MOLECULAR, MORPHOLOGICAL AND HISTOLOGICAL DIFFERENTIATION BETWEEN THE LESSER PUMPKIN FLY, *Dacus ciliatus* (Loew) AND THE GREATER PUMPKIN FLY, *Dacus frontalis* Becker

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Both of the lesser pumpkin fly, *Dacus ciliatus* (Loew) and the greater pumpkin fly, *Dacus frontalis* Becker are belonging to order Diptera family Tephritidae (Typetidae or Trupaneidae) a group of about 4000 known species, nearly about 80% of which have larvae develop in the seed bearing organs (flowers or fruits) of higher plants, and therefore known as fruit flies (White, 2000). The fruit flies in Egypt are not well known and the only comprehensive treatments are now very old, like Efflatoun (1924). *D. ciliatus* was recorded as a serious pest on cucurbitaceae since 1947 by Azab and Kira (1954), continued nearly till 1980 and disappeared then appeared again after 25 years in Egypt (Fetoh, 2003). The greater pumpkin fly, *D. frontalis* recorded in Egypt only by foreign scientists like Munro (1984), White (2000) and Carrol *et al.* (2002), In 1992 *D. frontalis* was recorded for first time as a serious pest on cucurbitaceous plants in Libya (Abo-Geshem *et al.*, 2003). Recently, Fetoh and Hegab (2007) recorded *D. frontalis* as a pest on cucurbitaceae.

Both flies are serious pests that cause high loss in yield and cause damage sometimes reached 100%. According EPPO (2009) both species could be arranged as highly serious agricultural quarantine pests under rank A1. Generally, accurate identification of insect species is essential, especially in the sibling species, in order to give right information for ecology, biology and control methods also in quarantine restrictions (Drew and Hancock 1994). Molecular biology as a new approach helps to classify and control pests in clear, easy and quick manner. The main objective of the present work is differentiating between both of the lesser pumpkin fly, *D. ciliatus* and the greater pumpkin fly, *D. frontalis* by comparative taxonomy throughout molecular characterizations and variations in protein using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and esterase profile using electrophoresis (EST-PAGE) as well as morphological characters and histological sections to facilitate identification methods, control measures and the agricultural quarantine applications.

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MATERIALS AND METHODS

Insect samples preparation for morphological and histological differentiations

To maintain stock cultures of both insects *D. ciliatus* and *D. frontalis* were collected from infested marrow fields from Ismalia governorate during January 2009, placed in plastic containers with sandy layer. The full-grown larvae, which pupate in the sand, were collected and transferred to adult rearing cage (30x 30 x 30 cm) with metal frames having mash screen at all sides. Small marrow fruits were used for eggs deposition and larvae rearing. Flies were reared for five successive generations. The late 3rd larval instars of both flies (jumping larvae) were cut from their upper and lower ends for cross sections and examined under stereomicroscope for histological illustration. Samples of adults required for microscopic preparations of whole mounting were killed in 70% ethanol, then washed with distilled water and treated with KOH solutions, then dehydrated in alcohol and mounted in Canada balsam for morphological description.

Sample preparation for electrophoresis

Preparation for total protein assay was carried out according to method of Lowry *et al.,* (1951) for different stages (eggs, larvae, pupae and adults) for *D. ciliatus* and *D. frontalis*. Electrophoresis process was carried out as described by Laemmli (1970) using pre-stained high molecular weight standard marker. After electrophoresis, gels were stained with silver stain and destained according to the method of Hitchocock and Brown (1983). The stained gels were photographed and examined for presence or absence of visualized bands. The same steps were followed for esterase electrophoresis using β- naphthyl propionate as substrate according to Sims (1965). Concentration of protein (conc. %), relative fragmentation (Rf), and similarity coefficient (sim. co.) were calculated according to Nei and Li (1979) and commonality percentage (com.%) were calculated according to Haymer and McInnis (1994).

