Comparative Biological Aspects of Two Strains from the Egg Parasitoid, *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae) in Egypt

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ABSTRACT

Two geographical strains from the egg parasitoid, *Trichogramma evanescens* Westwood (TE) were derived from parasitized egg-masses of the lesser sugar-cane borer, *Chilo agamemnon* Bles., collected from sugar cane fields at Komombo (Aswan Governorate – Upper Egypt) (UET) and from rice fields at Sakha (Kafr EL-Shiekh Governorate – Lower Egypt; (Delta)) (LET). The study was carried out to check whether the two strains are subspecies, geographical eco-types or two species through estimating their thermal requirements, cross-mating test and DNA analysis, developmental periods, rate of parasitism, emergence rates and sex ratio of both UET and LET using, the grain moth, *Sitotroga cerealella* Oliv. eggs as host were obviously influenced by different temperatures. Developmental thresholds (t), for the period pupa to adult (stage 2) (8.3 and 10.7 °C) for the two strains were relatively higher than the (t) for the period, egg to adult (stage 3) (7.1 and 6.4 °C) and much higher than the period, egg to pupa (stage 1) (2.8 and 1.2 °C), respectively. Recorded values were higher for UET than for LET at stages 1 & 3, while it was *vice versa* in stage 2. The heat sum requirement for the development (k) of UET was 31.6 day-degree higher than that for LET. Random Amplified Polymorphic DNA polymerase Chain Reaction (RAPD-PCR) and cross-mating tests proved that the strains belong to the same species, *T. evanescens*.

Key Words: *Trichogramma evanescens*, Strains, Thermal requirements, DNA, Egypt

INTRODUCTION

The relationship between temperature and rate of development in insects is linear over most of the range of temperatures to which they are exposed. Exceptions to this occur near high temperatures that prove deleterious or lethal to the insects, and near the developmental threshold (t) of the insect. The developmental threshold is defined as that temperature below which no measurable development occurs. The amount of heat required to complete development from egg to adult (k) is considered a thermal constant. The parameters (t) and (k) can be estimated by least squares regression analysis of the effect of temperature on the rate of development (Andrewartha and Birch, 1954).

In case of biological control, the thermal requirements, *i.e.* (t) and (k) of natural enemies are one among many attributes (*e.g.* fecundity, searching capacity, host preferences, etc.) that acting in concert with environmental factors will influence the outcome of attempts for biological control of a host. The thermal requirements of a natural enemy may determine its rate of success, or failure, in the biological control of a given host population. Different parasitoid species attacking pests in different geographical areas and also, genetic, behavioral, and bio-systematic differences among populations of parasitoids described under one species were found. Each species has several hosts and that one host species can be common to several parasitoid species (Gonzalez *et. al.* 1979 and 1990, Neuffer, 1988, Unruh *et. al.* 1989 and El-Heneidy *et al.*, 2003).

Today, *Trichogramma* species (Hymenoptera: Trichogrammatidae) are the most widely used insect natural enemy in the world (Li-Ying. 1994), partly because they are easy to mass rear and they attack many important crop insect pests. *Trichogramma evanescens* Westwood, as a species of the trichogrammitid group has many local or specialized races, and four have been distinguished by Shchepetil *et al.*, (1975) as adapted to special groups of hosts. Within these races, there are ecological forms differing in environmental requirements. Different requirements for heat and moisture and other environmental factors (solar) are limiting their occurrence (Shchepetil *et al.*, 1975). *T. evanescens* dispersal was affected by cumulated solar radiations at temperatures above 15 degrees C. (Fournier and Boivin, 1999).

