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Toxicity And The Possible Biological Effects For Three Insect Growth Regulators Against Flesh Fly, *Sarcophaga Dux* (Diptera: Sarcophagidae).

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ABSTRACT

This study aims to evaluate the effectiveness of three types of IGRs and recording inhibition effect that leads to preventing larvae from reaching adulthood. This was carried out by exposing the 2nd instar larvae flesh fly, Sarcophagadux to different concentrations of the tested IGRs using feeding and dipping methods. The results showed that Pyriproxyfen compound was more effective in inhabiting of emergence of adult flies than Diflubenzuron and Azadirachtin by about 2.44 and 2.73 fold using feeding method, also, by about 1.03 and 2.66 fold respectively using dipping method. The IC50value for the three compounds was 4.17, 10.19 and 11.44 ppm with feeding treatment and 14.87, 15.33 and 29.25 ppm respectively with dipping treatment. Therefore, the feeding method was more effective in comparison to dipping method.In addition, the results revealed morphological deformations in the treated larvae and all growth stages leading to the death of fly without completing its life cycle.

Keywords: *Sarcophaga dux,* insect growth regulators, Toxicity, Biological effects, flesh fly

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INTRODUCTION

Flesh fly (Diptera :Sarcophagidae) is closely related to humans and this family is known to enter houses for colonizing the decomposed corpses (Pohjoismaki, 2010). It depends on living and dead tissues to complete its life cycle (Watson and Dallwitz, 2003). This family of flies is attracted to many kinds of dead vertebrate remains including humans (Nishida, 1984). Among the 2000 types globally known of this family, 327 types are found in the United States. Species of this family have several habits of feedings some are parasitical, others are predatory. A lot of these species are considered pathogens factors and they also could be vector-borne diseases (Pape and Dahlem, 2010 ;Teixeira and Guimaraes, 1999).

Due to the habit of flesh flies feeding, the most common problem is to cause myiasis in both humans and animals. Myiasis disease is known as the infection of vertebrate animals with flesh fly larvae for a certain period. It feeds on living or dead tissues of the infected animal, the body fluids or the digested food (Sukontason, et al., 2000). In addition, there are other species of Sarcophagidae which can cause different types of myiasis that infect humans (Tuygun et al. 2009; Kokcam and Saki 2005; Yildirim et al., 2008; Droma et al. 2007).

In Saudi Arabia,(Alotaibi *et al*., 2016) had recorded a case of myiasis in a 7 months old female baby in Jazan province in which her body was covered with ulcers and lesions. Another case of myiasis was identified in Makkah province for a 4 years old boy (Badri et al., 2016). Moreover, the myiasis disease was diagnosed on the shoulders of 40 years old man which was caused by Sarcophgaspp (Zaglool *et al*., 2013).

Furthermore, many of the vector-borne diseases control strategies have been focused on using traditional chemicals insecticides. However, the frequent, intensive and unregulated use of these compounds has been accompanied with various problems. Despite their expensive costs, they have a wide range of side effects on humans and other non-target organisms which is caused by either direct or indirect exposure through their impact on natural food chains. Moreover, some dangerous insects such as mosquitoes could gain resistance against these used chemical components (Tehri *et al*., 2015).

This study aims to address recent approaches to flies control by evaluating the effectiveness of Insects Growth Regulators (IGRs) against the various stages of flesh flies, *Sarcophaga dux* which is the dominant species in Jeddah province as a safe strategy of control.

MATERIALS AND METHODS

- Flies rearing

The sample of flesh flies have been collected from different locations in Jeddah province and the dominant specie *S.dux*was identified using taxonomy keys, dissection methods and molecular level technique. Also, a colony was established under lab conditions according to (Amoudi*et al*., 1992, 1994) method to get abundant number from larvae and adult insects to conduct experimental trials.

