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A Study of The Biological Effect of Egy Pride on The Quantitative Genetic Variation of The Cucurbit Fruit Fly, Dacus Ciliatus (Diptera: Tephritidae)

Shatha, A. Mahdi[1], Hussein, M. Prism[*2], Ashwak, T. Humadi[3]
Department of Biology, College of Education for Pure Sciences, University of Diyala, Diyala, Iraq

Abstract. *This study investigates the influence of the insecticide Egy Pride on the quantitative genetic variation and wing morphology of the Cucurbita fruit fly, Dacus ciliatus, a pest of agricultural significance. Geometric morphometrics were applied to analyze the shape and size of right wings from 30 specimens, half of which were treated with Egy Pride. The analysis revealed a 67% match rate in landmark coordinates between treated and untreated specimens, with notable deviations at landmarks 2, 6, 8, 9, and 15. Treated specimens exhibited variations in wing shape and size, with a central size measurement of 868.6 microns compared to 850.8 microns in the control group. Statistical analysis showed a Mahalanobis distance of 9.49, indicating differentiation between the groups. Classification rates based on wing morphology were 74% for treated and 53% for untreated specimens, with an 83% rate when based on the Mahalanobis distance. The study suggests that Egy Pride induces quantitative genetic variation and morphological changes in D. ciliatus, potentially affecting their flight and mobility. These findings highlight the unique properties of Egy Pride in pest control and its impact on the genetic and morphological aspects of targeted species.*

Keywords: *Dacus ciliatus , Tephritidae, Geometric morphometric, Landmarks.*

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I. INTRODUCTION

The family of cucurbit fruit flies, Tephritidae, is one of the most important families of economic insects in the world, and includes more than 4,800 species belonging to 500 genera dispersal all over the world [1]. There are about 250 economically important species widely distributed in the world[2]. The cucurbit fruit flies (Diptera: Tephritidae) is an invasive pest of cucurbit crops worldwide[3]. The economic importance of these insect pests lies in the direct damage caused by the females inside the host fruits through their larvae that feed on the pulp of the fruits[4]. cucurbit fruit flies (Diptera: Tephritidae) are among the most widespread pests in the world [5][6]. In the Kingdom of Saudi Arabia, the *Dacus ciliatus* fly is considered one of the most important and widespread pests of cucurbit fruits, and it is also widespread in the islands of the Atlantic Ocean, Africa and East Asia [7]. The adult insect of *D. ciliatus* (Loew) is a oligotrophic pest of cucurbits (cucumbers, squash and melons), but its larvae feed on the pulp of these fruits, causing significant losses in cucurbit crops[8]. Since the fruits of cucurbit crops (Cucurbitaceae) have great economic importance, being of high nutritional and commercial value, they are vulnerable to a large number of insect pests, including the *D. ciliatus* fly, which is one of the most dangerous pests[9]. Chemical control is one of the methods of controlling flies using types of insecticides to control them, such as organophosphates, carbamates, and others[10]. Among the insecticides presently used to control the cucurbit fruit fly are deltamethrin, dimethoate, trichlorfon, spinosad, acetamiprid and malathion, which kill insects by contact and ingestion with food [11]. He has presently used the insecticide Egy Pride, and it gave great results in controlling the dispersal of many species of economically important insects. It also affects other insects and parasites and kills them, in addition to that it does not affect humans, animals and plants, and it is an environmentally friendly chemical pesticide. The purpose of this study is to evaluate the biological effect of Egy Pride on the quantitative genetic variation of the cucurbit fruit fly, *D. ciliatus*, and to use it to control it, and thus to recommend reducing the use of chemical pesticides that pollute the environment and are pathogenic to humans.