T. evanescens is the most common, native and widely distributed egg parasitoid species in several field, vegetable and fruit crops in Egypt. Besides, it has been recorded in different Egyptian habitats. The wasp plays an efficient role (its rate of parasitism reaches 90 and sometimes 100% during the period August – October) against the stem borers of sugar-cane, maize and rice plantations, particularly, the lesser sugar-cane borer, *Chilo agamnenon* Bles. (Lepidoptera: Crambidae) in the three field crops

and the European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Pyralidae) in maize fields. It was also recorded on many other lepidopterous pests on the fruit trees; olive, apricot and peach (Hegazi, *et al.*, 2004). *T. evanescens* was mass reared and used successfully in Egypt through different programs for controlling; *C. agammenon* in sugar-cane fields (El-Heneidy *et al.*, 1991), in rice fields (Soliman and Ewaise, 1997), the corn borers in maize fields (El-Mandarawy *et al.*, 2004), the cotton pink and spiny bollworms in cotton fields (Khidr *et al.*, 2003 and El-Heneidy *et al.* 2004) and the olive pests; *Palpita unionalis* Hb. and, *Prays oleae* (Bern.) (Hegazi, *et al.*, 2004).

T. evanescens (TE) has been recorded in Egypt by several authors as an efficient bio-control agent against *C. agammenon* in two different agro-ecosystems, almost at the same period of the year, August through October, in rice and maize fields in Northern Egypt (latitude 31.00N and average climatic conditions of 25.9 °C and 70.8% R.H.) and against the same pest in sugar-cane fields in Southern Egypt, >1000 km. apart and (latitude 24.20N and average climatic conditions of 31.3 °C and 37.5% R.H.).

Because of the extremely different climatic conditions between Upper- and Lower-Egypt and consequently expected different thermal requirements and developmental thresholds of the parasitoid in both regions, this study was carried out to check whether the two strains are subspecies, geographically eco-types or two species through estimating thermal requirements, cross-mating test and DNA analysis.

MATERIALS AND METHODS

Two TE strains were derived from parasitized egg-masses of the lesser sugarcane borer, *C. Agamemnon,* collected from sugar cane fields at Komombo (Aswan Governorate – Upper Egypt) (UET) and from rice fields at Sakha (Kafr EL-Shiekh Governorate – Lower Egypt; (Delta) (LET). Both UET and LET were mass reared on the eggs of the grain moth, *Sitotroga cerealella* Oliv. under the laboratory conditions of 25±1 °C and 65±5% R.H. and a photoperiod of 8L : 16D. *S. cerealella* eggs offered to the parasitoid were always less than 24 h old.

Rearing of *S. cerealella* and *T. evanescens*

S. cerealella was reared on wheat grains after being heated for four hours at 200 °C. Rearing took place in a rearing cage contained 10 trays, filled with wheat grains (6 kg/tray). Eggs of *S. cerealella* were scattered on wheat surface (6 gm/tray) and the trays were kept in the cage until moths' emergence. Emerged moths were placed into an ovipositional cage, a wire cylinder. Deposited eggs were received in a plastic tray, placed under the ovipositional cage. Fresh eggs were collected daily. The eggs were glued on cardboard cards (2x5cm) to facilitate exposing them to the parasitoid (Hassan, 1981 and Shoeb, 2000).

The two strains of TE were mass cultured separately by enclosing freshly emerged adults (< 24 h after emergence) into transparent plastic jars (1 kg each) containing the cards carrying fresh *S. cerealella* eggs (< 24 h old) and honey droplets to serve as food for the parasitoid adults. The jars were kept under the above mentioned laboratory conditions for few days until the death of TE adults and / or the *S. cerealella* eggs turned black (successful parasitization), then the cards containing parasitized host eggs were removed and kept for further experimentations.

Thermal requirements of the two strains of *T. evanescens*

Developmental periods

Measurements of the effect of temperature on the development of the two strains; UET and LET as well their thermal requirements were studied at three developmental stages (from egg to pupa, from pupa to adult and from egg to adult). Four constant temperatures 15, 20, 25 and 30 °C were used. Small cards (2x5 cm), each carried about one hundred fresh host eggs were exposed individually to a single female for 6 h for each strain into glass tubes then the cards were removed and placed separately in an incubator at one of the tested temperatures, 15 replicates from each strain for each temperature degree were used.

Parasitoid adults of both UET and LET were checked daily and new fresh host egg cards were replaced in each tube until the death of adults. Number of parasitized eggs (black ones) was counted for estimating percentage of parasitism. Developmental periods were partitioned and estimated at the three periods; 1) egg to pupa (exposure date to the date of eggs when turned black), 2) pupa to adult and 3) egg to adult at each constant temperature. Percentages of adult emergence and sex ratio of the two strains at each tested temperature were also recorded.

