



Susceptibility of screwworm fly, *Chrysomya albiceps* to commonly used pesticides in Jeddah Governorate

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Abstract: The widespread of the screwworm *Chrysomya albiceps* in the Kingdom of Saudi Arabia has begun to be alarming, and that controlling it and limiting its infestation to more animals has become a very important matter, and in this study, the level of sensitivity to some of the compounds used in the control programs in Jeddah by feeding and immersion methods was measured. The results showed that the treatment using the feeding method was more effective than the immersion method, according to the LC₅₀ values. The results showed that the compound Protec (LC₅₀=0.388ppm) was the most effective compound tested, followed by the compound Cyber Safe (LC₅₀=0.632ppm), then the compound Diuracid (LC₅₀=3.253ppm), while the compound Actyl (LC₅₀=33.624ppm) was the least tested compound. Efficacy against the second instar larvae of screw fly, and when comparing groups, the results showed that the group of pyrethroid compounds was more effective compared to the phosphorous compounds. As for comparison within the same group, The results showed that the Protec compound was more effective than the Cyber Safe compound by about 1.629 and 1.75 times within the group of pyrethroid compounds, while Diuraside compound was more effective than the Akicle compound by about 10.34 and 9.95 times within the group of phosphorous compounds by feeding and immersion methods, respectively. In general, the current study showed that screwworm larvae possess a high level of tolerance against a group of organophosphorus compounds, and it is recommended to limit their use and search for more effective alternatives against the screwworm that are safe for humans and the environment.

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1. Introduction

The screwworm *Chrysomya albiceps* is one of the dangerous pests that attack wounds in humans and all warm-blooded animals, including farm animals (sheep, goats, cows, and buffaloes) and wild animals (deer, field rabbit, stray dogs, jackals, ... etc.). Its danger lies in completing its life cycle, especially its larvae, which are considered obligatory parasites on living tissue, especially wounds from the umbilical cord of newborn animals, wounds resulting from operations of shearing wool, numbering, castration, removal of horns, or caused by tick bites, or the fights of males among themselves within the herd of animals or as a result of Friction of the bodies of animals with the barbed wire surrounding the fields (Spradbery and Kirk 1992; Marinho et al., 2006; Al-Ghamdi et al., 2015).

Some studies also indicated that the screwworm fly has a role in spreading Anthrax by transmitting the pathogen bacteria *Bacillus anthracis* (Bassonet et al., 2018).

The pathological condition resulting from infection of animal wounds or natural openings by the larvae of this pest is known as myiasis, and neglecting to treat such wounds may lead to the death of animals, especially newborns, as well as causing invisible losses to wild animals (Reigada et al., 2005).

The screwworm *C. albiceps* is one of the main species responsible for causing myiasis in Saudi Arabia (Badawi 1994; Alahmed, 2001; Setyaningrum and Al Dhafer 2014; Al-Shareef, 2016).

It is worth noting, that the screwworm fly, and through the greater economic losses it causes in livestock in addition to its great impact on public health, has received great attention in the multiplicity of ways to combat it. Conventional chemical pesticides are one of the most common and widely used methods, which has given this pest a characteristic Resistance against many of them, which requires the implementation of laboratory experiments to measure the level of sensitivity to the

pesticides used in the control programs and this is what this study aimed at.

2. Materials and Methods

Flies Rearing

Random samples of colored flies were collected from different locations in Jeddah Governorate, and the Screwworm was identified using the classification keys (Shaumaretal., 1989) and the confirmation of the identification at the molecular level using polymerase chain reaction (PCR). The predominant species was *C.albiceps*. A colony was established under laboratory conditions for the dominant species, according to the method (Alhuraysi et al., 2021; Singh and Kaur, 2017) to obtain sufficient numbers of larvae to carry out research and study experiments.

Compounds tested

The following compounds were used:

1-The organophosphate insecticide Duracide (Tetramethrin: 15.2 % w/w) and Actikil (Pyrimiphos methyl 5%EC)

2-The pyrethroid insecticides Project. (ALPHACYPERMETHRIN 10% EC) and Cyper Safe (CYPERMETHRIN 10% W/V)

These compounds were obtained by direct purchase from the local market.

