

FIELD AND LABORATORY EVALUATION OF ENTOMOPATHOGENIC FUNGI AGAINST SUCKING INSECTS INFESTING POTATO IN EGYPT

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INTRODUCTION

Potato, *Solanum tuberosum* (L.) is considered as a major export crop in Egypt (Abu El-Naser *et al.*, 1971 and Mariy *et al.*, 1999), ranking after cotton and rice crops. It is infested with many insect pests causing sever damages in the field (Hemeide, 1976). Whiteflies and aphids are the most important sucking pest insects that are distributed all over the world damaging nearly all agricultural and horticultural crops in the field. The sweet potato Whitefly *Bemisia tabaci* was among the major pests of crops throughout the world. Direct crop damages occur when whiteflies feed on plant phloem, remove plant sap and reduce plant vigor. High populations of whitefly may cause plant death. Whitefly also excretes honeydew, which promotes sooty mold (*Cladosporium sphaerospermum*) that interferes with photosynthesis and may lower harvest quality. Plant viruses also can be transmitted by whitefly. Aphids are also important agricultural pests and distributed all over the world damaging nearly all agricultural crops. They decreased crop yields in the field. The parthenogenesis is a common phenomenon in aphid so a small numbers of individuals produce progeny and develop within few days harming plants by sucking plant sap and/or with honeydew which promotes sooty mold and/or by transmitting plant pathogenic viruses. It had been controlled by different chemical insecticides which pollute the environment.

The entomopathogenic fungi have long been known to cause epizootics among certain insects under both laboratory and field conditions (Watson *et al.*, 1996 and Reithinger *et al.*, 1997). As an alternative to chemical control or as part of IPM programs, there is resurgence to the use of microbial insecticides for biological control of insect pests (Castillo *et al.*, 2000). The entomopathogenic fungi,

Paecilomyces fumosoroseus and *Verticillium lecanii* are effective at controlling whiteflies (Sanderson 1996). These organisms, however, are either not available commercially or are not labeled for use in greenhouses. *P. fumosoroseus* is a naturally occurring fungus that infects and kills several kinds of insects. Infected insects will be covered with a rosy-tan to smoky-pink (or gray) fungal mass (Sanderson 1996). Entomopathogenic fungi, however, do not offer stand-alone pest-control capabilities and are best used in conjunction with a program of conventional insecticides or insect growth regulators (Sanderson 1996).

The present study deals with bioassay of *P. fumosoroseus* and *V. lecanii* (entomopathogenic fungi) in controlling the sweet potato whitefly *Bemisia tabaci* and aphids (*Aphis gossypii* and *Myzus persicae*) and application of both against these pests in an experimental field of potato at two governorates, (Alexandria and Ismailia) during 2006-2007.

MATERIAL AND METHODS

Laboratory studies

Insect culture

- a- Whitefly *B. tabaci* was reared on leaves of potted potato plants grown inside cylindrical glass cages (15 cm in diameter, 22 cm in length) covered with muslin. To get the fourth instar nymph, plants infested with adult whitefly *B. tabaci* were kept for two days to allow egg laying, then the adults were collected. The first instar nymph hatched from the egg and reached the fourth instar nymph after two weeks.
- b- Aphids (*A. gossypii* and *M. persicae*) inoculated onto the potato leaves of potted plants (15 cm in diameter and covered with cylindrical glass cage 15 cm in diameter, 22 cm in length and covered with muslin).

Isolation and propagation of entomopathogenic fungi

Preparation of fungus inoculums Entomopathogenic fungi *V. lecanii* (originally isolated by Abd El-Gawad, 2000) and *P. fumosoroseus* (originally isolated by Abd El-Gawad, 2007). Conidiospores suspension was sprayed on *B. tabaci*, *M. persicae* and *A. gossypii* and incubated at 25 °C and 65± 5 % R.H. Following the host death and sporulation, conidiospores were harvested from cadavers and then transferred to Petri dishes containing potato – dextrose agar media (PDA). Isolated fungus was

grown using autoclaved potato – dextrose agar media (PDA) enriched with 1% peptone, 4% glucose, 2% yeast and 5 gm streptomycin (Rombach *et al.*, 1988).

