RESULTS

I-Pathological Studies:

A-Isolation and identification of the causal organisms:

Sixty-two samples of infected wheat seedlings were collected from wheat fields of seven governorates which showed wilting and root-rot symptoms i.e. Gharbiya, Minufiya, Behiera, Alexandria, Kafr El-Sheikh, Kalyobiya and Dakahliya.

Data in Tables (1,2) reveal that, 74 isolates of fungi were identified from the sixty-two infected wheat samples.Five species of fungi were represent the total isolates with different frequencies.These species were *Fusarium semitectum* (40 isolates), *Fusarium solani* (12 isolates),*Fusarium oxysporum* (10 isolates), *Epicocum* sp. (7 isolates) and *Alternaria* sp.(5 isolates).

B- Frequencies of the identified pathogens:

Out of the *Fusarium* isolates, the fungus *Fusarium semitectum* scored the highest number of isolates and frequencies (54 % of the total isolates),followed by *Fusarium solani* (16.2%),Then *Fusarium oxysporum* (13.5 %),of the total isolates. It could be noticed that *Epicocum* sp. scored (9.5 %) frequency, while *Alternaria* sp. showed the lowest percent of frequency (6.8 %).

II-Varietal Resistance:

Data in Table (3) and illustrated in Figure (1) clear the response of five bread wheat cultivars i.e. Gemmeiza-9, Gemmeiza-10, Sakha-94, Giza-168 and Sids-1 to wilt and root-rot diseases caused by *Fusarium semitectum*. The determination was carried out at Gemmeiza Research Station in 2005/2006 growing season under green-house conditions. Inoculum of the pathogen was mixed with sterilized soil at the rate of (25 gm /1 kg soil) pre, post and survival percentages of wheat plants was detected after 15, 30 and 90 days.

Data reveal that all the tested cultivars showed moderate values of survival percentages ranged from 45.00-68.33%. Gemmeiza-9 cultivar showed the highest values of survival plants (68.33%), followed by sakha-94 (61.67), Gemmeiza-10 (55.00) ,then Giza-168 (51.67) .No significant differences were found between Gemmeiza-10 and sakha-94, and between Gemmeiza-10 and Giza-168. The cultivar Sids-1 was the most affective one (45.00%).

Table (1) The total fungi isolated from infected wheat plants from different governorates of Delta and their frequencies.

Governorate	Isolated fungi	No. of isolates	Frequency (%)
Gharbiya	*Fusarium semitectum*	7	46.67
	Fusarium solani	5	33.33
	Epicocum sp.	2	13.33
	Alternaria sp.	1	6.67
Total		15	
Behiera	*Fusarium semitectum*	10	58.83
	Fusarium solani	3	17.65
	Fusarium oxysporum	2	11.76
	Alternaria sp.	2	11.76
Total		17	
Alexandria	*Fusarium semitectum*	6	75
	Fusarium solani	1	12.5
	Alternaria sp.	1	12.5
Total		8	
Kafr El-Sheikh	*Fusarium semitectum*	5	62.5
	Fusarium oxysporum	2	25
	Epicocum sp.	1	12.5
Total		8	
Minufiya	*Fusarium semitectum*	5	41.67
	Fusarium oxysporum	3	25
	Epicocum sp.	3	25
	Alternaria sp.	1	8.33
Total		12	
Kalyobiya	*Fusarium semitectum*	3	42.86
	Fusarium solani	1	14.29
	Fusarium oxysporum	3	42.86
Total		7	
Dakahliya	*Fusarium semitectum*	4	57.14
	Fusarium solani	2	28.57
	Epicocum sp.	1	14.29
Total		7	
Total		74	

$$\text{Frequency \%} = \frac{\text{No. of isolates for each specie}}{\text{Total no. of isolates}} \times 100$$

Table (2) Isolated fungi from different governorates and its frequency.

Isolated fungi	No. of isolates	Frequency(%)
Fusarium semitectum	40	54
Fusarium solani	12	16.2
Fusarium oxysporum	10	13.5
Epicocum sp.	7	9.5
Alternaria sp.	5	6.8
Total	74	---

Table(3) Varietal response of some bread wheat cultivars to artificial inoculation with *F.semitectum*..

Cultivars	Pre-emergence %	Post-emergence %	Survival plants %
Gemmeiza-9	15.00 c	16.67 bc	68.33 a
Gemmeiza-10	25.00 a	20.00 b	55.00 bc
Sakha-94	23.33 ab	15.00 c	61.67 ab
Giza-168	21.67 b	26.67 a	51.67 cd
Sids-1	26.67 a	28.33 a	45.00 d
L.S.D at 0.05	3.50	4.55	7.45

