BIODEGRADATION OF PIRIMIPHOS-METHYL USING TWO TYPES OF MICROORGANISMS ISOLATED FROM FAYOUM GOVERNORATE SOIL

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ABSTRACT

The results showed that the half-life of the pirimiphos-methyl ranged between 2.1 and 1.57 day in non- sterilized soil under 27˚C and moisture content 25 and 50% respectively. Also, at the same temperature degree in sterilized soil the half- life of the pirimiphos-methyl reached about 30.1 and 3.8 day under moisture content 25 and 50% respectively. The half- life of pirimiphos- methyl soil under 35˚C was about 2 and 1.8 day in non- sterilized soil for both tested moisture content 25 and 50% respectively. In sterilized soil, the half-life of the same insecticide under 35˚C was about 6.01 and 3.5 day for 25 and 50% moisture content, respectively.

Two strains of microorganisms were isolated from the soil one of them a positive gram bacteria strain and the other is a fungi strain. The breakdown of pirimiphos– methyl through these organisms was investigated in a liquid media. The degradation rate of pirimiphos-methyl was faster after first, second and third day by the fungi than the bacteria strain under 35˚C incubation temperature. On the other hand the degradation by bacteria was accelerated up to the fourth day of incubation. These effects may be due to the increase of the extracellular esterase activity, which tend to increase from the second day of incubation period. Generally, the degradation of pirimiphos-methyl by the two tested microorganisms was higher under 35˚C than 27˚C. The glucose consumption by the bacteria strain seemed to be correlated to the increase in the esterase activity up to the third day of incubation period. No significant differences were found between the control and the fungi in its glucose consumption.

Key Words: Biodegradation- pirimiphos - methyl-microorganisms

INTRODUCTION

Now thousands of different commercial pesticides are available on the market. At the present time, however, there is a growing consensus that conventional chemical pesticides are by no means the panacea they were once considered to be, and that in many cases their indiscriminate use has created for more problems than it has solved. This realization has come gradually and not without controversy, as the quotation from **Laws (1992)**.

The persistence of pesticides in the environment is one of the important factors that determine their efficacy and their impact on non target species. If pesticides degraded rapidly to harmless substances through natural processes there would be little opportunity for them to spread throughout the food web through feeding relationships, and for their concentrations to increase through biological magnification. The persistence of many pesticides depends on the surrounding environment compartments (soil, water, air, plant and animal). Soil

constitute is a major environmental compartment on the persistence and degradation of pesticides.

Martinez Toledo *et al* (1992) investigated the effect of two selected organophosphorus insecticides pirimiphos-methyl and chlorpyrifos on soil microflora in an agricultural loam decreasing aerobic dinitrogen fixing bacteria and dinitrogen fixation. **Racke and Coats (1988a) Racke *et al* (1990)** explained the resistance of chlorpyrifos to enhanced biodegradation in soil. Results indicate that chlorpyrifos is not susceptible to enhanced microbial degradation and repeated chlorpyrifos application should have no effect on its persistence or efficacy. **Getzin and Rosefield (1968)** showed that all of the pesticides degraded faster in non- sterile soils. Some insecticides decomposed much faster in irradiated soil than in autoclaved soil. **Cink and Coats (1993)** studied the effect of the soil moisture in the persistence of chloropyrifos. They also found that the temperature did not effect the degradation of chloropyrifos. **Aly *et al*. (1987)** studied persistence of aldicarb, chlorpyrifos and methomyl in 3 different types of soil common in Egypt. They found that, degradation of aldicarb was faster in silty clay loam, followed by sand-clay loam and clay-loam, while chlorpyrifos degraded fastest in clay- loam, followed by silty-clay loam and sandy-clay-loam. Methomyl degraded fastest in sandy-clay-loam, followed by silty clay loam and clay loam.

This study was carried to determine the persistence of pirimiphos-methyl under sterilize and non-sterilized soil. Moreover, this study aimed to emphasize the role of low tolerant isolates of microorganism on the biodegradation of pirimiphos-methyl in soil under different levels from moisture and temperature.