The cultures were incubated at 25 °C for 10 days. Cultures with fully developed conidiospores were washed by sterilized distilled water mixed with 0.05 % Tween – 80 to obtain the stock spore suspension. Spores suspended in sterilized water were counted with a haemocytometer.

Production of conidiospores for field application Fungal conidia (*P. fumosoroseus* and *V. lecanii*) were produced on a barley substrate composed of 50 gm of barley, 35 ml distilled water and 2 ml pressed sun flower oil. The barley mixed with water and oil was autoclaved in Erlenmeyer flasks (300 ml) for 20 min at 121 °C. Immediately after the lumps of grain were destroyed by shaking the flasks vigorously, the flasks were cooled to room temperature and inoculated with 1 ml of conidia suspension with 10^6 spores/ml, then incubated for 2-3 weeks in the dark at 25 ± 1 °C. The conidia were harvested by suspending them in 50 ml of 0.05% Tween 80. The suspension was filtrated through a double layer of muslin and the desired concentration for field application was obtained by the addition of sterile distilled water. Total spores were counted before application in the field using a haemocytometer.

Treatments The two experimental treatments evaluated were the *P. fumosoroseus* and *V. lecanii* entomopathogenic fungi on the *B. tabaci* (Adult and nymph) and aphids (*A. gossypii* and *M. persicae*). Five desired serial concentrations of 10^4 , 5×10^4 , 10^5 , 5×10^5 and 10^6 spores/ ml for *P. fumosoroseus* and *V. lecanii* entomopathogenic fungi were prepared.

The following procedures were followed:

1- The whitefly *B. tabaci*

a- Adult stage The uninfested leaves of potato plants were transferred to the laboratory and cleaned by paper towels. The petri-dishes 30mm in diameter (with holes at its wall side covered with wire gauze for ventilation) were prepared with a layer of 3mm of 1.5 % water agar for maintaining a relative humidity at 100%. Leaf discs (2.5 cm in diameter) are smeared with 2 ml spore suspensions of different concentrations, allowed to dry for 2min before exposure to the test individuals. One treated leaf disc is kept in a Petri dish. Adults of whitefly were carefully immobilized by frozen for 5 sec., about 15-20 individuals were placed in each Petri dish. A rubber band was used to fasten the two parts of the Petri dish together. The control was tested with sterilized distilled water. The exact number of

treated adults in each container was recorded within 10 min when they recovered from the cooling shock. Percentage mortality was assessed 3 days after inoculation to the end of the experiment (Nada 1999).

b- Nymphal stage Fifty square plastic trays (10x10 cm), each with 4 compartments one, furnished with moistened filter paper on the bottoms were used. One tray was used for each concentration as well as the control for each fungus. The numbers of reared fourth nymphal instars were counted on each potato leaf and recorded before applying each treatment. Leaves were smeared with 2 ml spores suspension of each concentration for 10 sec., then allowed to dry for 2 min. One leaf was inoculated in each of the plastic trays compartments, with the ventral side of the leave upward and kept at 25°C. Percentage mortality in each compartment was assessed 3 days after inoculation to the end of the experiment. The control was tested with sterilized distilled water (Nada 1999).

2- Aphids (*A. gossypii* and *M. persicae*)

Untreated potato leaf discs (2.5 cm in diameter) were smeared with 2ml spores suspension of different concentrations for 10 sec., then allowed to dry for 2 min before infesting with the test insects. The Petri dishes were bottomed with 3mm layer of 1.5 % water agar. One treated leaf disc was kept in a petri dish. Pieces of cheese cloth were used to fasten the two parts of the dish together and to prevent aphids escaping; the petri dish was keep at inverted position. Ten replicates from each concentration as well as the control were prepared with 10 apterous adult aphids for each Petri dish, and kept in large tray, with moistened filter paper at the bottom, using sterilized distillated water, in order to keep continuous humidity inside each petri dish. Percentage mortality was assessed in each petri dish 3 days after inoculation to the end of the experiment (Sewify 1989).

3- The effectiveness of the different treatments was expressed in terms of LC_{50} and Slope values at 95 % fiducially limits. Statistical analysis of the obtained data based on the analysis of variance and linear regression analysis (Finney, 1971). In addition, polynomial regression procedure in COSTAT program was carried out.

