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The effect of aqueous plant extracts of tobacco on Third larvae of house fly (*Musca dmestica* L., (Diptera: Muscide)

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Abstract
In this study crude leaf extract of Tobacco (*Nicotina tabacum* L., Solanaceae) was tested for larvicidal potential against third larvae of House fly (*Musca domestica* L.) by feeding method at five concentrations (1000ppm, 2000ppm, 3000ppm, 4000ppm and 5000ppm) at ambient temperature (25±2 C°) and relative humidity (RH 50±5 %). The percentage mortality of larvae was found to increase with higher concentrations of plant extracts which indicate a direct relationship between the dose and percent mortality. The LC_{50} value of aqueous extract of tobacco was found to be 2.300ppm. Moreover, significant reduction in pupation and adult emergency percent in addition to various morphological abnormalities of larvae, pupa and adult flies were detected post treatment of the third larvae with different concentration of tobacco extract. The present results revealed that aqueous leaf extracts of tobacco have control potential against house fly and may be an effective economic alternative to conventional synthetic insecticides.

Keywords: House fly, plant extract, larvae, LC_{50}

Introduction
Musca domestica L., is a severe health threat to live stocks and human beings by transmitting several infections disease [1]. It acts as significant mechanical carries of pathogenic bacteria such as *Escherichia coli*, *salmonella* sp., *Staphylococcus aureus, Shigella* sp., vibrio cholera [2]. However, the immature stages have several industrial and medical applications [3, 4]. Such challenging condition requires a management strategy to interfere with the insect development in order to maintain adult stage population as lower as possible through controlling larval stage population [5].
Control of *Musca domestica* normally depend on chemical compounds, because of their quickly action and easy application. Unluckily the resistance of *Musca domestica* to chemical compounds have increased [6]. Besides, chemical insecticides also have toxic side effects to animals, humans and environment [7]. However, alternative strategies for house fly control essential. therefore, the environmental friendly and biodegrable natural insecticides from plants origin have been receiving attention as an alternative green insecticide for controlling insect pests [8].
Many plant formulation and extracts have been evaluated for their toxicity, repellency and growth regulatory to various dipteran flies especially to house fly. Petrolium-ether extracts of *Griffonia simplicifolia* and *Zanthoxylum xanthoxyloides* has been evaluated for its toxicity to house fly [9]. Seed extracts of *Griffonia simplicifolia* prompted a very strong regulatory effect against the second larval stage of the house fly [10].
The essential oils from herbs or plants are recognized for exhibition of acute toxicity, oviposition deterrent, anti-feeding against a wide range of insect pests, including house fly [11, 12]. Additionally, plant essential oils considered for controlling house fly because of their selectivity, greatly toxicity for insects, target specify, minimal environmental effects and safe to human [13, 14].
Nicotina tabaccum, also recognized as tobacco consist of thousands of components, the chief ones being nicotine, tar, and carbon monoxide. Nicotine is a chemical agent intobacco and also very toxic. The greatest valuable part of the plant used is nicotine, which is found in all parts of plant except the seed. The concentration of nicotine increases with age of the plant. Amature plant has about 64% nicotine in leaves, 18% in stem, 13% in the root and 5% in

flowers. The chemical structure of nicotine consist of pyrrodine and pyrimidine ring. It is gradually becomes brown after it is exposed to air or light. Soluble in chloroform, alcohol, kerosene, water, and some fixed oils [15]. In this regard [16] reported that the extracts of tobacco plant residues had a significant effect to damage the nymph stages, the growth period of immature stages and the productivity of female peach aphid(*Myzus persicae).* Although, several researches have been investigated the mosquitocidal activity of the leaves of *Nicotina tabaccum* against several mosquitoes species [17-20]. Nicotine is active against piercing-sucking insects such as white flies, leafhoppers, mites, thrips and aphids [21]. Alkaloid compounds such as nicotine, anabasine, methyl anabasine, had pesticides activity against other dipteran insects like *Culex pipens* larvae and other larvae of some hexapods, and the efficacy of against the larvae of Culex and mosquitoes using ten larvae of the early fourth stage was evaluated [22]. Although, the mosquitocidal effect of leaf and seed of *Nicotina tabaccum* on the mortality of different developmental stages of *Anopheles gambiae* was investigated and regardless of plant parts used, increasing concentrations of both extracts resulted in increasing mortality of larvae, pupae and adults of *Anopheles gambiae* [23].

