FIELD AND LABORATORY EVALUATION OF DIFFERENT CONTROLLING AGENTS AGAINST THE POTATO TUBER MOTH *PHTHORIMAEA OPERCULELLA* ZELLER INFESTING POTATO IN EGYPT

HANY A. S. ABD EL-GAWAD[1] AND MAGDA M. SABBOUR[2]

[1]*Biological Control Research Dept., Plant Protection Institute, A.R.C., Dokki, Giza, Egypt.*

[2]*Pests and Plant Protection Dep., National Research Centre, El-Tahrir Street- Dokki, Giza-Egypt*

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INTRODUCTION

Potato tuber moth (PTM), *Phthorimaea operculella* Zeller is considered one of the most serious pests on plants of family Solanaceae (Mariy *et al.*, 1999). The larvae behave as leaf miners especially in the young instars, the older instars act as stem borers transferring from the leaves to the stem causing a serious damage to the plant, and when transferred to the store they cause a loss of the crop weight. This pest causes sever damages to the potatoes during harvest and in the store (Hemeide, 1976).

Oils give multiple effects such as increased adult mortality, lowered oviposition rate or interfere with larval development and adult emergence (Messina and Renwick, 1983). It is generally agreed that insect mortality from vegetable oils is not only due to oxygen starvation from the physical blockage of respiration but also to insecticidal effects of some oil components particularly the triglycerides, oleic, linoleic and arachidonic acids. Singh *et al.*, (1978) proposed that oil causes progeny mortality through partial or complete failure of embryos development in the eggs rather than reduced oviposition and increased adult mortality. The use of edible oils is the most suitable because of its commercial availability, low cost besides being non-toxic to humans and do not affect seed germination and do not create off flavors in cooked products (Abdel-Latif, 2003).

Egg parasitoids of the genus *Trichogramma* were widely used in controlling different lepidopteran pests world wide. PTM was controlled by *T.*

brasilensus on tomato in Chile (Loo and Aguilera, 1983) and by *T. chilonis* on potato in field and store in India (Pokharkar and Jogi, 2000).

The entomopathogenic fungi have long been known to cause epizootics among certain insects under both laboratory and field condition (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 present study deals with bioassay of garlic oil (plant extract), *Paecilomyces fumosoroseus* and *Nomuraea rileyi* (entomopathogenic fungi) in controlling larvae (L3) of (*P. operculella*) and application of garlic oil, *T. evanescens*, *P. fumosoroseus* and *N. rileyi* against *P. operculella* in an experimental field of potato at two governorates, (Alexandria and Ismailia) during 2006-2007.

MATERIAL AND METHODS

Laboratory studies

Insect and parasite culture

a-The potato tuber moth *P. operculella,* was cultured in the laboratory at 29 ± 2°C and 65 ± 5 % RH according to the method described by Haiba (1990). The larvae were fed on potato tubers, whereas adults were fed through a small plastic cover containing a piece of cotton soaked with 5% sugar solution.

b-*Trichogramma evanescens* was cultured in the laboratory according to the method described by Abd El-Gawad and Abd Al-Aziz (2005).

Isolation and propagation of entomopathogenic fungi

Preparation of fungus inoculums Entomopathogenic fungi *N. rileyi* (originally isolated by Abd El-Gawad, 2000) and *P. fumosoroseus* (originally isolated from *Sesamia cretica* during the recent study by Abd El-Gawad). Conidiospores suspension was sprayed on *P. operculella* larvae and incubated at 25 ℃ 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 ℃ 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 of *N. rileyi* were produced on a rice and fungal conidia of *P. fumosoroseus* were produced on a barley substrate which contained 50 gm of rice or barley, 35 ml distilled water and 2 ml pressed sun flower oil. The rice or barley mixed with water and oil was autoclaved in Erlenmeyer flasks (300 ml) for 20 min at 121 ℃. 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 ℃. 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 three experimental treatments evaluated were the plant extract (pure garlic oil); *N. rileyi* and *P. fumosoroseus* entomopathogenic fungi on the potato tuber moth *P. operculella* larvae. Five desired serial concentrations of 0.25, 0.5, 1.0, 2.0 and 4.0 % for plant extract and 10^4, $5x10^4$, 10^5, $5x10^5$ and 10^6 spores/ ml for *N. rileyi* and *P. fumosoroseus* entomopathogenic fungi were prepared.

