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Utilization of certain plant extracts and entomopathogenic fungi for controlling the black fig fly, *Lonchaea aristella* on fig trees.

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Abstract: Impact of plant extracts viz: *Centaurium spicatum*; *Prunus laurocerasus*; *Pyracantha coccinea*; *Sorbus aucuparia* as well as entomopathogenic fungi viz: *Beauveria bassiana* ; *Metarhizium anisopliae* and *Verticilium lecanii* as insecticide alternatives against the black fig fly, *Lonchaea aristella* Becker (Diptera: Lonchaeidae) was determined in both laboratory and field experiments. It can be concluded that treatments with plant extracts and entomopathogenic fungi have significant effect on the biology of this pest. Generally increased concentration decreased adult emergence; fecundity and egg hatchability percentage. *C. spicatum* 4% extracts has a highly significant affected on the duration of larval stage development then afterwards *P. laurocerasus*; *P. coccinea* and entomopathogenic fungi *B. bassiana*,. Conventional chemical insecticides are usually sprayed against fig insect pest. So, heavy and frequent insecticide applications are needed for their control. This can lead to problems of toxic residues and pollution of the environment. Therefore, the present work aimed to avoid the overuse and mis-use of conventional chemical insecticides as well as to investigate some save alternatives such as natural plant extracts and entomopathogenic fungi to reduce the level of *L. aristella* infestation in fig orchards consequently, increase the monetary value of yield. This may offer a reliable role in exploring integrated pest control programme in fig orchards.
Keywords: Plant extracts, entomopathogenic fungi, the black fig fly, fig orchards.

Introduction

Ficus carica L. (Moraceae) has been cultivated for a long time in various places worldwide for its edible fruit. Remnants of figs has been found in excavations of sites dating as far back as at least 5,000 B.C. *F. carica* presumed to originate from Western Asia and spread to the Mediterranean by humans. It is an important world crop today. Major producers of edible figs include Turkey, Syria, Egypt, Morocco, Spain, Greece, California, Italy, Brazil and other places with typically mild winters and hot dry summers[1,2,3]. Fruits can be eaten raw, dried, canned, or in other preserved forms.

Fig trees are attacked by several insect pests mainly stem borers, scale insects, mealy bugs, and fruit flies[4,5].

The black fig fly, *Lonchaea aristella* Becker *(*Diptera: Lonchaeidae) is considered a danger fly on fig trees (*Ficus carica*). The main fig part of this pest is the fruits. Fruits most injured are those at the ends of the branches which clearly appeared to the fly. The damage caused with insect pest occurs as a result of boring the fruits and reduction the fruit crop, reducing quality and diminishing their commercial value. Conventional

chemical insecticides are usually sprayed against fig insect pest. So, heavy and frequent insecticide applications are needed for their control. This can lead to problems of toxic residues and pollution of the environment[6]**.** Mean that, the fig fruits contaminated with high levels of insecticide residues are rejected in most markets. This situation dictates the need for safe, locally available and less expensive materials for pest control. The application of tested materials for the control of fig fruit fly can be a possible alternative.

Therefore, the present work aimed to avoid the overuse and mis-use of conventional chemical insecticides as well as to investigate some save alternatives such as natural plant extracts and entomopathogenic fungi to reduce the level of *L. aristella* infestation in fig orchards consequently, increase the monetary value of yield. This may offer a reliable role in exploring integrated pest control programme in fig orchards.

Experimental

1. Laboratory evaluation of different treatments on the biological aspects of *L. aristella*.

Laboratory experimental had been carried out to investigated the role of plant extracts viz. *Centaurium spicatum* ; *Prunus laurocerasus*; *Pyracantha coccinea*; *Sorbus aucuparia* as well as entomopathogenic fungi viz. *Beauveria bassiana*; *Metarhizium anisopliae* and *Verticilium lecanii* compared with Triazophos (Hostathion 40% EC) insecticide on the biological aspects of *Lonchaea aristella* Becker on fig tree.