From these points of view, the aim of this work was to study the effect of *Nicotine tobaccum* as larvicides for controlling the house fly population.

2. Materials and Methods.

2.1 Rearing of flies

Field populations of house fly were collected by using sweep nets from garbage dumps in Bakarajo District, Sulamani Government, Kurdistan region/Iraq, and colonized at the Crop Protection Department insectary laboratory, Bakrajo Technical Institute, Sulaimani Polytechnic University. The rearing method was that of [24]. Adult flies were placed in screened mesh cages (40x30x30 cm) supplied with food consisting of sugar solution 10% and powdered milk. Barn and milk was prepared at a weight ratio1:3 and 50 grams of this mixture were placed on the small plastic plate as an oviposition site. The flies reared under laboratory conditions: at 25±2Cº, 50±5 % relative humidity (RH), and 12:12 light: dark photoperiod. Flies larvae was bioassayed beginning with the second generation of adult flies produced by field collected flies.

2.2 Preparation of plant extracts

Naturally dried leaves of the tobacco plant (*Nicotina tabacum*) obtained from local market, leaves dried in oven at 40 ºC for 24 h and powdered in a mortar and pestle. The powdered form of plant leaves materials were extracted using distilled water. Aqueous extraction was achieved by adding 50gm powdered leaves to 1000 ml distilled water to prepare stock solution, the mixture was shake continuously for 24 hours using a rotary shaker at laboratory temperature and filtered through whatman filer paper.

2.3 Bioassays:

Feeding technique:

The feeding method was carried out according to the method described by [25]. From stock solution, concentration of 1000, 2000, 3000, 4000, and 5000 ppm was prepared. To test the larvicides, portions of artificial larval rearing medium containing (300gm powdered milk, 500 gm. wheat bran and 15 gm yeast then mixed together with 500 ml of distil water) were treated with different concentration of the tobacco plant extract. Fifty grams of larval rearing medium treated with different concentration of extract were put in a container (100ml polystyrene cups). Untreated media served as a control group. Triple replicate of 25 third larvae for each concentration and so for control group trials were conducted. Each cup was covered with muslin cloth to prevent of escaping of larvae and held at 25±2Cº, 50±5 % relative humidity (RH), and 12:12 light: dark photoperiod. Mortality of larvae followed by the exposure was recorded after 24 h. (Lethal concentration) LC_{50} was calculated using Karbers method [26].

The mortality of larvae of house flies and the number of formed pupae and adults emerging were recorded. House flies scored as survival if they were capable to emerge from puparium.

3. Results

The bioassay showed that out of 25 3rd larvae of house fly exposed to *Nicotina tabacum* at 24 hours, 3 larvae of 25 larvae died when exposed to 1000ppm of plant extracts of tobacco. When exposed to 2000ppm of aqueous leaf extracts of tobacco 8 larvae died out of 25 larvae. When larvae exposed to 3000ppm, 4000ppm and 5000ppm of aqueous leaf plant extracts of tobacco 14, 21, 25 larvae respectively died out of 25 larvae. The exposures of 3rd house fly larvae of house fly to aqueous plant leaf extracts of tobacco caused significant mortality in dose dependent manner Table.1.

The plant leaf extracts of tobacco (*Nicotina tabacum*) seems to be effective in controlling the domestic insect house fly larvae (*Musca domestica* L.). The nicotine and the related alkaloids anabasine and nornicotine which are found in tobacco, induces extremely insecticidal effects in controlling the house fly larvae. Table1 shows the percentage mortality rate of 3rd larvae of house fly when treated with different concentration of tobacco with concentrations 1000ppm, 2000ppm, 3000ppm, 4000ppm and 5000 ppm for (24 h). There is a proportional increase in mortality with increase in concentrations. At low concentration of leaf plant extract 1000ppm, the mortality very less (12%) compared to other concentrations. The highest mortality was recorded in 3000 ppm, 4000ppm, and 5000 ppm at 24 hours while the percentage of mortality were (66%, 84% %,100%) respectively in comparison with control which was (0%) Table1. The extracts was found to be quite effective against *Musca domestica* larvae as 100% mortality was observed at 5000 ppm. The LC_{50} for 3rd larvae was 2300 ppm at 24 hours.