The following procedures were followed

1-The experimental design consisted of four replicates, each of ten 3rd instar larvae of the potato tuber moth *P. operculella* larvae.

2-In case of the treatments by plant extract the larvae were allowed to feed on treated potato leaves which were dipped for one minute in the tested concentrations and then left to dry before being offered to tested larvae and adults for a feeding period of 48 hours in the case of plant extract. The larvae were starved for 6 hours before transferring onto treated leaves, in order to obtain rapid simultaneous ingestion. Surviving larvae after experimental treatments were transferred to other clean cups containing untreated potato leaves until pupation.

3-For the treatments with entomopathogenic fungi, the tested larvae were immersed for 10 seconds in the tested concentration. Then, larvae were transferred onto untreated potato leaves.

4-Control tests were conducted using the same source of food, but dipped in water only.

5-Experiments on the plant extract were carried out under laboratory conditions of 26±2 ºC and 65±5 % R.H., while experiments with entomopathogenic fungi were carried out at 26±2 ºC and 85±5 % R.H. and all replicates were checked daily to estimated percent larval mortality.

6-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.

7- Five egg clusters of *P. operculella*, eggs less than 24 hours old dipped into *N. rileyi* and *P. fumosoroseus* entomopathogenic fungi suspension at concentration of 10^7 spores/ml, then left to dry and exposed to 20 mated female of *Trichogramma evanescens* in glass tube for 30 minutes. The parasitized eggs were incubated at 26 ± 1°C for 1, 3, 5 and 7 days. Control was dipped in water only. Each treatment was performed seven times. The treated host egg masses were kept at 26 ± 1°C until emergence of parasitoid adults. The percentage of infestations was calculated after seven days. Percentage of parasitoid emergence inside the host was calculated. The percentage of eggs reductions were calculated as the following equations:

$$\% \text{ of reduction} = \frac{\% \text{ of egg emergence in the control} - \% \text{ of egg emergence after treatments}}{\% \text{ of egg emergence in the control}}$$

Field studies An area of about one feddan was chosen at each of Alexandria [at Eben-Malek farm at El –Nobaryia farm (N. R. C)] and Ismailia (in El- Kasaseen) governorates. This area was divided into 6 treatments and a control. Potato tubers were sown on October 2006, 13th, 15th and February 2007, 13th, 15th in Alexandria [at Eben-Malek farm at El –Nobaryia farm (N. R. C)] and Ismailia (in El- Kasaseen) governorates, respectively. All plots received the normally recommended agricultural practices. Experimental treatments started on December 2006, 4th, 6th and March 2007 20th, 22nd at Alexandria and Ismailia governorates, respectively. The plant extract (pure garlic oil) (*G*) was repeated 4 times at 15-days intervals by a rate of 2 %, *T. evanescens* (*T.e.*) was repeated 4 times at 15-days intervals by a rate of 120,000 parasitoids/feddan/release, the entomopathogenic fungi *P. fumosoroseus* (*P.f.*) and *N. rileyi* (*N.r.*) were repeated each 4 times at 15-days intervals by a rate of 10^7 spores / ml, the entomopathogenic fungi *P. fumosoroseus* was repeated 2 times

at 15-days intervals by a rate of 10^7 spores / ml followed by *T. evanescens* was repeated 2 times at 15-days intervals by a rate of 120,000 parasitoids/feddan/release (F1) and the entomopathogenic fungi *N. rileyi* was repeated 2 times at 15-days intervals by a rate of 10^7 spores / ml followed by *T. evanescens* was repeated 2 times at 15-days intervals by a rate of 120,000 parasitoids/feddan/release (F2) for controlling potato tuber moth *P. operculella.* Sampling started on December, 11th, 13th 2006 and March, 27th, 29th 2007 and continued weekly until February, 12th, 14th 2007 and June, 12th, 14th 2007 in Alexandria and Ismailia governorates, respectively. The efficacy of different treatments was measured on the number of survived larvae for potato tuber moth *P. operculella* on 100 leaves.