Plant extracts:

The plant materials (*C. spicatum* ; *P. laurocerasus*; *P. coccinea*; *S. aucuparia*) were collected from Sant Catherin (South Sinai, Egypt). The plant leaves were dried in shade place; about 250 g of each dried leaves of each tested plants were defatted in soxhelt using ethanol solvent. The ethanol extracts were evaporated under vacuum at about 45º c till dryness. The water emulsions were prepared by mixing five drops of Tween – 80 as emulsifier with 1g of plant extract, then water was added up to 100 ml to obtain 1% concentration of plant extract. The other desired dilutions were prepared from the stock emulsion (1%)[7]**.**

Entomopathogenic fungi:

Metarhizium anisopliae (Metchinkoff) Soroken isolated from larvae and adults of *Scrobipalpa ocellatella* and *Beauveria bassiana* (Balsamo) Vuillemin isolated from *Cassida vittata*[8] were grown on Peptone media (10g Peptone, 40g Dextrose, 2g yeast extract, 15g Agar and 500 ml. Chloramphenicol). The media was autoclaved at 120 ºc for 20 minutes, and poured in Petri- dishes (10 cm diameter x 1.5 cm). Then incubated the fungi and kept at 24 ±2˚c and 65± 5 % RH. The fungal isolates were re-cultured every 14 – 30 days and kept at 4˚c.

Preparing of concentrations

Spores of fungal isolates harvested by rising with sterilized water 0.5 % Tween 80 from 14 day old culture Peptone media. The suspensions were filtered through cheese cloth to reduce mycelium clumping. The spores were counted in the suspension using a Haemocytometer (0.1 mm x 0.0025 mm^2). The concentrations were used $1x10^4$spore /ml.

Triazophos (Hostathion 40% EC):

Triazophos is a kind EC - Emulsifiable concentrate, category as foliar insecticide, broad spectrum of organophosphorus pesticides, have strong tag and stomach poison effect, insecticidal effect is good, kill the eggs, strong permeability, Used for prevention and treatment of a variety of crops and Product by Bayer Group of Companies in India.

Insect culture and assay:

A laboratory colony of the *Lonchaea aristella* Becker (Diptera: Lonchaeidae) was reared at the laboratory of the National Research Centre. This colony was started by collecting samples of highly infested fig fruits (*Ficus carica*) from Ashmant village, Banisuif Governorate. Newly emerged larvae were kept in 10 cm

long plastic cups each containing 1fruit covered with muslin and rubber band under the laboratory conditions at 27±5 °C and relative humidity of 65±5 % R. H.

To study the effect of different treatments on the various biological aspects of *L. aristella*, two pairs of the newly emerged flies were taken from the stock culture and kept in cups containing 1 fig fruit treated with different treatments by dipping methods for 10 second then air- dried before being placed in a cups covered with muslin under the same laboratory conditions. The flies were left for mating and laying eggs. The fecundity, average no. of eggs/ female was observed. Each experiment was repeated ten replicates.

To study the effect of different treatments on egg stage, the fruits with eggs on them were collected from stock culture in new plastic cups and kept at laboratory conditions previously described. The percentage of hatching was calculated by counting the empty egg shells on the surface of the fruits.

The development of the larval and pupal stages was followed up and the duration of larval and pupal stages was recorded from the time of egg hatching until emergence of adults. Also, the percentages of adult's emergence were recorded.

2. Field evaluation of different treatments on *L. aristella* at Ashment region Banisuif governorate.

Field experimental had been carried out to investigate the role of *Centaurium spicatum* extract 4% and *Beauveria bassiana* ($1x10^4$ spores/ml) was used, compared with Triazophos (Hostathion 40% EC) insecticide with 0.005 concentration on the number of adult of *L. aristella* by trap, an orchard of fig trees, *Ficus carica* L. naturally infested with the *L. aristella* was chosen at Ashment region Banisuif Governorate. The trees were 9-10 years old and about 3 m in height. Plastic trap contained roughage soft + Black honey + Buminal and chemical components of Bumenal are shown in table (1) then add the treatment were used from the 18th of May 2014 till 13th of July 2014. Twelve traps were hanged randomly in the sides of trees at 1.5 -2 meters height. Each trap was far from each other by about 50 meters. The traps were inspected weekly and number of flies on each trap was counted.