Experimental Bioassay:

Feeding bioassay method

This test was conducted according to the method of Vagiriangadeh et al., (2007) with some modifications, which is a simulation of the method of spraying garbage containers, waste, and carrion with pesticides to control insects. The larval feeding environment (lamb's liver) was mixed with a series of selected pesticide concentrations. 50 g of the treated larval environment were placed in glass beakers (capacity 400 ml). Five replicates/concentrations were used with 20 second age larvae for each replicate, as well as the control (control), which was fed on sheep liver treated with distilled water only. Then cover each beaker with nylon tulle that is secured with a rubber band. The number of dead larvae was calculated, and then the larvicidal activity of the tested compounds was estimated based on the percentage of treated larvae after 24 hours of exposure.

Dipping method

The test was performed according to the method of Sukontason et al., (2004) with some modifications. The second instar larvae of the screw fly were exposed to a series of concentrations of selected compounds, which simulates the method of dipping the animals in a pesticide solution to get rid of external parasites by placing the larvae in a net of the mound and then gently dipping them in the concentration solutions for 30 seconds while the

control larvae were immersed in water. Five replicates were used, each with 20 larvae for each focus, as well as the control. Larvae were transferred after immersion into clean glass beakers containing the larval feeding environment. The number of dead larvae was recorded to determine the percentage of death 24 hours after treatment.

Statistical Analysis

Completely randomized design (CRD) was used in the experiments and analyzed using ANOVA, and the least significant difference test (LSD) was used at the level of significance ($P < 0.05$) to compare the selected concentrations and their corresponding death rates using SAS software, and the half-concentration was also determined. Larval killer and toxicity curves were plotted using LDP-line specialized statistical software.

3. Results

The sensitivity level of *C.albiceps* second instar larvae was measured by two feeding methods and by dipping method for some conventional insecticides. The tested conventional pesticides were Duracide and Actikil from the Organophosphorus (OP) group and Cyper Safe and Project from the group Organic Pyrethroides (PY).

The larval virulence activity of the tested pesticides was evaluated against the second instar larvae of *C. albiceps* after 24 hours of larval treatment, as these compounds have toxic effects. Therefore, the LC_{50} scale was used, which is the concentration of the compound needed to kill 50% of the treated larvae kill 50% of larvae.

The results shown in Table (1, 2) showed that the death percentages of the second instar larvae of *C. albiceps* treated with Duracide were directly proportional to the concentration, as the death rates ranged between 14.43-93.81% by feeding method and 11.34-87.63% by immersion method at Concentration 1 - 10 ppm. Also, in the case of measuring the sensitivity of the second instar larvae of *C. albiceps* treated with Actikil compound, it was found that the death rates ranged between 16.50-93.81% by feeding method and 6.19-88.66% by immersion method at concentrations ranging between 20-60 parts per million. Also, the percentage of deaths of second instar larvae of *C. albiceps* treated with Cyper Safe ranged between 12.25 - 93.88% by feeding method and 15.31-85.71% by immersion method at concentration 0.3-1.5 ppm (Table 3).

On the other hand, the results in Table (4) showed a direct proportion between the tested concentrations of Project pesticide and the percentage of deaths of the second instar larvae of the treated *C. albiceps* fly, where the death rates ranged from 10.20 - 94.90% by feeding method and from 13.27 -86.74% Immersion method at concentrations of 0.1-1 ppm.

By studying the LC-P lines Figs (1 and 2) and obtaining the values of LC₅₀ and LC₉₀, it is clear that there is a difference in the sensitivity level of the second instar larvae of *C. albiceps* exposed to different concentrations of the tested pesticides. In the case of the phosphorous pesticide Duracide, the concentrations needed to kill 50 and 90% of larvae after 24 hours of treatment are 3.2526, 10.2466 ppm

by feeding method and 3.7455, 12.8519 by immersion method, respectively, while in the case of Actikel the values of concentrations needed to kill 50 and 90% of larvae after 24 hours Of the treatment are 33.6242, 60.869 ppm by feeding method and 37,244, 62.0146 by immersion method, respectively (Table 5).

Table 1: Susceptibility levels of 2nd larval instars of Screwworm *Chrysomya albiceps* to Duracide following continuous exposure for 24 hr by using feeding and Contact bioassay method.

Concentrations (ppm)	Larval mortality ^a (%)	
	Feeding method	Contact method
1	14.43±1.25 ^a	11.34±1.33 ^a
3	34.02±1.39 ^b	34.02±1.49 ^b
5	69.07±1.04 ^c	61.86±1.69 ^c
8	84.54±1.66 ^d	78.35±1.80 ^d
10	93.81±1.83 ^e	87.63±1.11 ^e
LSD	9.5	8.1
P	0.0001	0.0001

^a: Five replicates, 20 larvae each, Larval mortality in control= 0 - 3%

Means followed by different letter(s) are significantly different from each other (p < 0.05) by LSD test

Table 2: Susceptibility levels of 2nd larval instars of Screwworm *Chrysomya albiceps* to Actikel following continuous exposure for 24 hr by using feeding and Contact bioassay method.