-Tested compounds:

Three types of non-conventional insecticides (IGRs) were used and evaluated against flesh fly, *Sarcophaga dux* as a safe control strategy. These types are:

Insecticide commercial name	Percentage of the active ingredient
Difoxflowable	Diflubenzuron 10%
Admiral	Pyriproxyfen 10%
Amen	Azadirachtin 1%

-Preparation of standard solutions:

The standard solutions needed for the experiments were prepared using 1 ml of liquid form insecticide or 1 g of powder form insecticide and then added to 100 ml of water. The solutions were mixed well to get the standard solutions which differ in its effectiveness depending on the active ingredient in the tested insecticide. After that a series of concentrations were prepared according to the world health organization (WHO, 2005).

-Bioassay of the selected IGRs against the larvae of flesh flies, *s.dux*:

Two methods were used to evaluate the effectiveness of IGRs against larvae of flesh flies:

1- Dipping method in which the2nd instar larvae were immersed in a series of different concentrations for five seconds according to (Esmaeel, 1995). After that the larvae were placed in a container containing a feeding environment (sheep liver) where the larvae were placed on top of the liver. Then the container was covered with a muslin cloth and fitted with a rubber band. Thereafter, the effect of the insecticide was monitored on a daily basis by recording numbers of the dead larvae, the emerged pupi and adult from the treated larvae and the inhibition percentage of flies for each insecticide.
2- Feeding method according to (Vazirianzadeh et al., 2007) method with some modification as 20 g from the larvae feeding environment (sheep liver) were treated with different concentrations of the tested IGRs and transferred to a new plastic container. The experiment was replicated three times with 10 2ndinstar larvae for each experiment. Each container was covered with a muslin cloth tightly fitted with a rubber band. In addition, control trails were set up where the larvae feeding environment was not treated. Then the numbers of the dead larvae, the emerged pupi and the emerged adult insects were recorded.

- Statistical analysis:

The results of the larvicidal activity assessment for the tested compounds were analysed based on the percentage of adult emergence inhibition after the treatment of larvae according to (Finney, 1971) method using LDP line software to obtain the different statistical values with the lower and upper confident limits as well as *the P*< 0.05 was taken to manifest statistical significance.

RESULTS AND DISCUSSION

The larvicidal activity was evaluated for the three types ofIGRs which are Azadirachin, Pyriproxyfen and Diflubenzuron. These insecticides were applied against the 2nd instar larvae of flesh flies, *S.duxm* using two methods for the bioassay which are the dipping and the feeding method (Table 1 and 2).

Initially, the results of feeding method treatment showed that the Difluenzuron compound caused the highest mortality in larvae(20-40%) with concentration of 5-25 ppm . Followed by the Pyriproxyfen compound which caused 13.33-36.67% of larvae mortality when 1-20 ppm concentration was used. Whereas, Azadirachtin compound showed the lowest value of mortality (6.67- 30%) with concentration of 5-30 ppm (Table 1). Moreover, these concentrations revealed adult inhibition between 35.31-82.65% when Difluenzuron was applied, 20.62-92.78% in Azadirachtin and 10.23-84.47% in Pyriproxyfen (Table 1).

According to IC50 vlue (Concentration in which inhibit the emergence of 50% of adults). The IC50 of Difluenzuron compound was 10.19 ppm, whereas, the IC50 of Azadirachin and Pyriproxyfen were 11.44 ppm and 4.17 ppm respectively (Table 3). Therefore, these results confirm that the Pyriproxyfen compound is the most effective against the S.dux larvae, followed by Difluenzuron and Azadirachtin by about 2.44 and 2.74 fold respectively.