II. METHODS

Breeding the fly in the laboratory

40 specimens of adults of the species *D. ciliatus*, in addition to a quantity of infected cucurbit fruits containing the larval stage of the insect, were collected from farmer farms in the Al-Wajhiya district of Al-Muqdadiya district / governorate in the period extending between 3/29 and 4/7/2023, Diyala, for the purpose of raising them in the laboratory. Under controlled environmental conditions appropriate to the growth of the life cycles of this species of insect in the insect breeding laboratory of the Department of Biology / College of Education for Pure Sciences / University of Diyala for the purpose of obtaining several generations of them. For the purpose of breeding the insect, boxes made of transparent organic glass (Perplex) 1.5 mm in dimensions of 40 x 40 x 40 cm^3 have been prepared, open at the top and covered with a transparent white cloth for the purpose of air entry. One of the sides of each box was modified to make a (sleeve) of white felt cloth, 40 cm long and 25 cm in diameter, for the purpose of controlling the contents of the box and perpetuating the colony.[12] after making some modifications to it and the different species of insect belonging to the same order and family. Inside each box, an amount of sawdust was placed in which full-grown larvae could not reach a height of 2 cm[13]. The boxes were placed under a temperature of 2 ± 25 m^o and a relative humidity ranging between 70-65% and a lighting period ranging from 8-16 hours of light and darkness, as the adults of the insect were distributed with the infected fruits in all the breeding boxes. Petri dishes with a diameter of 12 cm and a depth of 2 were used, inside which 3 grams of sugar mixed with 1 gram of dry yeast were placed, and the dishes were placed inside each box. He also placed in each box dishes containing medical cotton saturated with 10% sugar solution, which was prepared by adding 10 grams of powdered sugar to 100 ml of distilled water. The boxes were provided with fresh cucurbit and cucumber fruits, with 10 fruits in each box, in order for the females to lay eggs in them and to be a medium for the growth of the three larval stages of the insect. The damaged fruits were replaced with fresh, uninfected

ones, as well as the sugar and sugar solution with yeast every 3 days for the purpose of perpetuating the population colonies of the species. Corn grains are also sprayed daily with water using a daily plastic water sprinkler for the purpose of maintaining the humidity and controlling the appropriate temperature for the growth of the insect's roles and to reduce the period of pupation for mature larvae, which ranges between 5-7 days [14]. Two days after their emergence, 40 specimens from the first-generation adults of each species (20 males and 20 females) were transferred to new breeding boxes prepared for this purpose. They were presented with a sugar solution with yeast and dry sugar, in addition to fresh cucurbit and cucumber fruits, for the purpose of obtaining another generation, with the same steps, until the adults emerge. second generation. As 40 specimens of each species were transferred to new boxes so that the steps of the breeding process could be repeated again for the purpose of multiplying the number of insects and obtaining larvae, pupae and adults of the third generation on which the study was conducted.

Treatment the study specimens with the insecticide Egy Pride

The method of [12] was followed in the laboratory treatment of *D. ciliatus* specimens, once using (1) ml of the insecticide Egy Pride after adding one liter of distilled water to it, and another time using (1) ml of the same pesticide without mixing it with water for the purpose of treating the control specimen. Then the mixture was placed in a transparent plastic vial of 1.5 liters, and the treatment began by taking the most advanced larvae that fall from the infected fruits after 7 days of infection with forceps and placed them in a transparent plastic box with perforated lids to allow air to pass through and containing medical cotton inside it for the purpose of preventing these larvae. After that, the larvae taken were sprayed with the pesticide, and they were observe for the purpose of taking The non-dead larvae and placing them in a new plastic box to be treated also with the pesticide. After that, those pupae are monitored to take the adult and resulting insects after the end of the process of molting and the adults come out. After that, the resulting adults were taken and placed inside a 5-liter transparent plastic container containing a petri dish, which in turn contained sugar mixed with dry yeast, and another petri dish containing medical cotton saturated with 10% sugar solution. The food medium of the specimens was sprayed with insecticide and they were observe to take specimens that will be compared with specimens not treated with pesticide in this study. The control specimen was also treated with the pesticide only without adding water to it in the same way as the treatment with the pesticide mixed with water.

Specimens Processing

In this study, 15 right wings of Egy Pride treated specimens and 15 right wings of untreated specimens were used for the purpose of quantitative genetic comparison in wing shape and size between *D. ciliates* specimens using Geometric Morphometric of wing shape and size. It followed the method of [15] to prepare the glass slides for the wings to be examined, where 15 specimens treated with the above pesticide and 15 untreated specimens were taken, each of which was placed separately in transparent plastic cups with tight lids and left without feeding until it you die and dries completely. After that, the right wing was removed from the treated and untreated insect specimens, separately, using microdissection forceps, then the wing was placed between two slides of two bottles, and the edges of the two slides were attached by adhesive paper tape. After completing the preparation of all slides, they were photographed using a digital microscope after it was connected to a laptop containing a digital camera with a magnification of 1.3 megapixels and equipped with UV rays.