MATERIALS AND METHODS

Degradation of pesticides in soil:

The soil samples were taken from Dar Al-Ramad farm, Faculty of Agriculture, Cairo University, Fayoum branch. Samples of soil, 10g each were placed in glass flasks at a moisture content of 25 or 50% with distilled water. Each group of samples was divided into two groups, one was left as non sterilized soil and the other was sterilized by using autoclave at 125˚C for 20 min. The non- sterilized soil was treated with pirimiphos-methyl 50% EC. at the rate of 40ppm. The sterilized soil was treated with the same concentration under UV sterilized Laminar- flow. Each group of soil samples was divided to two groups, one was incubated at 27˚C ± 2˚C and the other incubated at 35˚C ± 2˚C. At time intervals of 0, 1, 2, 3, 4, 5, 8, 12, 18, 25 and 39 days samples were taken for residues analysis.

Extraction and clean up of pirimiphos- methyl from soil

The insecticide residues were extracted and purified. Fifty gram of soil was taken, and placed in 500 ml flask and shacked with 50 ml distilled water followed by 400 ml of 20% acetone in n-hexane fore 10 minutes. An equal volume of distilled water was added to the aliquot. A portion of extract was placed in a separating funnel and shaken for one min, allowing the n-hexane and water layer to separate and run off the water layer including any interfacial emulsion. The n-hexane layer was dried by passing though anhydrous sodium sulphate and then evaporate by using a rotary evaporator on a water bath at 40˚C. The residues were redissolved in 2ml of acetone then transferred to a 5ml

vials. After the evaporation of acetone using a light stream of air, the samples were kept under refrigeration until used. The recoveries from control samples fortified with a concentrate of primiphos- methyle for soil was 95% to 106%

Isolation of pesticides tolerant microorganisms from soil.

Mineral agar media was prepared and supplied with 0.5 g/l glucose as a carbon source. The agar plates were treated with pirimiphos-methyl at a concentration of 40 ppm. One gram of soil was added to 250 ml sterilized water and shaken to make a homogeneous suspension, 250 µL from the suspension was added at the surface of the agar plate. The inoculated plates were incubated at 27˚C until the microorganisms are grown. Two isolates were screened and chosen after the inoculation of agar plates from all possible growing microorganisms on the surface of different plates. One was a G.+ organisms, motile, long rods, producing no color, mucoid growth on plates and aerobic in its nature. The other which was found to spread over the surface of most plates, has a mycelia type of growth, produces planets of spores on plates surface, white color, not acceptable to gram stain, aerobic and non motile. It was a type of fungi. The two isolates were used proceedingly as tolerant microorganisms to tested pesticides.

Degradation of pirimiphos-methyl in liquid media

Mineral liquid media was used to evaluate the biological performance of the isolated soil microorganisms to degrade the pesticide under investigation. Pirimiphos-methyl was added to 100 ml sterilized liquid media in 250 ml Erlenmeyer flasks to final concentration of 40 or 100ppm. The flasks of each concentration were divided into two groups, the first was incubated with microorganisms at 27˚C and the second was incubated under 35˚C for 1,2,3,4 and 7 days. Three replicates were taken at each incubation period as well as untreated samples (control). The PH value, glucose consumption, total protein in cells and liquid media were determined according to **Lowrey *et al.,* (1952)** and esterase activity after **Gomori (1953).** The bacteria inoculum was prepared by growing the organisms on Malik liquid medium for 24 hours at 30˚C after which the cell counts was measured using the turbidity method described by **Ellen (1994)** and pesticides residues were determined. **Swelam (1996).**

HPLC analysis

The residue analysis of pirimiphos- methyl was determined using HPLC according to **Swelam (1996).**

RESULTS AND DISCUSSION

The residue of Pirimiphos- methyl in non-sterilized soil incubation under 27 ˚C and 25% moisture content ranged between 83.3 ± 2.9 % after the first day of the incubation period and 67 ± 2.1% after 18th day. After 25 and 39 days the residue of pirimiphos-methyl could be detected. Under the same temperature and 50% moisture content, no residue of pirimiphos-methyl was detected from the 12th day. This results may indicate that the moisture content play a role in pirimiphos-methyl degradation in soil. In sterilized soil the residue of pirimiphos-methyl was ranged between 91.7 ± 2.8 and 40±1.5% after 1st and 39th days, respectively, under 25% moisture. The residue decreased more under 50% moisture content where it ranged between 95 ± 5 and 4.7 ± 1.5% after the first and 39th day, respectively, (**Table 1).** The thermal effect enhanced the