Data in table) (3) indicates that when *Musca domestica* 3rd larvae were treated with different doses of leaf extracts of tobacco concentrations (1000ppm, 2000ppm, 3000 ppm, 4000ppm, and 5000ppm) a drastic decrease observed in pupation, pupation ratios of 3rd larvae of house fly in the treated groups were drastically decreased as the concentration of extracts were increased which was (88%, 68%, 44%, 16% and 0%), similarly a high decrease in percentage of pupa emerged in to adult house fly and ultimately percent mortality. Application of selected doses under test plant leaf exhibited a high range of morphological abnormalities of larvae (Fig.1. b, c, d) larvae with dark cuticle**,** deformate shrink larvae**,** larval pupal intermediate, and irregular body shapes. Morphologic malformation of pupa (Fig.2.b) metalized and dark brown pupa, shirked and few of them extra elongated pupa, reduction in size, condensed appendages and failure to metamorphose in to adult house fly, curved pupa. Malformations of Ault flies(Fig.3.a, b, c)

emerging adults were deformed failed to emerge fully from the puparium, poorly developed wings, some emerged flies with deformed wings or lose one wings. It was observed that during the course of metamorphosis, the propensity for development of treated larvae to pupa decreased with the rise in concentration of leaf extracts of the tobacco plant (Table1). The larvae treated with the extract of tobacco leaves at 5000 ppm exhibited 100% mortality. At lower concentration, some larvae continuously develop.

4. Discussion

The accumulation and biomagnifications of synthetic compounds into different non-target organisms, including humans through the food chain with increased risk of the development diseases or disease syndromes has promoted to explore for relatively safer and more potential molecules for better insect-pests management.

The results obtained from the present study with aqueous extracts of tobacco leaves indicated that exhibited higher insecticidal potential. The LC_{50} value of tobacco leaves recorded in this study may due to the crude nature of aqueous extracts of tobacco used in the present study. The crude plant extracts contain many active as well as inactive compounds which may act synergistically to enhance a specific bioactivity or antagonistically to mask certain activities.

The results showed that aqueous of leaf extracts of tobacco (*Nicotina tabacum*) exhibited its toxicity against third instar larvae of house fly (*Musca domestica* L.) at 24 hours. Mortality of the third larvae was dependent as mortality increased with increased with the in concentrations leaf extract of Tobacco. This finding corroborates with finding of authors [27, 28] who reported on the larvicidal activity of aqueous leaf extracts showed positive effect on third instar larvae of house fly studied. The effect of aqueous tobacco leave extracts using 2500ppm had100% death. This is in agreement with those who reported on the efficacy of *Nicotina tabacum* extracts against the larvae of (*Anopheles* and *Culex*) mosquitoes [29].

The mortality of 3rd larvae of house fly treated with higher concentration of aqueous extracts of tobacco on 3rd larvae of house fly treated higher concentration with 4000ppm, 5000ppm was (84% and 100%) death but using 1000ppm, 2000ppm and 3000 ppm some of larvae pupated and emerge as adult house flies. This present work corroborates with findings of [30] who reported the effect of the aqueous tobacco leaf extracts using 4mg/l and 5mg/l had 100% death, and leaf extracts caused mortality even though a delayed mortality was observed at the lower concentrations 1mg/l, 2 mg/l, 3mg/l respectively and some of larvae treated with 1mg/l pupated and emerged as flies.

The control showed no larval mortality at 24 hours. They all pupated and emerged as flies. This also corroborates with work of [31, 32] they reported on the efficacy of wood vinger against the larval of house fly and also in agreement with [33] who evaluated the efficacy of *Nicotiana tabacum* extracts against the larvae of *Anopheles* and *Culex* mosquitoes**.**

Neem plant extract of affect the growth of target insects in several ways, which has already been stated by [34, 35]. Various plant products have been reported to disturb the normal growth pattern of insects [36-39].