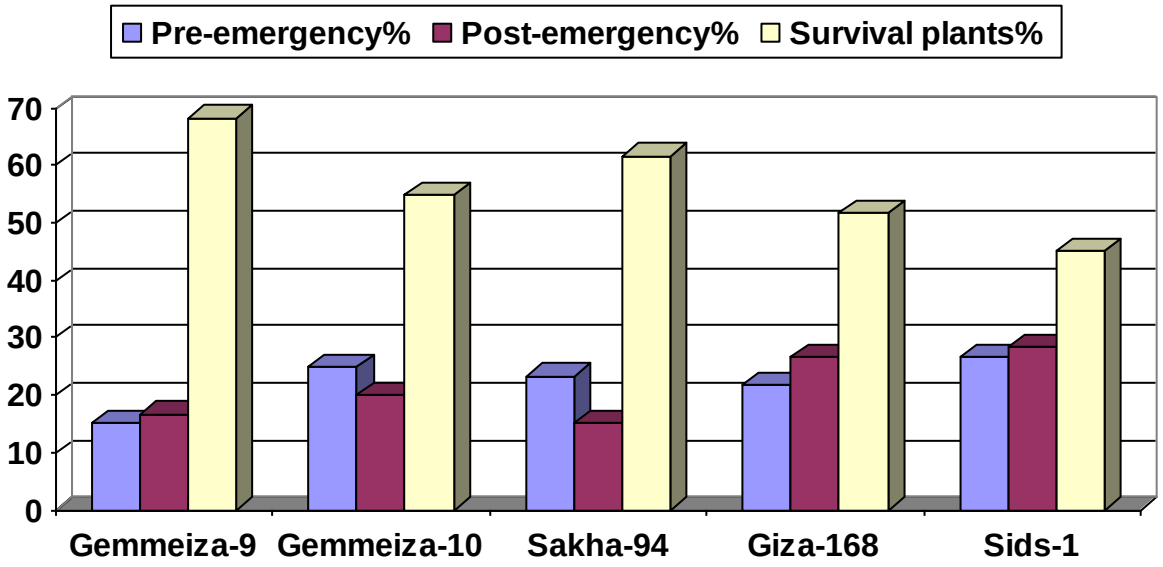

Fig.(1) Varietal response of some bread wheat cultivars to artificial inoculation with *F.semitectum*.

III-Biological Control Studies:

A-*In vitro* Experiments:

1-Effect of the tested bio-control agents on radial growth of *Fusarium semitectum*:

Out of the identified fungi which isolated from the collected samples, the most common fungus *Fusarium semitectum* was chosen for biological control experiments.Four antagonistic fungi i.e. *Trichoderma viride, Trichoderma hamatum, Gliocladium virens* and *Gliocladium deliquenscens* as well as two isolates of *Bacillus subtilis* (1 and 2), three isolates of *streptomyces* spp. (1,2 and 3) and one isolate of *Pseudomonas fluorescens* were used. The antagonistic effect of the previous bio-control agents was detected on PDA media as a percentage of inhibition.

a- Fungal antagonists:

Data in Table (4) and illustrated in Figures(2,3,6) show the efficiency percentage of the fungal bio-control agents against the radial growth of *Fusarium semitectum.* In general, significant differences were found, which *Trichoderma* spp. were the most effective than *Gliocladium* spp. in reducing the radial growth of *F. semitectum*. Which *Trichoderma viride* showed the highest percentage of efficiency,(76.67%) followed by *Trichoderma hamatum* (74.56%).While *Gliocladium virens* and *Gliocla*d--*ium deliquenscens* were appeared (65.89%) and (64.11%), respectively.

Table(4)Antagonistic effect of some bio-control agents fungi on radial growth of *Fusarium semitectum*.

Bio-control agents	Mean of radial growth(cm)	Efficiency (%)
Trichoderma viride	2.10 c	76.67
Trichoderma hamatum	2.29 c	74.56
Gliocladium virens	3.07 b	65.89
Gliocladium deliquenscens	3.23 b	64.11
Control	9.00 a	0.00
L.S.D at 0.05	0.19	---

b-Bacterial antagonists:

Data in Table (5) and illustrated in Figs (4,5,6) reveal the antagonistic effect of the bacterial bio-control agents, *Bacillus subtilis*, *Streptomyces* spp. as well as *Pseudomonas fluorescens* against the pathogenic fungus *Fusarium semitectum*.

Significant differences were found between all the bacterial antagonists as well as between them and the control treatment. *Streptomyces* isolate no.2 was the most effective one releazing the highest inhibition zone (1.37), followed by *Bacillus subtilis* isolate no.1 (1.20), then *Streptomyces* no.1(1.13). While the remained bacterial antagonists occupied the last ranking,were *Bacillus subtilis* no.2 (0.86), *Pseudomonas fluorescens* isolate (0.35) and *Streptomyces* isolate no.3 (0.23).

Table (5) Antagonistic effect of different bacterial isolates on radial growth of *F.semitectum*.