Furthermore, the effective concentration of Difluenzuron (5-25 ppm), Azadirachtin (5-30 ppm) and Pyriproxyfen (1-20 ppm) compounds caused mortality between 6.66-30, 3.33- 26.66 and 3.33-16.66 sequentially (Table 2) when dipping method treatment was applied. Also, the concentration of these compounds showed 18.37-69.39, 7.22-58.78 and 10.91-64.62 of adult inhibition respectively. The IC50 value

which was obtained from toxicity curve (Fig.1 and Table 3) revealed that the Pyriproxyfen compound (14.83 ppm) was more effective against larvae of flesh flies, followed by Difluenzuron compound (15.33 ppm) while the Azadirachtin the (29.26 ppm) the lowest effective. The indicator resistance ratio showed that the Pyriproxyfen was more effective against growth stage of S.dux flesh flies compared to Difluenzuron and Azadirachtin by about 1.03 and 2.66 time respectively.

Therefore, the results indicated that treatment of larvae of flesh flies using feeding method was more effective than the dipping method. This was confirmed when results of both methods were analysed based on IC50 value, revealing that the flies were more sensitive to the tested compounds (Pyriproxyfen, Difluenzuron and Azadirachtin) by about 3.565, 1.504 and 2.557 fold respectively (Fig.2).

The variations between larvae of flesh flies in terms of their sensitive to the tested compounds may be due to the differences in the concentrations of active ingredients for each insecticide. These results are correspondent to other studies that used the IGRs group. For instance, (Baeshen, 1998) found that the differences between concentrations of active ingredient of some IGRs led to the variance in sensitivity level between larvae of Sarcophagahaemorrhoidalis. (Cetin et al. 2009) showed that treatment of flesh flies larvae using feeding method was more effective than dipping method with application of Novalaron compound. In addition, the IGRs group showed obvious effect when they tested against house flies. One of these studies showed that the Azadirachtin was more effective than Difluenzuron against 2nd instar larvae feeding method which was applied according to LC90 and LC50 value (Vazirianzadeh *et al*., 2007). Also, (Sulaiman et al., 2008) confirmed that the concentration between 0.5 – 2.5 mlg/l of Difluenzuron and Pyriproxyfen caused 98.5% of adult inhibition when feeding method treatment applied on 1st instar larvae of house flies.

The current results also illustrated that the tested compounds did not have high percentage of death in the larval stage, however its impact continued to the later stage included pupa and adult insect which usually measured according to Larvicidal activity that lead to inhibition of some adult emergence. These delayed effects including morphological deformations such as body segments contraction, emergence of intermediate stage between larvae and pupa or between pupa and adult, body of adult relativity smaller and wings twisted (Fig.3).This might due to interfering IGR compounds with the normal physiological process during metamorphosis stages as they act as an inhibitor for chitin synthesis (Tunaz and Uygun, 2004) and disrupt the endocrine system of insects (Olmstead and Leblanc, 2003). These observations were similar to other laboratory studies. For example, (Baeshen, 1998) found that some flesh flies could not grow to adult stage and other was unable to fly including obvious morphological deformations in flies body.

In general, the use of non-conventional compounds from the IGRs is an effective strategy that could facilitate controlling flesh flies and reduce its damages. Furthermore, it could help to overcome the problems related to the use traditional chemical compounds in the control programmes.

Table 1: The percentage inhibition of adult emergence following treatment of *S. dux* larvae with the IGRs Compounds by using feeding bioassay methods

Tested Compounds	Concentrations (ppm)	Larval mortality[a] (%)	Pupae produced (%)	Adults hatched	Adult [b]emergence Inhibition
Difoxflowable Diflubenzuron	5	20	80	64.69	35.31
	10	26.67	73.33	54.49	45.51
	15	30	70	40.82	59.18
	20	36.67	63.33	30.61	69.39
	25	40	60	17.35	82.65
Admiral Pyriproxyfen	1	13.33	86.67	89.77	10.23
	5	20	80	76.55	23.45
	10	23.33	76.67	49.12	50.88
	15	26.67	73.33	29.64	70.36
	20	36.67	63.33	15.53	84.47
Amen Azadirachtin	5	6.67	93.33	79.38	20.62
	10	13.33	86.67	55.67	44.33

