EFFECTS OF RHIZOBIOPHAGES AND FUNGICIDES ON GROWTH OF *BRADYRHIZOBIUM JAPONICUM* AND *RHIZOBIUM MELILOTI* AS WELL AS NODULATION AND GROWTH OF SOYBEAN AND ALFALFA

SHAHABY, A.F.[1], NADIA F. EMAM[1], M.E. HASSAN[2], M.M. EL-SAWI[2] AND M.K. ZAHRA[1]

1 Department of Microbiology, Faculty of Agriculture, Cairo University,Giza, Egypt.

2 Agricultural Microbiology Res. Dept., Soil, Water and Environ. Res. Institute, Agric. Res. Centre, Giza, Egypt.

(Manuscript received 11 November, 1997)

Abstract

The effects of rhizobiophages and fungicides (Rizolex and Benlate) on *Bradyrhizobium japonicum* and *Rhizobium meliloti* as well as nodulation, N_2-fixation and growth of soybean and alfalfa were investigated. Both phage-and fungicide resistant rhizobia and bradyrhizobia mutants were secured. *Rhizobium* and *Bradyrhizobium* strains showed variable sensitivity to phages. Rhizobiophages effective on rhizobia strains produced clear, round and variable size of plaques. strains and their phage-resistant isolates exhibited marked variations in their responses to different concentration of the tested fungicides depending on the strain and the type of fungicide used and its level of application. Rizolex at 75 ppm concentration decreased the growth of rhizobial strains and their phage-resistant isolates. No growth was obseved for all tested strains when the lethal level (125 ppm) of Rizolex was used. All rhizobial strains and their phage-resistant isolates showed good growth at 700 and 800 ppm of Benlate, but 900 ppm reduced the growth of all tested strains. Benlate at 1200 ppm concentration was lethal for all strains. Inoculation of both legumes with their specific rhizobia promoted nodulation, growth and increased nitrogen content of plants. Increases in plant parameters depended on the type of inoculant with or without phage, Rhizolex or both. The highest nodulation, growth and nitrogen content were recorded when parent strains were applied without any additives compared to their phage-resistant isolates (PRI), Rizolex-resistant isolates (RRI) and phage-Rizolex-resistant isolates (PRRI). Addition of phage, Rhizolex or both to the parent strains or their resistant isolates caused more decreases in all plant parameters. Inoculated plants receiving fungicides recorded the least values of nodulation, plant biomass and nitrogen content. The numbers of *R. Meliloti* TAL380 and *B. Japonicum* USDA 218 sharply decreased after 50 and 45 days of planting, respectively; and more decreases were recorded at the second period of analysis, 90 and 75 days, in the same order. Marked reductions in rhizobia populations were recorded when phage was added to its homologous rhizobia. The phage particles in the inoculated treatments increased at 45-and 50-day periods compared to the initial levels then sharply decreased with progressive growth. Numbers of phages were particularly high in treatments received phages.

INTRODUCTION

The occurrence and number of rhizobial strains in soil are affected by chemical, physical and biological properties of the soil as well as environmental factors. Strains of rhizobia are commonly introduced to soil to increase their population and the amount of nitrogen fixed. Rizobiophages are considered as one of the important biological factors negatively affect the numbers and activity of rhizobia. They directly lead to lysis of rhizobial cells resulting in reducing their population in soil (Uchiumi *et al.*, 1995). In addition, they indirectly affect the ability of rhizobia to fix nitrogen due to the formation of phage-resistant strains which might have less or no nitrogen fixation capacity (Abebe *et al.*, 1992).

The use of fungicides to control seed and root diseases has now become an integral part of leguminous crop production. Unfortunately, the use of these chemicals may be incompatible with rhizobia to ensure adequate nodulation and nitrogen fixation. Alternatively, fungicides may affect the legume growth leading to low yield. An indirect effect of decreased legume growth will be reflected on the succeeding crops where legume are used as green manure (Ali-Khan and Zimmer, 1980). Revellin *et al.* (1993) evaluated several commercial fungicides for their effects on survival of *Bradyrhizobium japonicum*, nodulation and yield of soybean under greenhouse and field conditions. They found a small or no effect on the survival of *B. japonicum* and on the nodulation and yield of the legume plant. Isolation of rhizobial strains resistant to rhizobiophage and fungicides or both may increase legume productivity.

The present study aimed at isolation of rhizobiophage and/or fungicide-resistant *Rhizobium meliloti* and *B. japonicum* mutants. The effect of specific parent rhizobial strainss and their homologous phage and fungicide-resistant isolates or both on growth, nodulation and nitrogen fixation of alfalfa and soybean plants was examined.

MATERIALS AND METHODS

Rhizobial strains

Four strains of *Rhizobium meliloti* and six strains of *Bradyrhizobium japonicum* were used in the present study. *Rhizobium meliloti* ARC1 and ARC2 strains were obtained from Agricultural Research Center (ARC), Giza, Egypt; *R. meliloti* A2 from Rhizobia Research Laboratory, Canada; *R. meliloti* TAL380 from NifTAL project, Hawaii, USA; *B. japonicum* USDA 110, 138, and 218 from USDA Beltsville,

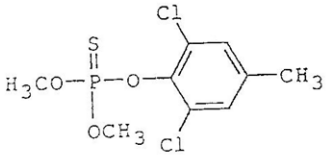
Maryland, USA; *B. japonicum* TAL397 from NifTAL project; *B. japonicum* ARC500 from ARC and *B. japonicum* UK 3407 from Rothamsted Experimental Station, London, UK. The strains were maintained on slants of yeast extract mannitol (YEM) agar medium (Vincent, 1970).

Plants

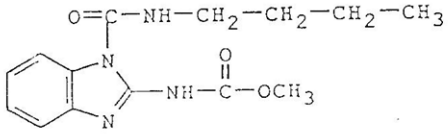
Seeds of soybean (*Glycin max, L. Merr.*) cv. Crawford and alfalfa (*Medicago sativa*) cv. El-Wady El-Gadead were kindly provided from Legume Crops Research Section, Field Crops Research Institute, ARC, Giza, Egypt. Seeds of each cultivar were chosen to be similar in size and weight as possible. The germination rate of seeds was initially determined and was found ca. 90% for each. Seeds were carefully washed with tap water to ensure the removal of any traces of pesticides possibly added for pest control during the storage period.

Fungicides

Two agrochemicals namely; Rizolex "Tolcofos-methyl", O-(2,6-dichloro-4-methylphenyl)-0, O-dimethyl-phosphorothioate) and Benlate "Benomyl", (N-1-(butylcarbomyl) benzimidazole-2-methylcarbamate) were kindly provided by the Central Pesticides Laboratory, ARC, Giza, Egypt. The following are the structural formula of the tested fungicides:



Rizolex



Benlate

Soil samples

Soil samples were collected from the rhizosphere of different leguminous and non-leguminous plants from upper Egypt. For the greenhouse experiment, clay loam soil from the top 20 cm layer was obtained from Malawi, El-Menia governorate. Soil was air dried and crushed to pass a 2.0 mm sieve, then mechanical chemical analyses were carried out according to Black *et al.*, (1965). Soil physico-chemical characteristics were: croase sand 1.7%, fine sand 21.3%, silt 25.3%, clay 51.7%,

$CaCO_3$ 2.67%, total carbon 1.84%, total nitrogen 0.197%, E.C. (dSm^{-1}) 1.2 and pH 7.2. Cations and anions (meq L^{-1}) were: Ca^{++} 2.1, Mg^{++} 4.9, K^{+} 0.06, Na^{+} 4.7, $H\bar{C}O_3$ 3.3, Cl^{-} 1.4 and SO_4 7.06.