Bio-control agents	Mean of radial growth(cm)	Efficiency(%)	Inhibition zone (cm)
Bacillus subtilis 1	4.63 cd	48.56	1.20 b
Bacillus subtilis 2	4.81 c	46.56	0.86 c
Streptomyces sp. 1	4.70 c	47.78	1.13 b
Streptomyces sp. 2	4.00 d	55.56	1.37 a
Streptomyces sp. 3	6.11 b	32.11	0.23 e
Pseudomonas fluorescens	5.40 bc	40.00	0.35 d
Control	9.00 a	---	0.00
L.S.D at 0.05	0.71	---	0.11

Fig.(2):Antagonistic effect of 2 *Trichoderma* species against *F.semitectum*.
T.hm = *T.hamatum* **T.v. = *T.viride*.**

Fig. (3): Antagonistic effect of 2 *Gliocladium* species against *F. semitectum*
G.v. = *G.virens* **G.d. =*G. deliquenscens***

Fig.(4) : Antagonistic effect of two isolates of *Bacillus subtilis* and one isolate *Pseudomonas fluorescens* against *F. semitectum*.
F= *Fusarium semitectum* **B.1= *Bacillus subtilis* no. 1**
B.2 = *Bacillus subtilis* no. 2 **Ps.f = *Pseudomonas fluorescens***

Fig. (5) : Antagonistic effect of 3 isolates of *Streptomyces* spp. against *F.semitectum*
F= *Fusarium semitectum* **S.1= *Streptomyces* sp.1**
S.2= *Streptomyces* sp.2 **S.3= *Streptomyces* sp.3**

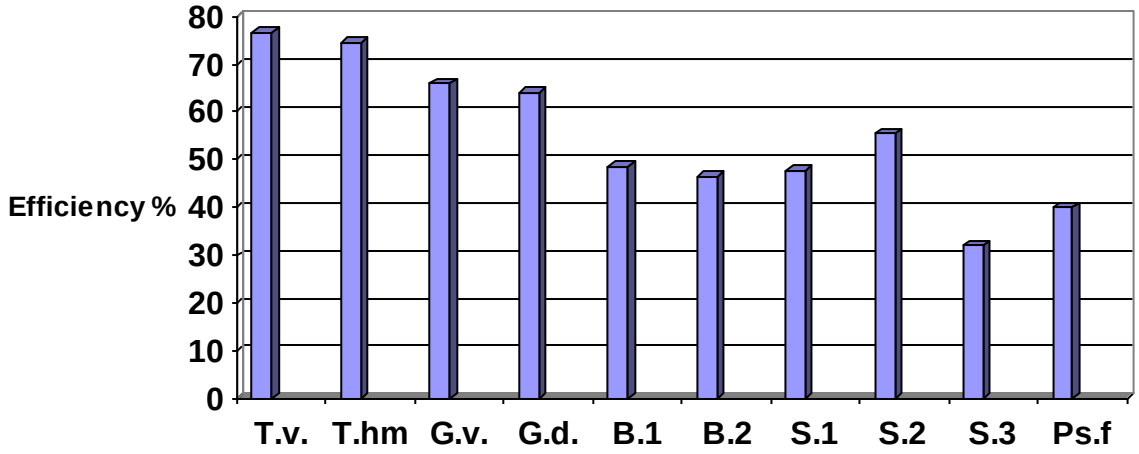

Fig.(6) Efficiency of fungal and bacterial antagonists against *Fusarium semitectum*..

C- Effect of culture filtrates of bio-control agents on radial growth of *F.semitectum*.

The culture filtrates of the fungal and bacterial bio-control agents were tested for suppressing the radial growth of *Fusarium semitectum*. Three dilutions were prepared from the culture filtrate of each bio-control agent such as 1/10, 1/20, and 1/30 v/v .In general,all the tested bio-control agents with different dilutions showed antagonistic effect against *Fusarium semitectum* but with varying degrees(Table 6) and illustrated in Fig.(7).The dilution of 1:10 proved to be the most effective one in reducing the radial growth of *Fusarium semitectum* concerning with all of the bio-control agents. There are positive correlation between the dilution of filtrate and radial growth of *F.semitectum* and vice versa. The fungal bio-control agents scored the highest percentage of afficacy comparing with bacterial bio-control agents. *Trichoderma viride* scored the highest percentages of efficacy with all dilutions in reducing the radial growth of *Fusarium semitectum* (94.44, 93.00 and 88.11%).While *Gliocladium virens* (94.44, 91.89 and 85.56 %) and *Trichoderma* hamatum (94.11, 90.33 and 82.56%) occupied the second and third ranking for their efficacies with all dilutions, respectively. *Gliocladium deliquenscens* came in the fourth rank in this respect (93.22, 88.11 and 79.22 %) with the three dilutions, respectively. Concerning with the bacterial antagonists, *Bacillus subtilis* 1 and 2 were more effective than *Pseudomonas fluorescens* in reducing the radial growth of *Fusarium semitectum.*

Table (6) Effect of culture filtrates of some fungal and bacterial bio-agents on radial growth of *F.semitectum* at different dilutions.