Concentrations (ppm)	Larval mortality ^a (%)	
	Feeding method	Contact method
20	16.50±1.58 ^a	6.19±1.29 ^a
30	37.11±1.88 ^b	28.87±1.83 ^b
40	59.79±1.73 ^c	56.70±1.42 ^c
50	79.38±1.64 ^d	77.32±1.31 ^d
60	93.81±1.49 ^e	88.66±1.82 ^e
LSD	8.5	5.9
P	0.0001	0.0001

^a : Five replicates, 20 larvae each Larval mortality in control= 0 - 3%

Means followed by different letter(s) are significantly different from each other (p < 0.05) by LSD test

On the other hand, the results showed in Table (6) the values of the concentrations needed to kill 50 and 90% of the larvae after 24 hours of treatment with the Cyper Safe and Project pyrethroid pesticides, they reached 0.6324, 1.3337 ppm by the feeding method and 0.6673, 1.7247 by the immersion method. For the pesticide Cyper Safe while it was 0.3878, 1.105 ppm by feeding method and 0.3815, 1.444 by immersion method of the pesticide Project, respectively.

These obtained results confirm that the treatment through feeding was more effective against the second instar larvae of the screw fly compared to the immersion method by about 1.151, 1.108, 1.055, and 1.016 times (Folds) for the tested pesticides Protec, Cyber Safe, Duracid and Actikel, respectively.

According to the relative resistance index RR and LC₅₀ values, the results showed that the pyrethroid insecticide Protec was the most effective tested against the second instar larvae of the Snail fly, followed by the pyrethroid insecticide Cyber Seif and then the phosphorous insecticide Duracid, while the phosphorous insecticide Acticle was the least effective insecticide tested against the second instar larvae of the snail form of the fly (Figures 1, 2 and Table 7).

In general, it can be said that the response of the second instar larvae of the screw flies to the tested pesticides Protec, Cyber Safe, Duracid and Actikel depends entirely on the type of pesticide used, the active substance, its proportion, method of action and the extent of its effective concentrations, and this is confirmed by those differences in the percentages of larval death and their increase in direct proportion with the increase user focus.

Table 3: Susceptibility levels of 2nd larval instars of Screwworm *Chrysomya albiceps* to Syper Safe following continuous exposure for 24 hr by using feeding and Contact bioassay method.

Concentrations (ppm)	Larval mortality ^a (%)	
	Feeding method	Contact method
0.3	12.25±1.47 ^a	15.31±1.30 ^a
0.5	32.65±1.85 ^b	33.67±1.27 ^b
0.8	58.16±1.44 ^c	54.08±1.75 ^c
1	83.67±1.61 ^d	76.53±1.43 ^d
1.5	93.88±1.44 ^e	85.71±1.98 ^e
LSD	11.7	10.5
P	0.0001	0.0001

^a: Five replicates, 20 larvae each, Larval mortality in control = 0 - 4%

Means followed by different letter(s) are significantly different from each other (p < 0.05) by LSD test

Table 4: Susceptibility levels of 2nd larval instars of Screwworm *Chrysomya albiceps* to Project following continuous exposure for 24 hr by using feeding and Contact bioassay method.

Concentrations (ppm)	Larval mortality ^a (%) Mean±SE	
	Feeding method	Contact method
0.1	10.20±1.29 ^a	13.27±1.68 ^a
0.3	27.55±1.27 ^b	33.67±1.59 ^b
0.5	55.10±1.46 ^c	59.18±1.63 ^c
0.8	82.65±1.71 ^d	75.51±1.91 ^d
1	94.90±1.53 ^e	86.74±1.49 ^e
LSD	6.8	8.8
P	0.0001	0.0001

^a: Five replicates, 20 larvae each Larval mortality in control= 0 - 4%

Means followed by different letter(s) are significantly different from each other (p < 0.05) by LSD test