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	15	16.67	83.33	41.24	58.76
	25	23.33	76.67	17.53	82.47
	30	30	70	7.22	92.78

a. Five replicates, 20 larvae each. *b* . Inhibition of adult emergence in control 0-3%

Table 2: The percentage inhibition of adult emergence following treatment of *S. dux* larvae with the IGRs Compounds by using dipping bioassay methods

Tested Compounds	Concentrations (ppm)	Larval mortality[a] (%)	Pupae produced (%)	**Adults hatched**	Adult [b]emergence Inhibition
Difoxflowable Diflubenzuron	5	6.66	93.33	81.63	18.37
	10	13.33	86.66	61.22	38.78
	15	20	80	51.01	48.99
	20	26.66	73.33	40.82	59.18
	25	30	70	30.61	69.39
Admiral Pyriproxyfen	1	3.33	96.66	89.09	10.91
	5	6.66	93.33	74.72	25.28
	10	10	90	61.83	38.17
	15	13.33	86.66	49.56	50.44
	20	16.66	83.33	35.38	64.62
Amen Azadirachtin	5	3.33	86.66	92.78	7.22
	10	6.66	93.33	82.47	17.53
	15	13.33	86.66	72.15	27.85
	25	23.33	76.77	61.85	38.15
	30	26.66	73.33	41.22	58.78

a Five replicates, 20 larvae each. *b* . Inhibition of adult emergence in control 0-4%

Table3: Effects of the IGRs (Diflubenzuron, pyriproxyfen and Azadirachtin) against *S. dux* by using dipping bioassay methods

Tested Compounds	**Statistical parameters**			
	IC_{50} L. limit- U. limit	**IC_{90} L. limit- U. limit**	**Slope**	**Calculated [c] $(Chi)^2$**
Feeding Method				
Difoxflowable Diflubenzuron	**10.19** 8.49 - 11.82	**52.36** 38.03 - 88.74	1.80	5.15
Admiral Pyriproxyfen	**4.17** 3.44 -5.08	**30.01** 21.60 - 46.25	1.49	3.40
Amen Azadirachtin	**11.44** 10.24 - 12.66	**32.36** 27.69 - 39.76	2.83	3.73
Dipping method				
Difoxflowable Diflubenzuron	**15.33** 13.46 -17.70	**65.09** 46.94 - 109.99	2.04	0.46
Admiral Pyriproxyfen	**14.87** 11.96 - 19.66	**137.36** 78.36 - 337.44	1.32	4.32
Amen Azadirachtin	**29.26** 25.10 - 36.22	**107.62** 74.77 - 191.53	2.26	4.09

C .Tabulated **$(Chi)^2$** = 7.8 larger than calculated at 0.05 level of significance indicates the line is good fit and the data are significantly homoge

