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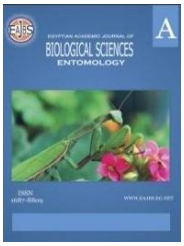

# Genetic Fingerprint Techniques and Biological Aspects of Three *Trichogramma* Populations Inhabiting Different Agroecosystems

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## ABSTRACT

The present work was initiated to study genetic differences by fingerprint technics and determine biological traits for the three *Trichogramma* populations inhabiting different Egyptian agroecosystems, including Abo Qurqas, Minia Governorate (*Trichogramma* M), Kharga, New Valley Government (*Trichogramma* NV), and Armant, Luxor Governorate (*Trichogramma* Lux). Molecular techniques, RAPD, and ISSR markers were used to distinguish the three *Trichogramma* populations. Results indicated that the three *Trichogramma* populations were separated into two clusters with individual RAPD and ISSR markers. Cluster I included TM and TLux groups, while Cluster II included only the TNV group. Biological studies included the effect of five host *Sitotroga cerealella* (SC) densities on three egg parasitoids, TM, TNV, and TLux populations. Biological criteria include percentages of parasitism, successive parasitized eggs, adult emerged from parasitized eggs, and female emerged parasitoids. Results indicated that the maximum female emerged parasitoids% was 56.12 ±15.27 in the TM group (F value= 4.49**). However, the minimum female emerged parasitoids% was 28.02 ±3.98 in the TLux group.

So, the present study highlights the need to integrate morphogenetic and biological descriptions for rapid and accurate identification of *Trichogramma* species within diverse Egyptian agroecosystems, thereby promoting effective and sustainable pest control.

## INTRODUCTION

Biological control, utilizing natural enemies like parasitoids, is an essential part of integrated pest management (IPM) techniques. The genus *Trichogramma* (Hymenoptera: Trichogrammatidae) is recognized as highly effective and extensively utilized egg parasitoids, playing a significant role in controlling a wide array of agricultural pests, particularly those belonging to order Lepidoptera (Smith, 1996). Their effectiveness in managing economically impactful pests has driven their extensive implementation in global agroecosystems, where their success depends on genetic diversity, host preference, and specific environmental conditions' adaptability (Pinto and Stouthamer, 1994a). Knowledge of the genetic structure and biological characteristics of *Trichogramma* populations across various agroecosystems is fundamental for enhancing their efficacy and ensuring optimal

application. Identifying the appropriate *Trichogramma* species for field release is a critical step in biological control programs utilizing these parasitoids (Sayed *et al*., 2011). A significant advancement in *Trichogramma* systematics was the recognition of male genitalia as a key identifier, now the standard method. Supporting features include body color, wing venation, and antennae. However, due to the difficulty in accurately identifying females, male specimens are essential for reliable species determination (Pinto and Stouthamer, 1994b; Knutson, 1998). Traditional *Trichogramma* taxonomy, relying heavily on male genitalia morphology, is a laborious and specialized process (Pinto, 1999; Samara *et al*., 2008), compounded by the minute size of these egg parasitoids wasps (<1mm in length).With the capacity to parasitize eggs from diverse insect orders, particularly Lepidoptera, *Trichogramma* species are extensively mass-reared and deployed in contemporary biological control initiatives (Smith, 1996; Samara *et al*., 2008). However, accurate identification of *Trichogramma* species is essential for the effectiveness of biological control programs (Stouthamer *et al*., 1999; Samara *et al*., 2008). Matching *Trichogramma* species or strains to the appropriate pest is crucial and extensively discussed (Stouthamer *et al*., 1999). Multiple studies have demonstrated cases of biological control projects that were early failed due to misidentification of natural enemies (Hassan, 1994). To effectively select *Trichogramma* strains for mass production and field release, it is vital to discern differences between populations from varying geographical origins, thereby enhancing our understanding of their characteristics. The use of DNA fingerprinting for genetic variation analysis has emerged as a vital method in both taxonomic classification and the investigation of genetic and evolutionary processes in insect species (Loxdale and Lushai, 1998). Among the most employed DNA markers are random amplified polymorphic DNA (RAPD) analysis and inter-simple sequence repeat (ISSR). Global augmentation programs targeting Lepidopteran pests often employ inundative releases of various *Trichogramma* egg parasitoids species (Hymenoptera: Trichogrammatidae), effectively preventing larval damage by targeting the pest's egg stage (Puneeth and Vijayan, 2014).*Trichogramma* species are readily reared in laboratory settings using hosts like Angoumois grain moth *Sitotroga cerealella* (Olivier, 1789) (Lepidoptera: Gelechiidae), Mediterranean flour moth *Ephestia kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae) and Rice moth *Corcyra cephalonica* (Stainton, 1866) (Lepidoptera: Pyralidae). Understanding biological traits, such as parasitization rate and longevity, is crucial for successful biological control outcomes (Oliveira *et al*., 2003; Puneeth and Vijayan, 2014). Accurate knowledge of *Trichogramma* biology is necessary for their optimal application in crop protection strategies (Pizzol *et al*., 2012). Therefore, comparative studies focusing on the genetic and biological attributes of *Trichogramma* populations from diverse agroecosystems are vital for identifying the most effective strains for targeted pest management strategies. So, the present work has been conducted to study genetic differences by fingerprint techniques (RAPD and ISSR) and determine biological traits for the three *Trichogramma* populations inhabiting different Egyptian agroecosystems at Minia, New Valley, and Luxor Governorates.

## MATERIALS AND METHODS

### *1-Trichogramma* Collection and Rearing:

Egg parasitoids *Trichogramma* supplied from three laboratories established in different geographical regions (Governorates) of Egypt include:

**a)** *Trichogramma* Minia collection (TM) from Abu Qurqas, Minia Governorate.
**b)** *Trichogramma* New Valley (TNV) from Kharga, New Valley Governorate.
**c)** *Trichogramma* Luxor collection (TLux) from Armant, Luxor Governorate.