Data Collecting

After completing the process of photographing the wings, the data for each picture was collected separately using the ready-made software, Collecting Landmarks for Identification and Characterization. The data collected from pictures of the wings are in the form of Landmarks, which are anatomical points placed at the intersections of the longitudinal veins with the transverse veins and the ends of the longitudinal veins. He used these points to distinguish between populations of species belonging to the same genus[16]. In this study, 15 landmarks were used that were placed in the form of numbered points between these intersections of the veins of the wings through the COO unit that is included in the program, and it is a unit for setting the coordinates parameters. species, then the data is transferred to the MOG unit, through which translation, scaling and rotation processes are performed. These processes are applied to the feature numbers in order to obtain the central size of each wing, the partial warp and the relative warp, as well as the differences in the apparent shape of each wing. In this study, the central size of the right wing was adopted in the comparison between specimens. It is the isometric amount of the wing that is calculated from the square root of the sum of the squares of the distances between the center of the polygon and each feature fixed on the wing[17].

Soft wares

Fixed to the wing were the parameters obtained from the COO unit, which included central size, partial warp as well as relative warp. As for the analysis data for the basic compounds, the principal component analysis was obtained from the MOG unit, and the discriminate analysis data from the PAD unit. As well as the analysis of variance data for the central size obtained from the COV unit, while the symmetry data for wing shape were obtained from the ASI unit. All of the mentioned units are included in the ready-made program related to the quantitative genetic side, which is Collecting Landmarks for Identification and Characterization, which is available on the website: http://www.mpl.ird.morphometrics.

III. RESULT AND DISCUSSIONS

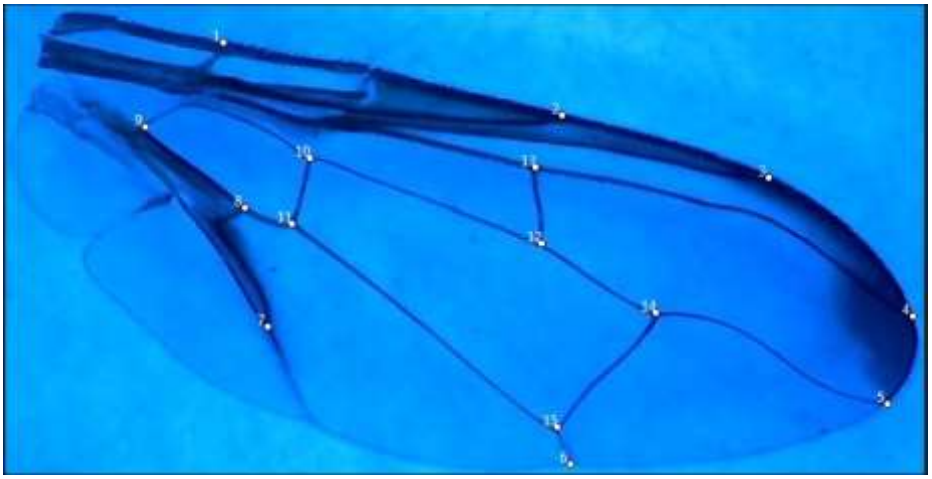

Figure (1) The right wing of the cucurbit fly *D. ciliatus* before control with Egy Pride, showing the coordinates of the features, magnification (X45)

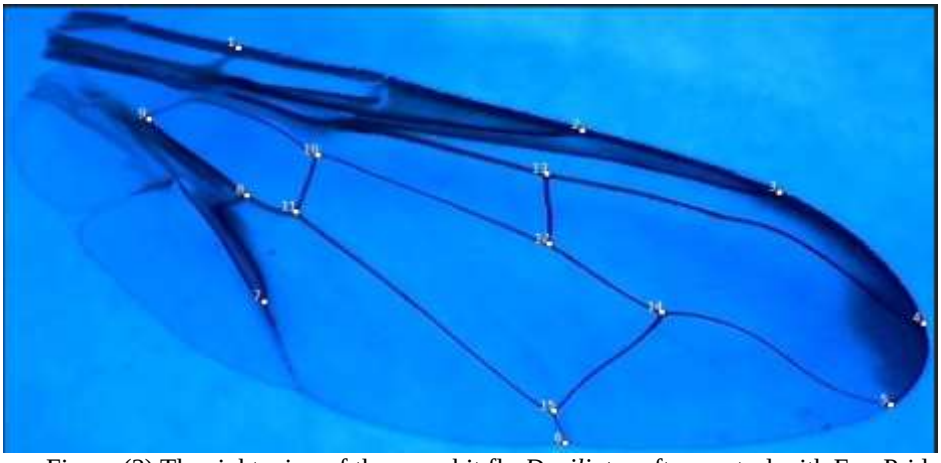

Figure (2) The right wing of the cucurbit fly *D. ciliatus* after control with Egy Pride, showing the coordinates of the features, magnification (X45)