Media

Yeast extract mannitol (YEM) broth medium (Vincent, 1970) was used for growing rhizobial strains as well as for the isolation and studying the effect of phages on survival of rhizobial cells. Congo red yeast extract mannitol agar medium (CR-YEM) was prepared by adding 10 ml of 1/400 aqueous solution of congo red to each liter of yeast extract mannitol agar medium.

Enrichment and isolation of rhizobiophages

Two phages specific for *R. meliloti* TAL 380 and *B. japonicum* USDA 218 strains were isolated and characterized by EL-Sawi (1998). Rhizobiophages were isolated from the rhizosphere soil of alfalfa and soybean plants and purified through five successive single plaque isolations. Phage titer assay was performed using the double layer technique (Adams, 1959).

Phage stock

High titers of phage stocks $>10^{10}$ pfu ml^{-1} were obtained by infecting exponentially growing liquid culture of rhizobial strains that was employed for the original phage isolation with a sufficient suspension of the phages to produce confluent lysis. The phage was stored in YEM broth at 4°C with few drops of 0.5% chloroform.

Determination of host range to rhizobiophages

Four strains of *R. meliloti* and six strains of *Bradyrhizobium japonicum* were examined for host specificity. Plates containing basal layers of agar were seeded with the different exponentially growing cultures of the tested rhizobial strains which suspended in semi-solid layer. Shortly after the agar solidified, the plates were spotted with one drop (0 . 05 ml) of phage suspension containing ca. 10^8pfu ml^{-1}. Plates were incubted at 28°C for 24-72 hr. depending upon the growing rate of the tested strains. Plates were examined for lysis (plaque formation) after the incubation period and compared with origainal rhizobial host of the phage.

Isolation of phage-resistant rhizobial isolates

One ml of a rhizobial culture containing $2x10^8$ cells ml^{-1} was mixed with 1 ml

of phage containing 4 x 10^9 particles ml^{-1}. After 15 min incubation, all rhizobia which can adsorb phage are infected. One tenth ml of the adsorption mixture was placed on the surface of an agar plate and spread uniformly with a glass rod until all of the liquid has been adsorbed by agar. The mixture was incubated for 24 hours at 28°C. A single isolated colony was picked from this plate, suspended in 1 ml of broth and a loopful restreaked on another plate. Two repeatitions of this procedure ensure isolation of pure strain of the variant free from contaminating phages as this strain showed no plaques using the double layer agar method (Adams, 1959; Ackermann and Dubow, 1987).

Lysis induction of phage-resistant rhizobial isolates

The UV induction of phage-resistant rhizobial isolates was completed according to Kowalski (1966). A 10 ml suspension of each phage-resistant rhizobial isolates containing 10^8 cells ml^{-1} was centrifuged at 6000 rpm for 30 min and the pellet re-suspended in 5 ml of 0.85% saline. Each suspension was transferred to a petri-dish and agitated by hand during irradiation with UV lamp (Philips 40 W) at a distance of 40 cm for 30 seconds. After 24 hours of incubation at 28°C in darkness, lysates were sterilized with chloroform and centrifuged at 6000 rpm for 30 min. One ml of the supernatant was used for each 5 ml of rhizobial strain suspension to estimate the titer of phage particles induced. Plaques number was scored for each phage-resistant rhizobial strain using the double layer agar-plate technique (Adams, 1959).

Effect of fungicides on growth of rhizobial strains and their phage-resistant isolates

The fungicides, Rizolex and Benlate, were tested under laboratory conditions to determine their effect on the growth of different rhizobia and their phage-resistant isolates. Rizolex was used at concentrations of 25, 50, 75, 100, and 125 ppm, while Benlate was applied at 700, 800, 900, 1000, 1100 and 1200 ppm. The concentrations were obtained by thoroughly mixing the appropriate amounts of each fungicide individually with YEM medium then, the mixture was poured into petri-dishes. The poured plates were surface dried in laminar flow. Standard inocula of parent and their phage-resistant isolates were spotted individually on plate surface by microppiette (each drop equals 20 µl). Inoculated plates were incubated at 28°C for 3-5 days.

Greenhouse experiments

Two experiments were performed under greenhouse conditions to investigate the effect of individual inoculation with parent strains, *R. meliloti* TAL 380 and *B. japonicum* USDA 218 and their phage-resistant, fungicide-resistant and phage-fungicide-resistant rhizobial isolates on nodulation, growth and N_2-fixation of alfalfa and soybean plants.

Pots of 30 cm diameter filled with 10 Kg clay loam soil. Prior planting, pots were amended with superphosphate (15.5% P_2O_5) at the rate of 100 kg fed^{-1} and potassium sulfate (48% K_2O) at the rate of 50 kg fed^{-1}. After 15 days of planting, urea (46.5% N) was applied at the rate of 15 Kg fed^{-1}. The pots were divided to four sets: 1) Parent strain (P.S.) set where pots were inoculated with 10 ml of the cultures of either *R. meliloti* TAL 380, at the level of 5.2 x 10^9 cfu pot^{-1} or *B. japonicum* USDA 218, at the level of 4.8 x 10^9 cfu pot^{-1}, 2) Phage-resitant isolate (P.R.I.) set where pots were inoculated with 10 ml of the cultures of phage-resistant isolate of either *R. meliloti* TAL 380, at the level of 4.0x10^9 cfu pot^{-1} or *B. japonicum* USDA 218, at the level of 4.3x10^9 cfu pot^{-1}, 3) Rizolex-resistant isolate (R.R.I.) set, where pots were inoculated with10 ml of the cultures of Rizolex-resistant isolate of either *R. meliloti* TAL 380, at the level of a 5.0 x 10^9 cfu pot^{-1} or *B. japonicum* USDA 218, at the level of a 6.2x10^9 cfu pot^{-1}, and 4) phage-Rizolex-resistant isolate (P.R.R.I.) set, where pots were inoculated with 10 ml of the cultures of phage-Rizolex-resistant isolate of either *R. meliloti* TAL 380, at the level of 6.2 x 10^9 cfu pot^{-1} or *B. japonicum* USDA 218, at the level of 6.5 x 10^9 cfu pot^{-1}.

Each set was divided into four subsets as follows: a) inoculated with parent strain of either *R. meliloti* TAL 380 or *B. japonicum* USA 218, b) as (a) + 10 ml of specific phage for either *R. meliloti* TAL 380 at the rate of 6.0 x 10^8 pfu pot^{-1} or *B. japonicum* USDA 218 at the rate of 8.2x10^8 pfu pot^{-1}, c) as (a) +Rizolex, and d) as (a) + specific phage for either *R. meliloti* TAL 380 or *B. japonicum* USD 218 + Rizolex. Rizolex was added to seeds of c and d subsets at the rate of 3.0g Kg^{-1} seeds (recommended dose). Six pots were left without inoculation as a control.

Alfalfa and soybean seeds were surface sterilized according to Vincent (1970). Seeds of alfalfa or soybean were planted at the rates of 12-14 and 6-8 seeds per pot, respectively. Seedlings were then thinned to 10 and 5 seedlings for alfalfa and soybean, respectively, after 10 days of planting.

A complete randomized block design was used with 6 replicates. Pots were ir-

rigated with tap water when needed. Sampling was done twice after 50 and 90 days for alfalfa, 45 and 75 days for soybean.