The formula of Henderson and Tilton (1955) was used to calculate the reduction rate among populations of the targeted potato tuber moth *P. operculella* pest in the field after application with the six tested treatments.

Yield assessments

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

RESULTS AND DISCUSSION

Insecticidal effect

1- Effect of garlic oil

Obtained results presented in Table (1) show the daily corrected mortality percentages due to the treatments by the plant extract (garlic oil) on 3rd instar larvae of PTM. The percent mortality after 6 days post treatment on larvae (L3) of *P. operculella* ranged from 40.0 to 95.0 % at concentrations of 0.25 to 4.00 % of the plant extract. It is evident from Table (1) that the percent mortality increased as a result of increasing the concentration of plant extract used, *i.e.*, the plant extract with different concentrations exhibited toxic effect against the tested larvae. The LC_{50} values for larvae of *P. operculella* were calculated after 3 and 4 days from treatment as 0.93 and 0.55 % for plant extract (Table 2). The obtained results are in agreement with the findings of Sabbour and Ismail (2001) showed that plant extract was the most toxic against the potato tuber moth *P. operculella.*

2- Effect of the entomopathogenic fungi

The daily corrected mortality percentages resulting from the treatment of 3rd instar larvae of the potato tuber moth *P. operculella* were shown in Table (1). Larval moralities at higher concentrations of $5x10^5$ and 10^6 spores/ ml. after 7 days

TABLE (I)

Corrected mortality percentages for 3th instar larvae of *Phthorimaea operculella* fed on potato leaves treated with the plant extracts and also for pest larvae dipped in different concentrations of the entomopathogenic fungi.

Larvae and adults of *P. operculella*								
	% cumulative mortality after days of treatment							
Concentration	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
%	**Garlic oil (Plant extract)**							**Surviving larvae reached the pupal stage**
0.0	0.0	0.0	0.0	0.0	0.0	2.5		
0.25	10.0	17.5	22.5	30.0	32.5	40.0		
0.5	17.5	30.0	37.5	50.0	55.0	67.5		
1.00	22.5	42.5	52.5	65.0	72.5	82.5		
2.00	27.5	55.0	65.0	77.5	82.5	90.0		
4.00	35.0	65.0	77.5	82.5	87.5	95.0		
Spores/ml	***Paecilomyces fumosoroseus* (Entomopathogenic fungi)**							
0.0	0.0	0.0	0.0	0.0	0.0	2.5	2.5	
10^4			5.0	10.0	17.5	25.0	32.5	
$5x10^4$			10.0	17.5	27.5	35.0	45.0	
10^5			17.5	27.5	40.0	50.0	62.5	
$5x10^5$			27.5	40.0	52.5	65.0	72.5	
10^6			40.0	52.5	65.0	77.5	85.0	
Spores/ml	***Nomuraea rileyi* (Entomopathogenic fungi)**							
0.0	0.0	0.0	0.0	0.0	0.0	2.5	2.5	
10^4			5.0	10.0	15.0	22.5	30.0	
$5x10^4$			10.0	15.0	25.0	30.0	40.0	
10^5			15.0	25.0	35.0	45.0	57.5	
$5x10^5$			22.5	35.0	47.5	57.5	67.5	
10^6			35.0	47.5	60.0	72.5	80.0	

post treatment were (72.5 and 67.5 %) and (85.0 and 80.0 %) for (*P. fumosoroseus* and *N. rileyi)*, respectively. At lower concentrations of 10^4, $5x10^4$ and 10^5 spores/ ml. the larval and adult moralities were (32.5 and 30.0 %), (45.0 and 40.0 %) and (62.5 and 57.5 %), respectively. After 5 and 6 days (at which the LC_{50} was calculated), the percent mortality ranged between (17.5 and 15.0 %) to (65.0 and 60.0 %) and (25.0 and 22.5 %) to (77.5 and 72.5 %) when using the concentrations of entomopathogenic fungi (*P. fumosoroseus* and *N. rileyi)* at 10^4 to 10^6 spores/ ml., respectively. The LC_{50} was recorded as (3.04154E+05 and 4.76945E+05) and (1.14399E+05 and 1.80881E+05) spores/ ml., respectively. (Table 2). These larvae were susceptible to fungal infection. Besides, as the concentrations of the entomopathogenic fungi increased, the larval moralities were also increased. These results are in complete accordance with those previously shown by Sabbour (1992) and Hafez *et al.*, (1994).