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Figure (3) The cucurbit fruit fly, *D. ciliates*

Quantitative genetic comparison of wing shape and size between D. ciliatus treated and untreated specimens with insecticide Egy Pride

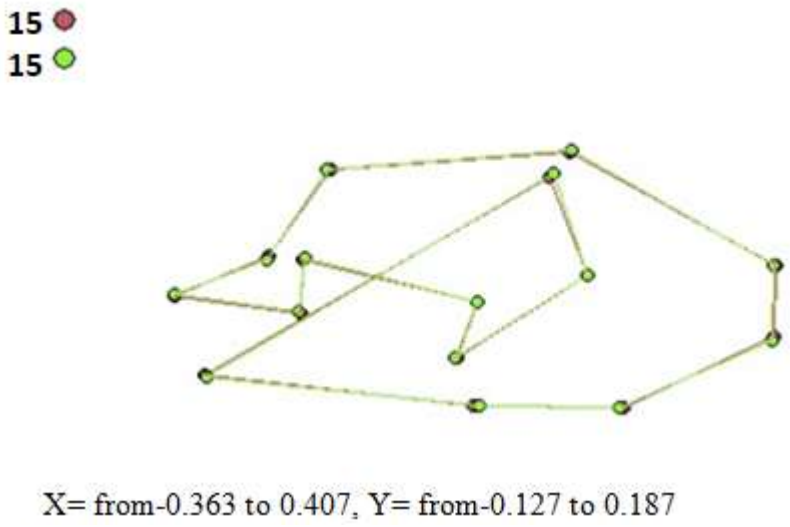
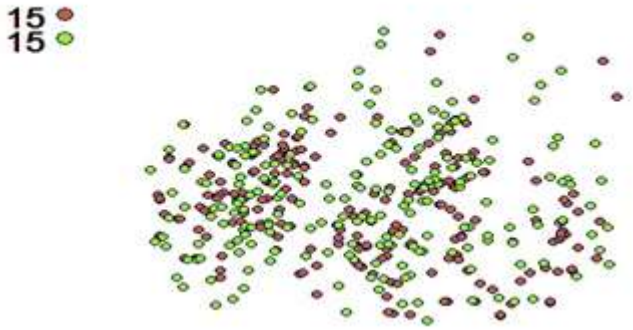


Figure (4) rate coordinates of the features in the right wing of the cucurbit fly *D. ciliatus* before and after treatment with the insecticide Egy Pride (the red color represents the specimens before treatment, and the green color represents the specimens after treatment).

Figure (4) shows the rate coordinates of the features for 30 right wings of *D. ciliatus* fly before and after treatment with the pesticide Egy Pride. And when the matching process was carried out between the specimens using the geometric morphometric of wing shape and size, it was noticed that the right wings before and after treatment with the pesticide were completely identical in most of the specimens, except for landmarks 2, 6, 8, 9 and 15 that did not match. It was found that the percentage of consistency between the coordinates of the features of the specimens amounted to 67%, and the cause for this percentage is due to the clear effect of the pesticide on the specimens of the species treated with it. Among the studies carried out in this field is the study conducted by[18] related the research in feature extraction technology to identification fruit flies using the geometric morphometric of wing shape and size. The researchers were able to identify 4 species belonging to the genus *Bactrocera*, namely *B. dorsalis*, *B. cucurbitae*, *B. tau* and *B. Scutellata* and noticed a clear variance between the diagnosed species by mismatching some of the coordinates of the 17 features fixed on the right wings of the specimens. They emphasized that the cause for the difference is due to the significant impact of c hemical pesticides on them in the areas from which the study specimens

were collected, which led to a difference in the geometric measurements of the wings.



X= from-365.676 to 433.324, Y= from-425.676 to 219.324

Figure (5) The diffusive figure of the distribution of *D. ciliatus* specimens before and after treatment with the insecticide Egy Pride along the factor analysis PCs of the shadow space coordinates derived from the coordinates of the original features of each wing (red and green colors represent specimens of the species before and after treatment, respectively).