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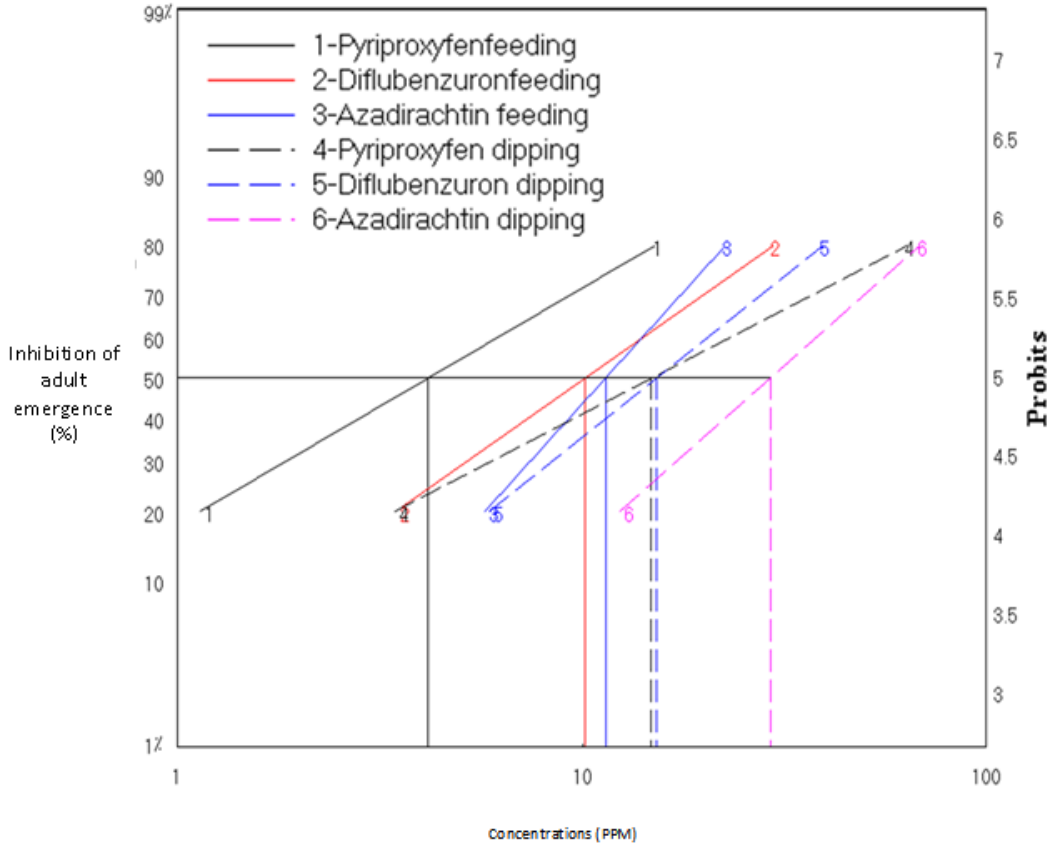

Fig 1: The relation between concentrations of the IGRs (Diflubenzuron, pyriproxyfen and Azadirachtin) and the percentage of inhibition of adult emergence after treatment of *S. dux* larvae by using feeding & dipping bioassay methods.

