicide carbendazim treatment and the highest (13.7%) was observed in pathogen control. The root rot incidence ranged between 4.17% and 5.9% in antagonists treated pots .The seed treatment followed by soil application was the superior treatment in both antagonists than other two treatments. After 60

days of sowing ,the lowest disease incidence (3.26%)was recorded with *P.putida* seed plus soil application and the highest disease incidence was 16.8% in pathogen control .Low root rot incidence (3.26 -5.60 %) was recorded with the antagonists treated plots. In general, seed and soil treatment showed the lowest disease incidence comparing with the other treatments. It could be noticed that up to 90 days from sowing there were significant differences between treatments in reducing disease incidence. Seed and soil treatment was the best in reducing disease incidence.

Table-3: Effect of *Pseudomonas fluorescens* and *P. putida* as powder formulations on *Rhizoctonia* root rot of chickpea when applied to seed ,soil or seed and soil treatments.

Treatments/ strain	Percent of root rot after sowing		
	30 days	60 days	90 days
Seed treatment			
P.fluorescens	5.53 b c	5.33 c	1.16 c d
P.putida	5.90 b	5.60 c	0.96 d
Soil treatments			
P.fluorescens	5.23 c	5.26 c	1.33 b c
P.putida	5.83 b	5.43 c	1.46 a b c
Seed and soil treatment			
P.fluorescens	4.86 d	3.63 d	0.53 e
P.putida	4.16 e	3.26 d	0.63 e
Cordendazin(2.59/kg)	3.00 f	10.13 b	1.63 a b
Control (infested with *Rhizoctonia*)	13.70 a	16.80 a	1.66 a
LSD 0.05	0.30	0.53	0.32

Means followed by the same letter are not significantly different according to Duncan's multiple range test (P=0.05).

Data in Table (4) reveal that, there was no significant differences between the treatments of antagonists against *Fusarium* wilt since there is no wilt incidence was observed after 30 days from sowing .After 60 days, the minimum wilt percentage (1.33%) was recorded with seed plus soil treatment with powder formulation of *P.putida* followed by *P.fluorescens*(1.43%) .The highest wilt incidence percentage (9.06 %) was recorded with pathogen control. In other treatments ,the percent wilted plants ranged between 1.83-2.66 %.After 90 days, the minimum wilted plants was 3.33 % recorded with *P.fluorescens* seed plus soil treatment followed by *P.putida* (3.6%)with the same application method. The highest percentage of wilt incidence (15.1%)recorded with pathogen control. Specific PGPR have been screened as biocontrol agents for microbial plant pathogens (deBoer *et al.* ,2003) Biological control of soil-borne diseases is known to result from the stimulation of induced systemic resistance(ISR) in the host plants (vanLoon *et al.* ,1998) .Also, it has being postulated that an additional mechanism of plant growth promotion by PGPR could be their altering of microbial rhizosphere communities (Ramos *et al.* ,2003) Rhizobacreia from the genus *Pseudomonas.* provide an excellent example of a combination of multiple

mechanisms for effective biocontrol including direct antagonism and induction of plant resistance (Haas and Keel,2003).Also, exposure of roots to non-pathogenic rhizosphere bacteria including *Pseudomonas* spp. Can induce resistance, including enhanced production phytoalexins, production of stress-related proteins and degradative enzymes (vanLoon *et al.* , 1998) . T he growth promoting rhizobacteria significantly reduced infection by *R.solani* on chickpeas. Seed bacterization with fluorescent pseudomonas reduced the number of wilted plants in wilt sick soil (*F.oxysporum* f. sp. *ciceri*). Kumar,(1998) reported that, colonization of chickpea roots by strains of *P.fluorescens* and *P.putida* significantly reduced wilt disease caused by *F.oxysporum* f. sp. *ciceri*. *Pseudomonas* sp. isolates were effective in controlling *Pythium aphanidermatum* causing damping-off of hot pepper when applied as seed treatment.

Table-4: Effect of *Pseudomonas fluorescens* and *P. putida* as powder formulations on *Fusarium* wilt incidence of chickpea when applied to seed ,soil or seed and soil .