Plants were gently uprooted and soil particles were carefully removed by shaking and washing in tap water. Number and dry weight of nodules, dry weight and nitrogen content of plants were determined (Piper, 1950). Numbers of *R. Meliloti* and *B. japonicum* and their specific phages per gram of soil were estimated as well.

Enumeration of *R. meliloti* TAL 380 and *B. japonicum* USDA 218

Counts of *R. meliloti* and *B. japonicum* were determined in the different soil treatments by using plant infection technique using modified leonard's bottle-jar (Leonard, 1944). Ten grams of soil were suspended in 90 ml of sterile distilled water. The suspension was serially diluted (ten folds), then 1 ml of specific dilution was used for seedlings inoculation. Seeds of alfalfa and soybean were surface sterilized by 95% ethanol (1 min) and 0.2% HgCl (3 min) followed by five successive washes with sterile distilled water. Also, 600 ml of nutrient solution (Jensen, 1942) were placed into each jar before planting seeds. After germination (2-4 days), seedlings were inoculated with 1 ml suspension of a particular soil dilution. After 4-6 weeks of growth in greenhouse, plants were examined for nodulation.

The MPN of *R. meliloti* and *B. japonicum* in soil were estimated on the basis of the presence of nodules formed at a given dilution using the tables of Brokwell (1963).

Determination of rhizobiophage

After removing the plants, ten grams of soil were ground in a mortar, then mechanically suspended in 90 ml of YEM broth medium and homogenized for 10 min. After setting the particles, the suspension was centrifuged at 10,000 rpm for 15 min, the supernatant was filtered through a 0.45 μm pore size membrane filter (Minisart P). The soil filtrates were assayed for the presence of phage by the plaque formation on *R. meliloti* TAL 380 and *B. japonicum* USDA 218 as an indicator strains using the standard double-layer technique (Adams, 1959). Plates were incubated at 28°C for 24 hours, then plaque forming units were counted.

Statistical analysis

Data obtained were subjected to statistical analysis using analysis of variance according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Isolation of phage-resistant rhizobia

Five phage-resitant isolates of rhizobia strains were isolated. These isolates were found to be resistant to their respective phages. Three representative phage-resistant rhizobial isolates (*R. meliloti* AFC 1 and TAL 380 and *B. japonicum* USDA 218) were selected for inoculation experiment later.

Colonies of some phage-resistant isolates were typically rough, others were smooth or intermediate; they were opaque while others were transparent, whereas other showed yellow or pink color (Table 1). These morphological characteristics of phage-resistant isolates were similar to their parent strains for some isolates but not the same for others. Cells of the isolated colonies were gram-negative and short rods similarly to their parent strains (Table 1).

Table 1. Morphological changes in rhizobial colonies in parent and their phage-resistant isolates.

Rhixobial strains	Morphological characterization					
	Parent strains			phage - resistant isolates		
	Appearance	Transparency	Color	Appearance	Transparency	Color
B. japonicum: USDA 218	Smooth	Transparent	White	Rough	Opaque	Pink
R. Meliloti : ARC 1: TAL 380	Smooth Smooth	Transparent Transparent	White White	Intermediate Smooth	Transparent Transparent	Yellow Yellow

These data are similar to those of Kleczkowska (1971) and Abdel-Wahab (1977) who reported that some phage-resistant colonies did not differ in appearance from those of the parent strains, while others differed in various aspects. Abebe *et al.*, (1992) isolated numerous phage-resistant variants from the wild type of *B. japonicum* USDA 123.

Lysis induction of phage-resistant isolates

The previously chosen three phage-resistant isolates were irradiated by UV lamp. Data indicated that all rhizobial strains might be highly-resistant and no cell lysis occurred.

Therefor, it was clear that the UV-dose used had no effect on the susceptibility of the tested phage-resistant isolates of rhizobia. The obtained results are in

agreement with those of Abdel-Wahab (1977) who found that UV-induction of four *R. trifolii* phage-resistant isolates produced no plaques. However, another eleven phage-resistant isolates were lysogenic and produced pfu ranged from 10^4 to 10^6 ml^{-1}. Also, Abebe *et al.*, (1992) isolated numerous phage-resistant variants from the wild type of *B. japonicum* USDA 123 and found that two of them were lysogenic.

Effect of fungicides on growth of rhizobial strains and their phage-resistant isolates.

Data recorded in Table (2) showed that the rhizobial strains and their phage-resistant isolates exhibited marked variations in their responses to different concentrations of the tested fungicides depending on the strain and the fungicide. All rhizobial strains and their phage-resistant isolates showed good growth at 25 ppm Rizolex with no variations among them. Increasing the concentration of fungicide to 50 and 75 ppm was accompanied by parallel decreases in the growth of all tested rhizobia and their phage-resistant isolates.

Table 2. Effect of fungicides on growth or rhizobial strains and phage-resistant isolates on "YEM" solid medium.

Rhizobial strains and phage-resistant isolates	Concentration of fungicides (ppm)										
	Rizolex					Benlate					
	25	50	75	100	125	700	800	900	1000	1100	1200
P.S. of *B. japonicum* USDA 218	+++	++	+	-	-	+++	+++	++	+	+	-
P.R.I. of *B. japonicum* USDA	+++	++	+	-	-	+++	+++	++	+	-	-
P.S. of *R. meliloti* ARC 1	+++	++	+	-	-	+++	+++	++	+	+	-
P.R.I. of *R. meliloti* ARC 1	+++	++	+	-	-	+++	+++	++	+	-	-
P.S. of *R. meliloti* TAL 380	+++	++	+	+	-	+++	+++	++	+	+	-
P.R.I. of *R.Meliloti* TAL 380	+++	++	+	-	-	+++	+++	++	+	-	-

Where: +++ abundant growth, ++ moderate growth, + slight growth, - no growth, P.S. parent strains and P.R.I. Phage-resistant isolate.

When the tested organisms exposed to Rizolex at the level of 100 ppm, all failed to grow except *R. meliloti* TAL 380 (parent), while the highest level of Rizolex (125 ppm) was lethal for all tested strains.

Concerning the other fungicide (Benlate), it was clear that it had less deleterious effect on the tested organisms as compared to Rizolex. All rhizobial strains and their phage-resistant isolates showed good growth at both 700 and 800 ppm levels. Increasing the fungicide concentration to 900 ppm decreased the growth rate of all tested organisms and such decreases were more pronounced by the application of

1000 ppm of Benlate. All rhizobial strains and their phage-resistant isolates failed to grow at 1100 ppm of the fungicide with the exception of the parent strains of *B. japonicum* USDA 128 and *R. meliloti* TAL 380 and ARC1. The highest level of Benlate (1200 ppm) was lethal for all tested strains. These results are in harmony with those reported by Diatoff (1986) and Kapusta and Rouwenhorst (1973) who found that fungicides had an inhibitory effect on the growth of different strains of rhizobia grown in pure cultures. The depressive effect of the fungicides depends on the type of the fungicide, its concentration, the strain of rhizobia and pH of the medium.

Alfalfa pot experiment

Plant nodulation

Data given in Table (3) showed that the uninoculated plants had the lowest numbers and biomass of nodules among all plants under investigation. Nodulation of

Table 3. Nodulation status of alfalfa plants as affected by inoculation with parent *R. meliloti* strain (P.S.) and its phage (P.R.I.) as well as fungicde-resistant isolates (R.R.I.) and phage-fungicide resistant isolates (P.R.R.I.).