Biocontrol Agents	Mean of radial growth(cm) dilution 1:10	Efficiency (%)	Mean of radial growth(cm) dilution 1:20	Efficiency (%)	Mean of radial growth(cm) dilution 1:30	Efficiency (%)
Trichoderma viride	0.50^{f}	94.44	0.63^{G}	93.00	1.07^{h}	88.11
Trichoderma hamatum	0.53^{f}	94.11	0.87^{f}	90.33	1.57^{f}	82.56
Gliocladium virens	0.50^{f}	94.44	0.73^{G}	91.89	1.30^{G}	85.56
Gliocladium	0.61^{e}	93.22	1.07^{e}	88.11	1.87^{e}	79.22

deliquenscens						
Bacillus subtilis 1	0.73[d]	91.89	1.37[d]	84.78	2.13[d]	76.33
Bacillus subtilis 2	0.87[c]	90.33	1.53[c]	83.00	2.47[c]	72.56
Pseudomonas fluorescens	1.33[b]	85.22	2.03[b]	77.44	3.17[b]	64.78
Control	9.00[a]	0.00	9.00[a]	0.00	9.00[a]	0.00
L.S.D at 0.05	0.05		0.13		0.08	

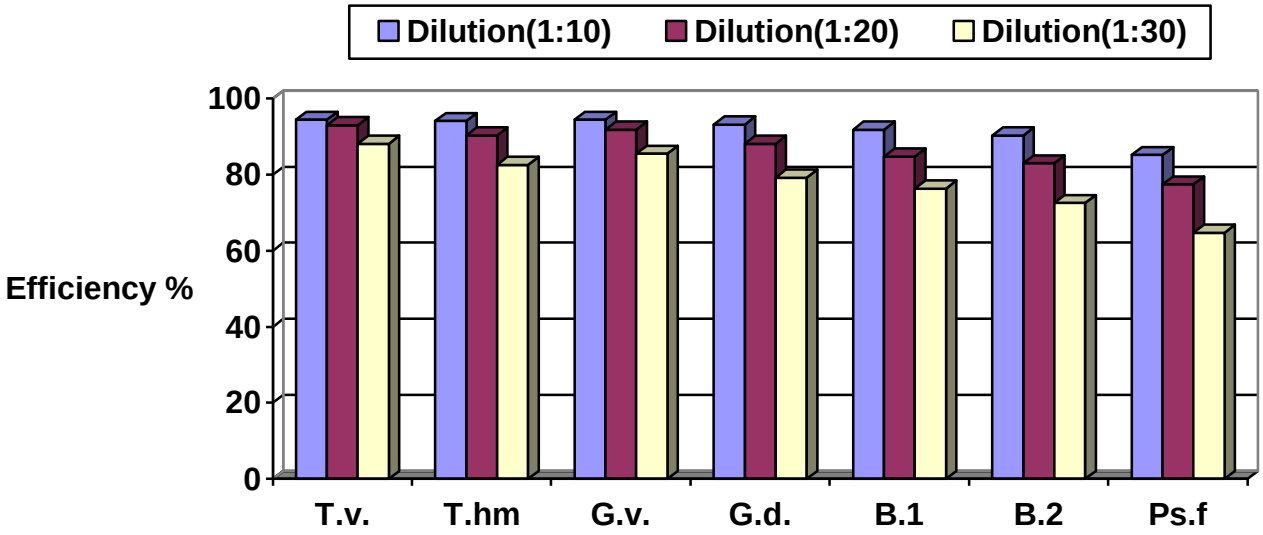

Fig.(7) Efficiency of culture filtrates of fungal and bacterial antagonists against *Fusarium semitectum* at different dilutions.

B-Green-House Experiments:

Experiments of biological control against *Fusarium semitectum* were carried out under green-house conditions at Gemmeiza Research Station in 2005/2006 growing season.Three methods of application were used to control *Fusarium semitectum*.The bio-control agents were prepared in two forms :- (1) cultured in wheat bran medium, (2) cultured in broth media,the first form of bio-control agents was mixed with talc-powder and carboxyl-methyl cellulose while the second form was used for seed-soaking.

Data in Table (7) and illustrated in Figures (8,9,10) show the data of the fungal antagonists as seed-soaking and soil treatments by inoculated wheat bran and talc-powder. Seed-soaking treatment showed the highest values of survival plants percentages comparing with the other treatments. Also, *Trichoderma* spp. proved to be the most effective than *Gliocladium* spp. with all the methods of application. *Trichoderma viride* scored the highest values of survival plants with seed soaking (83.33 %), wheat bran (75.00%) and talc-powder (68.33%),respectively.Similar results were obtained by *Trichoderma hamatum* which came in the second rank with seed-soaking (76.67%), inoculated wheat bran (73.33%) then talc-powder (65.00%),respectively. *Gliocladium virens*, and *Gliocladium deliquenscens* came in the third and fourth ranking and take similar trends with all of the used methods of application.

Table (7) Effect of different treatments from some antagonistic fungi on wilt and root rot diseases caused by *F.semitectum*.