Treatments/ strain	Percent of root rot after sowing		
	30 days	60 days	90 days
Seed treatment			
P.fluorescens	0.03	1.83 d e	5.23 c
P.putida	0.00	2.33 b c	4.20 c d
Soil treatments			
P.fluorescens	0.00	2.16 c d	7.26 b
P.putida	0.00	2.66 b	6.86 b
Seed and soil treatment			
P.fluorescens	0.00	1.43 e f	3.33 d
P.putida	0.00	1.33 f	3.63 d
Cordendazin(2.59/kg)	0.00	2.33 b c	4.03 c d
Control (infested with fusarium)	0.00	9.06 a	15.10 a
LSD 0.05	N. S.	0.49	1.40

Means followed by the same letter are not significantly different according to Duncan s multiple range test (P=0.05).

Assay for saprophytic growth of *Fusarium oxysporum* f.sp. *ciceri* and *Rhizoctonia solani*.

The saprophytic growth of the pathogens was significantly reduced by *P.fluorescens* and *P.putida* (Table-5).In general, the application of *P.fluorescens* and *P.putida* powder formulations as soil or as seed and soil was effective in reducing the saprophytic growth of *F.oxysporum* f. sp. *Ciceri* and *R. solani* than seed treatment alone. The saprophytic growth of *F. oxysporum* f.sp. *ciceri* in infested control was 84.8% ,whereas in soil treated with powder formulation of *P.fluorescens* and *P.putida* was 33.6 and 34.5% respectively, and it was 36.03 and 35.36 % when applied as seed plus soil treatments respectively. The saprophytic growth when the two antagonists applied as seed treatment was 47.5 and 51.7% respectively, and 71.8% with fungicide carbendazim treatment. The application of *P.fluorescens* and

P.putida powder formulations to soil or to seed and soil was effective in reducing the saprophytic growth of *R. solani* than seed treatment alone. The saprophytic growth of R.solani in infested control was 85.66 %, whereas in soil treated with powder formulation of P.fluorescens and P.putida was 27.33 and 26.46 % respectively, and 24.46 and 25.26 % when applied as seed and soil treatments respectively., and 65.76 % with fungicide carbendazim treatment. In addition to disease reduction, to evaluate formulation effectiveness, the ability of various formulations to reduce the saprophytic growth of pathogens (*F.oxysporum f. sp. ciceri* and *R. solani*) appears to be a reliable and more easily performed technique than setting up disease assay (Lewis *et al.*, 1995, Lewis and Larkin, 1997). Biocontrol of soil-borne diseases is known to result from the reduction of saprophytic growth of the pathogen and then of the frequency of root infection through microbial antagonism (vanLoon *et al.*, 1998). There are many examples of bacteria that can suppress the growth of pathogenic fungi in the rhizosphere.Effective colonization of the root is a key factor determining the ability of these bacteria to exert biocontrol. A number of these bacteria produces antifungal metabolities, including antibiotics, extra cellular enzymes, and HCN (Brimecombe *et al.*, 2001).

Table-5: Effect of *Pseudomonas fluorescens* and *P. putida* powder formulations on saprophytic growth of *F. oxysporum* f sp. *ciceri and R.solani* in soil after 45 days of sowing .

Treatments	Saprophytic growth of the pathogens(%)	
	F. oxysporum f sp. *ciceri*	*R. solani*
Seed treatment		
P.fluorescens	47.50 d*	78.23 b
P.putida	51.70 c	67.83 c
Soil treatments		
P.fluorescens	33.60 g	27.33 e
P.putida	34.50 f g	26.46 e
Seed and soil treatment		
P.fluorescens	36.03 e	24.46 f
P.putida	35.36 e f	25.26 f
Corbendazin (2.59/kg)	71.80 b	65.76 d
Control(infested)	84.80 a	85.66 a
LSD 0.05 %	1.47	1.10

***Means followed by the same letter are not significantly different according to Duncan's multiple range test (P=0.05).**

REFERENCES

AMER , G. A.(2007).Bioencapsulation of biocontrol agents in biopolymer gel matrix for increasing its survival stability and biocontrol activity .Minufiya J.Agric. Res. ,32.(2) : 347- 358.