Figure (5) shows the method of distribution of *D. ciliatus* specimens before and after treatment with the pesticide, Egy Pride, using the basic compounds analysis of the landmark points fixed on the right wings between the intersections of the longitudinal veins with the transverse veins and the ends of the longitudinal veins, which are 15 landmarks in each wing. The red dots represent the features of the right wings of the specimens before treatment, while the green dots represent the features of the right wings of the specimens after treatment. And it was found by observing the method in which the distribution was made that the specimens are very close to each other, and most of them are completely identical with the presence of a discrepancy in the shape and size of the wing for some of the treated specimens that are far from each other, and the cause for this discrepancy is due to their influence on the pesticide. Among the studies and research conducted in this field is the study carried out by [19] related the study of the quantitative genetic relationship between three populations of the old-world screwworm *Chrysomya bezziana*, whose specimens were collected from different regions in Iraq. In that study, the researchers were able to compare 90 right wings of males and females of the above species using the geometric morphometric of wing shape and size. The results of the study indicated that there is a clear difference in the shape and size of the right wing between insect specimens. The two researchers also carried out the analysis of the basic components of 18 landmarks from the landmark points installed at the intersections of the transverse veins with the longitudinal veins of the right wings of all specimens, and it was noted through this that a large convergence occurred between some specimens of the north and the middle and a divergence between the specimens of the north and the middle on the one side and the specimens of the south on the other side.

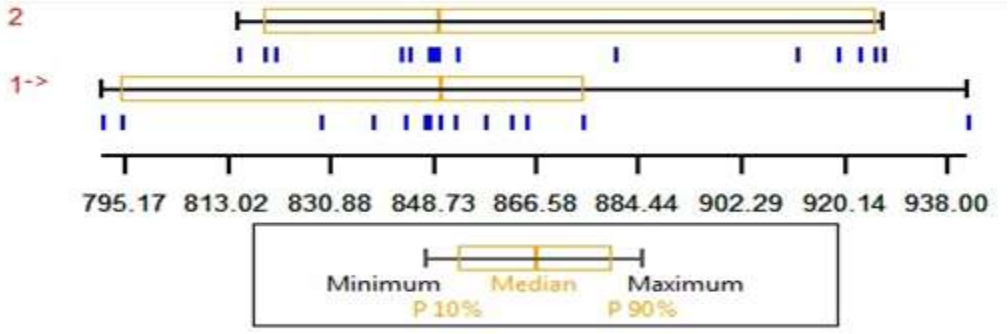


Figure (6) The central size of the right wings of the species *D. ciliatus* before and after treatment with the pesticide Egy Pride (each box in the figure indicates the middle group distributed between spring 10% and spring 90%, and the blue lines at the bottom of each box represent the wings, as well as the numbers 1 and 2 shown in the figure are specimens of the species before and after treatment).

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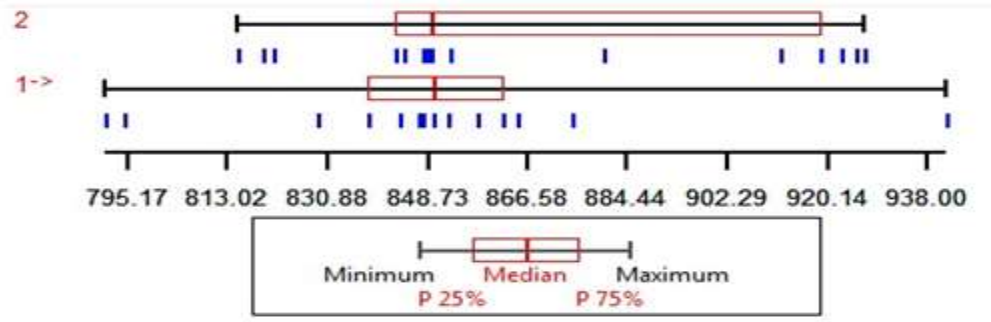

Figure (7) central size of the right wings of the species *D. ciliatus* before and after treatment with the fungicide Egy Pride, and each box in the figure indicates the middle group distributed between spring 25% and spring 75% (the blue lines at the bottom of each box represent the wings, as well as the numbers 1 and 2 shown in the figure are specimens of the type before and after treatment.

Table (1) Comparison of the central size of the right wing between *D. ciliatus* specimens before and after treatment with the pesticide Egy Pride.