Table (1). Chemical screening of Buminal

Glycosides	(+)
Sterol or tri-terpene	(-)
Protein	(+)
Tannin	(-)
Saponin	(+++)
Flavonoids	(-)
Coumarin	(+)

3. The impact of different treatments on the average fig fruits yield and economic returns.

Collected fruits normal, infected and falling on the ground, the fruits were divided according to the degree of quality as follows:

1. Fruits healthy (normal)
2. False pangs
3. Infected fruits with larvae
4. Infected fruits with egg

5. Fruits falling and moldy

Then the cost of one used of pesticides and extract for one feddan was calculated, to can calculated the net cash return per feddan after deduction of the cost to compared between the insecticide and plant extract as alternative insecticides used from two dimension economic and environment.

4. Statistical analysis

Data were statistically analyzed by ANOVA using the Instat V2.03 computer programme test and mean values were separated and analyzed by using Duncan's multiple range tests[9] at probability = 5% in the field experiments and 1% in laboratory experiments.

Results and Discussion

1. Laboratory evaluation of different treatments on the biological aspects of *L. aristella*

Effect on adult and egg stages:

New adults from stuck culture have tacked; fruits treaded with different extracts and kept under plastic cups and their emergence percentage and Average no. of eggs/ female was followed up. When the adult emergence, percentage of *L. aristella* was considered (Table 2), it was show that the average of adult emergence was minimum percentage from 24.7 % and 28.2 % at *Centaurium spicatum* 4% and *Beauveria bassiana* respectively. While the average Adult emergence increased to 61.8% and 92.3 % at *Pyracantha coccinea* 1% and control, respectively.

Hatchability percentage increased from 21.1% and 25.6 at *C. spicatum* 4% and *B. bassiana* respectively to reach to 44.9 and 93.7% at *P. coccinea* 1% and Control respectively.

It can be concluded that treatments with extracts effect on the biology of this pest. Generally increased concentration decreased Adult emergence; fecundity and Hatchability %.

Table (2). The role of different treatments on biological aspects of *L. aristella* on fig fruits.

Treatment	Conc. %	Adult emergence %	Average no. of eggs/ female	Hatchability %	Larval stage in days	Pupal stage in days
Centaurium spicatum	4.0	**24.7**	16.2±1.2c	**21.1**	6.9±4.3c	3.4±0.7c
	2.0	29.7	19.8±1.3bc	28.6	8.3±0.7bc	7.2±0.6a
	1.0	59.5	21.2±1.4b	28.8	9.7±0.6b	8.1±0.7a
Prunus laurocerasus	4.0	31.0	20.3±1.2b	24.1	8.2±0.3bc	4.8±0.3c
	2.0	38.7	21.0±1.5b	29.1	9.3±0.3b	7.6±0.9a
	1.0	63.1	24.4±1.6b	40.8	12.3±0.3a	8.5±0.3a
Pyracantha coccinea	4.0	30.4	20.7±1.6b	26.0	8.3±0.9bc	4.2±0.7c
	2.0	37.8	21.2±1.8b	40.9	10.7±0.3b	7.6±0.8a
	1.0	**61.8**	29.8±1.1b	**44.9**	12.7±1.2a	8.5±0.7a
Hostathion 40%	0.005	28.8	20.8±1.3b	27.2	7.4±4.3c	5.6±0.6bc
Beauveria bassiana	$1x10^4$	**28.2**	20.1±2.1b	**25.6**	9.5±0.4 b	6.3±0.3bc
Metarhizium anisopliae	$1x10^4$	35.1	22.2±3.1b	33.2	12.0±0.4a	7.5±0.6b
Verticilium lecanii	$1x10^4$	39.2	25.2±3.9b	35.2	10.5±0.4b	7.8±0.7a
Control	0.0	**92.3**	72.3±5.3 a	**93.7**	13.5±0.4a	9.4±0.3a

Mean within a column followed by the same letter are not significantly different at 1% level.