Biocontrol Agents	Seed soaking			Wheat bran			Talc powder		
	Pre-emergency %	Post-emergency %	Survival %	Pre-emergency %	Post-emergency %	Survival %	Pre-emergency %	Post-emergency %	Survival %
Trichoderma viride	13.33^{c}	3.33^{b}	83.33^{a}	15.00^{b}	10.00^{b}	75.00^{a}	18.33^{b}	13.33^{b}	68.33^{a}
Trichoderma hamatum	18.33^{bc}	5.00^{b}	76.67^{b}	16.67^{b}	10.00^{b}	73.33^{ab}	18.33^{b}	16.67^{b}	65.00^{ab}
Gliocladium virens	23.33^{b}	3.33^{b}	73.33^{bc}	18.33^{b}	11.67^{b}	70.00^{bc}	21.67^{b}	15.00^{b}	63.33^{b}
Gliocladium deliquenscens	25.00^{b}	3.33^{b}	71.67^{c}	20.00^{b}	11.67^{b}	68.33^{c}	21.67^{b}	16.67^{b}	61.67^{b}
Control	38.33^{a}	16.67^{a}	45.00^{d}	36.67^{a}	21.67^{a}	41.67^{d}	33.33^{a}	23.34^{a}	43.33^{c}
L.S.D at 0.05	**7.05**	**7.95**	**5.00**	**5.80**	**6.30**	**3.45**	**5.95**	**5.00**	**4.90**

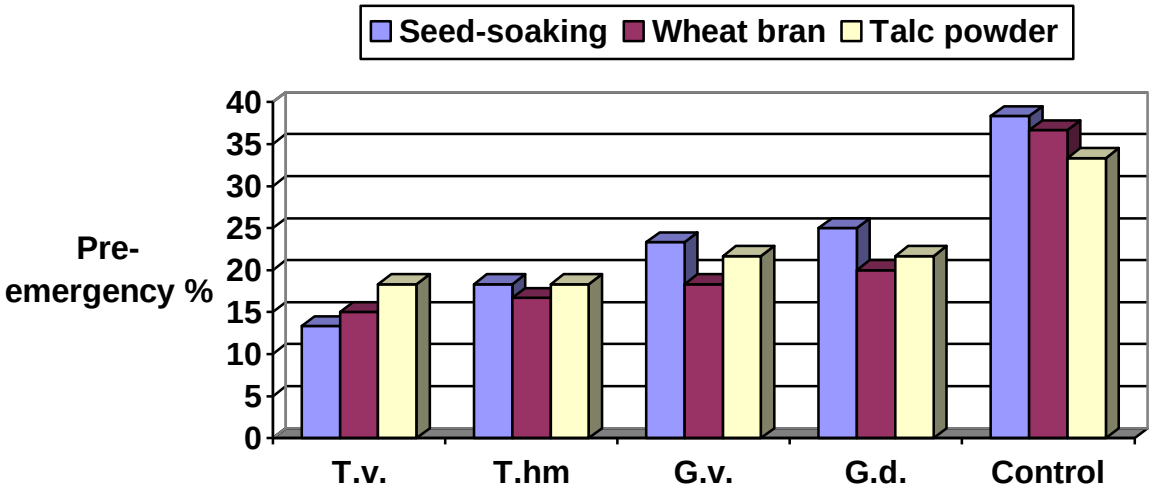

Fig. (8) Effect of certain fungal bio-control agents applied either as seed-soaking or wheat bran and talc powder formulation on percentage of pre-emergency of wheat seeds grown in soil infested by *F.semitectum*.

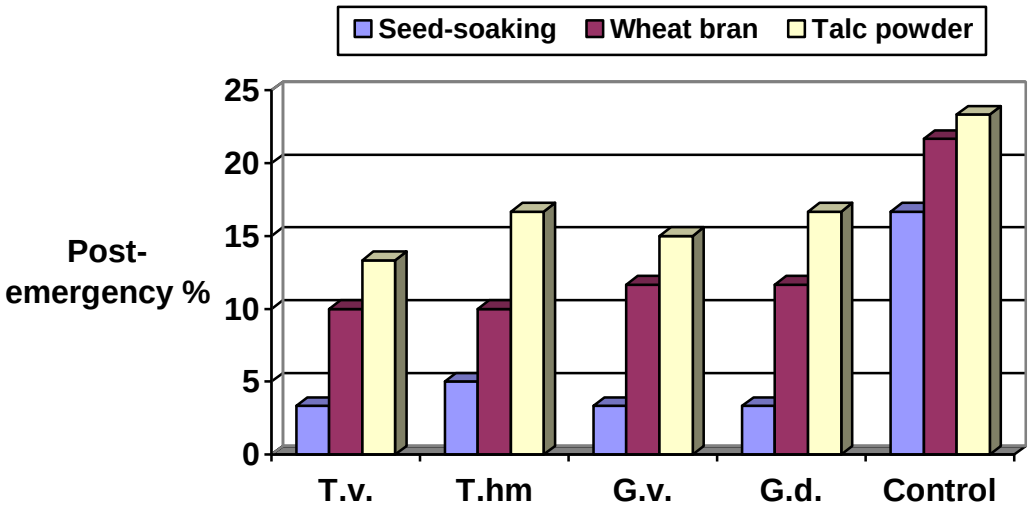

Fig.(9) Effect of certain fungal bio-control agents applied

either as seed-soaking or wheat bran and talc powder formulation on percentage of post-emergency of wheat plants grown in soil infested by *F.semitectum*.