TABLE (II)

Values of LC_{50} and slopes for the tested compounds

Treatments	Days after treatment	LC_{50}	Slope	Fiducial limits at 95% of LC_{50}	
				Lower	Upper
Phthorimaea operculella (larvae)					
Garlic oil	3	0.93 %	1.23	0.74	1.16
	4	0.55	1.25	0.42	0.69
P. fumosoroseus	5	3.04154E+05	0.64	1.99259E+05	5.19938E+05
	6	1.14399E+05 Spores/ml	0.74	77868.08	1.66856E+05
N. rileyi	5	4.76945E+05	0.63	3.00035E+05	9.09305E+05
	6	1.80881E+05 Spores/ml	0.67	1.21809E+05	2.80367E+05

TABLE (III)

Percent hatching of parasitized eggs after laboratory treatment.

Treatments	Emergence %							
	1	reduction %	3	reduction %	5	reduction %	7	reduction %
P. fumosoroseus	89.98	3	91.89	2	92.88	2	95.53	2
N. rileyi	83.55	9	87.45	6	88.34	6	90.22	7
control	92.12		93.54		94.99		97.98	

Data in Table 3 show , after one day of that the percentage of parasitoid emergence were decreased to 89.98 and 83.55 % after one day of treatments of the PTM eggs by , *P. fumosoroseus* and *N. rileyi*, respectively as compared to 92.12% in the control. After 3 days of parasitism the data showed that higher percent of parasitoid emergence was (91.89%) after *P. fumosoroseus* treatments as compared to 93.54% in the control. After five days the percentage of reduction ranged between 2 and 6 %. After seven days the percentage of emergence were 95.53 and 90.22 % after treating *P. operculella* eggs with, *P. fumosoroseus* and *N. rileyi*, respectively, the percentage of reduction reached 2 and 7 % (Table3). The same results were obtained by Borah and Basit (1996), Radhika (1998) and El-Mandrawy *et al.*, (2004).

Field studies

1- Application of the garlic oil, *T. evanescens*, *P. fumosoroseus* and *N. rileyi* against *P. operculella* in potato field

It is clear from obtained results (Figs. 1 and 2) that damage caused to potato plants by the PTM was higher in control than any of the tested 6 treatments. In case of summer plantation, the overall average of damage caused by PTM larvae was reduced by 65.19, 68.96, 71.18, 74.67, 76.72 and 85.01% and 67.84, 70.47, 73.20, 77.42, 79.25 and 87.23% due to the applications of the entomopathogenic fungi, *N. rileyi, P. fumosoroseus* the entomopathogenic fungi *N. rileyi* followed by *T. evanescens*, the entomopathogenic fungi *P. fumosoroseus* followed by *T. evanescens*, *T. evanescens* and the plant extract (pure garlic oil) in Alexandria and Ismailia fields, respectively during 2007. Also in case of late plantation, the resultant damage in the 6 treatments took the same trend as reduced by 68.36, 72.02, 73.08, 77.15, 77.92 and 88.19 % and 69.78, 74.08, 75.80, 80.22, 81.45 and 88.94 % in Alexandria and Ismailia fields, respectively during 2006-2007. The obtained results by the plant extract (pure garlic oil) are in agreement with the findings of Sabbour and Ismail (2001) and Salem, (1992) where it was effective against the potato tuber moth *P. operculella.*

Results of field release of the parasitoid *T. evanescens* as biocontrol agent agreed with those reported from Chile by Loo and Aguilera (1983) and from India by Pokharkar and Jogi (2000).

The entomopathogenic fungi have been used in the protection of potato plants. In this respect Amonkar, *et al.*, (1979) and Sabbour, (1992) showed that *B. bassiana* could to control the potato tuber moth.

The entomopathogenic fungi followed by *T. evanescens* obtained results that are in agreement with the findings of El-Mandrawy *et al.*, (2004).