degradation of pirimiphos-methyl in the sterilized soil. Also the break down rate of the tested pesticide was faster at 35˚C after the first three days than at 27˚C, The percent residue of pirimiphos-methyl under 35˚C and 25% moisture content was ranged between 75 ± 13.4 and 2.33 ± 58% after 1st and 25th day incubation in non-sterilized soil. Also under the same degree and 50% moisture the residue of pirimiphos-methyl proved 65±8.7 and 8,2.1% after the 1st and 8th day, respectively. After the 12th day no pirimiphos-methyl residue could be detected. On the sterilized soil, the residue of the pirimiphos-methyl was detected after the 39th day as 11.3 ±1.2 under 25% moisture and 8.7±3.2 under 50% moisture (**Table 1).**

The half-life of pirimiphos-methyl ranged between 2.1 and 1.57 day in non-sterilized soil under 27˚C and moisture content 25 and 50% respectively. Also, at the same temperature degree in sterilized soil the half-life of pirimiphos-methyl was about 30.1 and 3.8 day under moisture content i.e. 25 and 50%, respectively. The half-life of pirimiphos-methyl in non-sterilized soil under 35˚C was about 2 and 1.8 day under both tested moisture content 25 and 50% respectively. In sterilized soil, the half-life of the tested insecticide under 35˚C was about 6.02 and 3.5 day for 25 and 50% moisture content, respectively. These results agreed with those reported by **Lichtensten *et al.* (1964), (1968), Walker and Stojanovic (1973), Getzin and Rosefield (1968) and Naumann (1959).**

The physical and chemical properties of the soil such as soil temperature, moisture and pH, the physicochemical properties of the pesticide, and finally span of time during which pesticide remains in contact with the soil , affect the adsorption, desorption and biological activity of pesticides. Our results agree with those of **Mulbry *et al.* (1996**).

Effect on the Glucose consumption.

Results in **Table 2** indicate that the bacteria strain under 27˚C increased the glucose consumption significantly (P≤0.01) in pirimiphos-methyl at a concentration of 40 and 100ppm. In case of the fungi strain (**Table 2)** results show significant increase in glucose consumption after the 1st day under 35˚C for both (40 and 100 ppm) pirimiphos-methyl. After the 7th day the glucose consumption decreased significantly (P ≤0.01). Under 27˚C the results indicate no significant differences in glucose consumption between the control and treated fungi.

Table 1

Table (2) The percentage of glucose consumption by the bacteria and fungi strain under 27°C and 35°C in medium treated with Pirimiphos methyl

Isolate	Temperature °C	Concentration (ppm)	Sampling intervals in days				
			1	2	3	4	7
Bacteria	27	0	13±6^{b}	38.9±1.6^{cde}	57.9±.2^{f}	65.2±.2^{i}	70.1±.8
		40	16.9±1.8^{bc}	60.1±.2g	69.1±1.1^{g}	72.3±1.1^{j}	72±0
		100	6.7±.5^{a}	28.7±2.8^{b}	68.7±2.1^{g}	70.7±.5^{j}	71±0
	35	0	24.6±0^{ef}	39.7±7.5^{cde}	48±3.4^{e}	46.4±3.2^{cde}	47.5±.4
		40	16.2±0^{bc}	21±0^{a}	47.7±1.5^{de}	53.6±1.4^{fgh}	43.1±1.1
		100	4.5±.1^{a}	42.6±.3^{e}	46.1±2.9^{de}	42.4±0^{bc}	48±3.4
Fungi	27	0	24.2±.7^{ef}	37.4±.3^{cde}	41.7±.3^{cd}	54.6±1^{gh}	58.8±0
		40	39.6±0^{g}	42.9±.1^{e}	49±0^{e}	58.3±.5^{h}	52.9±2.1
		100	28.3±2.8^{f}	40.4± 3.8de	43.9±.1cde	48.9±1.8def	52.9±.1
	35	0	7.4±2.4^{a}	36.2±0^{cd}	39.7±6.8^{bc}	50.4±5.5^{efg}	42±0
		40	22.5±.9de	49.9±.1^{f}	31.2±1.3^{a}	44.4±2.9^{cd}	53.1±2.2
		100	19.5±.2^{cd}	39.9±1.1^{cde}	44±5.8^{cde}	47.3±8^{cde}	46.7±4