RESULTS AND DISCUSSION

Molecular differentiation between D. ciliatus and D. frontalis using SDS-Page and EST-Page

Results of quantitative protein pattern are given in Table (1) and illustrated in Figs (1 and 2) showed representative SDS-PAGE (silver- stained) patterns for protein of *D. ciliatus* and *D. frontalis.* The lesser pumpkin fly, *D. ciliatus* showed 13 visualization bands. These reactive bands ranged between 200.00 and 14.30 kDa, have relative fragmentation (Rf) ranged from 0.04 to 0.91 and conc. % varied between 1.22 and 28.1.While, the greater pumpkin fly, *D. frontalis* has 12 bands only. These bands are also located between 200.00 kDa and 14.30 kDa have Rf ranged from 0.04 to 0.82 and conc.% varied between 1.15and 33.50, similarity% was 67.10, similarity coefficient was 0.60 and commonality % between the two species was 44.00.

Results of quantitative esterase

pattern are given in Table (2) and illustrated in Figs (3 and 4). These results showed esterase (EST-Page) profile patterns of the two species of genus *Dacus*. The first species was *D. ciliatus* which has seven esterase bands after the electrophoresis process. These reactive bands have Rf. values ranged from 0.23 to 0.76 and conc.% varied between 2.40 and 21.8. The second species was *D. frontalis*, which has also seven esterase bands. These reactive bands have Rf. values ranged from 0.24 to 0.78 and conc. % varied between 0.64 to 20.6, respectively, similarity % was 59.8, similarity coefficient was 0.71 and com.% was 28.6, respectively.

Protein SDS-PAGE and esterase PAGE were carried out in all stages for both species (eggs, larvae, pupae and adults), there was no difference appeared among gels of the tested samples, this will help to differentiate and distinguish between *D. ciliatus* and *D. frontalis* using any insect stage although they are resemble in all immature stages (eggs, larvae, pupae) as well as all Tephritidae flies, especially the harmful stage (larvae) which are typical and identical in color, size, behavior, number of instars in addition to infestation symptoms.

White (2000) stated that all fruit flies are classified using adult stage only; while Zhijian *et al*. (1997) stated that electrophoresis is the most simple and quite powerful tool for identification and provides biochemical means for species identification. The utilization of enzyme species as careful controlled Electrophoretic analysis separate proteins into fractions that have species-specific mobility (Nilima *et al*., 1987). This approach (molecular biology tools) in Taxonomy and quarantine was reported by many several authors i.e. Ahmed (1985) who gave classification for genus *Gerbillus* from rodents using protein and enzymes profiles. Nilima *et al.* (1987) who utilized PAGE to detect esterase characterization and variation among adults of three species of white flies, *Bemisia tabaci* (Gen.), *Trialeurodes tabutilonea* (Hald.) and *T. vaporariorum* (West.). Fetoh (2005) using the same techniques and methods to discriminate between the lesser pumpkin fly, *D. ciliatus* and the peach fruit fly, *Bactrocera zonata* (Diptera: Tephritidae) in Egypt. Furthermore the using of molecular characterization SDS- PAGE is faster than DNA-DNA hybridization and elaborate phenotypic comparison between different species and subspecies (Khan *et al*., 1996 and Hassanian and Rabie 2003).

Morphological differentiation between D. ciliatus and D. frontalis

The greater pumpkin fly, *D. frontalis* is very similar to the lesser pumpkin fly, *D. ciliatus* and these two species can be difficult differentiate (White, 2000). Scientists for long time were considered *D. frontalis* as subspecies of *D. ciliatus* till Munro (1984) who separated and classified it as separated species.

The following key and Figs (5-6) are showing the comparison faces between *D. ciliatus* and *D. frontalis*:

Key to species of Genus Dacus (Adults)

a. Distance between compound eyes 0.5 mm; katatergites colorless, anatergites yellow; femur of mid leg uniform color (brawn)…… *ciliatus*.

b. Distance between compound eyes 0.9 mm; katatergites and anatergites yellow; femur of mid leg with two colors, mid femur yellow and hind femur brawn…….. *frontalis*.