Thermal requirements

Thermal requirements of the two strains of TE; UET and LET were calculated. Developmental rate (y) = (1/total developmental period in days) was

regressed against temperature (x) in the form of the equation: y = Ax +B where A is the slope of the regression line and B is the y-intercept (Camphell *et al.*, 1974). The developmental threshold (t) and the total day-degree (k) were calculated as follows: t = 1/A and k = - B/A. The developmental thresholds of the three periods; 1, 2 and 3 were estimated.

Random Amplified Polymorphic DNA polymerase Chain Reaction (RAPD-PCR)

DNA amplification by PCR is one of the most reliable techniques currently used in molecular biology. It allows rapid detection of the presence or absence of a target DNA sequence in any genetic material. In this technique, DNA is amplified *in vitro* by a series of polymerization cycles consisting of three temperature-dependent steps (Denaturation, annealing and extension) resulting in target DNA amplification (Mullis *et al.*, 1986 and Rychlik *et al.*, 1990).

The two strains; UET and LET were mass reared separately and newly emerged adults were freezed and maintained in a freezer until obtaining adequate numbers required for the DNA test.

Reactions of the two tested materials; UET and LET were performed in a total volume 50 µl reaction buffer (100 mM KCl, 100 mm Tris HCl pH 8.3) 3.0 mm $MgCl_2$, 200 mm dNTPs (Promega Biotec. Inc.) 50 p/mole primers and 0 .2 µl Taq Polymerase (Hot Start). This reaction was added to 0.1 µl genomic DNA. All reactions tubes, pipette tips, micro pestles and water were irradiated with UV light to destroy possible contaminating surface DNA (Ou *et al.*, 1991). Irradiation treatment was 20 minutes at 2.5 cm from the bulbs of a Gene linker (Biorad, inc.) UV light source. Tubes containing mixes were placed in a thermcycler (Perkin-Elmer 2400) and DNA was amplified using the temperature cycle (modified from Black *et al.*, 1992). Amplification of the DNA was performed by placing the tubes containing the reactions in a Perkin Elmer thermal cycler 2400. RAPD PCR performed in 50 µl reaction volumes for 30 cycles. After the reaction mixture was mixed with DNA loading buffer and electrophoreses on 1% Agarose gel. (These procedures were carried out by the specialists in the Genetic Engineering Research Institute, Agricultural Research Center, Giza, Egypt).

Cross mating

This experiment aimed to check if the two strains belong to the same species, TE or not. Parasitized host eggs from both UET and LET were separated individually in glass tubes early before adults' emergence. Emerged adults were sexed by use of a binocular. Small cards (2x5 cm), each carried about 50 fresh host eggs, were exposed individually for 6 h into glass tubes to couples of male UET + female LET and vice versa male LET + female UET for cross mating and eggs laying. 15 replicates were applied.

Statistical Analysis

Obtained data were statistically analyzed using T-, F-, Variance tests and linear regression using Advanced Graphed 2.11 Software.

RESULTS AND DISCUSSION

Effect of temperature on some biological parameters of the egg parasitoid, *T. evanescens*

Developmental periods, rate of parasitism, emergence rate and the sex ratio of the two strains, UET and LET of the egg parasitoid TE using the eggs of *S. cerealella* as host were obviously influenced by different temperatures.

Developmental periods: Increase of temperature shortened the developmental periods of wasp species (Table 1). As shown in the table, the time required to develop from egg to pupa (stage 1) did not differ significantly between UET and LET in most cases, while that for the pupa to adult (stage 2) and total developmental from egg to adult (stage 3) were influenced significantly by both the temperature and the strain. They prolonged in stage 2 from 5.06 - 6.13 days at 30 °C to15.84 -18.47 days at 15 °C and in stage 3 from 8.2 – 9.2 days at 30 °C to 22.17 – 26.18 days at 15 °C, almost 3-folds in both cases. Generally, they lasted also about 2-3 times the first developmental period (stage 1) at each temperature in all cases. Developmental periods of UET required always longer time than that of LET.