Field studies An area of about half feddan was chosen at each of Alexandria [at El-Esraa farm at El -Nobaryia farm (N. R. C)] and Ismailia (in El- Kasaseen) governorates. Each area was divided into three treatments and a control. Potato tubers were sown on October 2006, 10th, 12th and February 2007, 10th, 12th in Alexandria and Ismailia governorates, respectively. All plots received the normally recommended agricultural practices. Experimental treatments started on December

2006, 1st, 3rd and March 2007 12th, 14th at Alexandria and Ismailia, respectively. The entomopathogenic fungi *P. fumosoroseus* (*P.f.*) and *V. lecanii* (*V.l.*) were repeated each 4 times at 15-days intervals by a rate of 10^7 spores / ml, and the entomopathogenic fungi *P. fumosoroseus* was repeated 2 times at 15-days intervals by a rate of 10^7 spores / ml followed by *V. lecanii* (F) was repeated 2 times at 15-days intervals by a rate of 10^7 spores / ml for controlling the *B. tabaci* (Adult and nymph) and aphids (*A. gossypii* and *M. persicae*). Sampling started (after application compared with the control) on December 2006, 8th, 10th and March 2007, 19th, 21st and continued weekly until February 2007, 9th, 11th and June 2007, 4th, 6th in Alexandria and Ismailia governorates, respectively. The efficacy of different treatments was measured on the number of survived individuals for the *B. tabaci* and aphids (*A. gossypii* and *M. persicae*) on 10 plants.

The formula of Henderson and Tilton (1955) was used to calculate the reduction rate among populations of the targeted sweet potato whitefly *B. tabaci* and aphids (*M. persicae* and *A. gossypii*) in the field after application with the three tested treatments.

Yield assessments

Data presented yield weight in kgs for treated and untreated plots.

RESULTS AND DISCUSSION

Effect of *P. fumosoroseus* and *V. lecanii*

1- The whitefly *B. tabaci*

a- Adult stage The calculated LC_{50} values were shown in Table (1). The mortality of whitefly *B. tabaci* exposed to the different concentrations of fungal isolates showed that the most active fungus was *P. fumosoroseus* ($LC_{50}= 26485.73$ spores/ml), followed by *V. lecanii* ($LC_{50}= 39844.44$ spores/ml).

b- Nymphal stage Data in table (1) indicate that the most active fungus was *P. fumosoroseus* ($LC_{50}= 1.31364E+05$ spores/ml), followed by *V. lecanii* ($LC_{50}= 2.28807E+05$ spores/ml).

2- Aphids (*A. gossypii* and *M. persicae*)

The more effective fungus was *P. fumosoroseus* ($LC_{50}= 60035.65$ and $1.00419E+05$ spores/ml respectively), followed by *V. lecanii* ($LC_{50}= 78810.71$ and $1.54549E+05$ spores/ml) as shown in table (1).

These pests (whitefly and aphids) were found susceptible to fungal infection. Besides, as the concentrations of the fungus *P. fumosoroseus* or *V. lecanii* increased, the whitefly and aphids mortalities were also increased. These results are in complete accordance with those previously recorded by Weinzierl and Tess 1989; Tanada and Kaya 1993; Vandenberg 1995; Mahr 1997 and Loureiro and Moino JR. 2006 for controlling the whitefly *B. tabaci* and aphids (*A. gossypii* and *M. persicae*) by different strains of fungi. Other study showed *P. fumosoroseus*, *M. anisopilae*, and *V. lecanii* are effective at controlling whiteflies and aphids (Sanderson 1996).

TABLE (I)
Values of LC₅₀ and slopes for the tested entomopathogenic fungi.

Treatments	LC ₅₀	Slope	Fiducial limits at 95% of LC ₅₀	
			Lower	Upper
<i>B. tabaci</i> (adults)				
<i>P. fumosoroseus</i>	26485.73	0.78	15606.84	39496.60
<i>V. lecanii</i>	39844.44 Spores/ml	0.73	24340.28	58772.42
<i>B. tabaci</i> (nymphs)				
<i>P. fumosoroseus</i>	1.31364E+05	0.62	85274.98	2.04160E+05
<i>V. lecanii</i>	2.28807E+05 Spores/ml	0.62	1.49614E+05	3.79897E+05
<i>A. gossypii</i>				
<i>P. fumosoroseus</i>	60035.65	0.62	35652.36	92130.94
<i>V. lecanii</i>	78810.71 Spores/ml	0.62	48887.47	1.20091E+05
<i>M. persicae</i>				
<i>P. fumosoroseus</i>	1.00419E+05	0.61	63394.92	1.54918E+05
<i>V. lecanii</i>	1.54549E+05 Spores/ml	0.55	95813.58	2.59370E+05