These results agree with that found by[10]. *Curcuma longa* extracts control the peach fruit fly by reduced adult progeny and highly percent mortality, reported by[11]. *Ceratitis capitata* was affected by used *Azorella cryptantha*[12].

Effect on developmental stages:

Newly hatched larvae, produced from stock culture and puted on treated fruits with treatments were allowed to develop. The results obtained in Table (2) show that tested extracts and entomopathogenic fungi used effected on the average larvae stage duration in days and decreased it from 13.5 days in control to 6.9days when fruits treated with *C. spicatum* 4%; 9.5 days with *B. bassiana* while it was 7.4 days when treated with Hostathion 40% . Pupal stage in days decreased from 9.4 days in control to 3.4; 5.6 and 6.3 days when fruits treated with *C. spicatum;* Hostathion 40% and *B. bassiana* respectively. From the foregoing results, it can be concluded that *C. spicatum* 4% extracts has a highly significant affected on the duration of larval stage development then afterwards *P. laurocerasus*; *P. coccinea* and entomopathogenic fungi *B. bassiana*, So that can recommend controlling this pest use these concentrations.

The biology of the Lonchaeidae is differently, although primarily associated with living in plant tissue, including trees and herbs[13,14]. The larvae of *Silba* and *Lamprolonchaea* usually develop in fruits and vegetables, sometimes being pests of commercial crops [(15)]**.** However, larvae of the latter genus are usually secondary invaders of injured fruit and other plant material. [16]evaluated entomopathogenic fungi, *Beauveria bassiana*, *Metarhizum anisopilae* and *Lecanicillium lecanii*, against adults of the olive fly *Bactrocera oleae* (Gmelin) under laboratory conditions. He found that oral bioassays caused higher mortality than the used contact bioassays. [17]studied the Biology of Fruit flies (Diptera: Tephritoidea).

2. Field evaluation of different treatments on *L. aristella* at Ashment region Banisuif Governorate.

Results in Table (3) show efficacy of safe locally available and less expensive petroleum ether extract 4% of spicked centaury, *Centaurium spicatum*; entomopathogenic fungi, *Beauveria bassiana* $1x10^4$spore and Hostathion 40% EC with 0.005 were assessed against *L. aristella* under field condition at Ashment region Banisuif Governorate through May 2014. The number of adults found on trap ranged from 2.0 to 6.4 flies/ trap. After two month the number of adults/ trap was ranged from 4.3 to 8 flies /trap. These results agreement with that found by [18]that the Botanical insecticides and entomopathogenic fungal (EPF) insecticides are highly effective, safe and ecologically acceptable in general**.** [19]stated that the entomopathogenic fungi showed a high effect on some insect pests either in the laboratory and field experiments.

3. Effect of different treatments for controlling fig fruit fly, *L. aristella* on the figs yield.

The results in table (4) showed that mixed tested compounds were affected on *L. aristella.* The no. of adults reached to 1.44 adult /trap when treated with Hostathion 40% EC , also, plant extract+ Hostathion 40% EC it was reach to 1.20 , plant extract +entomopathogenic fungi it was1.33 adult /trap while it was 2.30 adult /trap in control.

Table (3). Effect of different treatments on *L. aristella* at Ashment region Banisuif Governorate.

Date	No. of adults/ trap			
	Control	**Hostathion 40% EC**	***Centaurium spicatum***	***Beauveria bassiana***
18/5/2014	**6.4a**	**2.4b**	**2.0b**	**2.5b**
25/5/2014	3.7a	1.5b	1.2b	1.8b
1 /6/2014	8.0a	4.0b	3.5b	4.2b
8/6/2014	11.4a	6.0b	5.6b	6.3b
15/6/2014	**15.0a**	**8.6b**	**7.9b**	**9.1b**
22/6/2014	11.0a	7.4b	7.2b	7.6b
29/6/2014	19.4a	11.0b	9.0b	11.3b
6/7/2014	9.4a	5.6b	4.9b	6.1b
13/7/2014	**8.0a**	**4.5b**	**4.3b**	**5.0b**

Mean within a row followed by the same letter are not significantly different at 5% level.