Parasitism rate: Highest parasitism rates (87.05 & 78.20 % for UET and LET, respectively) were recorded at 25 °C, while the lowest (61.9 & 60.87 % and 61.95 & 59.2 %) were found at 15 and 30 °C, respectively (Table 1). Parasitism rates were always higher in case of UET by a range between 1.7 and 10.6 %.

Emergence rate: Highest emergence rate (87.21 & 74.31 for UET and LET, respectively) was also recorded at 25 °C, while the lowest (60.10 & 51.94 % and 59.21 & 56.05 %) were found at 15 °C and 30 °C, respectively (Table 1). Emergence rates were always higher in case of UET by a range between 5.4 and 14.8 %.

Sex ratio: As shown in table (1), highest sex ratio, about 2 female: 1 male was recorded at 25 °C and relatively less at 20 °C., while it was about 1: 1 at

Table (1): Effect of temperature on some biological aspects of two strains of the parasitoid *T. evanescens*

Temp. (°C)		15	20	25	30
Developmental period (days)					
Stage 1 (egg – pupa)	LET	7.18 ± 1.00	4.0 ± 0.49	3.76 ± 0.48	3.05 ± 0.24
	UET	8.27 ± 2.60	4.17 ± 0.38	3.82 ± 0.64	3.06± 0.24
Stage 2 (pupa – adult)	LET	15.84 ± 0.60	10.6 ± 0.59	6.0 ± 0.57	5.06 ± 0.59
	UET	18.47 ± 0.61	11.86 ± 0.74	8.1 ± 0.80	6.13 ± 0.63
Stage 3 (egg – adult)	LET	22.17 ± 0.04	14.4 ± 0.63	10.2 ± 1.54	8.2 ± 0.77
	UET	26.18 ± 1.01	17.2 ± 0.68	11.3 ± 1.56	9.2 ± 0.68
Parasitism rate %	LET	60.87 ± 4.89	69.4 ± 4.37	78.20 ± 4.76	59.2 ± 3.29
	UET	61.9 ± 3.67	77.6 ± 4.35	87.05 ± 6.58	61.95 ± 3.56
Emergence rate %	LET	51.94 ± 1.64	59.31 ± 2.70	74.31 ± 2.33	56.05 ± 2.48
	UET	60.10 ± 2.58	65.89 ± 3.68	87.21 ±2.20	59.21 ± 2.68
Sex ratio (F : M)	LET	1.2 : 1	2.0 : 1	2.2 : 1	1 : 1
	UET	1.1 : 1	1.9 : 1	2.1 : 1	0.9 : 1

N = 15, LET = Lower Egypt TE, UET = Upper Egypt TE

15 °C and 30 °C.

All biological parameter data at 20 °C were more or less closer to that at 25 °C than to the other two degrees 15 and 30 °C (Table 1). It seemed that, at 25 °C that was the most suitable one for the two eco-types in all the cases measured. Abd El-Hafez (1995) reported that the optimum temperature for fecundity ranged between 22 and 28 degrees °C and that highest numbers of progeny of TE were produced at 25 °C and 28 °C.

Thermal requirements of the two strains of *T. evanescens*

As shown in the table (2) and the figures (1 & 2), the developmental thresholds (t) for the period pupa to adult (stage 2) for the two strains (8.3 and 10.7 °C) were relatively higher than the (t) for the period egg to adult (stage 3) (7.1 and 6.4 °C) and much higher than the period egg to pupa (stage 1) (2.8 and 1.2 °C). Recorded values were more or less higher for UET (2.8 and 7.1 °C) compared to that of LET (1.2 and 6.4 °C) at the two stages 1 and 3, while it was vice versa in stage 2 (10.7 for LET compared to 8.3 for UET).

As shown in table (2), the values of k differed according to the strain as well the developmental stage. The lowest heat sum requirements (k) (83 and 88.7 day-degree) were estimated for the developmental stage (1) followed by (134.3 and 82 day-degree) for the developmental stage (2), while the highest (224 and 192.4 day-degree) were found for the developmental stage (3) for UET and LET, respectively.