Two generations of egg parasitoids were reared on *Sitotroga cerealella* (Olivier,

1789) eggs at the Assiut University Biological Control Unit (AUBCU) (23±2°C, 75±5% RH, 16:8 L:D), Assiut, Northern Upper Egypt (375 Km South of Cairo).

**2. Molecular Genetic Identification:**

**2.1. Isolation of Genomic Deoxyribonucleic Acid (DNA) for three *Trichogramma* Populations:**

Genomic DNA was extracted according to **Stouthamer *et al*. (1999)** in Central Laboratories, Faculty of Agriculture, Assiut University, Assiut, Egypt.

**2.2. Distinguishing Genetic Differences Through Random Amplification of Polymorphic DNA (RAPD) and Inter-Simple Sequence Repeat (ISSR) Fingerprinting:**

**2.2.1. PCR-Based Amplification of RAPD and ISSR, and Gel Electrophoresis:**

The genetic variation and phylogenetic relationships of three *Trichogramma* populations were examined using RAPD-PCR with OPA-2 and OPA-3 primers, as described by Martorell *et al*. (2005). Also, four ISSR- PCR primers (HB, HB11, 17898 A, and 17898 B) were used, as shown in Table 1.

PCR reactions (25 µl) included GoTaq green master mix (Promega), 1 µl DNA, and 2 µl primer. RAPD-PCR: initial denaturation at 94°C (5 min), 45 cycles of 92°C (1 min), annealing at 36°C (1 min), extension at 72°C (2 min), and final extension at 72°C (10 min), 4°C hold. ISSR-PCR: 94°C (5 min), 45 cycles of 92°C (1 min), 38-44°C (1 min), 72°C (2 min), and 72°C (10 min), 4°C hold, respectively.

After separating 5 µl of amplified products on a 1.5% agarose gel in 0.5×TBE buffer, the gel was stained with 0.01% ethidium bromide and visualized under UV light. Fragment sizes were then determined by comparison to a 100-bp molecular marker.

**2.2.2. RAPD and ISSR Analysis:**

Using the respective primers, RAPD-PCR and ISSR-PCR generated fragments that were individually analyzed for band type determination. The size of each band was determined using Kodak Digital Science 1D (KSD1D 2.0, Rochester, USA) software, and the presences (1) or absences (0) of a band were recorded to generate a binary table. The data tables were exported into the NTSYS-pc software (Rohlf, 2000) for analysis. The normalized RAPD and ISSR patterns were further analyzed using Gel.

RAPD and ISSR similarity levels were determined using Pearson's correlation coefficient and clustered via UPGMA, generating dendrograms that illustrate pattern profile relationships as percentage similarity. The discriminatory power of these typing methods was quantified using Hunter and Gaston's (1988) numerical discriminatory index (D), all processed with Compare II version 2.5 (Applied Maths, St. Martens-Latem, Belgium).

**Table 1:** PCR primers (sequence and annealing temperature) of RABD and ISSR Markers used in the present study**.**

| Target gene | Markers | Primers Operon/Design | PCR primers sequence 10-mer in length - 5' to 3' | Annealing temperature (°C) |
|---|---|---|---|---|
| Genomic DNA | RAPD | OPA-02 | TGCCGCGCTG | 36 |
| | | OPA-03 | AGTCAGCCAC | 36 |
| | ISSR | HB | CAC ACA CAC ACA AC | 38 |
| | | HB11 | GTGTGTGTGTGTCC | 41 |
| | | 17898A | CACACACACACAAC | 44 |
| | | 17898B | CACACACACACAGT | 44 |

**3. Biological Studies of Three *Trichogramma* Collections:**

**3.1. *Trichogramma* Collection Colony:** As mentioned above, the three collected strains of *Trichogramma* were reared individually under laboratory conditions. The emerged adults of *Trichogramma* were kept 24 hr for mating. Mated females transferred for biological studies in Eppendorf Safe-Lock Tubes**,** 2.0 mL, Eppendorf Quality™, colorless, under laboratory conditions (23±2°c, 75±5% RH, L 16: D 8).

**3.2. *Sitotroga cerealella* Colony:** Mass production stages of the laboratory host (SC) were conducted as described by the Project Food Aid in Aswan **(**Mousa, 2018) with some modifications adapted in Biological Control Lab., Plant Protection Department, Assiut University, Assiut, northern Upper Egypt.

**3.3. Experimental Design:** Mated females were singly transferred to Eppendorf Safe-Lock Tubes**,** 2.0 mL, and supplied with the number of SC fresh eggs (5- 10- 20- 40 and 80 egg/ *Trichogramma* female) with (N= 10) for each tested *Trichogramma* collection. The old SC fresh eggs were <48 hr. Experiments were conducted under laboratory conditions and incubated for 24 hr (23±2°C and 70±5 RH %). Then, SC eggs were incubated under the same conditions. After three days, SC eggs were examined for initial parasitism (black eggs) **(**Fig. 1). Then, daily examination was conducted tell adult parasitoid emergence.