All these differences in the same trend with Carrol *et al*., (2002)

Histological differentiation between D. ciliatus and D. frontalis

Adult stages are only used for morphological differentiation between species belong to family Tephritidae (White, 2000). The cross section in upper and lower ends of 3rd larval instar (jumping larvae) is showing cephalopharyngeal skeletons, anterior and postior spircles (Figures 7-8). The following key is showing the cross sections larvae differences between *D. ciliatus* and *D. frontalis* **.**

Key to species of Genus Dacus (larvae)

a. Anterior spiracular tubes 14-16 in single irregular row; number of dorsal spiracular processes 14-19 , number of ventral spiracular processes 11-14 , number of lateral spiracular processes 3-9…… *ciliatus.*

b. Anterior spiracular tubes 14-15 in single irregular raw; number of dorsal spiracular processes 16-17, number of ventral spiracular processes 16-17, number of lateral processes 4-8…..*frontalis*.

All these differences in the same trend with Malan and Giliomee (1969).

SUMMARY

In Egypt the lesser pumpkin fly, *Dacus ciliatus* (Loew) and the greater pumpkin fly, *Dacus frontalis* Becker which belong to the genus *Dacus* family Tephritidae order Diptera were found infesting some vegetables like cucurbitaceous and solanaceous plants. Both flies resemble each other in infestation symptoms and all immature stages; furthermore adults have the same shape, size and color. For near time both of the adults and larvae were constructed. Keys for morphological characters of the two species appear in the thorax and mid femur leg in the adults. The cross sections in the 3rd larval instar also showed little differences. Electrophoresis for total protein (SDS-PAGE) in all stages of both species indicated the presence of 13protein bands in *D. ciliatus* and 12 protein bands in *D. frontalis* ranging between 200.00 kDa and 14.30 kDa, similarity percentage was 67.10, similarity coefficient was 0.60 and commonality coefficient was 44.00. Esterase isoenzyme pattern after electrophoresis showed the presence of 7 visualization bands in both species, which having similarity percentage was 59.80, similarity coefficient was 0.71 and commonality

percentage was 28.60. This could provide a new tool for the identification of any stage (egg, larva, pupa and/or adult) in an easy and quick manner thus helps in controlling and quarantine tools of both insects.

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Table (1): Quantitative protein pattern of the lesser pumpkin fly, *Dacus ciliatus* and the greater pumpkin fly, *D. frontalis*

Band Number	*Dacus ciliatus*			*Dacus frontalis*		
	Band occur-rence	Rf.	Conc.%	Band occur-rence	Rf.	Conc.%
1	+	0.04	7.24	+	0.04	7.67
2	+	0.09	6.77	+	0.08	5.09
3	+	0.14	1.22	+	0.13	7.04
4	+	0.19	2.11	+	0.19	1.42
5	+	0.25	11.70	+	0.25	11.00
6	+	0.38	10.40	+	0.34	8.08
7	+	0.40	10.50	+	0.41	10.50
8	+	0.54	28.10	+	0.48	33.50
9	+	0.62	3.36	+	0.61	2.45
10	+	0.68	1.87	+	0.64	1.15
11	+	0.80	2.00	+	0.75	6.38
12	+	0.84	8.47	+	0.82	5.85
13	+	0.91	6.21	-	-	-

Rf.= Relative fragmentation, Conc.%= Concentration %, (-) Absent, (+) Present, Sim. % =67.10, Sim. co. = 0.60, Com % =44.00.