Specimens	MCs	St.D	Va.	F	P	T	P	A.D
D. ciliatus (1)	850.8	32.7	1067.9	b-a= 1.5	b-a= 0.48	b-a= 1.4	b-a= 0.19	b-a= 17.9
D. ciliatus (2)	868.6	39.5	1564.1					

M.Cs : Mean central size
St.D : Standard deviation
Va. : Variance
The calculated F value
The calculated T value
A.D : Absolute differences
1: after treatment, 2: before treatment

Figure (6) indicate the results of the analysis of variation in the central size of the right wing in specimens of the cucurbit fruit fly species *D. ciliatus* before and after treatment with the pesticide Egy Pride. Each box, as shown in the figure, represents the middle group, which was distributed between spring 10% and spring 90%. It was observed that there is a clear, but small, variation in the central size of the wings, and the cause for this variation may be due to the significant effect of the insecticide on the adults. Figure (7) shows the results of the analysis of the variation in the central size of the right wing in the species of the species before and after the treatment, but the middle group here is distributed between spring 25% and spring 75%. It was also found, as in the preceding analysis, a slight variation in the central size of the wings, especially the specimens treated with the pesticide, as a result of its effect on the life cycles of the insect, as the rate central size of the wings of the treated and untreated specimens was 850.8 and 868.6, respectively; As shown in Table (1). While the value of variance for them amounted to 1067.9 and 1564.1, respectively, and both the F and T tests were used in this study for the purpose of comparison between treated and untreated specimens in the rate central volume of the right wing. And when performing a comparison procedure between them, it was noted that the value of F = 1.5 and the value of P = 0.48, while the value of T = 1.4 and the value of P = 0.19, while the value of the absolute difference was A.D = 17.9, and this result shows the presence of statistically significant differences between the specimens of the insects studied as a result of the effect of the pesticide used her specimens to be treated with. There are many international studies in the field of using the geometric morphometric

of wing shape and size, including the study conducted by [20] related the use of the geometric morphometric in identification the quantitative genetic diversity of *Dacus* and *Bactrocera* in orchids that were exposed in previous years to control with different types of chemical pesticides. After conducting a comparison between several species of the sexes, the researcher concluded, and after fixing 14 parameters on the right wing of the specimens of the species belonging to each sex between the ends of the longitudinal veins and the intersections of the transverse veins with the longitudinal veins, to the conclusion that the central size of the right wing in the species of the genus *Dacus* is greater than it is in the species of the genus *Dacus* genus *Bactrocera*. And that the reason for this difference may be due to the great effect of chemical pesticides on subsequent generations of species belonging to both unisex.

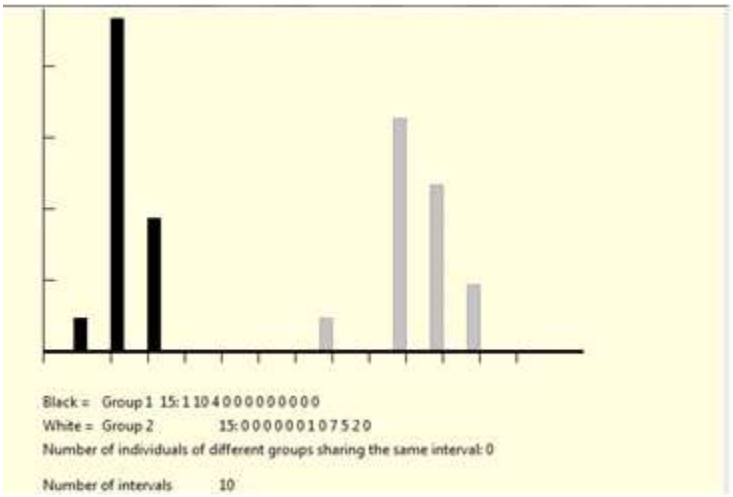

Figure (8) Discriminant analysis of the coordinates of the right wings in D. ciliatus specimens before and after treatment with the pesticide Egy Pride. (Black and white bar graphs represent pre- and post-treatment specimens, respectively).

Table (2) Comparison of the distance of the mhalanopes between *D. ciliatus* specimens before and after treatment with the pesticide Egy Pride.

Specimens	***D. ciliatus* (b)**	***D. ciliatus* (a)**
D. ciliatus (b)	0.00	
D. ciliatus (a)	9.49	0.00

(b) species specimens before treatment, (a) species specimens after treatment.