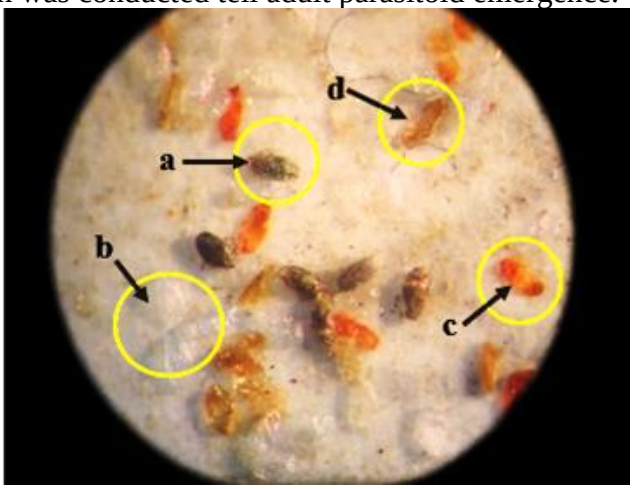


**Fig. 1:** Light micrograph of SC eggs parasitized and unparasitized (x= 16):
A-black egg =parasitism eggs, b- transparent eggs host larvae emerge from eggs, c- red eggs, and d- brown eggs = dead eggs.

**4-Data Analysis:**

Measurements of morphological and biological data were statistically analysed to calculate ANOVA, Correlation Coefficient, and Regression by using the SAS 9.1 software program (SAS, 2008).

## RESULTS

**1-Molecular genetic identification of *Trichogramma***

**1.1. Genetic Differentiation Fingerprinting Technique:**

Molecular differentiation of *Trichogramma* populations TNV, TM, and TLux was assessed using RAPD and ISSR-PCR fingerprinting.

**RAPD Marker:** Two ten-mer primers (OPA-2 and OPA-3) were used in RAPD-PCR to fingerprint selected strains. The resulting patterns showed 8-9 amplified fragments (90-1100 bp) per primer (Table 2, Fig. 2 a and b). Overall, 17 bands were detected, of which 82.35% were polymorphic and 17.65% monomorphic (Table 2).

**Table 2:** RAPD analysis used to assess the number of bands, monomorphism, and polymorphism (count and percentage) for the three *Trichogramma* populations

| Primer | Number of bands (a) | Number of monomorphic bands(b) | Number of polymorphic bands(c) | Monomorphism -b/a * 100% | Polymorphism -c/a * 100% |
|---|---|---|---|---|---|
| **OPA-2** | 8 | 0 | 8 | 0 | 100 |
| **OPA-3** | 9 | 3 | 6 | 33.33 | 66.67 |
| **Total** | 17 | 3 | 14 | 17.65 | 82.35 |

Fragment characterization from the two primers is visualized in Figure 2 (a, b), and primer and band details are summarized below:

**OPA-2:** The results of RAPD – analysis obtained by primer OPA-2 are illustrated in **(**Fig. 2 a). This primer reacted with all populations (TNV, TM, and TLux) and generated 8 fragments ranging in size between 90 to 900 bp. From the results, we can notice that the height reaction of this primer was TLux population, but the lowest reaction was with the TM population. The bands with sizes 900 and 500 bp were reacted only for the TNV population and the bands with sizes 220, 300, 400, and 600 bp were reacted only for the TLux population. Also, the band with size 800 bp was reacted only for the TM population by the primer (OPA-2). Results in Table 2, show that the polymorphic percentage was 100 %.

**OPA-3:** The results of RAPD-PCR analysis obtained by primer OPA-3 are illustrated in (Fig. 2 b). This primer reacted with all three *Trichogramma* populations generating 9 fragments ranging in size between 150 and 1100 bp. From the results, we can notice that the highest band (1100 bp) was reacted for the TNV population, and the lowest one (150 bp) was generated for the TLux population by this primer (OPA-3). On the other hand, the bands of sizes were 900 bp 400 bp, and 300 bp were found in all species. The highest reaction was found in the TNV population and the lowest one was found in the TM population. Results in Table 2, show that, the monomorphism and polymorphism percentages were 33.33% and 66.67% respectively.

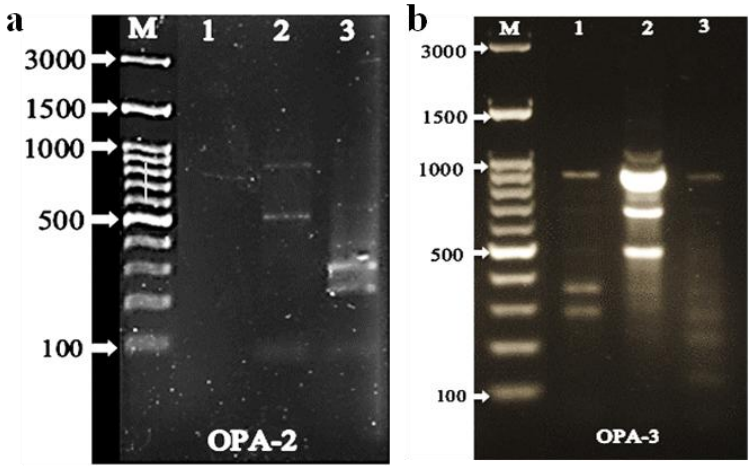


**Fig.2:** Agarose gel electrophoresis of RAPD products by:
**a**-OPA-2 primer and **b**- OPA-3 primer (lane M, 100- 3000bp DNA markers; lane 1, TM; lane 2, TNV and lane 3, TLux populations).

### 1.1.1. ISSR Marker:

ISSR-PCR amplification using four primers (Table 3) generated 35 bands and products 100-990 bp (Fig. 3 a-d). The number of amplified products per primer ranged from **5** (HB) to **15** (17898A), with 28 polymorphic (80%) and 7 monomorphic (20%) products detected.

**Table 3:** ISSR analysis used to assess the number of bands, monomorphism, and polymorphism (count and percentage) for the three *Trichogramma* populations.

| Primer | Number of bands (a) | Number of monomorphic bands(b) | Number of polymorphic bands(c) | Monomorphism - b/a * 100% | Polymorphism -c/a * 100% |
|---|---|---|---|---|---|
| **HB** | 5 | 0 | 5 | 0 | 100 |
| **HB11** | 6 | 6 | 0 | 100 | 0 |
| **17898A** | 15 | 0 | 15 | 0 | 100 |
| **17898B** | 9 | 1 | 8 | 11.11 | 88.89 |
| **Total** | 35 | 7 | 28 | 20 | 80 |

Fragment characterization from the four primers is shown in Fig. 3 (a-d), with primer and band details summarized below.