Treatments			Days post planting			
			Number of nodules (pot^{-1})		Dry weight of nodules (mg pot^{-1})	
			50	90	50	90
A		Uninoculated Control	24	28	34	36
	1	P.S. TAL 380	134	148	128	140
	2	P.S. TAL 380 + Phage TAL 380	97	105	99	110
	3	P.S. TAL 380 + Rizolex	118	130	113	125
	4	P.S. TAL 380 + Phage TAL 380 + Rizolex	94	102	89	95
B	5	P.R.I. TAL 380	120	142	114	134
	6	P.R.I. TAL 380 + Phage TAL 380	108	114	102	116
	7	P.R.I. TAL 380 + Rizolex	104	112	97	111
	8	P.R.I. TAL 380 + Phage TAL 380 + Rizolex	98	108	100	114
C	9	R.R.I. TAL 380	123	135	114	128
	10	R.R.I. TAL 380 + Phage TAL 380	107	115	103	117
	11	R.R.I. TAL 380 + Rizolex	116	122	111	123
	12	R.R.I. TAL 380 + Phage TAL 380 + Rizolex	105	112	101	109
D	13	P.R.R.I. TAL 380	117	133	113	126
	14	P.R.R.I. TAL 380 + Phage TAL 380	104	112	102	110
	15	P.R.R.I. TAL 380 + Rizolex	111	116	109	118
	16	P.R.R.I. TAL 380 + Phage TAL 380 + Rizolex	99	105	101	97
		L.S.D. 0.05	3.5	4.1	3.6	7.0
		0.01	4.8	5.5	4.8	9.4

Where: A= pots inoculated with parent strain of *R. meliloti* TAL 380; B= A + *R. meliloti* TAL 380 phage; C= A + Rizolex; D= A + *R. meliloti* TAL 380 phage + Rizolex.

uninoculated plants is due to the presence of alfalfa native rhizobia in the soil used. Generally, inoculation of alfalfa enhanced the nodulation process and led to significant increases in both number and dry weight of nodules. Inoculation of alfalfa with the parent strain TAL 380 alone resulted in the best nodulation among all treatments where 148 nodules pot^{-1} with dry weight of 140 mg pot^{-1} were recorded after 90 days of planting. These values represented increases of 429 and 289% over uninoculated plants. Alfalfa plants inoculated individually with either P.R.I., R.R.I. or P.R.R.I. -TAL 380 recorded lower numbers and dry weights of nodules compared to those plants inoculated with the parent strain TAL 380. The reduction in number and dry weight of nodules ranged from 4 to 10% after 90 days of planting. Addition of phage, Rizolex or both to the parent strain TAL 380 or any of its resistant strains (P.R.I., R.R.I. or P.R.R.I.- TAL 380), resulted in more decreases in both nodulation parameters. The average reductions in nodule number and biomass ranged from 21 to 25 and 17 to 23%, respectively compared to plants inoculated with P.S. strain only. Addition of phage TAL 380, Rizolex or both to the phage-Rizolex resistant isolate (P.R.R.I.) showed the highest rate of reduction in both nodule number (25%) and nodule biomass (23%) after 90 days of growth.

Plant dry weight

Data presented in Table (4) revealed that the uninoculated plants accumulated the least amounts of plant biomass being 7.62 g pot^{-1} after 90 days of planting. Inoculation of alfalfa led to significant increases in the plant dry matter production. These increases ranged from 10 to 35% compared to the uninoculated plants. Among inoculated treatments, the rate of increases, however, depended obviously on the type of inoculum and if it was applied with or without phage, Rhizolex or both. Inoculation with parent strain (P.S.-TAL 380) accumulated more amounts of plant materials and recorded the highest plant dry matter among all inoculated treatments being 10.25g pot^{-1} after 90 days of planting. Application of any of resistant rhizobial isolates used (P.R.I., R.R.I. or P.R.R.I.-TAL 380) or addition of phage, Rizolex or both to the inoculum reduced plant dry weight by 18% compared to plants received the parent strain (P.S.-TAL 380) after 90 days of planting. These decreases were depended on resistant isolates used with or without phage, Rizolex or both. Such findings were in accordance with those of nodulation and indicated that the rhizobial parent strain (P.S.-TAL 380) was more efficient in nodulation and enhanced the growth of alfalfa plant compared to their resistant isolates. Addition of phage, Rizolex or both to the inoculated plants negatively affected both nodulation and plant growth. This negative effect recorded 4-11% reduction in plant biomass compared to the parent strain

(P.S.) after 90 days of plant growth. The inferior plant biomass was obtained when the phage, Rizolex or both were added to the phage-Rizolex resistant isolate (P.R.R.I.) treatment.

Table 4. Dry weight nitrogen control of alfalfa plants as affected by inoculation with parent *R. meliloti* strain (P.S.) and its phage (P.R.I.) as well as fungicde-resistant isolates (R.R.I.) and phage-fungicide resistant isolates (P.R.R.I.).

Treatments			Number of nodules (pot^{-1})		Dry weight of nodules (mg pot^{-1})	
			Days post planting			
			50	90	50	90
A		Uninoculated Control	1.14	7.62	59	330
	1	P.S. TAL 380	1.87	10.25	120	533
	2	P.S. TAL 380 + Phage TAL 380	1.55	9.88	90	454
	3	P.S. TAL 380 + Rizolex	1.78	10.10	107	449
	4	P.S. TAL 380 + Phage TAL 380 + Rizolex	1.44	9.60	81	427
B	5	P.R.I. TAL 380	1.70	10.17	107	524
	6	P.R.I. TAL 380 + Phage TAL 380	1.62	9.65	99	439
	7	P.R.I. TAL 380 + Rizolex	1.58	9.35	91	411
	8	P.R.I. TAL 380 + Phage TAL 380 + Rizolex	1.50	8.36	74	322
C	9	P.R.I. TAL 380	1.82	10.20	108	515
	10	P.R.I. TAL 380 + Phage TAL 380	1.57	9.60	88	434
	11	P.R.I. TAL 380 + Rizolex	1.69	10.10	99	499
	12	P.R.I. TAL 380 + Phage TAL 380 + Rizolex	1.52	9.50	83	413
D	13	P.R.R.I. TAL 380	1.66	10.15	98	505
	14	P.R.R.I. TAL 380 + Phage TAL 380	1.53	9.95	85	453
	15	P.R.R.I. TAL 380 + Rizolex	1.61	10.05	94	494
	16	P.R.R.I. TAL 380 + Phage TAL 380 + Rizolex	1.45	9.60	78	418
		L.S.D. 0.05	0.09	3.6	7.0	0.09
		0.01	0.12	4.8	9.4	0.12

Where: A= pots inoculated with parent strain of *R. meliloti* TAL 380; B= A + *R. meliloti* TAL 380 phage; C= A + Rizolex; D= A + *R. meliloti* TAL 380 phage + Rizolex.

Plant nitrogen content

The changes in nitrogen content of alfalfa plants are given in Table (4). Generally, inoculation with rhizobia strains had beneficial effects on plant nitrogen content. Inoculation with the parent *R. meliloti* strain TAL 380 alone gave the highest plant nitrogen content among all inoculated treatments. After 90 days of planting, the nitrogen contents of plants inoculated with P.S.-TAL 380 (parent strain) were 102, 103 and and 106% higher than plants inoculated individually with its resistant

isolates P.R.I., R.R.I. and P.R.R.I.-TAL 380, respectively. Addition of phage, Rizolex or both to either parent strain (P.S.-TAL 380) or its resistant isolates significantly decreased the amounts of nitrogen accumulated in the growing plants and these decreases were 16-27% compared to plants inoculated with parent strain (P.S. TAL 380). It was clear that plants received Rizolex recorded the least plant nitrogen contents against the other treatments. This may be due to the harmful effect of the applied fungicide on the growth, multiplication and efficiency of the tested rhizobia. It was noticed that plant nitrogen contents were positively correlated with nodulation and dry weights of plants.