Pupation ratios in the treated groups were drastically decreased as the concentration of extracts was increased. Similar findings were reported by [40], they observed up to 95.97% decrease when third larvae of *Synthesiomyia nudiseta* (Diptera: Muscidae) with the *Cupresss macrocarpa* oil. Percent adult emergence was also decreased in the under test insects due to the established insect growth inhibitory(IGR) effect of the test plant extract. These results are in agreement with the finding of [40].

Deformed adults emerged from treated groups with aqueous leaf extract of tobacco. Similar finding reported by [41] who stated that intensity of deformed adults depend upon the concentration, more deformation was observed under the higher concentration doses [40]. also reported the similar results when 3rd larvae *Synthesiomyia nudiseta* (Diptera: Muscidae) treated with essential oil of *Cupresss macrocarpa*, they observed deformed and pigment larvae, larval pupal intermidiates, C shaped pupa, elongated and balloon shaped pupa, from most of the pupa adult failed to emerge, emerged adults were suffering in severe degree of morphological deformities and abnormalities. In the present study most of the abnormalities were noticed in the treated groups of under test insects. This indicates that an aqueous leaf extract of tobacco has insect growth inhibitory (IGR) effect. Silimar finding against *Musca domestica* have been pointed by [42].

Malformed winged adults of house fly were obtained. This was under the toxic effect of aqueous leaf extract of tobacco effect of under test, due to the hormonal misbalance during development process [43, 44]. Reported that hormonal misbalance and modification of ecdysteroid titre that consequently change the activity of lysosomal enzymes which in turn cause teratomorphic abnormalities in the treated groups.

5. Conclusion

The extracts of *Nicotina tabaccum* (tobacco) at high concentration (5000ppm) exhibited maximum toxicity to the third larvae of house fly. The effects of extract was dose dependent. The LC_{50} value of aqueous extract of tobacco was found to be 2.300ppm. Various results from this work shows that aqueous leaf extracts of *Nicotina tabaccum* (tobacco) led to considerable reduction in larvae, pupa and adult population of house fly and various malformations. *Nicotina tabaccum* could be suggested as a potential insecticide for control of *Musca domestica* that act as prospective vectors of human diseases.

Table 1: toxicity testing of aqueous extracts of tobacco (*Nicotina tabacum)*

Concentration (ppm)	No. of live larvae at (24h).	No, of dead larvae at (24h).	% larvae alive at 24 h.	%Mortality larvae at 24h.
0(control)	25	0	100	0
1000	22	3	88	12
2000	17	8	68	32
3000	11	14	44	66
4000	4	21	16	84
5000	0	25	0	100

The larvae of *M. domestica* (25 in each set) were treated with different concentrations of aqueous extracts of tobacco (*Nicotina tabacum*) for 24 hours. The experiments were conducted in triplicate.

Table 2: LC_{50} value of of aqueous of tobacco (*Nicotina tabacum*).

Concentration (ppm)	Concentration difference	No. of live larvae at 24h.	No. of dead larvae at 24h.	Mean death	Mean death x Concentration difference
0(control)	0	25	0	0	0
1000	1000	22	3	3	3000
2000	1000	17	8	5.5	5.500
3000	1000	11	14	11	11000
4000	1000	4	21	17.5	17000
5000	1000	0	25	23	23000
Total 60000					

The LC_{50} value of aqueous extracts of tobacco (*Nicotina tabacum*) for 24 hours has been determined according to the arithmetic method of Karber (1931).The calculation was done as following:

$$LC_{50} = LC_{100} - \frac{\sum \text{Mean death X Concentration difference}}{\text{No.of organisms per group}}$$

$$LC_{50} = 5000 - \frac{60000}{25}$$

LC_{50} = 2300 PPM.

Table 3: Effect of aqueous extracts of tobacco (*Nicotina tabacum)* on the metamorphosis of *Musca domestica.*

Aqueous extracts from leaves	Concentration (ppm)	Percentage of larvae developed in to pupae*	Percentage of larvae developed in to adult**
Nicotina tabacum	0	100 % (25/25)	100% (25 /25)
	1000	88 % (3/25)	86.4%(12 /17)
	2000	68 %(8/25)	70.5% (12 /23)
	3000	25 % (11/25)	54 %(6 /11)
	4000	10 % (4 /25)	25 % (2/4)
	5000	0 % (0/60)	0 % (0 /0)

*Each value in parenthesis is the number of total pupae developed from the larvae/total number of larvae.
** Each value in parenthesis is the number of total pupae developed to adult/the number of total pupae.