**HB:** The results of ISSR analysis with primer HB (Fig. 3a) revealed 5 amplified fragments (100-400 bp) in all *Trichogramma* populations. However, 280, 300, and 400 bp fragments were unique to the TNV population. This primer exhibited 100% polymorphism (Table 3).

**HB11:** ISSR analysis using primer HB11 (Fig. 3b) yielded 6 fragments (150-390 bp) in all *Trichogramma* populations. All fragments were monomorphic, resulting in 100% monomorphism, as indicated in Table 3.

**17898A:** ISSR analysis with primer 17898A (Fig. 3c) yielded 15 fragments ranging in size between (310-990 bp) in all *Trichogramma* populations. Specific bands were observed with sizes: 450, 510, 800, 900, and 990 bp were restricted only to the (TNV) population; 310, 370, 490, 500, and 570 bp to the (TLux) population; and 350, 400 bp to the (TM) population. Table 3, indicates 100% polymorphism for this primer.

**17898B:** The results of ISSR – analysis obtained by primer 17898B are illustrated in **(**Fig. 3 d). This primer reacted with all *Trichogramma* populations generating 9 fragments ranging in size between 360 and 700 bp. Also, bands with sizes 480, 490, and 600 bp were restricted only to the TNV population by the primer (17898B). The band with 670 bp size was monomorphic. Results in Table 3 show that the polymorphic % and monomorphic% were 88.89% and 11.11%).

**1.2. Genetic Similarity Matrix and Cluster Analysis:**

RAPD and ISSR marker presence/absence data were analysed using NTSYS-pc ver. 2.20c to calculate pairwise genetic similarities among the three *Trichogramma* populations.

**1.2.1. RAPD Marker:**

Genetic similarity data illustrated in Table 4, indicates that TNV and TLux are the most distinct *Trichogramma* populations (40% similarity). Conversely, TM exhibited the highest similarity to both TNV and TLux (42.9%).

The three *Trichogramma* populations were separated into two clusters; cluster I included TM and TLux populations, while cluster II included only the TNV population, as shown in (Fig. 4 a).

**1.2.2. ISSR Marker:**

The results of cluster analysis based on ISSR analysis are shown in Table 5. The highest similarity value recorded was 62.9% which was observed between TM and TLux populations, while the lowest similarity value (35%) was recorded between TNV and TLux populations. A dendrogram for the genetic relationships via ISSR analysis among the three *Trichogramma* populations results were carried out and are shown in (Fig. 4 b). The three *Trichogramma* populations were separated into two clusters; cluster I included TM and TLux populations, while cluster II included only the TNV population.

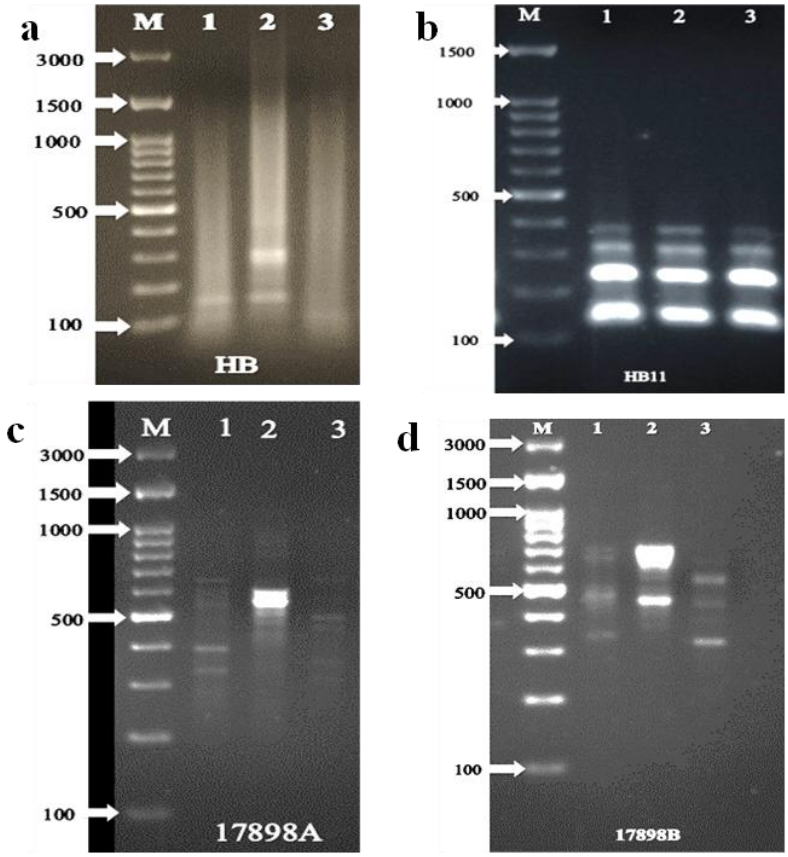


**Fig. 3:** Agarose gel electrophoresis of ISSR products by:
**a**-HB, **b**- HB-11, **c**- 17898A and **d**- 17898B primers (lane M, 100- 3000 bp DNA markers; lane 1, TM; lane 2, TNV and lane 3, TLux populations).

### 1.2.3. Combined RABD and ISSR Markers:

In Table 6, data showed that the lowest genetic similarity was observed between TNV and TLux populations (40.6%), while the highest value was observed when TM population was compared with that of TLux population (51.9%.). Also, the three *Trichogramma* populations were separated into two clusters (as mentioned above with individual RAPD and ISSR markers); cluster I included TM and TLux populations, while cluster II included only TNV population, as shown in **(**Fig. 4 c).