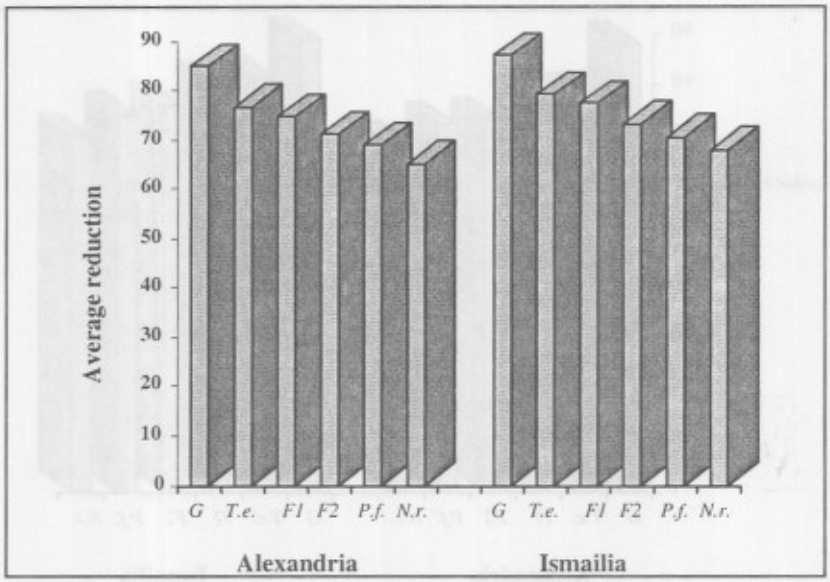

Fig.(1): Average reduction of treating potato plants with the plant extracts, *T. evanescens*, the entomopathogenic fungi *P. fumosoroseus* followed by *T. evanescens*, the entomopathogenic fungi *N. rileyi* followed by *T. evanescens*, entomopathogenic fungi *P. fumosoroseus* and *N. rileyi* on the population density of PTM larvae in summer plantation at each of Alexandria and Ismailia governorates during 2007 season.

The yield in the experimental field

Results presented in (Figs. 3 and 4) revealed that potato yield was affected by the tested six treatments. The treatment with the plant extract (pure garlic oil) gave the highest rate of potato yield followed by *T. evanescens*, the entomopathogenic fungi (*P. fumosoroseus)* followed by *T. evanescens*, *N. rileyi* followed by *T. evanescens*, *P. fumosoroseus* and *N. rileyi* in summer and late plantation at each of Alexandria and Ismailia governorates during 2006 and 2007 seasons.

SUMMARY

The present study deals with bioassay of garlic oil (plant extract), and the entomopathogenic fungi *Paecilomyces fumosoroseus* and *Nomuraea rileyi* in versus larvae (L3) of PTM and field application of garlic oil, *T. evanescens*, *P. fumosoroseus* and *N. rileyi* at two governorates, (Alexandria and Ismailia) during 2006-2007.