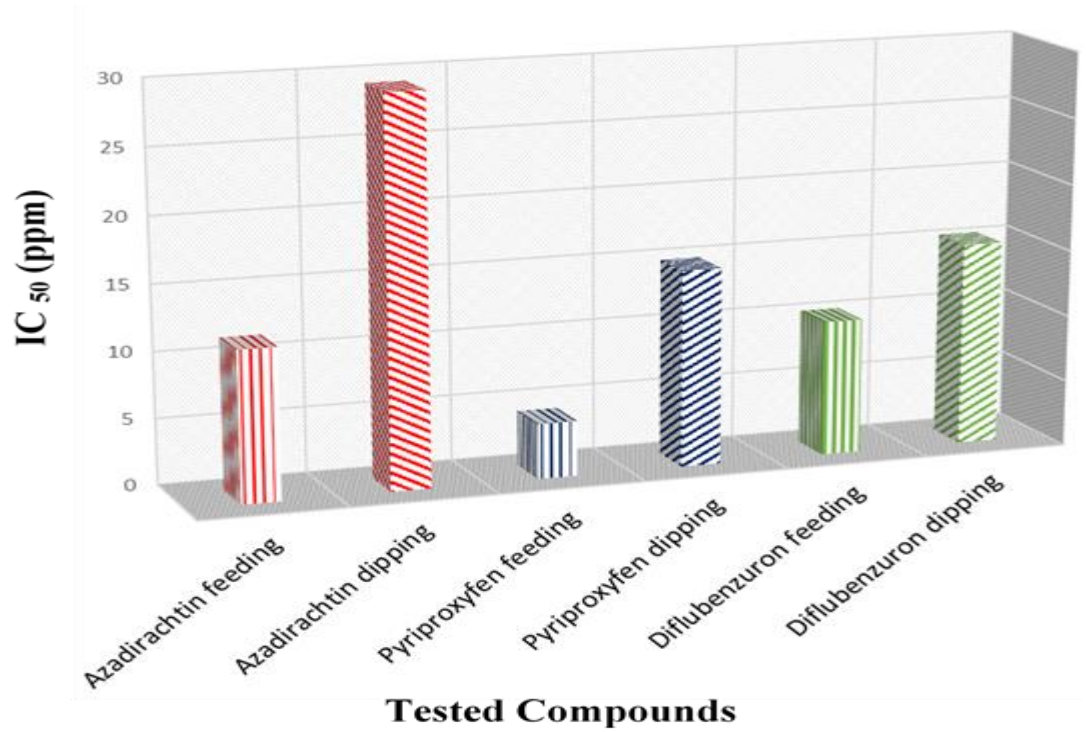

Fig 2: Toxicity values of the IGRs (Diflubenzuron, pyriproxyfen and Azadirachtin) against *S. dux* by using feeding & dipping bioassay methods

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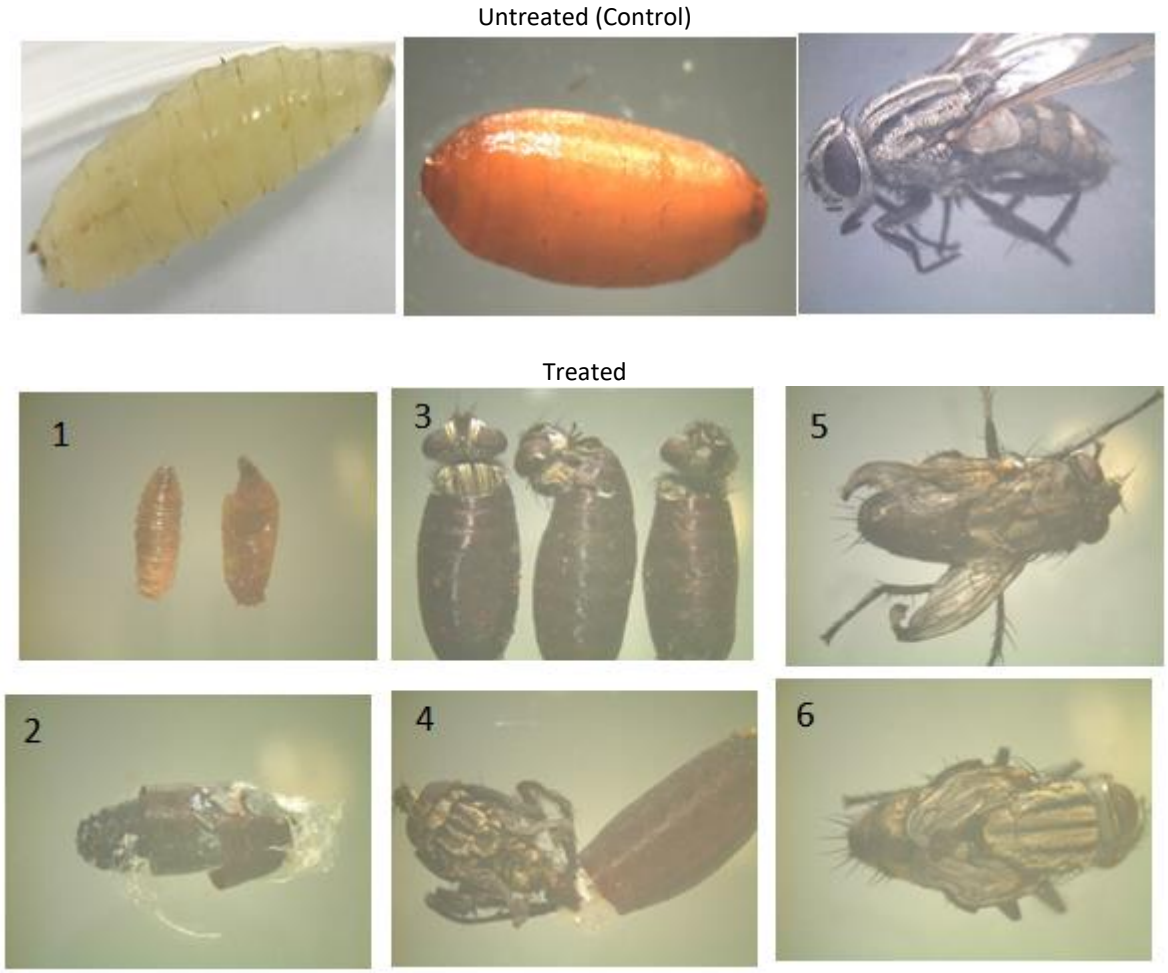