**Table 4:** Genetic similarity values calculated from the DNA fragments amplified from the three *Trichogramma* populations using two RAPD primers.

| Populations | TM | TNV | TLux |
|---|---|---|---|
| **TM** | 1.000 | | |
| **TNV** | 0.429 | 1.000 | |
| **TLUX** | 0.429 | 0.400 | 1.000 |

**Table 5:** Genetic similarity values calculated from the DNA fragments amplified from the three *Trichogramma* populations using four ISSR primers.

| Populations | TM | TNV | TLux |
|---|---|---|---|
| **TM** | 1.000 | | |
| **TNV** | 0.564 | 1.000 | |
| **TLUX** | 0.629 | 0.350 | 1.000 |

**Table 6:** Genetic similarity values calculated from the DNA fragments amplified from the three *Trichogramma* populations using two RAPD primers and four ISSR primers.

| Populations | TM | TNV | TLux |
|---|---|---|---|
| **TM** | 1.000 | | |
| **TNV** | 0.491 | 1.000 | |
| **TLUX** | 0.519 | 0.406 | 1.000 |

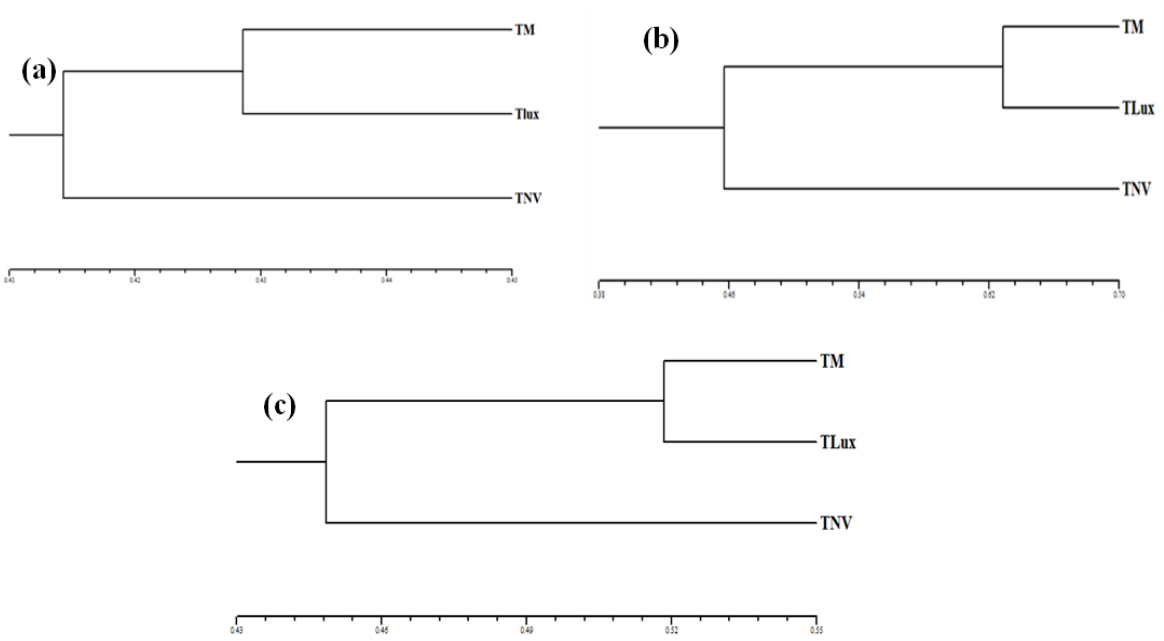


**Fig. 4:** Dendrogram demonstrating the relationship among the three *Trichogramma* populations, TM, TNV, and TLux, based on data recorded from polymorphism of: a- RAPD marker, b- ISSR marker, and c- combined RABD and ISSR markers.

**2-Biological Studies of *Trichogramma*:**

The present work aimed to study the effect of five host *S. cerealella* (SC) densities on three collected egg parasitoids, TNV, TM, and TLux populations. Biological criteria include percentages of parasitism, successive parasitized eggs, adult emerged from parasitized eggs, and female emerged parasitoids.

**2.1. Percentages of Parasitism:**

Data in (Table 7 and Fig. 5**)** show that, the host density affected on parasitism % of the three *Trichogramma* populations. Concerning *Trichogramma* from New Valley (TNV) the host density affected on the parasitism % with a highly significant (F value =7.070**). The maximum parasitism % reached (74.29± 8.28) with 5 host-density eggs and it reached the minimum percentage (23.39± 2.81) with 80 host-density eggs. An increase of the host density decreased the percentage of parasitism, and the correlation coefficient value (r) reached -0.527** and $R^2$ = 0.460 **(**Fig. 5a**)**. The same trend of results was in *Trichogramma* from Minia (TM) (F value = 10.240**). The maximum parasitism % reached (71.429±6.36) with 20 host-density eggs and it reached the minimum percentage (29.108±3.47) with 80 host-density eggs. The increase of the host density decreased the percentage of parasitism, and the correlation coefficient value (r) reached -0.611** and $R^2$= 0.544 **(**Fig. 5b**)**. Also, the *Trichogramma* from Luxor (TLux) F-value was 25.420**. The maximum parasitism % reached (92.00 ± 3.27) with 5 host-density eggs and it reached the minimum percentage (29.38 ± 4.98) with 80 host-density eggs. The increase of the host-

density decreases the percentage of parasitism and correlation coefficient value (r) reached -0.772** and $R^2$ = 0.768 **(**Fig. 5c**)**.