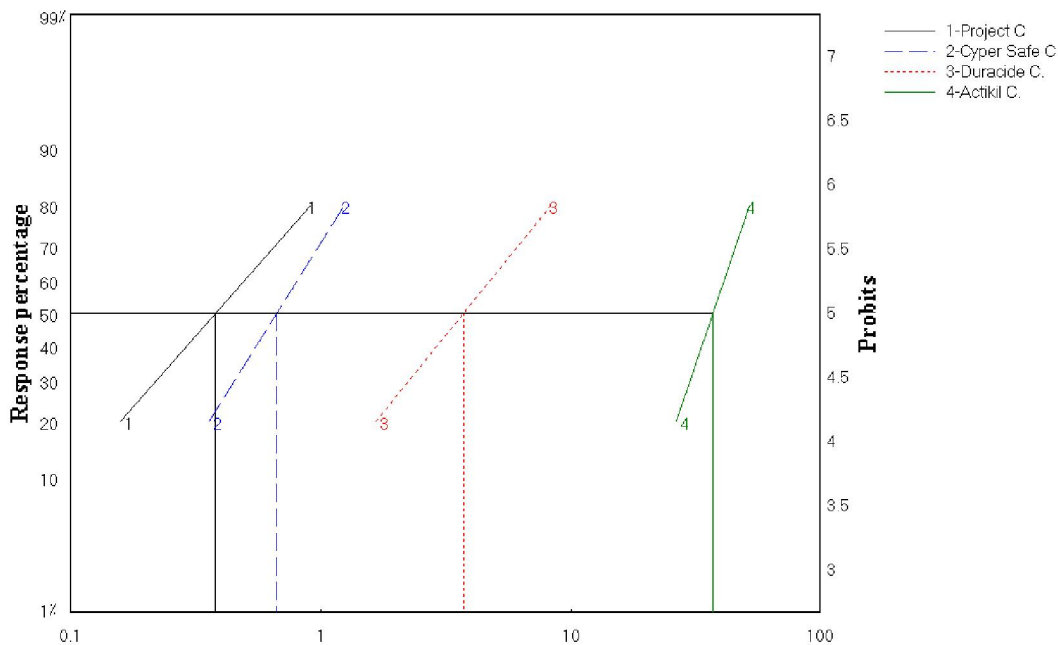


Fig.1: The relation between concentrations of Project, Cyper Safe, Duracide and Actikil and the percentage of larval mortality of Screwworm *Chrysomya albiceps* following continuous exposure for 24hr by using contact bioassay method.

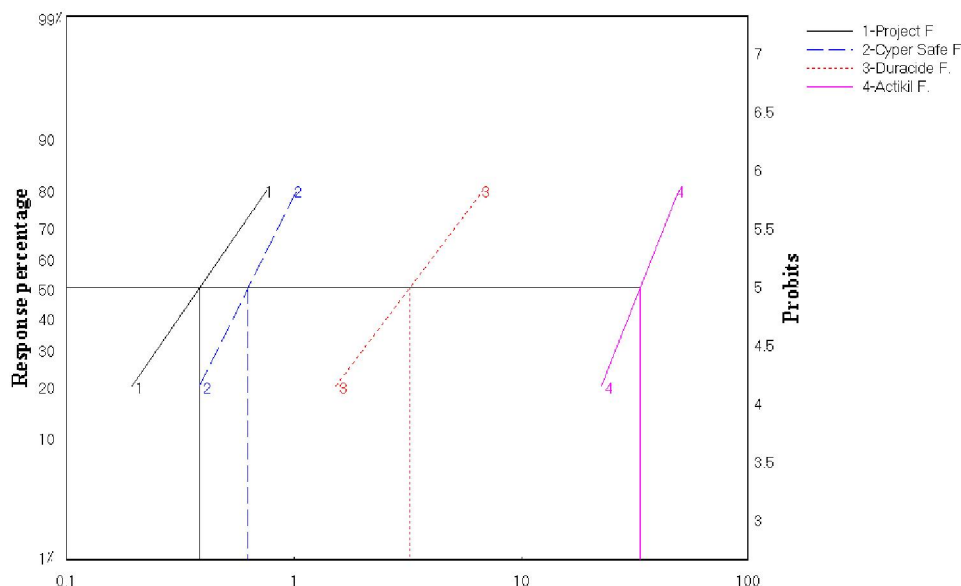


Fig.2: The relation between concentrations of Project, Cyper Safe, Duracide and Actikil and the percentage of larval mortality of Screwworm *Chrysomya albiceps* following continuous exposure for 24hr by using feeding bioassay method.

Table 5: Toxicity of Duracide and Actikil against the 2nd instar of Screwworm *Chrysomya albiceps* by using feeding and Contact bioassay method.