In general, the injurious effects of phage, Rizolex or both on alfalfa plants were more pronounced with plant nitrogen content (16-27%) and nodule biomass (17-23%) than plant biomass (4-11%) compared to the parent strain (P.S.). Furthermore, this depression influence on plant biomass, nodule biomass and nitrogen content reached 11, 23 and 27%, respectivily, when the phage, Rizolex or both were added to the phage-fungicide resistant isolate (P.R.R.I.) inoculated treatment.

Soybean pot experiment
plant nodulation

The results (Table 5) showed that the uninoculated plants had the lowest numbers of nodules among all plants under investigation being 10 and 14 nodules plant^{-1} after 45 and 75 days of planting, respectively. The corresponding values of nodule dry weight were 76 and 84 mg plant^{-1}. Nodulation of uninoculated plants is due to the presence of soybean native rhizobia in the soil used. Inoculation of soybean plants promoted the nodulation process and led to marked increases in both number and dry weight of nodules. Inoculation of soybean with the parent *B. japonicum* strain USDA 218 alone resulted in the best nodulation among all treatments. The number of nodules were 30 nodules plant^{-1} with dry weight of 257 mg plant^{-1} after 75 days of planting. Soybean plants inoculated individually with either P.R.I., R.R.I. or P.R.R.I.-USDA 218 without any additives recorded lower number and dry weight of nodules compared to plants inoculated with the parent strain USDA 218. The reduction in number and dry weight of nodules ranged from 28 to 43% after 75 days of planting. The highest reductions in nodule number (43%) and nodule biomass (28%) were scored when phage USDA 218, fungicide or both were added to the phage-fungicide resistant isolate treatment P.R.R.I. - USDA 218 after 75 days of planting.

Table 5. Nodulation of soybean plants as affected by inoculation with parent *B. japonicum* strain (P.S.) and its phage (P.R.I.) as well as fungicide-resistant isolates (R.R.I.) and phage-fungicide resistant isolates (P.R.R.I)

Treatments			Number of nodules (pot^{-1})		Dry weight of nodules (mg pot^{-1})	
			Days post planting			
			45	75	45	75
		Uninoculated Control	10	14	76	84
A	1	P.S. USDA 218	25	30	145	257
	2	P.S. USDA 218 + Phage USDA 218	18	23	99	218
	3	P.S. USDA 218 + Rizolex	20	25	107	227
	4	P.S. USDA 218 + Phage USDA 218 + Rizolex	17	20	94	212
B	5	P.R.I. USDA 218	12	19	84	194
	6	P.R.I. USDA 218 + Phage USDA 218	11	17	80	182
	7	P.R.I. USDA 218 + Rizolex	17	22	96	208
	8	P.R.I. USDA 218 + Phage USDA 218 + Rizolex	10	16	74	178
C	9	R.R.I. USDA 218	24	28	136	238
	10	R.R.I. USDA 218 + Phage USDA 218	16	21	91	215
	11	R.R.I. USDA 218 + Rizolex	20	25	108	225
	12	R.R.I. USDA 218 + Phage USDA 218 + Rizolex	15	19	90	210
D	13	P.R.R.I. USDA 218	14	17	89	185
	14	P.R.R.I. USDA 218 + Phage USDA 218	12	15	82	172
	15	P.R.R.I. USDA 218 + Rizolex	16	20	93	214
	16	P.R.R.I. USDA 218 + Phage USDA 218 + Rizolex	13	16	84	175
		L.S.D. 0.05	3.5	3.1	6.7	10.7
		0.01	4.7	4.1	9.0	14.3

Where: A= pots inoculated with parent strain of *B. japonicum* USDA 218, B= A + *B. japonicum* USDA 218 phage, C= A + Rizolex and D= A + *B. japonicum* USDA 218 phage + Rizolex.

Plant dry weight

The control plants accumulated the least amounts of plant materials (Table 6). Inoculation of soybean led to marked increases in plant biomass. These increases ranged from 13 to 44% compared to the uninoculated plants after 75 days of planting. Among inoculated treatments, the rate of increases depended on the type of inoculum and on presence of phage, Rhizolex or both. Inoculation with parent strain (P.S.-USDA 218) alone accumulated more plant materials and recorded the hgiehst plant biomass being 7.28 g $plant^{-1}$. Application of any of resistant isolates (P.R.I., R.R.I. or P.R.R.I.-USDA 218) or addition of phage, Rizolex or both to the inoculum decreased plant biomass compared to plants received the parent strain (P.S.-USDA 218). Such decreases ranged from 7 to 17%. Application of phage, Rizolex or both to

the phage-fungicide resistant isolate (P.R.R.I.) treatment gave the highest reduction (17%) in biomass. These data were similar to those of nodulation and suggested that the rhizobial parent strain (P.S.-USDA 218) was more efficient in nodulation and improved the growth of soybean plant compared to its resistant isolates. Addition of phage, Rizolex or both to the inoculated plants negatively affected nodulation and plant growth.

Plant nitrogen content

Inoculation with rhizobia strains significantly increased plant nitrogen content over control plants (Table 6). Moreover, inoculation with the parent *B. japonicum* strain USDA 218 alone gave the highest plant nitrogen uptake among all treatments. Nitrogen contents of plants inoculated with P.S.-USDA 218 (parent strain) were increased by 30, 3 and 31% after 75-days of planting compared with plants inoculated individually with its resistant P.R.I., R.R.I. and P.R.R.I. -USDA 218 isolates, respectively. Application of phage, Rizolex or both to either parent strain (P.S.-USDA 218) or its resistant isolates decreased the amounts of nitrogen accumulated from 12 to 31% compared to plants inoculated with parent strain (P.S.-USDA 218) after 75 days of planting. Reduction in nitrogen content was equal (31%) in soybean plants received either phage resistant isolate (P.R.I.) or phage-fungicide resistant isolate (P.R.R.I.). It was clear that plants received Rizolex recorded the least values of plant nitrogen content against the other treatments at boh periods of plant growth. Application of phage, Rizolex or both to the inoculated soybean plants negatively affected nodulation, plant biomass and nitrogen content.

In general, data presented indicated that inoculation had positive effects on root nodulation as indicated by significant increases in number and dry weight of nodules. The dry weight of plants was positively affected also with rhizobial inoculation. A similar trend of plant nitrogen content was also noticed. Alagawadi and Gaure (1988) mentioned that inoculation with *Rhizobium* increased the nodulation and nitrogenase activit y of check pea. The results obtained in the present study may point out to the importance of inoculation of both alfalfa and soybean plants with their selected and specific rhizobial strains in spite of the presence of indigenous rhizobia which is supported by the opinion of Young *et al.* (1986).

Inoculation of soil with rhizobiophages seemed to be an important factor affecting the nodulation, plant growth and nitrogen content of alfalfa and soybean plants under study. The soil treatments received phage indicated that the reduction in rhizobial numbers was associated with a decrease in number and dry weight of no-

dules as well as plant weight and plant nitrogen content. These data are in accordance with those of Emam (1989) and Ahmad and Morgan (1994), they reported that inoculation with phage and its homologous rhizobia significantly decreased the number and dry weigh of nodules. The effect of rhizobiophage in depressing nodulation by tested rhizobial strains and subsequently plant growth associated with decreases in number of cells at the plant root surface (Hashem and Angle, 1988 and 1990). The harmful effect of phages could be attributed to their indirect action on the plant. The obtained results do not contradict the pervious findings, since Vidor and Miller (1980) found that phage susceptible strains of rhizobia suppressed nodulation and nitrogenase activity.