### 3.2. Other Biological Parameters for The Three Parasitoids Populations TNV, TM, and TLux in Different Host (SC) Eggs Density:

Data in Table 8 and Fig. 6 show that the effect of host egg density on a percentage of successive parasitized eggs, adults emerged from parasitized eggs, and female emerged parasitoids, for the three parasitoids populations TNV, TM, and TLux. Concerning the successive parasitized eggs% of the TLux population was affected with a highly significant F value (3.97**), with a maximum average of 93.54 ±6.76 %, **(**Fig. 6a). Percentage of adults emerged from parasitized eggs was affected significantly in TM population (F value= 2.67*), with a maximum mean 106.30 ±6.61 %, (Fig. 6b). The maximum female emerged parasitoids% was 56.12 ±15.27 in TM group (F value= 4.49**) (Fig. 6c**)**. However, the minimum female emerged parasitoids% was 28.02 ±3.98 and 36.39 ±15.51in TLux and TNV groups, consequently.

**Table 7:** Parasitism percentages of parasitoids TNV, TM, and TLux populations in different host SC eggs density

| Host density | Parasitism % ± SD | | |
|---|---|---|---|
| | TNV | TM | TLux |
| **5** | 74.29± 26.19**a#** | 65.71 ± 24.43**a** | 92.00 ± 10.33**a** |
| **10** | 51.43± 24.23**b** | 65.71±21.53**a** | 79.00 ± 15.24**ab** |
| **20** | 42.86± 24.02**ab** | 71.43±20.10**a** | 68.50 ±21.86**b** |
| **40** | 55.71± 12.62**bc** | 29.64±15.65**b** | 44.50 ±21.14**c** |
| **80** | 23.39± 8.87**c** | 29.11±10.97**b** | 29.38 ± 15.74**d** |
| **Mean** | **49.54± 5.88B** | **52.32± 6.67B** | **62.68± 8.06A** |
| **F-value** | 7.070** | 10.240** | 25.420** |
| **r** | -0.527** | -0.611** | -0.772** |
| **$R^2$** | 0.460 | 0.544 | 0.768 |

#Average having the same letter in each row is not significant at a 5% level of probability, according to Duncan's multiple range test.

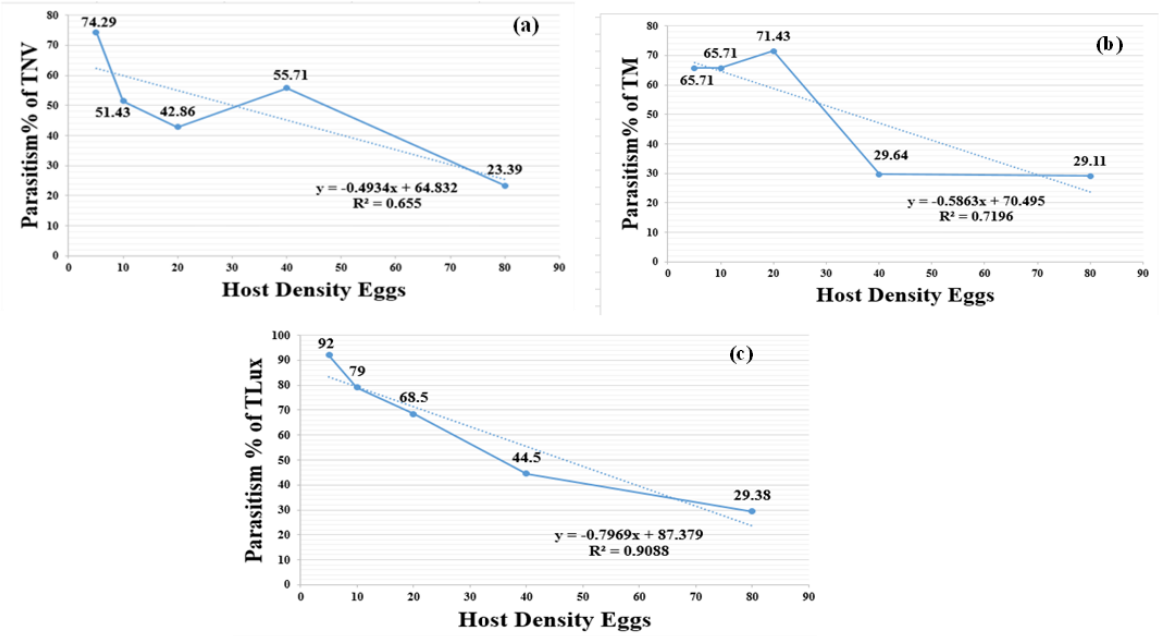


**Fig. 5:** The relationship between the host density of SC eggs and parasitism % of three *Trichogramma* populations: a- TNV, b- TM, and c- TLux.

**Table 8:** Biological parameters for the three parasitoids populations TNV, TM, and TLux in different host SC eggs density.

| Populations | Host density (n =10) | Parameters% | | |
|---|---|---|---|---|
| | | Successive parasitized eggs | Adults emerged from parasitized eggs | Female emerged parasitoids |
| TNV | 5 | 72.86 | 95.24 | 32.86 |
| | 10 | 91.02 | 113.29 | 17.14 |
| | 20 | 81.2 | 100.00 | 30.99 |
| | 40 | 81.67 | 103.04 | 41.72 |
| | 80 | 76.83 | 109.76 | 59.23 |
| | **Mean ±SD** | **80.72 ±6.78b#** | **104.27 ±7.30a** | **36.39 ±15.51b** |
| | **F value** | **1.69 ns** | **1.70 ns** | **3.01*** |
| TM | 5 | 85.00 | 104.76 | 59.05 |
| | 10 | 96.98 | 118.02 | 54.34 |
| | 20 | 95.91 | 102.81 | 79.08 |
| | 40 | 90.40 | 103.57 | 51.29 |
| | 80 | 90.78 | 102.34 | 36.87 |
| | **Mean ±SD** | **91.82 ±4.82a** | **106.30 ±6.61a** | **56.12 ±15.27a** |
| | **F value** | **1.40 ns** | **2.67*** | **4.49 **** |
| TLux | 5 | 93.50 | 107.50 | 24.49 |
| | 10 | 81.88 | 102.00 | 34.44 |
| | 20 | 96.70 | 105.10 | 29.18 |
| | 40 | 98.06 | 104.45 | 26.15 |
| | 80 | 97.55 | 101.25 | 25.80 |
| | **Mean ±SD** | **93.54 ±6.76a** | **104.06 ±2.51a** | **28.02 ±3.98b** |
| | **F value** | **3.97**** | **1.04 ns** | **0.68 ns** |