Field studies:

1- Application of *P. fumosoroseus* and *V. lecanii* against whitefly and aphids in potato field

It is clear from obtained results (Figs. 1 and 6) that damage caused to potato plants by the whitefly *B. tabaci* and aphids (*A. gossypii* and *M. persicae*) was higher in control than in any of the tested three treatments. In case of summer plantation, the overall average of damage caused by *B. tabaci*, *A. gossypii* and *M. persicae* was reduced by [(60, 55 and 49), (50, 45 and 39) and (48, 43 and 37 %)] and [(66, 60 and 55), (56, 50 and 45) and (54, 48 and 43 %)] due to the applications of the entomopathogenic fungi *P. fumosoroseus*, *P. fumosoroseus* followed by *V. lecanii* and *V. lecanii* in Alexandria and Ismailia governorates, respectively during 2007. Also, in case of late plantation, the resultant damage in the 3 treatments took the

same trend as reduced by [(69, 64 and 58), (59, 54 and 48) and (57, 52 and 46%)] and [(75, 69 and 64), (65, 59 and 54) and (63, 57 and 52%)] in Alexandria and Ismailia governorates, respectively during 2006-2007. In this respect many authors showed that the entomopathogenic fungi could be used to control the whitefly and aphids (Hall 1984; Fransen *et al.*, 1987; Sanderson 1996; Vidal *et al.*, 1998; Wraight *et al.*, 2000; Kirk *et al.*, 2001; Olson. *et al.*, 2002; Paine *et al.*, 2003; Goolsby *et al.*, 2004; John and Frank 2004 and Pickett *et al.*, 2004).

2- The yield in the experimental field

Results presented in (Fig. 7 and 8) revealed that potato yield was affected by the tested three treatments. The treatment with the entomopathogenic fungus *P. fumosoroseus* gave the highest rate of potato yield followed by the entomopathogenic fungi *P. fumosoroseus* followed by *V. lecanii* and entomopathogenic fungi *V. lecanii* in summer and late plantation at each of Alexandria and Ismailia governorates during 2006 and 2007 season.

SUMMARY

The present study deals with bioassay of *Paecilomyces fumosoroseus* and *Verticillium lecanii* (entomopathogenic fungi) in controlling the sweet potato whitefly *Bemisia tabaci* and aphids (*Aphis gossypii* and *Myzus persicae*) and application of both fungi against the same pests in an experimental field of potato at two governorates, (Alexandria and Ismailia) during 2006-2007.

Five desired serial concentrations of 10^4 , 5×10^4 , 10^5 , 5×10^5 and 10^6 spores/ml for each entomopathogenic fungus were tested. The obtained data showed that these pests (whitefly and aphids) were susceptible to fungal infection. Besides, as the concentrations of the fungi increased, the whitefly and aphids mortalities were also increased. Three treatments as field applications with the entomopathogenic fungi *P. fumosoroseus* (*P.f*) and *V. lecanii* (*V.I*) were repeated each 4 times at 15-days intervals by a rate of 10^7 spores / ml, and the entomopathogenic fungi *P. fumosoroseus* was repeated 2 times at 15-days intervals by a rate of 10^7 spores / ml followed by *V. lecanii* (F) was repeated 2 times at 15-days intervals by a rate of 10^7 spores / ml for controlling the *B. tabaci* (Adult and nymph) and aphids (*A. gossypii* and *M. persicae*). Spray was conducted during 2006 and 2007 seasons showed significant efficacy against whitefly and aphids.