The recorded values for (k) through the three developmental stages were found more or less higher in UET in the stages (2) and (3) (134.3 and 224 day-degree) than those for LET (82 and 192.4 day-degree), and vice versa in stage (1) (83 and 88.7 day-degree) for the strains, respectively. The heat sum requirement for the development of UET was 31.6 day-degree higher than the average heat sum requirement for LET (Table 2).

Table (2): Linear regression of developmental threshold against temperature for two strains of *T. evanescens*.

Strain	Stage	Regression Equation	t (°C)	k (day-degree)
UET	Egg –pupa	y = 0.01206x-0.03325	2.8	83
	Pupa-adult	y = 0.00745x - 0.06155	8.3	134.3
	Egg –adult	y = 0.00447x - 0.03173	7.1	224
LET	Egg –pupa	y = 0.01128x - 0.01333	1.2	88.7
	Pupa-adult	y = 0.12182x - 0.13002	10.7	82
	Egg –adult	y = 0.00519x - 0.03343	6.4	192.4

Obtained values of t and k in the case of the total developmental stage from egg to adult were relatively higher than that reported by Shoeb (2005) for TE using the same host, eggs of *S. cerealella*. Also, t values were (7.1 and 6.4 °C) for UET and LET, respectively versus (4.25) and respective k values were 224 and 192.4day-degree versus 141. Abd El-Hafez (1995) reported 10.44 °C as the value of t and 146.17 day-degree as k for the same parasitoid when the pink bollworm, *Pectinophra gossypilla* Saund. eggs were used as host.

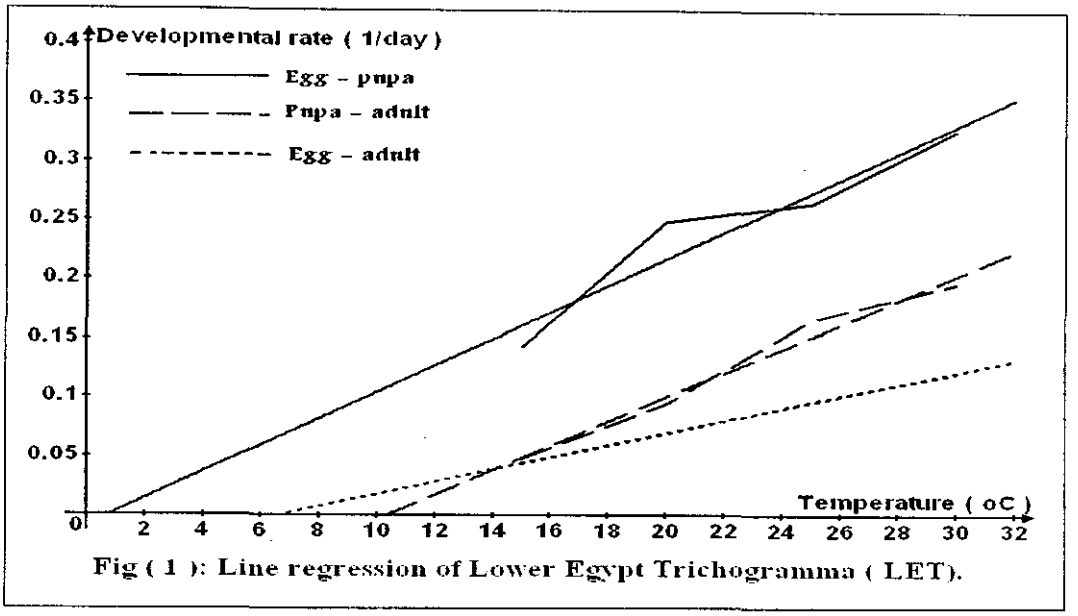

Fig (1): Line regression of Lower Egypt Trichogramma (LET).