#Average having the same letter in each column is not significant at a 5% level of probability, according to Duncan's multiple range test.

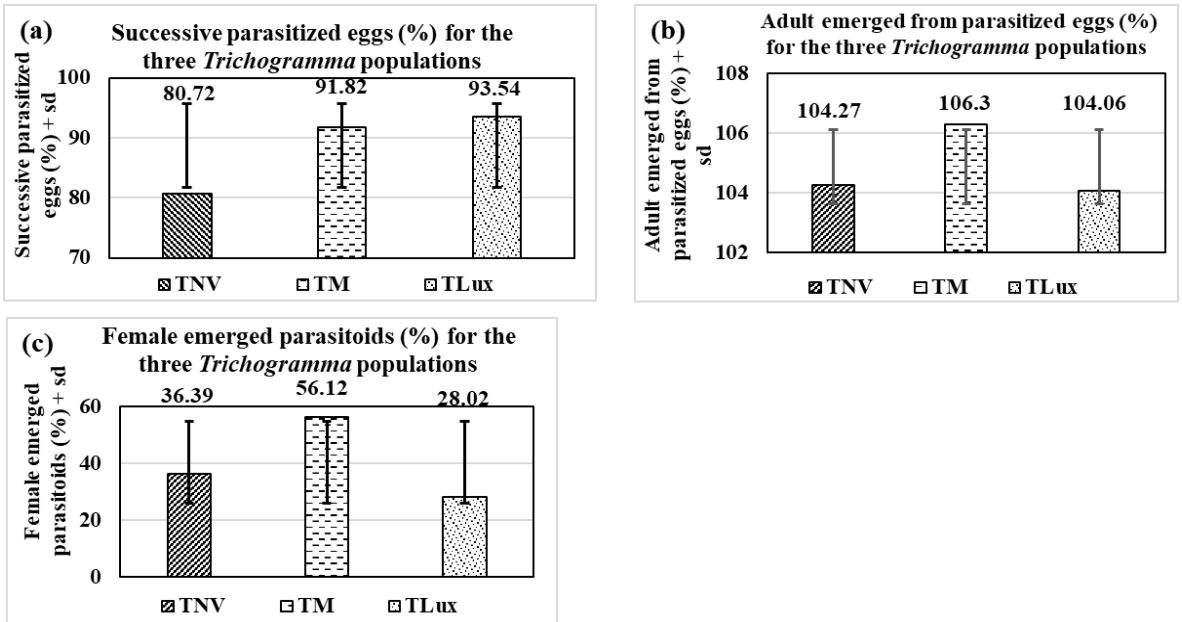


**Fig. 6:** The relationship between the host density of SC eggs and other biological parameters for three *Trichogramma* populations: a- successive parasitized eggs (%), b- adult emerged from parasitized eggs (%) and c- female emerged parasitoids (%).

## DISCUSSION

Molecular markers are essential for natural enemies to determine phylogenetic relationships, identify biotypes, and assess heritable variation for population genetics and ecological studies. RAPD and ISSR markers were used to distinguish three *Trichogramma* species from different Egyptian geographical zones. The efficacy of RAPD in discriminating between three *Trichogramma* species was investigated. ISSR markers, developed according to the methodology of Zietkiewicz *et al*. (1994), are employed to amplify DNA regions located between microsatellite sequences via PCR. Both RAPD and ISSR proved to be reliable species-diagnostic markers. ISSR, due to its high polymorphism, reproducibility, and low cost, is particularly useful for differentiating closely related individuals (Borba *et al*., 2005). As shown in the dendrogram (Fig. 4), generated from RAPD, ISSR, and combined RAPD/ISSR data, the three *Trichogramma* populations were clearly separated into distinct clusters. Results demonstrated an absence of correlation between the RAPD and ISSR profiles and the geographical origin of the *Trichogramma* strains under investigation. The results also showed that TM and TLux, both employed in sugarcane borer control, grouped into a single cluster, suggesting a relationship between the collected populations and the sugarcane agroecosystem.

Concerning, intensive and extensive search in different publications concerning the biological aspects of *Trichogramma* includes parasitism percentage, mortality of host eggs, parasitoids emergence, and development time (Broucheir and Smith, 1996; Gingras *et al*., 2002; Oliveira *et al.*, 2003; Grieshop *et al.*, 2007; Puneeth and Vijayan, 2014). The above-mentioned results indicated that the increase of egg host density from 5 to 80 eggs decreased significantly the parasitism% by TNV and TLux populations except by TM group, the parasitism% increased insignificantly from 5 to 20 eggs host density. These findings agree with Abdel-Galil (1978), who used egg parasitoid *Trichogramma evanescens* with a rate of 1:5 of host eggs *Heliothis armigera*. Also, one of the most important characteristics of effective parasitoids is the ability to find the lowest host density as reported by several authors (Hassan, 1994; Smith, 1996). So, such research, which indicated that lower densities one of the uses of *Trichogramma* spp., can be effective in the field, and could reduce cost (Parra and Zucchi, 2004; Van Driesche *et al.*, 2009).