Fig 3: Morphological effects of the IGRs (Diflubenzuron, pyriproxyfen and Azadirachtin) against *S. dux* by using feeding & dipping bioassay methods, the abnormalities ranged form :

1- Larvae were shrinkage in size and miss shape larvae.
2- A type of larval – pupal intermediate
3- A type of pupal– adult intermediate
4- Ecdysial failures of adult stage
5- Wings deformations (twisting wings)
6- Wings deformations (shrinkage wings)

REFERENCES

[1] Alotaibi, S.; D. Al shahrani and O.Alzomor (2016). Cutaneous Myiasis in Saudi Infant: A Rare Case Report. International Multispecialty Journal of Health. 2: 98-12.

[2] Amoudi, M. A.; Diab, F. M. and Abou-Fannah,S.S.M(1994). Development rate and mottality of immature *Parasarcophaga*(Liopygia) *ruficornis* (Diptera : sarcophagidae) at constant laboratory *temperatures. J.Med . entomol.*vol 31; 168-170.

[3] Amoudi, M. A.; M. Leclercq and R. Richet (1992). Rearing of a newly recorded dipterous fly *Parasarcophaga* (Liopygia) *ruficornis* (Fabricius) (Diptera: Sarcophagidae) from Saudi Arabia under laboratory conditions. Pakistan Journal of Zoology, 24; 181-186.

[4] Badri A. l. R.; A. l. T.Harbi; A.Tonnsi; A.Almatary and R.Hassanein (2016). Cutaneous Myiasis in a Child Scalp Caused by WohlfahrtiaMagnifica(Diptera: Sarcophagidae): A Case Report. MOJ Clin Med Case Rep 4(3): 00093. DOI: 10.15406/mojcr.2016.04.00093.

[5] Baeshen, Rowida Saeed Hassan(1998).Effect of Tow Juvenle Hormone Analogue on Production of the Flesh Fly Sarcophagahaemorrhoidlis .Master thesis, Dep.of Biological Science Faculty of Science, King Abdulaziz University ,K.S.A.

[6] Cetin, H.; Erler, F. and Yanihoglu A. (2009).Survey of insectgrowth regulator (IGR) resistance in house flies (Muscadomestica L.) from southwesternTurkey.I. of vector Ecotogy. 34 (2): 329-337.

[7] Droma, E. B.; A.Wilamowski; H.Schnur; N.Yarom; E. Scheuer and E.Schwartz (2007). Oral myiasis: a case report and literature review. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology, 103: 92-96.

[8] Esmaeel, Doa, M.A.(1995) Biological activities of insect growth regulators on plodiainterpunctella (Hb)(Order; Lepidopera-family;pyralidae)(M.Sc.D.) Biological Sciences and Geology,Faculty of education,AinShams University,Cairo,pp.45-87.

[9] Finney, D. (1971). Probit analysis. Cambridge Press, Washington. DC. 333 pages.

[10] Kokcam, I. and C. E. Saki (2005). A case of cutaneous myiasis caused by Wohlfahrtiamagnifica. The Journal of dermatology, 32: 459-463.