AMER , G. A. and Rania Z. El-Shennawy (2004).Corn flour powder formulation of antagonists for biocontrol of chickpea seed rot and wilt diseases. Minufiya J.Agric. Res., 29(4):799-811.

AMER , G.A. and R,S. Utkhede(2000).Development of formulations of biocontrol agents for management of root rot of lettuce and cucumber.Can.J. Microbiol.,46:809-816 .

Beattie , G.A.;S.E. Lindow (1995).The secret life of foliar bacterial pathogens on leaves.Annual Review of Phytopathology.,33:145-172.

Brimecombe,M.J.;F.A.De Leij and J.M.,Lynch.(2001).The effect of root exudates on rhizosphere microbial populations. In: Pinto R, Varanini Z, Nannipierei P, eds. *The rhizosphere*. New York: Marcel Dekker, 95-141.

Burges,H.D.(1998).Formulation of Microbial Biopesticides: Beneficial microorganisms,nematodes and seed treatments. Kluwer Academic Publishers, Netherlands, pp.412.

Connick , W.J.;D.J. Daigle; A.B.Pepperman; K.P. Hebbar; R.D. Lumsden; T.W. Anderson and D.C. Sands(1998). Preparation of stable granular formulations containing *Fusarium oxysporum* pathogenic to narcotic plants. Biological control, 13:79-84.

Cook and Baker(1983).The nature and practice of biological control of plant pathogens .Am.Phytopath.Soc.St.Paul.,MN .pp.533.

deBoer,M;P. Bom;F .Kindt; J.B.Keurentjer;I.van der Sluis;L.C. van Loon and P.A.H.M.Bakker.(2003).Control of *Fusarium* wilt of radish by combining *Pseudomonas putida* strains that have different disease-suppressive mechanisms.Phytopathology 93:626-632.

Fravel,D.R.; J.J. Marois;R.D. Lumsden and W.J. Connick,Jr.(1985). Encapsulation of potential biocontrol agents in an alginate-clay mixture.Phytopathol.75:774-777.

Hass,D. and C.Keel(2003).Regulation of antibiotic production in root-colonizing *Pseudomonas spp.* and relevance for biological control of plant disease.Annual Review of Phytopathology 41:117-153.

Kloepper,J.W.and M.N. Schroth (1981).Relationship of *in vitro* antibiosis of plant growth promoting rhizobacteria to plant growth and the displacement of root microflora.Phytopathology, 71: 1020-1024

Kumar,B.S.D.(1998).Disease suppression and crop improvement through fluorescent pseudomonads isolated from cultivated soils. World Journal of Microbiology and Biotechnology, 14:735-741.

Lewis,J.A. and R.P. Larkin(1997).Extruded granular formulation with biomass of biocontrol *Gliocladium virens* and *Trichoderma* spp. to reduce damping-off of eggplant caused by *Rhizoctonia solani* and saprophytic growth of the pathogen in soil-less mix.Biocontrol Science and Technology, 7: 49-60 .

Lewis,J.A. and G.C. Papavizas (1987).Reduction of inoculum of Rhizoctonia solani in soil by germ lings of Trichoderma hamatum. Soil Biol.Biochem. 19:195-201.

Lewis ,J.A.;R.D. Lumsden ;D.R. Fravel and B.S. Shasha(1995).Application of pregelatinized starch flour granule formulations of biocontrol fungi to control damping-off diseases caused by *Rhizoctonia solani*. Biol.Control, 5:397-404.

Lugtenberg ,B.J.J.;L.Dekkers ;G.V., Bloemberg(2001). Molecular determinants of rhizosphere colonization *by Pseudomonas*. Annual Review of Phytopathology ,39:461.

Ramos.; J.A.L. Garcia; A.Probanza ;M.L. Barrientos; F.J. Gutierrez Manero (2003). Alternations in the rhizobacterial community associated with European alder growth with PGPR strain *Bacillus licheniformis*. Environmental and Experimental Botany 49:61-68.