PH monitoring in liquid media

Generally, it could be suggested that the microorganism type and the temperature are the main factors which play the role in the change of pH value. Also, the results show that the pH value of the growth media tend to decrease in presence of the fungi strain more than the bacteria strain under 35˚C than 27˚C. The pesticides free media (control) show the lowest PH value (**Table 3**) after the first day as compared to pirimiphos-methyl treated media. This may be due to the weakness state of the microorganisms at the beginning of the incubation with the pesticides under investigation

Table (3): pH value profile during 7 days incubation of bacteria and fungi strain with different concentrations of pirimiphos methyl under 27˚C and 35˚C

Isolate	Temperature °C	Concentration (PPm)	Sampling intervals in days				
			1	2	3	4	7
Bacteria	27°C	0 ppm	03.85+.06^{c}	3.55+.02^{cdef}	3.62+.02^{c}	3.54+.05^{gh}	3.63+.01^{ef}
		40 ppm	4.52+.1^{a}	3.58+.07^{cde}	3.66+.04^{c}	3.63+.03^{efg}	3.68+.01^{cde}
		100 ppm	4.61+.09^{a}	4.3+.1^{a}	3.77+.04^{b}	3.66+.01cdefg	3.74+.02bcde
	35°C	0 ppm	3.17+.0^{1j}	3.24+.05^{j}	3.34+.1^{e}	3.48+.17^{h}	3.26+.02^{h}
		40 ppm	3.62+.02de	3.29+.01^{ij}	3.37+.06de	3.34+.03^{i}	3.23+.01^{h}
		100 ppm	4.07+.03^{b}	3.39+.02^{ghi}	3.34+.02de	3.32+.01^{i}	3.23+.02^{h}
Fungi	27°C	0 ppm	3.5+.13^{efg}	3.43+.04^{fgh}	3.61+.05^{c}	3.79+.02^{ab}	3.78+.04^{abcde}
		40 ppm	3.49+.08^{efg}	3.53+.29^{cdef}	3.98+.04^{a}	3.77+.02^{abc}	3.8+.03^{abcde}
		100 ppm	3.67+.03^{d}	3.61+0^{bcd}	3.95+.02^{a}	3.78+.01^{ab}	3.82+.04^{abcd}
	35°C	0 ppm	3.39+.03^{gh}	3.48+.17^{efgh}	3.34+.07^{e}	3.59+.09^{fgh}	3.47+.29^{g}
		40 ppm	3.32+.01^{hi}	3.62+.02^{bc}	3.35+.02^{e}	3.75+.05^{abcd}	3.69+.02^{cde}
		100 ppm	3.47+.02^{fg}	3.54+.01^{cdef}	3.47+.21^{d}	3.64+.02^{defg}	3.7+0^{bcde}

A, b, c, d, e, f, ------means in the same column per each item having different letter are significantly diferent (P≤0.01)Esterase activity.

Protein content:

The intracellular (mg/g) protein content of bacteria increased significantly after the 1st, 2nd, 4th and 7th days of incubation with 40 and 100ppm concentration, of primiphos-methyl at 27˚C; At 35˚C however, the increase was after 1st to seven days with 40ppm concentration but with 100ppm this increase was after 2nd to seven days only. The extracellular (mg/dl) protein contents decreased significantly after 1st, 3rd 4th and 7th days of incubation with 40ppm of pirimiphos-methyl at 27˚C while at the same temperature with 40ppm concentration the decrease was after 1st, 2nd 3rd and 7th days of incubation .The extracellular (mg/dl) protein content was increased after 2nd to 7th days of incubation with 40ppm concentration at 35˚C while at 100ppm it increased significantly at the 3rd ,4th and 7th days. At 35˚C the increase was significant after 1st to 7th days of incubation with both concentrations. The decrease was significant after 4th and 7th days with both concentration 40 and 100ppm at 27˚C. The extracellular (mg/dl) protein content was decreased significantly after 1st, 2nd, 3rd, and 7th days of incubation with primiphos-methyl concentration 40ppm at 27˚C but at 100ppm there was an increase in the protein content after 1st, 2nd, 4th, and 7th days. (**Table 4**).