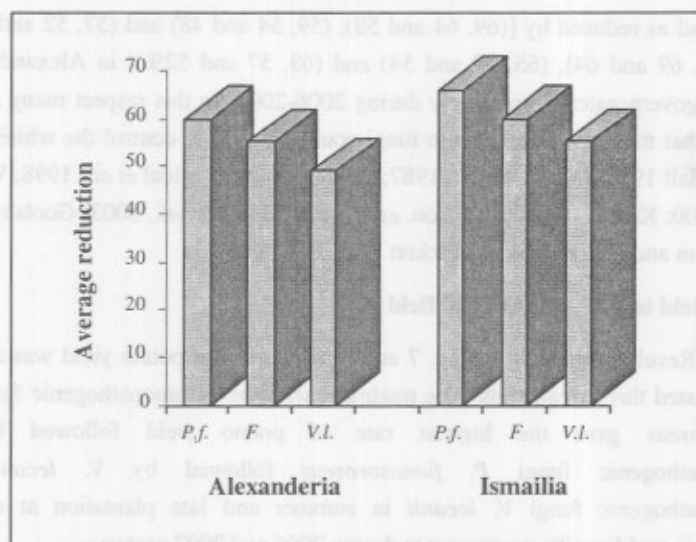


Fig. (1): Average reduction of treating potato plants with the entomopathogenic fungi *P. fumosoroseus*, *P. fumosoroseus* followed by *V. lecanii* and entomopathogenic fungi *V. lecanii* on the population density of *B. tabaci* in summer plantation at each of Alexandria and Ismailia governorates during 2007 season.

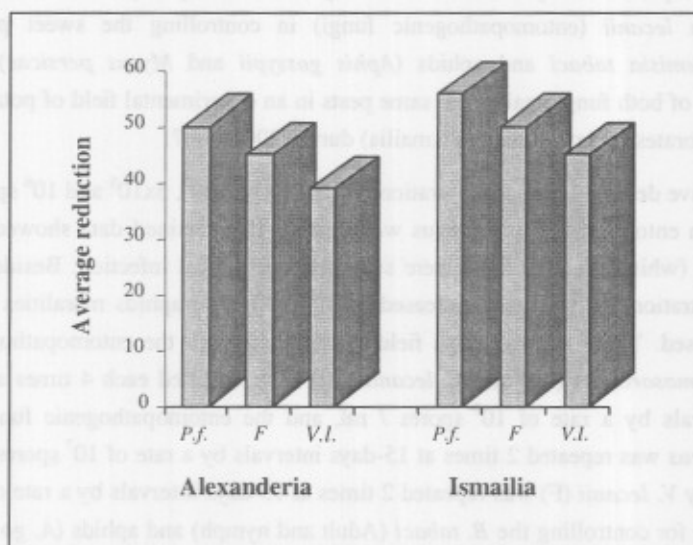


Fig. (2): Average reduction of treating potato plants with the entomopathogenic fungi *P. fumosoroseus*, *P. fumosoroseus* followed by *V. lecanii* and entomopathogenic fungi (*V. lecanii*) on the population density of *A. gossypii* in summer plantation at each of Alexandria and Ismailia governorates during 2007 season.

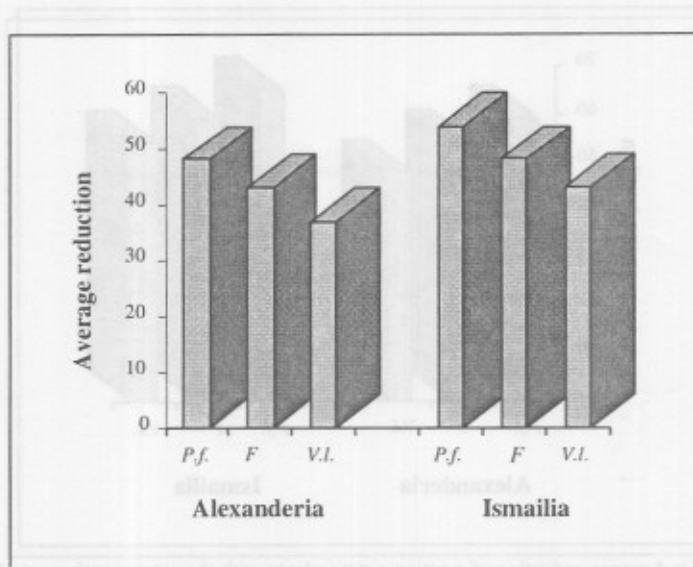


Fig. (3): Average reduction of treating potato plants with the entomopathogenic fungi *P. fumosoroseus*, *P. fumosoroseus* followed by *V. lecanii* and entomopathogenic fungi (*V. lecanii*) on the population density of *M. persicae* in summer plantation at each of Alexandria and Ismailia governorates during 2007 season.