[11] Masngi, S.; Lyatuu, E. and Kweka, E. (2011). Field and laboratory evaluation of bioefficacy of an insect growth regulator (Dimilin) as a larvicide against mosquito and housefly larvae. J. Tropical Medicine . V.2011:1-8. Entomol. 96 (3): 1016-1020.

[12] Nishida K. (1984). Experimental studies on the estimation of postmortem intervals by means of fly arvae infesting human cadavers. Japanese Journal of Legal Medicine 38: 24-41

[13] Olmstead AW, LeBlanc GA. (2003). Insecticidal juvenile hormone analogs stimulate the production of male offspring in the crustacean Daphnia magna. Environmental health perspectives.; 111(7):919-923.

[14] Pape, T. and G. A. Dahlem (2010). Sarcophagidae (Flesh Flies). In: Brown BV, Borkent A, Cumming JM, Wood DM, Woodley NE, Zumbado MA. Manual of Central American Diptera. NRC Research Press 2. 744 p.

[15] Pohjoismaki JLO, Karhunen PJ, Goebeler S, Saukko P, Sääksjärvi IE(2010). Indoors forensic entomology: Colonization of human remains in closed environments by specific species of sarcosaprophagous flies. Forensic Science International 199: 38-42.

[16] Slama, K. Romanuk, M. and SormF(1974). Insect hormones and bioanaloguesFirst ed. Wien. Newyork. Springer-Verlag. pp.96-263.

[17] Sukontason, K., Bunchoo, M., Khantawa, B., Piangjai, S. and Choochote, W.(2000). Musca domestica as a Mechanical Carrier of Bacteria in Chiang Mai, North Thailand. J. Vect. Ecol. 25: 114–117.

[18] Sulaiman, S.; Hidayatul, Sh; Mustaffa , M.S. and Jeffery, J.(2008). Effect of triflumuronand pyriproxyfen on Muscadomesticalharval stages in the laboratory. Iranian J arthropod-Borne Dis. 2(1):1-6.

[19] Tehri, K., and Singh, N. (2015). The role of botanicals as green pesticides in integrated mosquito management–A review, International Journal of Mosquito Research, 2 (1): 18-23 .

[20] Teixeira, D. M.; J. H Guimarães and N.Papavero (1999). Myiasis in man and animals in the Neotropical Region: Bibliographic database. Myiasis in man and animals in the Neotropical Region: Bibliographic database.

[21] Tunaz H, Uygun N. 2004. Insect growth regulators for insect pest control. Turkish Journal of Agriculture and Forestry; 28(60):377-387.

[22] Tuygun, N.; A.Taylan-Ozkan;G.Tanir and K. Y. Mumcuoglu (2009). Furuncularmyiasis in a child caused by Wohlfahrtiamagnifica (Diptera: Sarcophagidae) associated with eosinophilia. Turk J Pediatr, 51: 279-281.

[23] Vazirianzadeh, B; Jervis, M.A and Kidd, No.A.C (2007). The effects of oral application of cyromazine and triflumuron on house fly larvae. Iranian J. Arthropod – Borne Dis. 1(2): 7-13.

[24] Watson, L. and M. J. Dallwitz (2003). Onwards. British insects: the families of Diptera. Version: 28th August 2009.

[25] (WHO) World Health organization (2005) Guidelines for laboratory and field-testing of mosquito larvicides.WHO/ CDS/ WHOPES/ CDPP/13.

[26] Yildirim, I.; M. Ceyhan; A. B.Cengiz; C. E.Saki; E.Ozer; S.Beken and E.Cilsal (2008). What's eating you? Cutaneous myiasis (Wohlfahrtiamagnifica). Cutis, 82: 396-398

[27] Zaglool, D. A. M.; K.Tayeb; Y. A. W.Khodari and M. U. Farooq (2013). First case report of human myiasis with Sarcophaga species in Makkah city in the wound of a diabetic patient. Journal of natural science, biology, and medicine, 4: 225