Table (2): Quantitative esterase pattern of the lesser pumpkin fly, *Dacus ciliatus* and the greater pumpkin fly, *D. frontalis*

Band Num-ber	*Dacus ciliatus*			*Dacus frontalis*		
	Band occur-rence	Rf.	Conc.%	Band occur-rence	Rf.	Conc.%
1	+	0.23	21.80	+	0.24	0.64
2	+	0.31	9.00	+	0.31	17.70
3	+	0.42	10.40	+	0.40	17.30
4	+	0.53	2.40	+	0.50	17.80
5	+	0.59	22.70	+	0.58	17.70
6	+	0.65	17.70	+	0.66	8.24
7	+	0.76	16.00	+	0.78	20.6

Rf.= Relative fragmentation, Conc.%= Concentration %, (-) Absent, (+) Present, Sim.% = 59.80, Sim. co. = 0.71, Com % = 28.60.

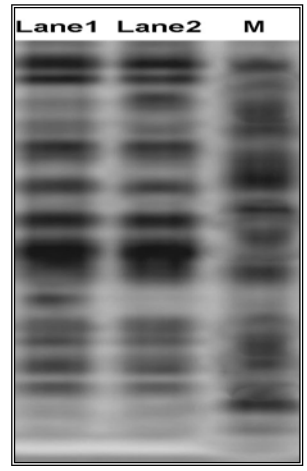

Fig. (1): SDS-polyacrylamide gel zymogram of denatured protein patterns of *D. ciliatus* and *D. frontalis*. Lane 1-2 represent samples of *D. ciliatus* and *D. frontalis*, respectively. Lane M represents the molecular size markers.

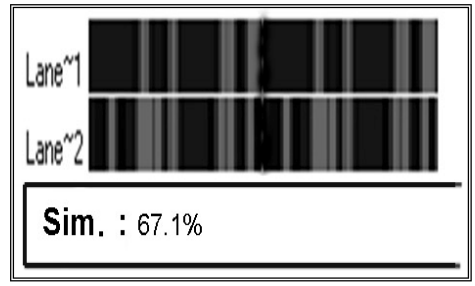

Fig. (2): Differences and similarity relationships among protein bands of *D. ciliatus* and *D. frontalis*, respectively.

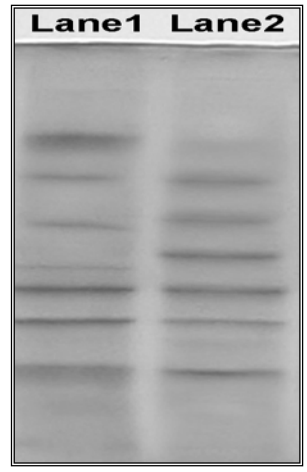

Fig. (3): Polyacrylamide gel zymogram of esterase isozyme patterns of *D. ciliatus* and *D.frontalis*. Lane 1-2 represents samples of *D. ciliatus* and *D. frontalis*, respectively.

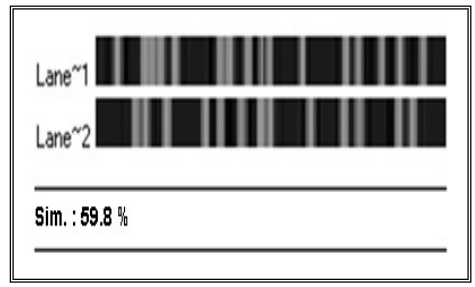

Fig. (4): Differences and similarity relationships among esterase bands of *D. ciliatus* and *D. frontalis*, respectively.