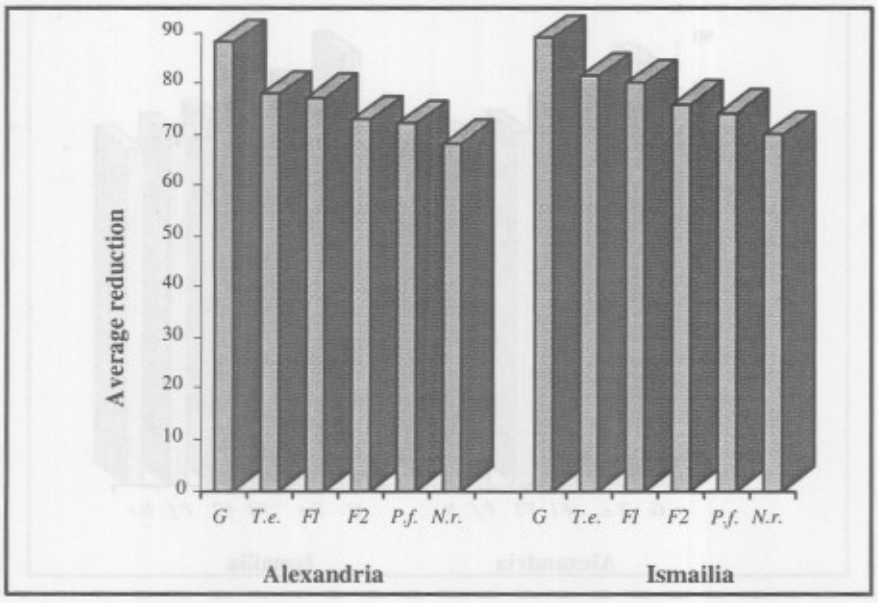

Fig.(2): Average reduction of treating potato plants with the plant extracts, *T. evanescens*, the entomopathogenic fungi *P. fumosoroseus* followed by *T. evanescens*, the entomopathogenic fungi *N. rileyi* followed by *T. evanescens*, entomopathogenic fungi *P. fumosoroseus* and *N. rileyi* on the population density of PTM larvae in summer plantation at each of Alexandria and Ismailia governorates during 2006 - 2007 season.

Five desired serial concentrations of 0.25, 0.5, 1.0, 2.0 and 4.0 % for plant extract and 10^4, $5x10^4$, 10^5, $5x10^5$ and 10^6 spores/ ml for entomopathogenic fungi were tested. The obtained data showed that the plant extract was toxic to the larvae. Also, these larvae were susceptible to fungal infection. Besides, as the concentrations of the fungi increased, the larval moralities were also increased. Six treatments as field applications with the garlic oil that was repeated 4 times at 15-days intervals by a rate of 2 %, *T. evanescens* (*T.e*) that was repeated 4 times at 15-days intervals by a rate of 120,000 parasitoids/feddan/release, the entomopathogenic fungi (*P. fumosoroseus* and *N. rileyi)* that were repeated 4 times at 15-days intervals by a rate of 10^7 spores / ml, the entomopathogenic fungi *P. fumosoroseus* was repeated 2 times at 15-days intervals by a rate of 10^7 spores / ml followed by *T. evanescens* was repeated 2 times at 15-days intervals by a rate of 120,000 parasitoids/feddan/release (F1) and the entomopathogenic fungi *N. rileyi* was repeated 2 times at 15-days intervals by a rate of 10^7 spores / ml followed by *T. evanescens* was repeated 2 times at 15-day intervals by a rate of 120,000 parasitoids/feddan/release (F2) for controlling potato tuber moth. Spray was conducted during 2006 and 2007 seasons showed efficacy against potato tuber moth PTM.