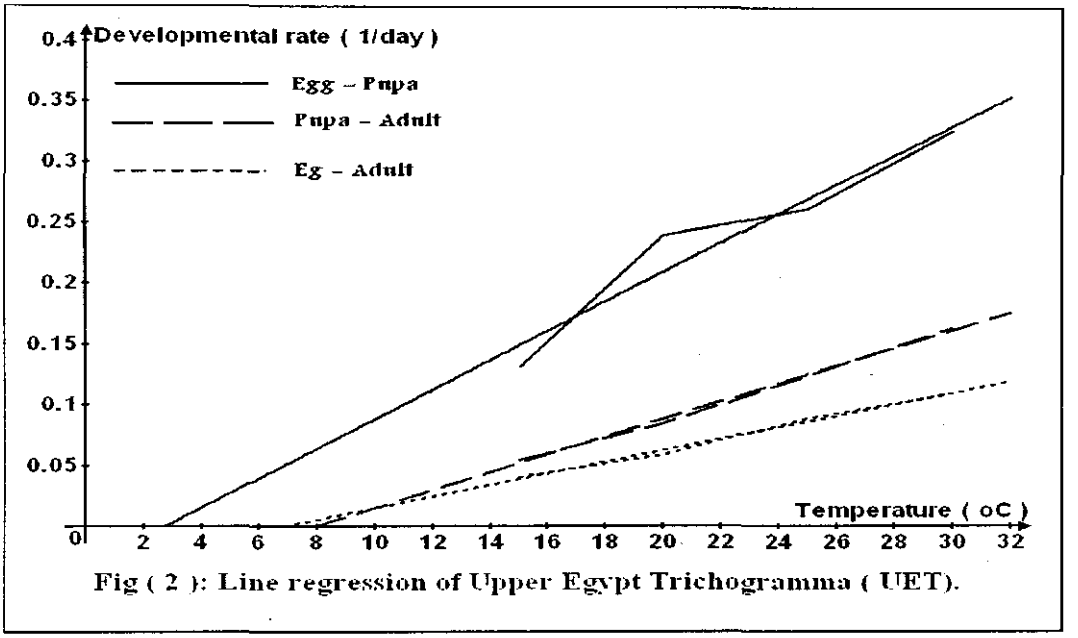

Fig (2): Line regression of Upper Egypt Trichogramma (UET).

Statistical analysis

According to the values of T-test, F-test and variances there were no significant differences in stage (3) (egg to adult) between the two strains at 20, 25 and 30 °C but at lower temperature (15 °C) there was a significant difference. Also temperature had a highly significant effect on the developmental period (egg to adult) for both the two strains.

There was no significant difference in stage (1) (egg to pupa) between the two strains at 20 and 25 °C. Also the temperature had no significant effect on the developmental period (stage, 1) for both the two eco-types.

There was no significant difference in stage (2) (pupa to adult) between the two strains in all tested temperatures. But the temperature had a significant effect on developmental period (stage, 2) for both the two strains.

There was no significant difference in parasitism rate between the two strains at 15 and 30 °C but at 20 and 25 °C there was a slight difference. Also, the temperature had significant effect on parasitism rate for LET and highly significant effect for UET.

The emergence rate had nearly the same statistical indications as that for the parasitism rate.

There was no significant effect of temperature on the sex ratio for the two strains; also there were no significant differences in sex ratio between the two strains in all tested temperatures.