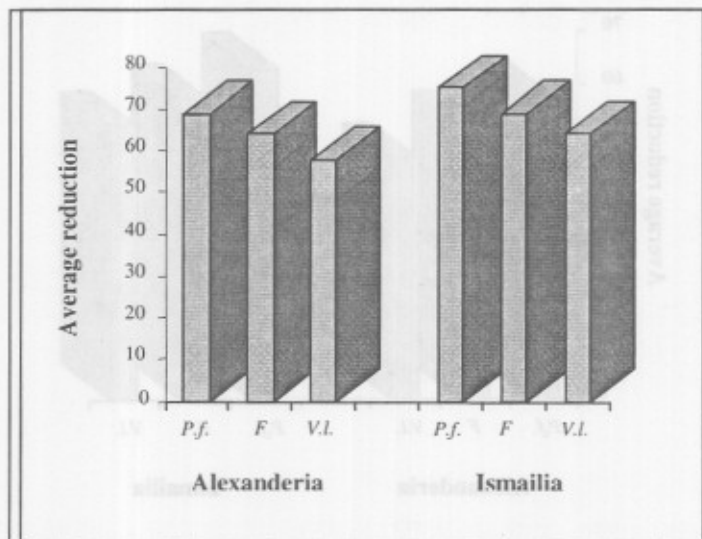


Fig. (4): Average reduction of treating potato plants with the entomopathogenic fungi *P. fumosoroseus*, *P. fumosoroseus* followed by *V. lecanii* and entomopathogenic fungi (*V. lecanii*) on the population density of *B. tabaci* in late plantation at each of Alexandria and Ismailia governorates during 2006 - 2007 season.

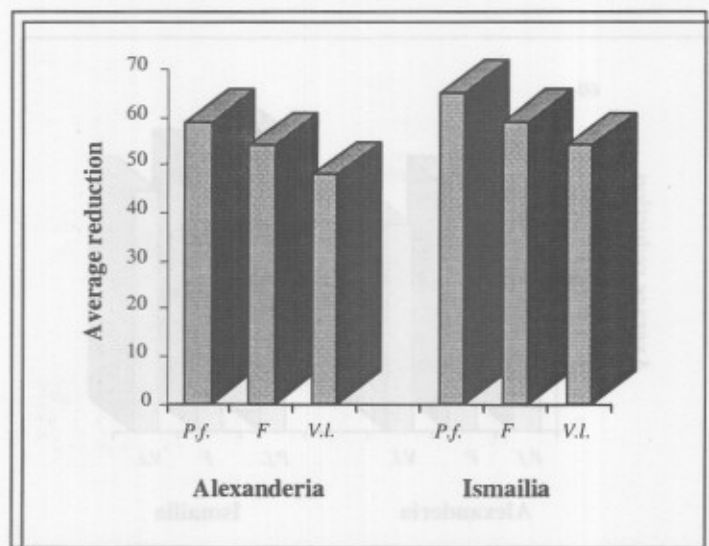


Fig. (5): Average reduction of treating potato plants with the entomopathogenic fungi *P. fumosoroseus*, *P. fumosoroseus* followed by *V. lecanii* and entomopathogenic fungi (*V. lecanii*) on the population density of *A. gossypii* in late plantation at each of Alexandria and Ismailia governorates during 2006 - 2007 season.