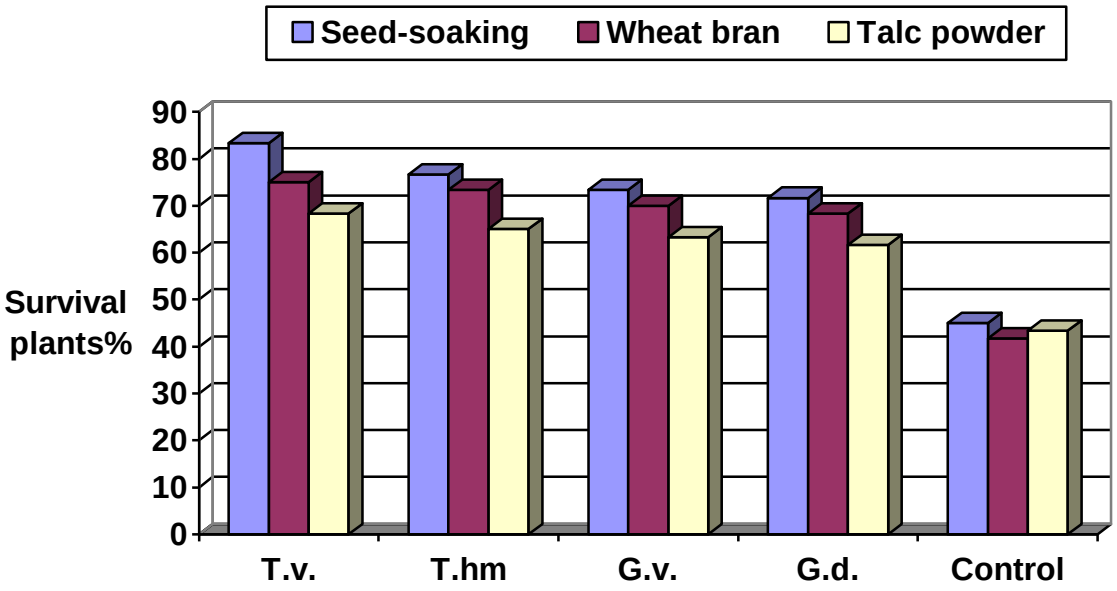

Fig.(10) Effect of ertain fungal bio-control agents applied either as seed-soaking or wheat bran and talc powder formulation on percentage of survival of wheat plants grown in soil infestedby *F.semitectum*.

Bacterial antagonistic effects on survival of wheat plants percentages were studied through two methods of application i.e. seed-soaking and mixtures with talc-powder.

Data in Table (8) and illustrated in Figs (11,12,13) show that both of the two methods of application showed moderate values of survival plant percentages, which ranged between 56.67-66.67 % with seed-soaking application and from 50-58.33 % with mixtures of talc-powder.In general, the isolates of *Bacillus subtilis* were more effective than *Pseudomonas fluorescens*. The isolate of *Bacillus subtilis* (1) was more effective either used as seed-soaking (66.67%) or as mixing with talc powder (58.33%).The isolate of *Bacillus subtilis* (2) came in the second rank with the two applications (63.33 and 55.00%) respectively. While *Pseudomonas fluorescens* isolate showed the lowest survival plant percentages in this respect (56.67 and 50.00%).

Table(8)Effect of two different treatments from some antagonistic bacteria on wilt and root-rot diseases caused by *F.semitectum*.

Bio-control agents	Seed-soaking			Talc powder		
	Pre-emergency %	Post-emergency %	Survival %	Pre-emergency %	Post-emergency %	Survival plants %
Bacillus subtilis 1	26.67 b	6.67 b	66.67 a	25.00	16.67	58.33 a
Bacillus subtilis 2	31.67 ab	5.00 b	63.33 a	25.00	20.00	55.00 a
Pseudomonas fluorescens	35.00 a	8.33 b	56.67 b	28.33	21.67	50.00 b
Control	38.33 a	16.67 a	45.00 c	33.33	23.34	43.33 c
L.S.D at 0.05	6.80	7.45	3.75	0.00	0.00	4.35