It is also concluded that plants received Rizolex recorded the least nodulation, plant biomass and nitrogen content in comparison to the other treatments. This may due to the harmful effect of the applied fungicide on growth and efficiency of the tested rhizobial strains. These data are in harmony with those obtained by other investigators (Catroux and Arnaud, 1991; Revellin *et al.*, 1993) who found that fungicides decreased the survival of rhizobial cells which is accompanied with parallel decreases in nodulation, growth and nitrogen content of the growing plants.

It was observed that alfalfa and soybean individually inoculated with the phage-resistant isolates of both *R. meliloti* TAL 380 and *B. japonicum* USDA 218 had the lowest nodulation, plant growth and plant nitrogen contents compared to the corresponding plants inoculated with the parent strains. These results are in agreement with those reported by Abdel-Wahab (1977) and Patel (1978) who found that phage-resistant isolates differed in their effectiveness and/or failure to form nodules with host legumes although they have been obtained from effective nitrogen fixing parent strains. Uchiumi *et al.*, (1995) reported that constructed lysogenic rhizobiophage of *R. leguminosarum* bv. *trifolii* formed completely functional nodules. There is variation among parent strains and their phage-resistant isolates in effectiveness. This may be due to that the phage may destroy the nodule bacteria and/or increase the proportion of ineffective strains in the soil (Patel, 1978).

Counts of *R. meliloti* TAL 380 and its homologous phage

Results revealed that numbers of rhizobia, in all treatment, markedly decreased after 50 days of planting compared to initial population (Fig.1). More reductions in rhizobial counts were recorded after 90 days after planting. In uninoculated soil, the number of rhizobial cells was $6.5x10^5$ cfu g^{-1} soil at zero time and decreased to $3.0x10^5$ and $6.5x10^4$ cfu g^{-1} soil after 50 and 90 days of planting, respectively. This trend was also observed in all inoculated treatments. Remarkable

decreases in soil population of *R. meliloti* strain TAL 380 (parent strain) and its resistant isolates were observed when the phage was applied along with its homologus rhizobia. Generally, the number of rhizobial cells in inoculated treatments decreased from $2x10^7$ cfu g^{-1} soil at the beginning to only $6.8x10^3$ - $4.2x10^5$ cfu g^{-1} soil after 90 days of planting.

Concerning the number of the homologous phage particles of *R. meliloti* strain TAL 380 and their resistant isolates (Fig. 1), it was noticed that in uninoculated treatment, the number of phage particles was very low at the beginning being $46x10^3$ pfu g^{-1} soil. No marked changes in titer of phage particles were recorded after 50 and 90 days of planting. These numbers approximated $48x10^3$ and $36x10^3$ pfu g soil-1 after both periods of growth. In inoculated treatments, the phage numbers markedly increased at 50 days period then sharply decreased. The numbers of phage particles, however, were higher in treatments received phage compared to those without phage. This trend was true at both periods of estimation.

Counts of *B. japonicum* USDA 218 and its homologous phage

The results illustrated in Fig. (2) showed that numbers of rhizobia sharply decreased after 45 days of planting compared to initial densities. The reduction in rhizobial counts was increased after 75 days of planting. The decreases in population of the tested rhizobia depended on the strain used (parent strain or its resistant isolates) and if the rhizobial inoculum used alone or in combination with phage, Rizolex or both. Clear reductions in parent *B. japonicum* strain USDA 218 population and its resistant isolates were recorded when the phage was applied alone with its homologous rhizobia. The population of rhizobia in inoculated treatments reduced from $6.2x10^7$ cfu g^{-1} soil at the beginning to 2.1×10^4 - 6.5×10^5 cfu g^{-1} soil at the end of the experiment.

With regard to the number of the homologous phage particles of *B. japonicum* strain USDA 218 and its resistant isolates, it was observed that, in uninoculated treatment, the number of phages was very low at initial estimation being $48x10^3$ pfu g^{-1} soil (Fig. 2). The changes in titer of phage particles were not significant at both periods of estimation. The numbers estimated by $52x10^3$ and $38x10^3$ pfu g^{-1} soil after 45 and 75 days of planting, respectively. Inoculation markedly increased the phage particles numbers at 45-day period then sharply decreased after 75 days of planting. Furthermore, the number of phages were higher in treatments received phage compared to those without phage.

The decreases in rhizobia numbers in soil may be due to that the rhizobiophage is one factor frequently proposed as a mechanism of change in rhizobial population and it has the ability to limit host number (Patel, 1978). The action of phages is at-

tributed to the lysis of rhizobial cell, thereby, reducing the number of rhizobia (Evans *et al.*, 1979). Thus, in the present study, marked decreases in rhizobial counts in soil were observed when the phage was applied along with homologous rhizobia. These results are in agreement with the findings of previous studies (Barnet, 1980). Also, it was observed that the reduction in rhizobial numbers increased with increasinge phage numbers. There is an agreement among certain researchers that the reduction rate of rhizobia is associated with phage concentration (Barnet, 1980).

In conclusion, the annual inoculation of differnt cultivated legumes with efficient and highly phage-resistant rhizobial strains should be taken into consideration to enhance nodulation and maximizing the legumes productivity.

Table 6. Biomass and nitrogen content of soybean plants as affected by inoculation with parent *B. japonicum* strain (P.S.) and its phage (P.R.I.) as well as fungicide-resistant isolates (R.R.I.) and phage-fungicide resistant isolates

Treatments			Biomass (g plant^{-1})		Nitrogen content (mg N plant^{-1})	
			Days post planting			
			45	75	45	75
		Uninoculated Control	1.75	5.05	50	149
A	1	P.S. USDA 218	2.77	7.28	97	273
	2	P.S. USDA 218 + Phage USDA 218	2.35	6.99	80	253
	3	P.S. USDA 218 + Rizolex	2.60	7.18	87	246
	4	P.S. USDA 218 + Phage USDA 218 + Rizolex	2.25	6.64	75	224
B	5	P.R.I. USDA 218	1.82	6.25	54	190
	6	P.R.I. USDA 218 + Phage USDA 218	1.79	5.95	52	177
	7	P.R.I. USDA 218 + Rizolex	2.20	6.85	72	223
	8	P.R.I. USDA 218 + Phage USDA 218 + Rizolex	1.79	5.74	48	166
C	9	R.R.I. USDA 218	2.75	7.20	96	265
	10	R.R.I. USDA 218 + Phage USDA 218	2.18	6.80	70	226
	11	R.R.I. USDA 218 + Rizolex	2.65	7.10	89	240
	12	R.R.I. USDA 218 + Phage USDA 218 + Rizolex	2.05	6.35	63	200
D	13	P.R.R.I. USDA 218	1.94	6.40	59	187
	14	P.R.R.I. USDA 218 + Phage USDA 218	1.80	5.70	30	173
	15	P.R.R.I. USDA 218 + Rizolex	2.15	6.55	68	213
	16	P.R.R.I. USDA 218 + Phage USDA 218 + Rizolex	1.90	5.92	57	179
		ex	0.12	0.17	8.77	9.06
		L.S.D. 0.05	0.16	0.22	11.80	12.20

Where: A= pots inoculated with parent strain of *B.japonicum* USDA 218, B= A + *B. japonicum* USDA 218 phage, C= A + Rizolex and D= A+ *B. japonicum* USDA 218 phage + Rizolex.