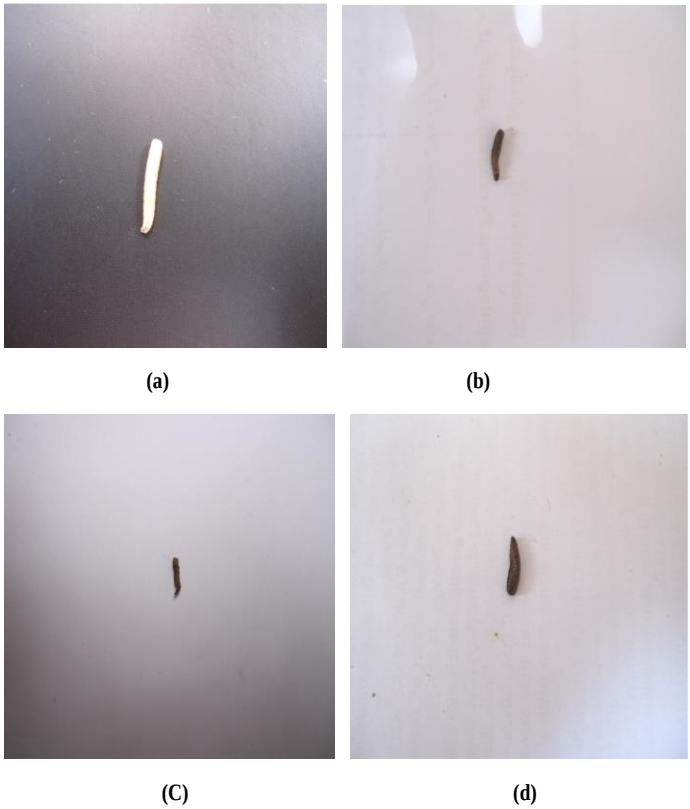

Fig 1: Normal and deformed larvae of *Musca domestica* treated with aqueous extracts of tobacco a) Normal larvae. b) Larvae with dark cuticle. c) deformate shrink larvae. d) Larval pupal intermediate

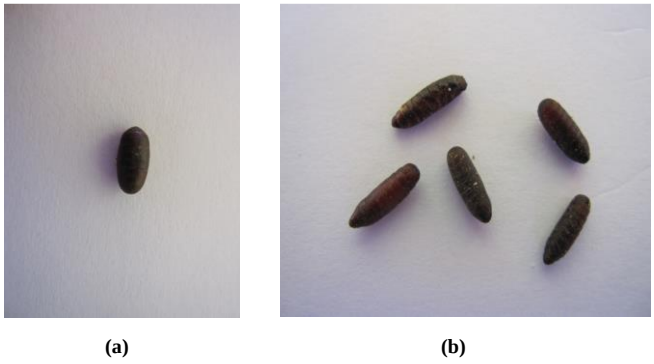

Fig 2: Normal & deformed of pupae *Musca domestica* treated with aqueous extracts of tobacco. a) Normal pupae. b) *Musca domestica* pupae showing the morphological change due the treatment.

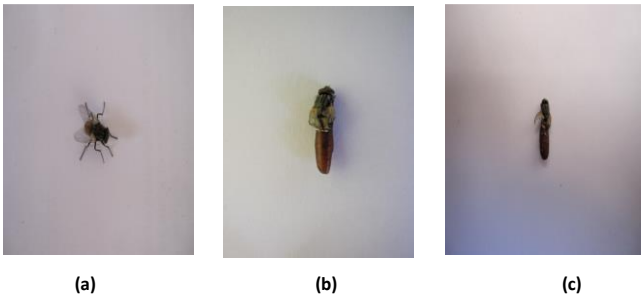

Fig 3: Normal & deformed adult of *Musca domestica* treated with aqueous extracts of tobacco. a) Normal adult fly b) Incomplete adult eclosion crumpted wing. c) Incomplete adult eclosion with crumpted wing. (one wing).

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