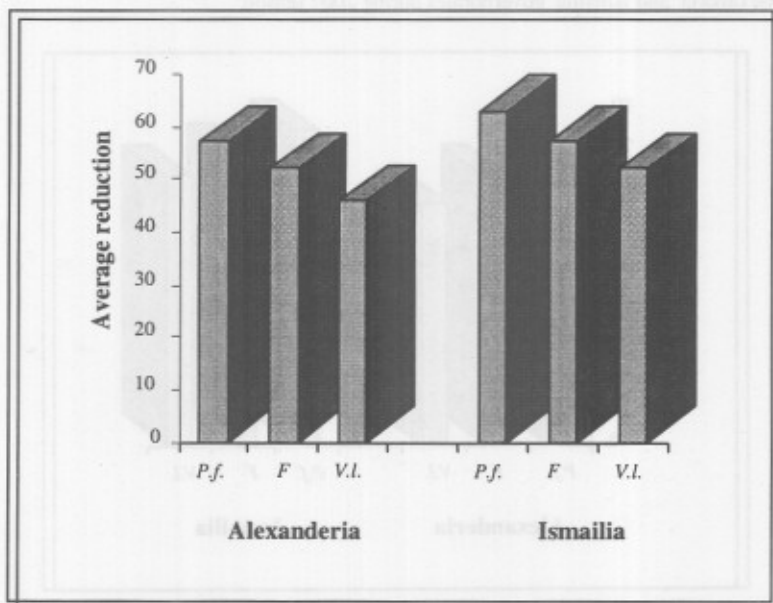


Fig. (6): Average reduction of treating potato plants with the entomopathogenic fungi *P. fumosoroseus*, *P. fumosoroseus* followed by *V. lecanii* and entomopathogenic fungi (*V. lecanii*) on the population density of *M. persicae* in late plantation at each of Alexandria and Ismailia governorates during 2006 - 2007 season.

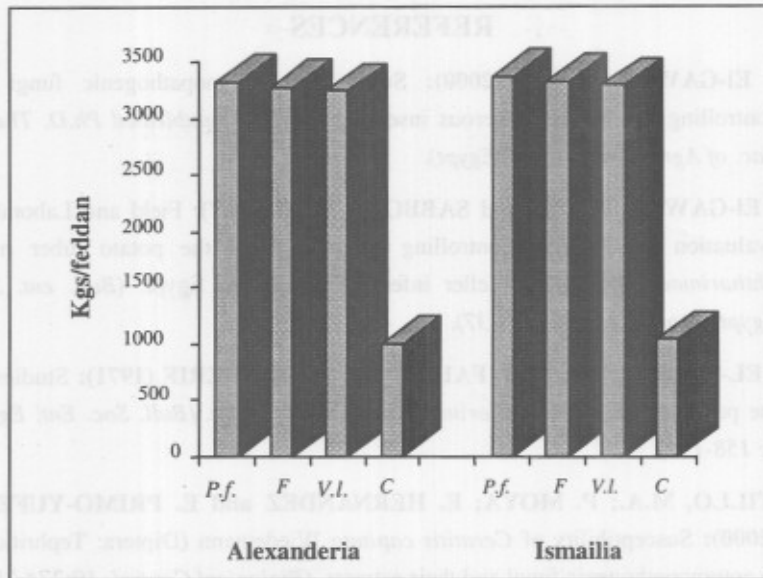


Fig. (7): Yield of potato tuber (Kgs/feddan) in the 3 treatments in summer plantation at Alexandria and Ismailia governorates during 2007 season.

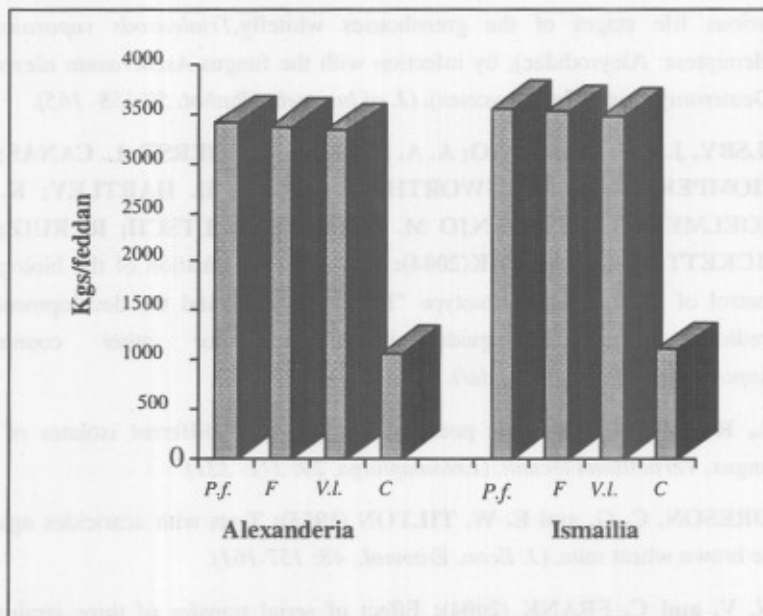


Fig. (8): Yield of potato tuber (Kgs/feddan) in the 3 treatments in late plantation at Alexandria and Ismailia governorates during 2006 - 2007 season.

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