The income of yield /feddan (pounds) was increased after application done from 9690 pounds in control to reach 21510 pounds when trap treated with Hostathion 40% EC, it was reached to 23710 when teated

with Hostathion 40% EC + *Centaurium spicatum*, while when mixed *Centaurium spicatum*+ *Beauveria bassiana* the income of yield /feddan (pounds) was reached to 21420 pounds, table 5. These results obtained that The monetary value of treatments was 14020 in treated with Hostathion 40% EC + *Centaurium spicatum* while it was 11400 pound in *Centaurium spicatum* + *Beauveria bassiana* compared with Hostathion 40% EC it was reach to 11820 pounds, table 5.

Methyl-eugenol has been used to trap oriental fruit fly, *Dacus dorsalis* Hendel as effective attractants[20]. [21]evaluated botanicals and entomopathogenic fungi for the control insect pests. The treatments also showed reducing pest population and also gave significant control over the treated check. He confirmed that the valuable rate of botanicals and EPF as components of Integrated Pest Management. [22]founded that all treatments Anthio 33%, Anthio 33% plus the plant extract of Antholyza, Anthio 33% plus entomopathogenic nematode, *Steinernema carpocapsae* and Antholyza plus the entomopathogenic nematode were controlled *Hesperophanes griseus* infesting fig orchards and all treatments significantly increased the average yield of fig. Anthio 33% + *A. ringens* was the most effective treatment caused an increase in average fig production /feddan.

Table (4). Effect of different treatments for controlling fig fruit fly, *L. aristella* on the figs yield.

Treatment	Mean no. of adults/ trap	Fruit quality	Grade quality	Price /Kg (piasters)	Yield /tree	Tree product / pounds
Hostathion 40% EC	1.44b	Normal	10	800	25	200
		False pangs	8	600	6	36
		Egg	6	400	4	16
		Larvae	4	200	2	4
		Falling fruits and moldy	0	00	2	00
Hostathion 40% EC + *Centaurium spicatum*	1.20b	Normal	10	800	29	232
		False pangs	8	600	5	30
		Egg	6	400	4	16
		Larvae	4	200	3	6
		Falling fruits and moldy	0	00	2	00
Centaurium spicatum + *Beauveria bassiana*	1.33b	Normal	10	800	26	208
		False pangs	8	600	4	24
		Egg	6	400	4	16
		Larvae	4	200	2	4
		Falling fruits and moldy	0	00	2	00
Control	2.30a	Normal	10	800	9	72
		False pangs	8	600	4	24
		Egg	6	400	3	12
		Larvae	4	200	3	6
		Falling fruits and moldy	0	00	2	00

Mean within a column followed by the same letter are not significantly different at 5% level.

Table (5): Effect of different treatments for controlling the fig fruit fly, *L. aristella* on the monetary value of treatments.

Treatment	Tree mass product / pounds	Cost of treatment /feddan (Pounds)	Feddan product/ Pound	Income of yield /feddan (pounds)	The monetary value of treatments
Hostathion 40% EC	256	250	21760	21510	11820
Hostathion 40% EC + *Centaurium spicatum*	284	250+180	24140	23710	14020
Centaurium spicatum + *Beauveria bassiana*	252	180+150	21420	21090	11400
Control	114	0	9690	9690	-

Used local natural cheap materials such as *Centaurium spicatum extracts and Beauveria bassiana* in comparison with Hostathion 40% EC insecticides reduced the number of *L. aristella* on fig orchards. All treatments are considered good candidates for reducing the population of *L. aristella* to minimum level in fig orchards and increasing the monetary value.

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