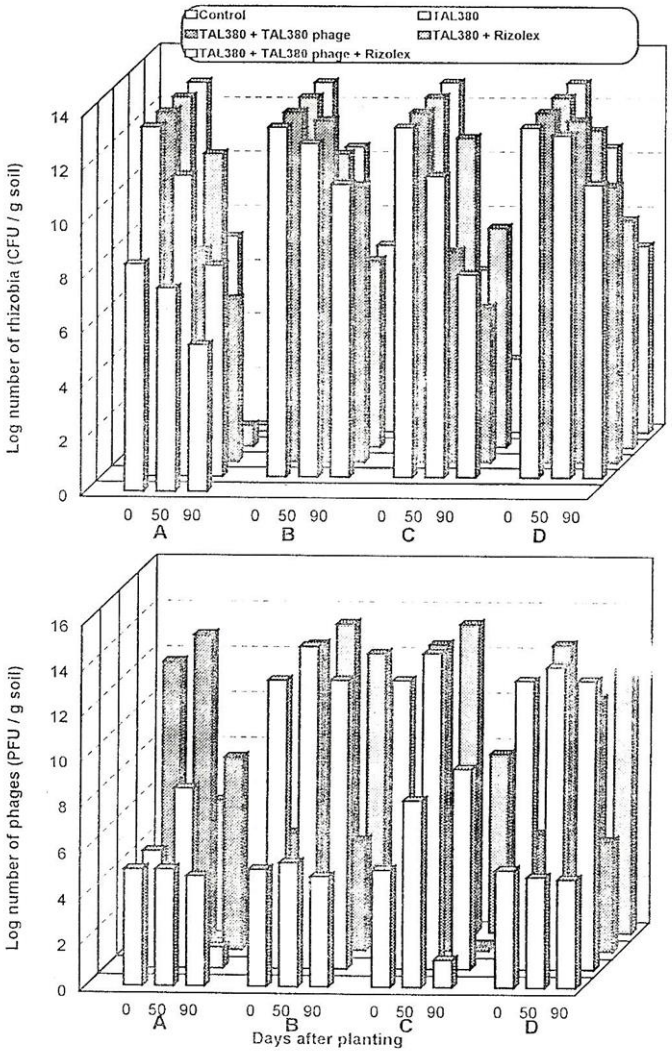


Fig. 1. Periodical change in counts of *R. meliloti* and its homologous phage in soil. A= pots inoculated with parent strain of *R. meliloti* TAL 380, B= A + *R. meliloti* TAL 380 phage, C= A + Rizolex and D= A + *R. meliloti* TAL 380 phage + Rizolex.

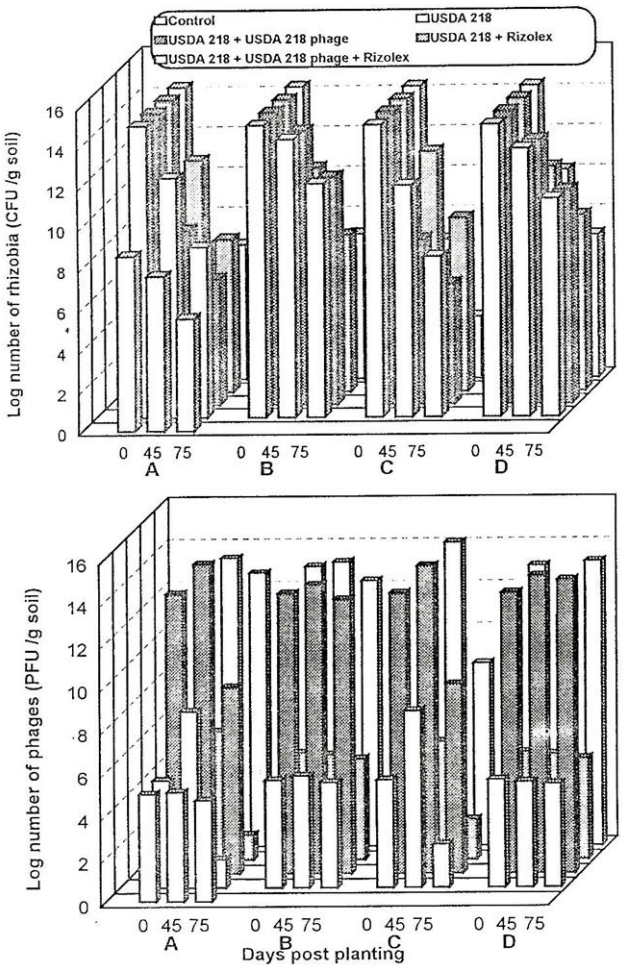


Fig. 2. Periodical change in counts of *B. japonicum* and its homologous phage in soil. A= pots inoculated with parent strain of *B. japonicum* USDA 218, B= A + *B. japoncium* USDA 218 phage, C=A + Rizolex and D= A + *B. japonicum* USDA 218 phage + Rhizolex.

REFERENCES

1. Abdel-Wahab, S.M. 1977. Genetic control of nitrogen fixantion in *Rhizobium trifolii strains*. Ph. D.Thesis, Fac. of Agric. (Microbiology), Cairo Univ., Cairo. Egypt.
2. Abebe, H.M., M.J. Sadowsky, B.K. Kinkle, and E.L. Schmidt. 1992. Lysogeny in *Bradyrhizobium japonicum* and its effect on soybean nodulation. Appl. Environ. Microbiol. 58 (10): 3360-3366.
3. Ackermann, H., and M. Dubow. 1987. Viruses of Prokaryotes.I. General Properties of Bacteriophages. CRC. Press. Inc. Florida. U.S.A. , pp. 49-50.
4. Adams, M.H. 1959. Bacteriophages. Wiley-Interscience Publishers, Inc., New York.
5. Ahmad, M.H., and V. Morgan. 1994. Characterization of cowpea (*Vigna unguiculata*) rhizobiophage and its effect on cowpea nodulation and growth. Biol. Fertil. Soils. 18:297-301.
6. Alagawadi, A.R., and A.C. Gaure. 1988. Associative effect of *Rhizobium* and phosphate solubilizing bacteria on the yield and nutrient uptake of chickpea. Plant and Soil. 105:241-246.
7. Ali-Khan, S.T., and R.C. Zimmer. 1980. Production of field peas in Canada. Publ. No. 710, Canada Department of Agriculture, Ottawa, Ontario.
8. Barnet, Y.M. 1980. The effect of rhizobiophages on population of *R. trifolii* in the root zone of clover plants. Can. J. Microbiol. 26:572-576.
9. Brokwell, J. 1963. Accuracy of plant-infection technique counting population of *Rhizobium trifolii*. Appl. Microbiol. 11:377-383.
10. Catroux, G., and F. Arnaud .1991. Compatibility of a soybean peat inoculant with some seed applied fungicides and microgranular insecticides. Toxic. Environ. Chem. 30:229-239.
11. Diatoff, A. 1986. Compatibility of systemic and non-systemic fungicides with *Rhizobium japonicum* applied to soybean seed. Soil Biol. Biochem. 18 (1): 121-122.
12. El-Sawi, M.M. 1998. Further studies on rhizobiophages in Egyptian soils. Ph. D. Thesis, Fac. of Agric. (Microbiology), Cairo Univ., Giza, Egypt.
13. Emam, Nadia, F. 1989. Growth and root nodulation of clover as affected by the specific rhizobiophage. Egypt. J. Appl. Sci. 4 (3): 454-462.

14. Evans,J., Y. M., Barnet, and J.M. Vincent. 1979. Effect of a bacteriophage on the colonization and nodulation of clover roots by a strain of *Rhizobium trifolii.* Can. J. Microbiol. 25: 968-973.