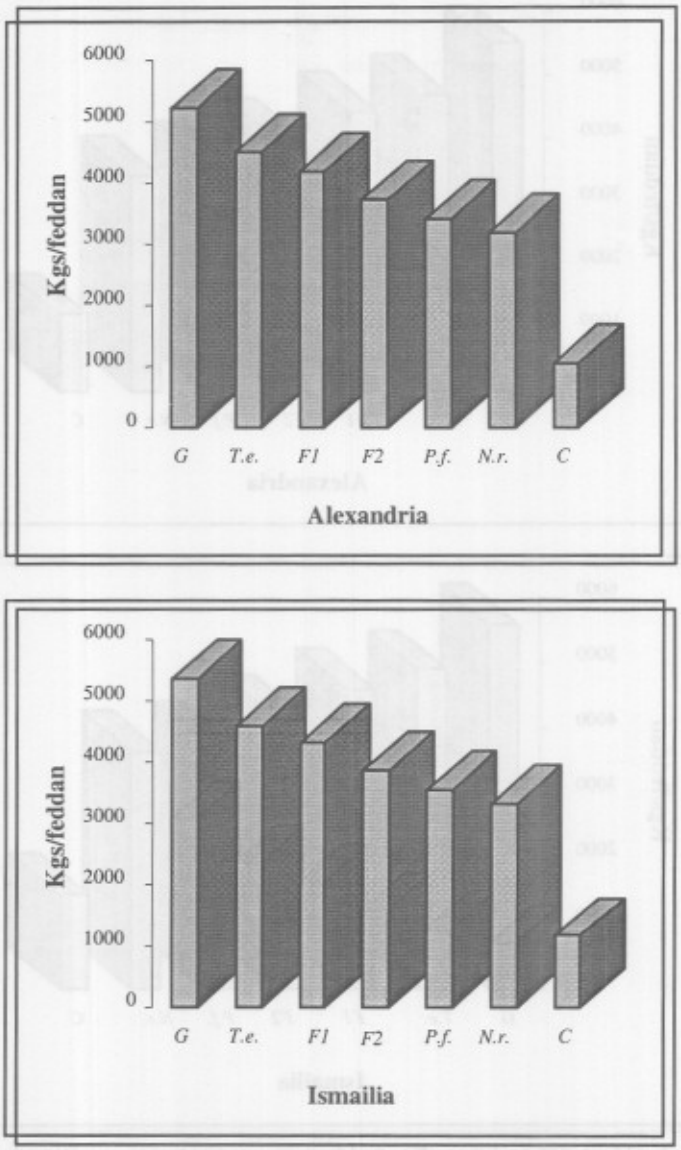

Fig. (3): Yield of potato tuber (Kgs/feddan) in six treatments in summer plantation at each of Alexandria and Ismailia governorates during 2007 season.

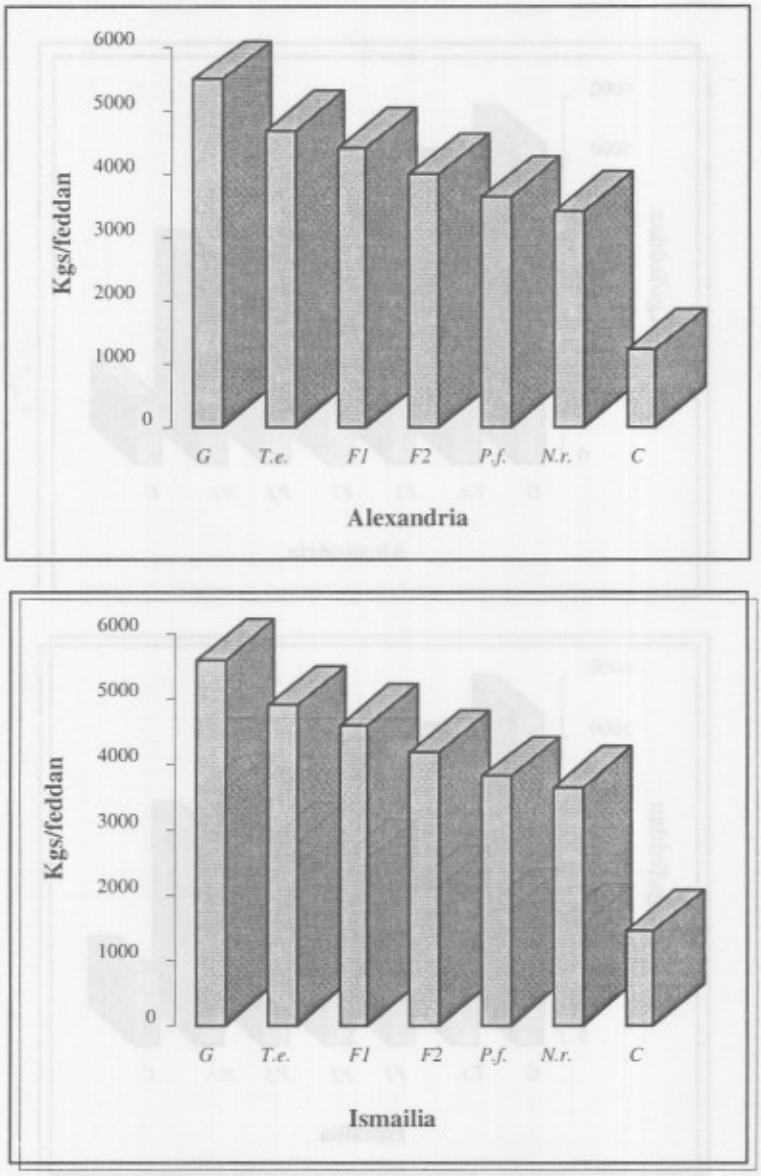

Fig. (4): Yield of potato tuber (Kgs/feddan) in six treatments in late plantation at each of Alexandria and Ismailia governorates during 2006 - 2007 season.

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