Esterase activity:

The extracellular esterase activity was recorded at both incubation temperatures. The esterase activity was increased significantly after 1st day incubation at 27˚C. The maximum activity was 8.86 ± 2.5 mm ol/h mg protein after the second day with 40ppm pirimiphos-methyl where the control value was 5.98 ± 7 mmol/h/mg protein. Under incubation temperature 35˚C and 40ppm pirimiphos-methyl the esterase activity reached 9.73 ± .8 mmol/h/mg protein against 5.89 ±.2 mmol/h/mg protein for the control (**Table 5).** The activity of the intracellular esterase was inhibited through the incubation of the bacteria strain with 40 and 100ppm pirimiphos-methyl under 27˚C. The incubation decreased significantly after the first and seventh day incubation of the bacteria with 40 and 100ppm of pirimiphos-methyl.

The intracellular esterase activity of fungi strain at 27˚C was decreased at 1st and 2nd day with 40 ppm concentration but showed an increase at 3rd, 4th and 7thdays.With 100ppm concentration the activity decreased at 1st, 2nd, 3rd and 4th days but increased in 7th day. The extracellular esterase activity was increased at 1st, 2nd, 3rd and 7th days with 40ppm concentration at 27˚C and decreased at the 4th day. The extracellular esterase activity decreased at 1st, 2nd, 4th and 7th days, but increased at 3rd day at the same conditions but with 100ppm.

Pirimipos-methyl biodegradation in liquid media:

The residue of 40 ppm pirimipos-methyl in control (without microorganism was ranged from 49.6 ± 1 % after one day to 6.6 ± 8%, when incubated under 27˚C. The determined residue after 7 days when incubated at 35˚C ranged from 50.3 ± 1.3% after one day to 20.7 ± 1% after seven days incubation. **Table 6** shows the same trend in case of the higher concentration 100ppm. **Walker and Stojanovic (1973)** found that malathion disappeared from natural soil more rapidly than from soil sterilized by a number of techniques, **Getzin (1968)** found that degradation rates for diazinon were similar in autoclaved and non-autoclaved soil, while Zinophos degraded faster in non -autoclaved soil than in autoclaved soil. They also stated that parathion persisted longer in autoclaved soil than in non-autoclaved soil.

Table 4

Table (5) Residue percenet of Primiphos-methyl and Aldicarb in Liquide media incubated with bacteria and fungi under 27 °C and 35 °C

Isolate	Temperatur °C	Concentration (ppm)	Ssmpling intervals in days				
			1	**2**	**3**	**4**	**7**
Free	**27**	**40**	49.6±1	40±1.9	39±1.5	25.9±1.2	6.6±.8j8
		100	44.3±.8	35.5±1.3	27.8±1.2	22.5±0.3	17.5±1.6
	35	**40**	50.3±1.3	43.3±1.3	33.7±1.3	25.4±2.3	20.7±1
		100	46.7±1.8	38.7±.3	24.3±.9	23.3±1.3	17.2±1
Bacteria	**27**	**40**	50.6±1.8	40.4±2	27.4±1.2	21.9±0.7	16.3±1.5
		100	34.9±2.2	35.1±1.5	28.8±1.5	25±2	14.7±5.7
	35	**40**	45.5±2.7	39.2±1.6	29.7±1.5	22.3±1.5	17.2±0.9
		100	41±2	36.5±1.3	21±1	17.3±2	10.2±7
Fungi	**27**	**40**	42.9±1.5	35.9±.7	36.3±1.1	36.8±3.6	20.5±1.6
		100	27.9±1.8	21.7±.9	22.1±.3l	27±0.7	12±0.9
	35	**40**	20.9±.5	20±.6	12.7±2.1	12.2±1.9	10±0.6
		100	19.3±1.3	14.3±2.2	7.5±1.6	5±1	4±0.5

A, b, c, d, e, f, ----------- means in the same column per each item having different letter are significantly different (P<0.01)

The interaction of the microorganism type and temperature shows enhancement of the degradation of the pirimipos-methyl through the presence of the bacteria or fungi strain under 35˚C The percent residue of 40ppm pirimipos-methyl, incubated with bacteria strain under 27˚C ranged from 50.6±1.8 to 16.3 ± 1.5% after 1 and 7 days, respectively. Under 35˚C the residue of pirimipos-methyl was 45.5±2.7 and 17.2±9% after 1 and 7 days respectively. The percent residue of 100ppm pirimipos-methyl at 27˚C was 34.9±2.2 after the 1 day and 14.7± 5.7% after 7 days. Under 35˚C the percent residue ranged between 41±2% and 10.2±7% after the first and 7th day respectively.