Table (3) ANOVA analysis of variance for right wing symmetry in *D. ciliatus* specimens before and after treatment with Egy Pride.

Source	**SS**	**DF**	**MS**	**F**	**Signification**
Modal	0.0006	3	0.000196	0.86	0.4731
Individual	0.0000	1	0.000023	0.10	0.7506
Side	0.0000	1	0.000036	0.16	0.6926
Side*I	0.0005	1	0.000528	2.33	0.1400
Residue	0.0054	24	0.000227		

Table (4) ANOVA analysis of variance for the similarity of right wing size in *D.ciliatus* specimens before and after treatment with Egy Pride.

Source	**SS**	**DF**	**MS**	**F**	**Signification**
Modal	0.0041	78	0.000052	0.92	0.6759
Individual	0.0016	26	0.000063	1.11	0.3178
Side	0.0008	26	0.000032	0.57	0.9600
Side*i	0.0016	26	0.000061	1.07	0.3695

Residue	0.0355	626	0.000057		

Figure (8) shows the discriminant analysis of the coordinates of the features in the right wings of the specimens of the cucurbit fruit fly species *D. ciliatus* before and after the treatment with the pesticide Egy Pride. These analyzes are used to make a comparison between individuals of the same species that belong to a particular genus for the purpose of detecting the variation occurring between them and thus knowing the causes leading to this variation. In this analysis, 30 right-wing specimens were used, 15 for untreated specimens, which are represented by black color, and 15 for treated specimens, which are represented by white color. It was noted in the aforementioned figure that the specimens of the untreated species are far from the specimens treated with the pesticide. The direct cause for this dimension is due to the effect of the pesticide on the specimens treated with it, which led to a clear difference in the geometric measurements of the right wing of it. The results of the study also showed in Table (2) that the distance between the central size of the right wings in the specimens of the species *D. ciliatus* before and after treatment with the pesticide Egy Pride is 9.49. When reclassifying based on the percentage of species specimens before and after treatment using the PAD unit, and based on the matching of the right wings of species specimens in both cases (treatment and non-treatment), it was found that the classification percentage for insects of the untreated species was 53% and for insects of the treated species 74%, meaning that the resulting discrepancy. The shape and size of the right wing of the treated specimens were higher than in the case of the untreated specimens. And when the classification process was repeated again for the same samples, but this time depending on the distance of the mahalanops and the match between the wings of all the treated and untreated specimens, it appeared that the classification rate was equal to 83%, meaning that 25 individuals out of 30 were completely identical. It was found that the cause for the discrepancy between the wings is due to the absence of any change in the morphology of the untreated specimens and the significant effect of the pesticide on the life cycles of the insect, which led to a discrepancy in the geometric measurements of the wing in the case of the specimens treated with the pesticide. Tables (3 and 4) show the ANOVA analysis of variance in the symmetry of the right wing shape and size of *D. ciliatus* specimens before and after treatment with the pesticide Egy Pride by the ASI unit within the components of the ready program. Through which it is known whether there is a discrepancy in the shape and size of the wing, as it was found that there are few significant differences between the wings of the treated and untreated specimens because of the effect of the pesticide on them, which is represented by the occurrence of a discrepancy in most of the geometric measurements of the wing, so that the size of the wing became slightly larger than it is in the natural state of the insect. Many studies have been conducted in this field, including the study by [21] by comparing the shape and size of the right wing in the two species *Chrysomya albiceps* and *Chrysomya megacephala* using the geometric morphometric of wing shape and size. The study concluded, after conducting the analysis of the basic compounds, that there is a discrepancy between the size of the wing in both species, as the researchers found that the size of the wing in the species *Ch. megacephala* is larger than in species *Ch. albiceps*. The researchers also mentioned that there are significant differences with statistical indications between the specimens of the two species, and they confirmed that the cause for these differences and significant differences is the effect of the two species on the different environmental conditions in the areas from which the study specimens were collected.

IV. CONCLUSION

This study was conducted on 30 specimens of the cucurbit fruit fly *D. ciliatus*. It shows the effect of the insecticide Egy Pride on the apparent shape of the wing of the specimens treated with it, and this in turn limits their ability to fly and move from one agricultural field to another, which is a feature that distinguishes this type of pesticide from other pesticides. The continuous use of Egy Pride pesticide also leads to quantitative genetic variation in the wing shape and size of the cucurbit fruit fly species *D. ciliatus.*

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