Statical parameters	Tested compounds			
	Duracide		Actikil	
	Feeding method	Contact method	Feeding method	Contact method
LC₅₀(ppm)	3.2526	3.7455	33.6242	37.244
95% (F. L.)	1.95- 4.56	3.28 - 4.22	31.56 - 35.63	35.31 - 39.19
LC₉₀(ppm)	10.2466	12.8519	60.869	62.0146
95% (F. L.)	8.21- 23.05	10.64 - 16.50	55.68 - 68.39	57.29 - 68.69
Slope	2.5717	2.3935	4.9724	5.7876
Calculated (Chi)²	6.68	3.6876	4.5517	0.0406
Tabulated (Chi)²	7.81	7.81	7.81	7.81
Resistanc ratio (R.R)	1.151		1.108	

Table 6: Toxicity of Cyper Safe and Project against the 2nd instar of Screwworm *Chrysomya albiceps* by using feeding and Contact bioassay method.

Statical parameters	Tested compounds			
	Cyper Safe		Project	
	Feeding method	Contact method	Feeding method	Contact method
LC₅₀(ppm)	0.6324	0.6673	0.3878	0.3815
95% (F. L.)	0.58 - 0.68	0.60- 0.73	0.20 - 0.59	0.33 - 0.43
LC₉₀(ppm)	1.3337	1.7247	1.105	1.444
95% (F. L.)	1.19- 1.54	1.47- 2.13	1.01 - 3.77	1.16 - 1.92
Slope	3.9544	3.1078	2.8184	2.2171
Calculated (Chi)²	4.9071	3.1422	17.7464	4.8398
Tabulated (Chi)²	7.81	7.81	7.81	7.81
Resistanc ratio(R.R)	1.055		1.016	

* 5 replicates, 20 Secondinstar larvae each.

Table 6: Comparison between the selected insecticides against the 2nd larval instars of Screwworm *Chrysomya albiceps* by using feeding and Contact bioassay method.

Tested methodes	Line name	LC ₅₀	RR*
Feeding	Project F	0.388	1
	Cyper Safe F	0.632	1.629
	Duracide F.	3.253	8.384
	Actikil F.	33.624	86.66
Contact	Project C	0.382	1
	Cyper Safe C	0.667	1.746
	Duracide C.	3.745	9.804
	Actikil C.	37.244	97.497

* Resistance Ratio (RR)

4. Discussion

In this study, the effectiveness of some insecticides used in the control programs in Jeddah Governorate against the second instar larvae of the screwworm was evaluated using the feeding method and through immersion, and this method was used by many researchers to evaluate the effectiveness of many chemical compounds and plant extracts against different types of insects (Somia et al, 2019 a and b; Abdullah, et al, 2019; Al-Ghamdi et al, 2015; Al-Ghamdi et al, 2014).

The results showed a direct proportion between the tested concentrations and death rates in the treated screwworm fly larvae. This may be due to the ability of high concentrations to bind to the target sites in the insect's body and cause a toxic effect. Perhaps higher concentrations are more tolerant and resistant to the enzyme degrading the active substances that the insect releases to destroy the pesticide and reduced its toxic effect on it as a defense by insects, and these results are consistent with many studies that showed an increase in the mortality rate.

The sensitivity of the screwworm flies differed according to the different compounds tested, where the pyrethroid pesticides were more effective than the phosphorous pesticides. The pesticide was more effective compared to Cybersafe, and the reason for this may be due to the difference in the active substances involved in the composition of these compounds, their ratio and method of effect. It could also be due to the history of their use and exposure to the insect, snails and other insects (Ajayi and Muse 2015 ; Fraternal, et al., 2015; Mahyoub, 2021).

Treatment through the feeding method was more effective than that through immersion. The reason for this may be because the insect's body rubs against the food treated with the pesticide, which facilitates the penetration of the active substances into the wall of the insect's body and causes the toxic effect of contact, in addition to the passage of the active substances into the body through

ingestion during feeding. Many previous studies have shown the effectiveness of the effect of pesticides and plant extracts through feeding better than the immersion method (Jang et al., 2002 and Cavalcanti et al., 2004).

Conclusions and Recommendations

Insect resistance against pesticides extended to all commonly used organophosphorus groups and pyrethroid compounds. In addition, there is evidence of the emergence of resistance, which can be formed against some insect growth regulators IGRs and even against the bacterial pesticide. We recommend the importance of carrying out biological evaluation experiments for the used pesticides. In the control programs to monitor and track the level of sensitivity, tolerance, or resistance of insects to the pesticides used in the control programs periodically and take the right decision towards the continuation of the use of these compounds if they are still effective or to stop their use and search for suitable alternatives to control disease vectors for humans and their domesticated animals.

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