Pirimipos-methyl was degraded through the fungi strain under 35˚C faster than under 27˚C. At the beginning of the incubation period, the biodegradation rate of pirimipos-methyl by fungi strain was faster than by bacteria strain, then tend to be slower until the 7th day. The percent residue of pirimipos-methyl 40ppm under 27˚C ranged from 42.9±1.5 to 20.5±1.6% for the 1st to 7th day, respectively. In the case of 35˚C the percent residue ranged between 20.9±5 and 10.0±6% for the 1st and the 7th day. **Table 6 Racke and Coats (1988a)** investigated comparative degradation of six organophosphorus insecticides in soil as affected by enhanced microbial degradation. **Racke *et al.*,(1990)**studied resistance of chlorpyrifos to enhance biodegradation in soil.

The degradation rate of pirimipos-methyl was faster during the all period of experiment by the fungi than the bacteria strain under 35˚C. On the other hand degradation by bacteria was accelerated up to the fourth day of incubation (**Table 6).** These effects may be due to the increase of the extracellular esterase activity, which tend to increase from the second day of incubation period. Generally, the degradation of pirimipos-methyl by the tested microorganisms was higher at 35˚C than at 27˚C with 40ppm concentration. The interaction of the two tested strains and the concentrations showed that the fungi degraded the pirimipos-methyl faster than the bacteria strain under the concentration of 40ppm and 100ppm. This mean that the biodegradation of pirimipos-methyl by the fungi strain depended on an enzymatic system difference from the esterase**. Martinez-Toledo *et al.* (1992)** reported that the fungal populations and denitrifying bacteria were not affected as a consequence of the addition of the organophosphorus insecticides such methylpyrimifos and chlorpyrifos to the agricultural soil, showing that these microorganisms can tolerate high amounts of those insecticides.

Table 6

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الهدم الحيوي لمبيد المثيل بريميفوس بواسطة نوعين من الكائنات الدقيقة المعزولة من تربة محافظة الفيوم

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أوضحت النتائج أن مكون الرطوبة والحرارة يسرع من هدم مبيد المثيل بريميفوس في التربة المعقمة. بلغت فترة نصف العمر لمثيل بريميفوس في التربة غير المعقمة حوالي ٢.١، ١.٥٧ يوم و ذلك على درجة حرارة ٢٧°م وعلى مستوى رطوبة ٢٥% و ٥٠% رطوبة نسبية على التوالي. في حالة التربة المعقمة ازدادت فترة نصف العمر الى ٣.٨ و ٣.١ يوم تحت نفس الظروف السابقة. تحت درجة حرارة ٣٥ °م كانت فترة نصف العمر للمبيد في التربة غير المعقمة ٢ و ١.٨ يوم علي مستوى رطوبة ٢٥% و٥٠% على التوالي، أما في التربة المعقمة كانت فترة نصف العمر لنفس المبيد ٦.٠١ و ٣.٥ يوم تحت تأثير درجة حرارة ٣٥ °م ومستويات رطوبة ٢٥% و ٥٠% على التوالي. تم عزل سلالتين من كائنات التربة الدقيقة إحداها كان موجب لصبغة جرام والأخر سلالة فطرية. تم اختبار هدم المثيل بريميفوس بواسطة هذه الكائنات في بيئة سائلة. كان معدل الهدم للمثيل بريميفوس بعد اليوم الأول والثاني والثالث أسرع بواسطة الفطر عن سلالة البكتيريا وذلك على درجة حرارة ٣٥ °م مقارنة بـ ٢٧ °م.

أرتبط استهلاك الجلوكوز بواسطة سلالة البكتيريا بزيادة نشاط أنزيمات الأستيريز حتى اليوم الثالث من التحضين لم تسجل أي اختلافات معنوية في استهلاك الفطر بين المقارنة والمعاملات بالمثيل بريميفوس.