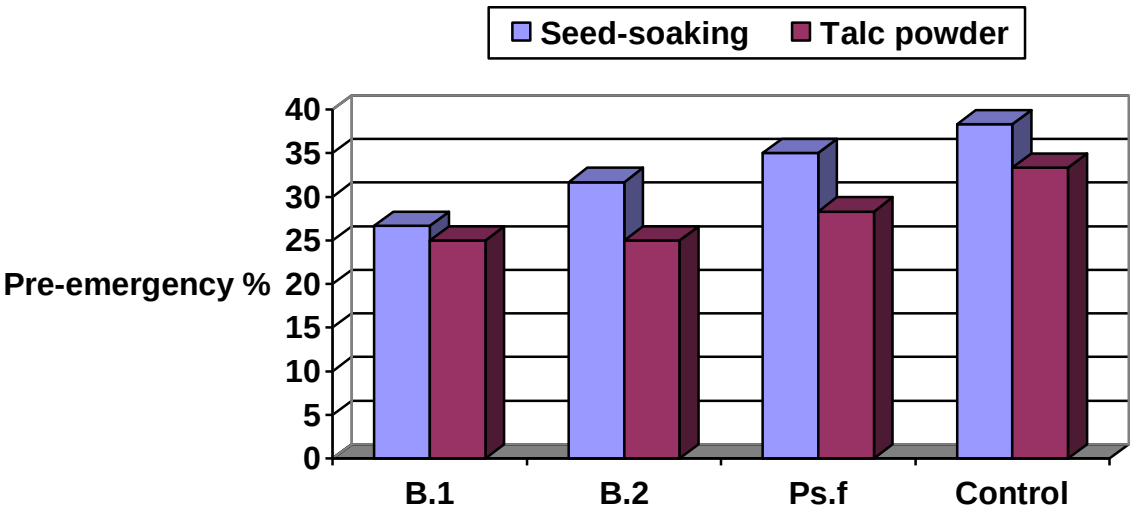

Fig. (11) Effect of certain bacterial bio-control agents applied

either as seed-soaking or talc-powder formulation on percentage of pre-emergency of wheat seeds grown in soil infested by *F.semitectum*.

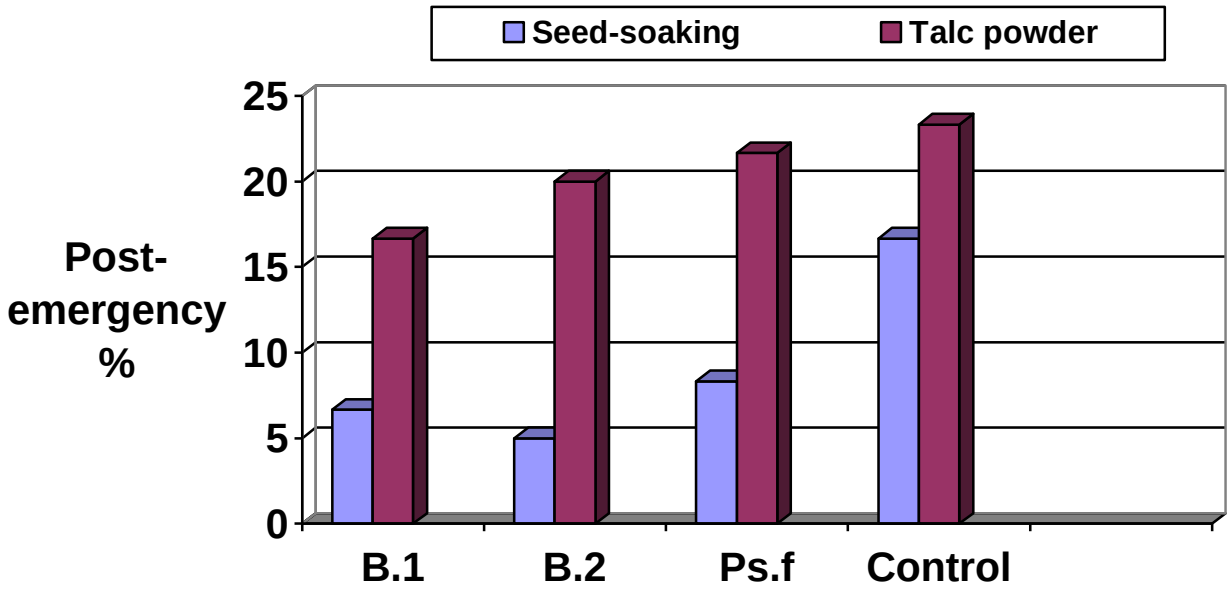

Fig. (12)Effect of certain bacterial bio-control agents applied either as seed-soaking or talc-powder formulation on percentage of post-emergency of wheat plants grown in soil infested by *F.semitectum*.

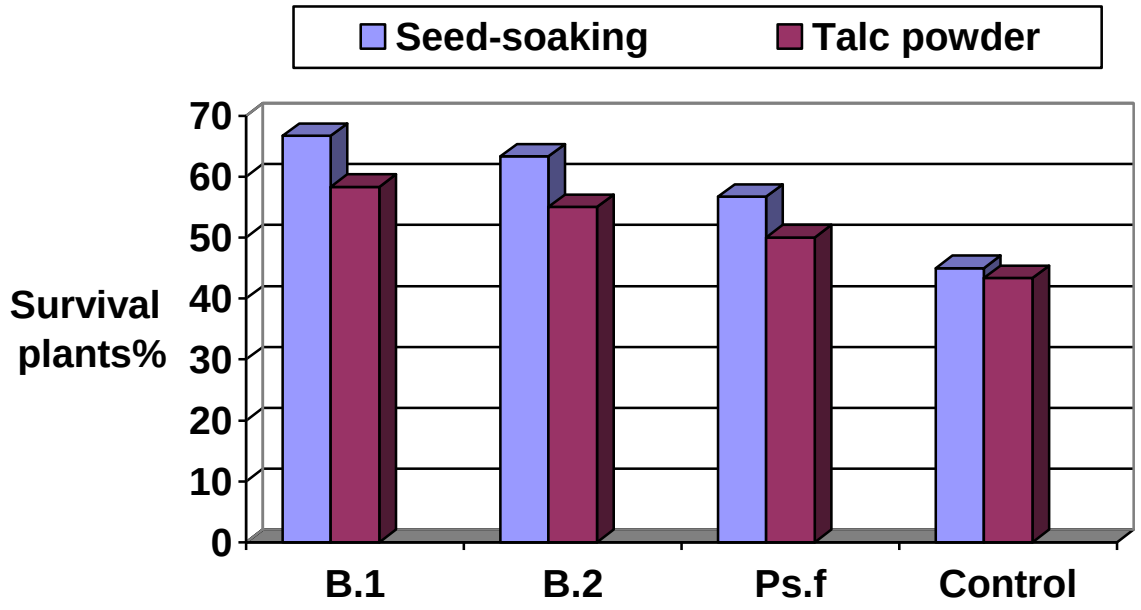

Fig. (13)Effect of certain bacterial bio-control agents applied either as seed-soaking or talc-powder formulation on percentage of survival of wheat plants grown in soil infested by *F.semitectum*.