The maximum parasitism% was 62.68± 8.06 by TLux followed by 52.32± 6.67 for TM. In this approach, the minimum parasitism% was 49.54± 5.88 by TNV. So, it's of interest to point herein that both TLux and TM *Trichogramma* are used in sugarcane fields against sugarcane borers, and TNV *Trichogramma* is used against date palm lepidopterous pests in which no pesticides are used. These findings agree with El-Heneidy and Shoeb (2007).

The present study examined the biological aspects of three *Trichogramma* populations (TNV, TM, and TLux), including successive parasitized eggs, adult emergence, and female parasitoids emergence. Female emergence is considered a key quality parameter, as supported by Singh *et al*. (2001), Ballal *et al*. (2005), and the International Organization for Biological Control (IOBC). However, Van Lenteren *et al*. (2003) and Ballal *et al*. (2005) established a 1:1 (female: male) sex ratio as a quality criterion for *Trichogramma brassicae*. According to Hassan and Zhang (2001), *T. brassicae* from German commercial suppliers had a higher female percentage than the IOBC standard. This observation aligns with the TM population, which showed a female-to-male sex ratio of 56.12 ± 15.27. Among parasitic Hymenoptera development by parthenogenesis can be subdivided into three categories include: Thelytoky (females only), deuterotoky (males and females), and Arrhenotoky (males only). Venkatesan *et al*., (2015) identified two different parthenogenesis *Trichogramma* including *T. evanescens* (Arrhenotokous), and *T. evanescens* (Thelytokous).

So, the TM population considered deuterotoky.

## CONCLUSION

Molecular techniques RAPD and ISSR markers can used to distinguish the three *Trichogramma* populations. The three *Trichogramma* populations were separated into two clusters with individual RAPD and ISSR markers. Cluster I included TM and TLux populations, while cluster II included only TNV population. The biological criteria studied by using five host *S. cerealella* (SC) densities indicated that the increase of egg host density from 5 to 80 eggs decreased significantly the parasitism % by TNV and TLux populations except for the TM population the parasitism% increased insignificantly from 5 to 20 eggs host density. Also, female emerged parasitoids parameter is considered as one of the most important quality parameters.

**List of Abbreviation**

| Abbreviation | Full name |
|---|---|
| AUBCU | Assiut University Biological Control Unit |
| DNA | Deoxyribonucleic Acid |
| IPM | Integrated Pest Management |
| IOBC | International Organization for Biological Control |
| ISSR | Inter-Simple Sequence Repeat |
| PCR | Polymerase Chain Reaction |
| RAPD | Random Amplified Polymorphic DNA |
| SC | *Sitotroga cerealella* |
| TLux | *Trichogramma* Luxor |
| TM | *Trichogramma* Minia |
| TNV | *Trichogramma* New Valley |
| UPGMA | Unweighted Pair Group Method with Arithmetic |

**Declarations:**

**Ethical Approval:** This study has been granted by the Research Ethics Committee of Faculty of Agriculture at Assiut University in accordance with Egyptian laws and university guidelines for the care of animals (approval no. 03-2025-0025).

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## ARABIC SUMMARY

### تقنيات البصمة الوراثية والجوانب البيولوجية لثلاث مجموعات من الترايكوجراما القاطنة في نظم بيئية زراعية مختلفة

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أجريت الدراسة الحالية لتحديد الفروق الجينية عن طريق تقنيات البصمات وتحديد الصفات البيولوجية لثلاثة مجموعات من طفيل البيض التريكوجراما *Trichogramma* التي تعيش في ثلاثة نظم بيئية زراعية مصرية مختلفة شملت أبو قرقاص بمحافظة المنيا (TM)، الخارجة بمحافظة الوادي الجديد (TNV)، وأرمانت بمحافظة الأقصر(TLux).

أستخدمت التقنيات الجزيئية عن طريق واسمات ال- RAPD و ISSR وذلك للتمييز بين مجموعات الترايكوجراما الثلاثة. أظهرت النتائج إلى أن مجموعات الترايكوجراما الثلاثة تم فصلها إلى مجموعتين مع واسمات RAPD و ISSR الفردية. تضمنت المجموعة الأولى مجموعات تريكوجراما المنيا TM والاقصر TLux، بينما تضمنت المجموعة الثانية مجموعة تريكوجراما الوادي الجديد TNVفقط.

اشتملت الدراسات البيولوجية تأثير خمس كثافات من بيض فراشة الحبوب *سيتوتروجا سيراييلا* المضيفة (SC) على كفاءة ثلاثة من طفيلات البيض تريكوجراما المنيا TM والوادى الجديد TNV والاقصر TLux. المعايير البيولوجية التي استخدمت شملت: النسب المئوية للتطفل ،والبيض الناجح في التطفل ، وأعداد الطفيلات الكاملة الذين ظهروا من البيض المتطفل ، ونسبة الإناث الناشئة عن التطفل. أظهرت النتائج أن أعلى نسبة للإناث الخارجة من الطفيليات بلغت 15.27± 56.12% لمجموعة TM (قيمة F = 4.49**). أما أقل نسبة للإناث الخارجة من الطفيلات فكانت 3.98 ± 28.02% لمجموعة TLux.

لذا، تسلط الدراسة الحالية الضوء على الحاجة إلى دمج الأوصاف المورفولوجية والبيولوجية لتحديد سريع ودقيق لأنواع الترايكوجراما داخل النظم البيئية الزراعية المصرية المتنوعة، وبالتالي تعزيز مكافحة الآفات الفعالة والمستدامة.

الكلمات المفتاحية: ISSR، RAPD، عائلة التريكوجراماتيدى، نسبة التطفل، النسبة الجنسية