Russo, A.; Y. Moennez-Loccoz ; S. Fedi ; P. Higgins ;A. Fenton; D.N. Dowling ; M. O,Regan and F. O'Gara (1996).Improved delivery of biocontrol *Pseudomonas* and their antifungal metabolites using alginate polymers. Appl. Microbiol. Biotechnol. 44:740-745.

Suslow,T.V.(1980).Growth and yield enhancement of sugar beets by pelleting seed with specific *Pseudomonas* spp. Phytopathol.News L.,12(9);40.

van Loon, L. C.; P.A.H.M., Bakker ; C.M.J. Pieterse (1998). Systemic resistance induced by rhizosphere bacteria. Annual Review of Phytopathology. 36:453-483.

Vidhyasekaran,P. and M. Muthamilan(1995).Development of formulations of *Pseudomonas fluorescens* for control of chickpea wilt. Plant Dis.,79:782-786.

Vidhyasekaran,P. and M. Muthamilan(1999).Evaluation of powder formulations of *Pseudomonas fluorescens* Pf-1 for control of rice sheath blight.Biocontrol Science and Technology,9:67-74.

Weller ,D.M.(1988).Biological control of soil-borne plant pathogens in the rhizosphere with bacteria. Annual Review of Phytopathology, 26:379-407.

Weller,D.M., and R.J.Cook(1986).Increased growth of wheat by seed treatment and implications of *Pythium* control. Can. J. Plant Pathol. 8,328-334.

Whipps, J. M. (2001).Microbial interactions and biocontrol in the rhizosphere. Journal of Experimental Botany,52 :487-511.

استخدام اللبن المجفف فى تنشيط حيوية بعض البكتيريا المقاومة لمسببات عفن الجذور و الذبول فى الحمص

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أجريت هذه الدراسة بغرض استخدام اللبن المجفف منـزوع الدسـم skimmed milk وشرش اللبن المجفف whey لتغذية وحماية بكتريـا ***P. flurescens* and *P. putida*** أثناء تشكيلها في صورة بودرة في وجود دقيق الارز وال vermiculite كمادة حاملـة . هـذه البودرة حسنت من قدرة البكتريا على البقاء حية لمدة طويلة في صورة بودرة وبأعداد كبيرة لمدة ١٨٠ يوم من التخزين تحت ظروف الغرفة . بعد ذلك بدا العدد في التناقص التـدريجي وببطـئ حتى ٦ شهور من التخزين . وبنهاية فترة التخزين لمدة عام تراوحت النسب المئوية لبقـاء هـذه

العزلات حية في البودرة مابين ٦١,٥% و٧٥,٩% لكلا العـزلتين *P.fluorescens* and *P.putida* على التوالي .

تشكيل البكتريا في صورة بودرة بهذه المكونات مكنها من زيادة قدرتها على التنافس في المنطقة المحيطة بالجذور rhizosphere competence في الحمص وباعـداد كبيـرة ازدادت تدريجيا حتى ٧٥ يوم من الزراعة . ثم بعد ذلك بدأ العدد في التنـاقص التـدريجي مـع وصول النباتات إلى مرحلة النضج بعد ٩٠ يوم من الزراعة .

إضافة هذه العزلات من البكتيريا في صورة بودرة إلى البذور او التربة او الإثنـين معـا لمقاومة الذبول الفيوزاريومي او عفن الجذور الرايزوكتوني في الحمص ادى إلى خفض معنـوي لنسبة الإصابة بهذه الأمراض . وكانت معاملة التربة أو معاملة البذور والتربة معا بتلك البـودرة من أحسن المعاملات كفاءة في تقليل نسبة الإصابة في كلا المرضين.

إضافة البكتريا المحملة في صورة بودرة إلى التربة أدى إلـى خفـض النسـبة المئويـة لـ sprophytic growth لأعداد الفطريات المسـببة للأمـراض *F. oxysporum* f sp. *ciceri and R.solani* والتي تعيش مترممة في التربة بصورة معنوية . وكانـت معاملـة التربة أو التربة والبذور معا من اكثر المعاملات كفاءة في خفض النسـبة المئويـة لقـدرة هـذه الفطريـات علـى المعيشـة ترمميـا فـي التربـة واكثـر فعاليـة مـن المبيـد الفطـري carbendazim(Bavistin) .