IV-Serological Studies:

Two methods of serology were followed in this study i.e.(1) crossed-immunoelectrophoresis technique (2) double diffusion test. Homologous reactions were carried out for each of *Fusarium semitectum, Trichoderma viride*, and *Pseudomonas fluorescens* to detect their antigenic structure. While heterologous reactions were performed to detect the common antigens between them. This procedures were followed to study the serological relation-ship between the best antagonistic bio-control agent, *Trichoderma viride* , the lowest one *Pseudomonas fluorescens* and the. pathogenic fungus *Fusarium semitectum*. Data inTable (9) and illustrated in Figs (14,15,16,17,18) reveal homologous and heterologous reactions using crossed-immunoelectrophoresis technique. Seven, five and four precipitin bands were detected in homologous reaction of each of *Fusarium semitectum, Trichoderma viride* and *Pseudomonas fluorescens*, respectively.While in heterologous reactions, two and one common antigens were detected between *F. semitectum* and *T. viride*, and between *F. semitectum* and *Ps. fluorescens*, respectively.

Table (9) Number of precipitin bands detected in homologous and heterologous reactions of *Fusariu semitectum,Trichoderma viride* and *Pseudomonas fluorescens* using crossed–immuno electrophoresis technique.

Antibodies / **Antigens**	*Fusarium semitectum*	*Trichoderma viride*	*Pseudomonas fluorescens*
Fusarium semitectum	7	2	1
Trichoderma viride	2	5	---
Pseudomonas fluorescens	1	---	4

Fig.(14) illustrate the homologous reaction between antibodies and antigen of *F.semitectum* using crossed–immunoelectrophoresis technique(CIE),seven precipitin bands were detected.
Ab= Antibodies Ag= Antigen

Fig.(15) illustrate the homologous reaction between antibodies and antigen of *Trichoderma viride* using crossed-immunoelectro phoresis technique(CIE),five precipitin bands were detected.
Ab= Antibodies Ag= Antigen

Fig.(16) illustrate the homologous reaction between antibodies and antigen of *Pseudomonas fluorescens* using crossed-immuno electrophoresis technique(CIE),four precipitin bands were detected.
Ab= Antibodies Ag= Antigen

Fig.(18) illustrate heterologous reaction between antibodies of *F.semitectum* and antigen of *Pseudomonas fluorescens* using crossed-immunoelectrophoresis technique(CIE),one precipitin bands were detected.
Ab= Antibodies Ag= Antigen

Fig.(19) illustrate the homologous reaction between antibodies and antigen of *F.semitectum* using ouchterlony double diffusion test.
Ab = Antibodies Ag = Antigen

Fig.(20) illustrate the homologous reaction between antibodies and antigen of *Trichoderma viride* using ouchterlony double diffusion test.
Ab= Antibodies Ag= Antigen

Fig.(21) illustrate the homologous reaction between antibodies and antigen of *Pseudomonas fluorescens* using ouchterlony double diffusion test.
Ab= Antibodies Ag= Antigen

Fig.(22) illustrate heterologous reaction between antibodies of *F.semitectum* and antigen of *Trichoderma viride* using ouchterlony double diffusion test, two precipitin line was detected.
Ab= Antibodies Ag= Antigen

Fig.(23) illustrate heterologous reaction between antibodies of *F.semitectum* and antigen of *Pseudomonas fluorescens* using ouchterlony double diffusion test, one precipitin line was detected.
Ab= Antibodies Ag= Antigen

Data in Table (11)compare between crossed-immunoelectrophoresis technique and double diffusion test in detecting the antigenic structure of *Fusarium semitectum*, *Trichoderma viride* and *Pseudomonas fluorescens*. It could be noticed that,crossed-immunoelectrophoresis technique showed highest numbers in homologous reactions of *Fusarium semitectum* (7), *Trichoderma viride* (5) and *Pseudomonas fluorescens* (4). While low numbers of precipitin lines were detected in double diffusion test 2, 2 and 3 for *F.semitectum*, *T.viride* and *Ps.fluorescens*, respectively.

Table (11) Comparison between crossed–immunoelectrophoresis technique and double diffusion technique.

Antibodies / **Antigens**	**Homologous reactions**	
	Crossed – immunoelectrophoresis technique	**Double diffusion technique**
Fusarium semitectum	7	2
Trichoderma viride	5	2
Pseudomonas fluorescens	4	3