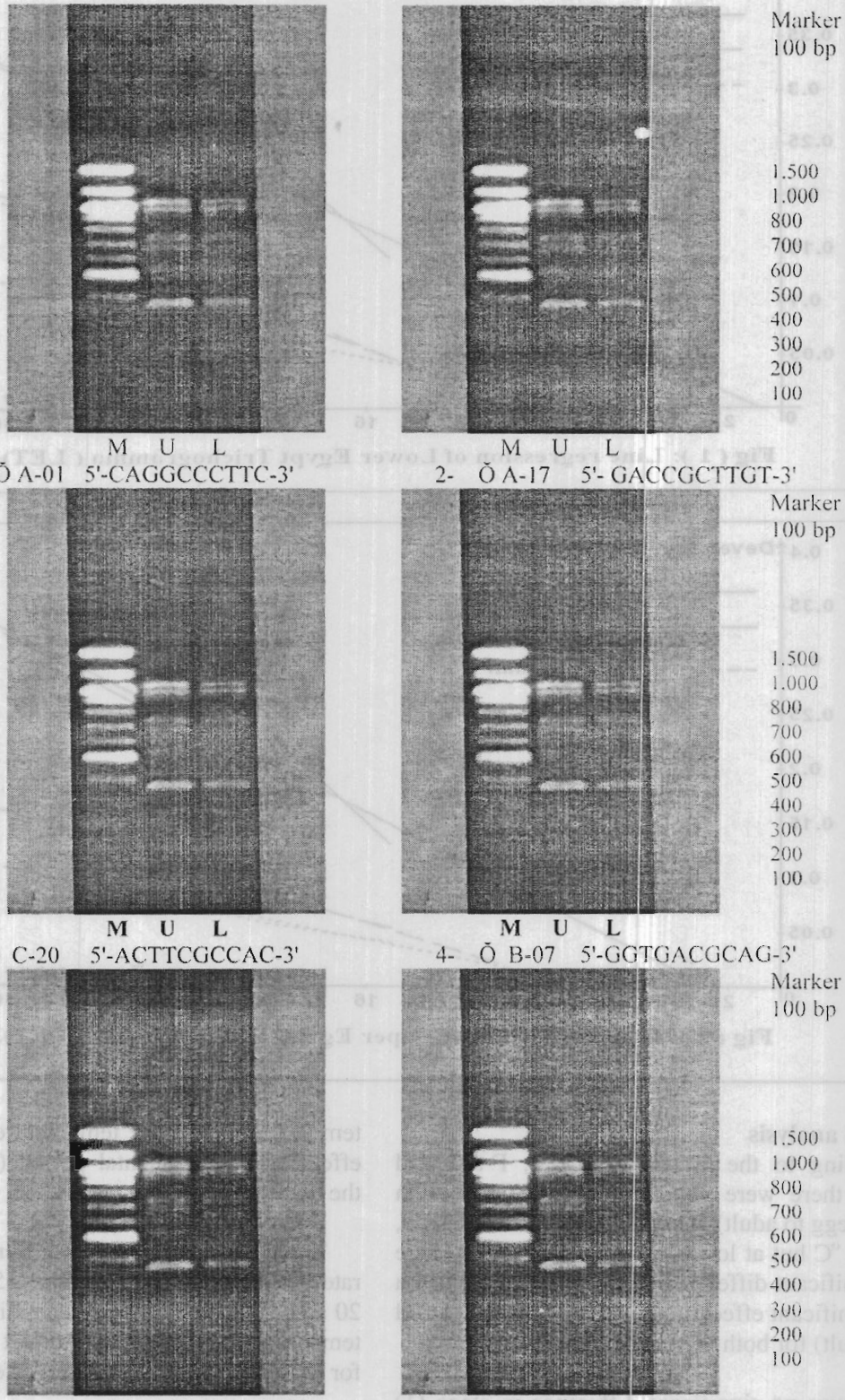

Fig. (3): DNA gel electrophoresis bands using 6 primers
Lane 1 (M) = DNA Master Lane 2 (U) = UET Lane 3 (L) = LET

DNA Test

Random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) was carried out using 6 primers showed that both samples of UET and LET look like the same identity (same species) because the same profile was obtained and there was no obvious genetic difference between both tested materials (Fig. 3).

Cross Mating Test

The cross mating test was carried out under laboratory conditions to check if the two strains belong to the same species TE and they represent two geographical eco-types or not. The test proved that both strains were the same species as they succeeded to mate and produce progeny completed their life cycle inside the eggs of *S. cerealella* for two generations. This result confirmed obtained data of the DNA analysis that there were no genetic differences between the two strains.

In general, natural parasitism rates by TE were always higher in the southern region (UET) where almost no pesticides are used, particularly in sugar-cane fields than in the northern region (LET) where the pesticides use in comparison is much higher in maize and rice fields. ET as a species of the trichogrammatid group has many local or specialized races adapted to special groups of hosts. Within these races, there are ecological forms differing in environmental requirements (Shchepetil *et al.*, 1975). Ram *et al.*, 1995 mentioned that strains of TE, collected from six different geographical regions in Eurasia, Russia were compared in terms of their biological and morphological characteristics and electrophoretic patterns. Significant differences were observed among the strains for biological characteristics such as the proportion of female progeny, developmental period, longevity, proportion of adults emerging and fecundity. Fournier and Boivin, 1999 reported that TE dispersal was affected by cumulated solar radiations at temperatures above 15 degrees °C.

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