15. Hashem, F.M., and J.S. Angle. 1988. Rhizobiophage effect on *Bradyrhizobium japonicum*, nodulation and soybean growth. Soil Biol. Biochem. 20: 69-73.

16. Hashem, F.M., and J.S. Angle. 1990. Rhizobiophage effect on nodulation, nitrogen fixation and yield of field-grown soybean (*Glycine max* L.Merr). Biol. Fertil. Soils. 9:330-334.

17. Jensen, H.L. 1942. Nitrogen fixation in leguminous plant. I-General characters of root nodual bacteria isolated from species of *Medicago* and *trifolium* in Australia. Proc. Linn. Soc. New South Wale. 67: 98-108.

18. Kapusta, G., and D.L. Rouwenhorst. 1973. Interaction of selected pesticides and *Rhizobium japonicum* in pure culture and under field conditions. Agronomy J. 65:112-115.

19. Kleczkowska, J. 1971. Genetically changes in *Rhizobium* bacteria and in their bacteriophages during coexistence. Plant and Soil. Sepecial Volume. pp 47-56.

20. Kowalski, M. 1966. Lysogeny in *R. meliloti.* Acta Microbiol. Polon. 15:119-128.

21. Leonard, I.T. 1944. Method of testing bacterial cultures and results of tests of commercial inoculants. U.S.D.A. Circ. No. 703, Washington.

22. Patel, J.J. 1978. Symbiotic effectiveness of phage resistant mutant of two strains of lotus rhizobia. Plant and Soil 49:251-257.

23. Piper, C.S. 1950. Soil and Plant Analysis. 1st Ed. Interscience Publishers, N.Y.

24. Revellin, C., P. Leterme, and G. Catroux. 1993. Effect of some fungicide seed treatments on the survival of *Bradyrhizobium japonicum* and on the nodulation and yield of soybean (*Glycin max.* (L) Merr.). Biol. Fertil. Soils 16:211-214.

25. Snedecor, G.W., and W.G. Cochran. 1980. Statistical Methods. Seventh. Ed., Iowa State Univ. Press, Ames. Iowa, USA, pp. 255-269.

26. Uchiumi, T., S. Higashi, and M. Abe. 1995. Nodule formation by clover-*Rhizobium* carrying chromosomal *nod* genes. J. Gen. Appl. Microbiol. 41 (1): 11-22.

27. Vidor, C., and R.H. Millerm. 1980. Relative saprophytic competence of *R. japonicum* strains in soils as determined by the quantitative fluorescent antibody technique (FA). Soil Biol. Biochem. 12: 483-487.

28. Vincent, J.M. 1970. A manual for the practical study of root nodule bacteria. In: International Biological Program. Handbook No. 15. Blackwell Scientific Publications. Ltd., Oxford and Edinburgh. U.K.

39. Young, N.R., D.M. Hughes, and L.R. Mytton. 1986. The response of white clover to different strains of *Rhizobium trifolii* in hill land reselling. A second trail. Plant and Soil. 94:279-284.

تأثير الريزوبيوفاج ومبيدات الفطريات على ريزوبيا البرسيم الحجازى وبرادى ريزوبيا فول الصويا وتكوين العقد والنمو لنباتات البرسيم الحجازى وفول الصويا

أحمد فاضل الشهابى١، نادية فهمى امام١، محمد السيد حسن٢،
مجدى محمد الصاوى٢، معوض كامل زهره١

١ قسم الميكروبيولوجى - كلية الزراعة - جامعة القاهرة - الجيزة - مصر.

٢ قسم بحوث الميكروبيولوجيا الزراعية - معهد بحوث الاراضى والمياه والبيئة - مركز البحوث الزراعية - الجيزة - مصر.

أجرى هذا البحث بغرض دراسة تأثير الريزوبيوفاج والمبيدات الفطرية مثل الريزولكس والبنلت على البكتريا العقدية ريزوبيا البرسيم الحجازى وبرادى ريزوبيا فول الصويا وتكوين العقد وتثبيت النتروجين الجوى ونمو كل من نباتات البرسيم الحجازى وفول الصويا. وقد تم عزل طفرات من الريزوبيا وبرادى ريزوبيا المقاومة للفاج أو المبيد الفطرى او لكلاهما معا. وكانت حساسية كل من سلالات الريزوبيا والبرادى ريزوبيا للفاج مختلفة. هذا وأعطى الريزوبيوفاج الفعال ضد سلالات الريزوبيا مجاميع فاج رائقة ومستديرة ومختلفة فى الحجم. واختلفت سلالات الريزوبيوم والعزلات المقاومة للفاج فى درجة استجابتها للتركيزات المختلفة من مبيدات الفطريات المستعملة. واعتمد ذلك على السلالة ونوع المبيد الفطرى المستخدم وتركيزة. هذا وأدى استخدام التركيز ٧٥ جزء فى المليون من الريزولكس الى تقليل نمو سلالات الريزوبيا وكذلك عزلاتها المقاومة للفاج. ولم يلاحظ اى نمو لسلالات الريزوبيا عند استخدام الجرعة المميتة من الريزولكس ١٢٥ جزء فى المليون ونمت جيدا كل سلالات الريزوبيا والعزلات المقاومة للفاج عند تركيز ٧٠٠، ٨٠٠ جزء فى المليون من البنلت ولكن استخدام التركيز ٩٠٠ جزء فى المليون قلل من نمو كل السلالات. أما التركيز ١٢٠٠ جزء فى المليون فكان مميتا لكل السلالات المختبرة.
أدى تلقيح كل من البرسيم الحجازى وفول الصويا بالريزوبيا الخاصة بها الى تشجيع تكوين العقد الجذرية ونمو النباتات وزيادة المحتوى النتروجينى للنبات. وكانت الزيادة فى هذه القياسا تعتمد على نوع اللقاح المستخدم وإضافة الفاج او المبيد الفطرى او الاثنين معا. وكانت اعلى قيم سواء لعدد العقد او وزنها الجاف أو النمو أو المحتوى النتروجينى للنباتات عند التلقيح بالسلالة الابوية فقط دون أى اضافات مقارنة بالعزلات المقاومة للمبيد الفطرى او المقاومة للفاج والمبيد الفطرى معا. وادت اضافة اى من الفاج او المبيد الفطرى أو الاثنين معا الى لقاح السلالات الابوية أو السلالة المقاومة للفاج الى نقص واضح فى كل القياسات مقارنة بالسلالات الابوية او العزلات المقاومة للفاج أو المبيد. سجلت النباتات الملقحة والمعاملة بالمبيد الفطرى اقل قيم للتعقيد ووزن النباتات والمحتوى النتروجينى لها. وحدث نقص حاد فى اعداد كل من ريزوبيا البرسيم الحجازى TAL 380وبرادى ريزوبيا فول الصويا USDA218 بعد ٤٥، ٥٠ يوم من الزراعة على التوالى. كذلك حدث نقص اكبر فى الاعداد فى الفترة الثانية من التقدير بعد ٧٥ ، ٩٠ يوم من الزراعة بنفس الترتيب. وايضا حدث نقص أكبر لأعداد الريزوبيا عند اضافة الفاج لها. زادت اعداد جزيئات الفاج بعد فترة ٤٥، ٥٠ يوم من الزراعة عند مقارنتها بالاعداد الاولية ثم حدث نقص شديد بتقديم عمر النباتات. وعموما كانت اعداد الفاج عالية وخصوصا فى المعاملات المضاف لها الفاج.