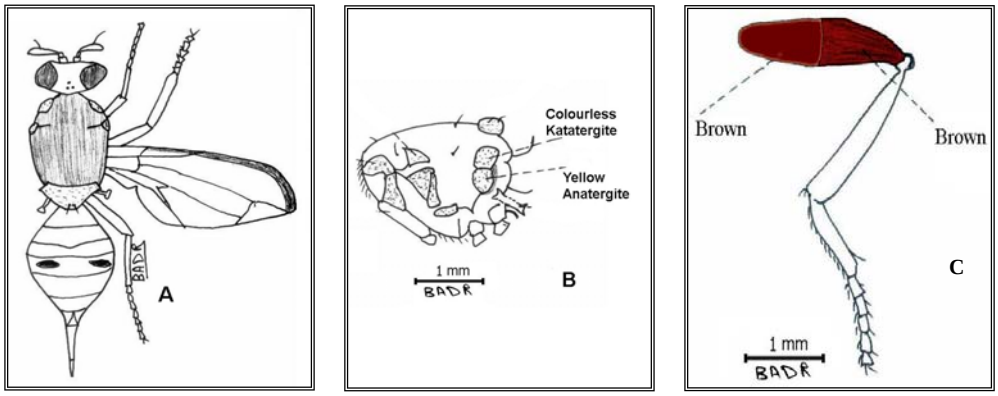

Fig. (5): The lesser pumpkin fly, *D. ciliatus*. A = Dorsal view, B= Lateral view and C = Mid leg.

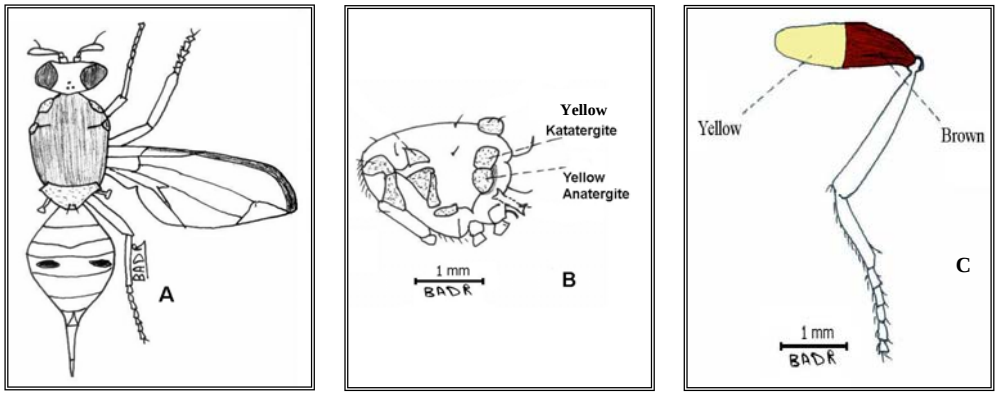

Fig. (6): The greater pumpkin fly, *D. frontalis.* A= Dorsal view, B= Lateral view and C= Mid leg.

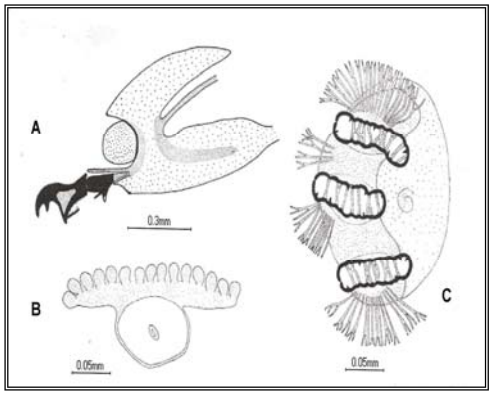

Fig. (7): Cross sections in the upper and lower ends of the 3rd larval instar of *D. ciliatus*. A = Cephalopharyngeal skeleton, B=Anterior spiracles and C= Posterior spiracles.

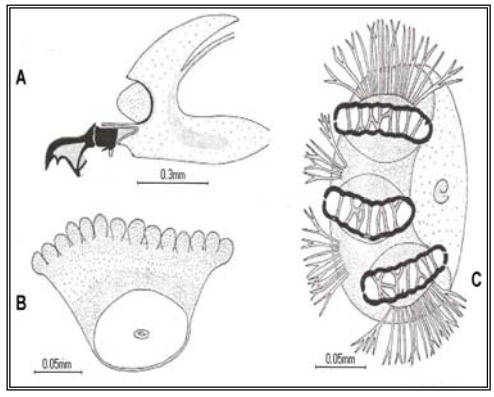

Fig. (8): Cross sections in the upper and lower ends of the 3rd larval instar of *D. frontalis*. A = Cephalopharyngeal skeleton, B=Anterior spiracles and C= Posterior spiracles.