



## Realized Heritability, Cross Resistance and Stability of Sulfoxaflor Resistance in the Cowpea Aphid, *Aphis craccivora* (Koch.)

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### Abstract

*Aphis craccivora* is a serious insect pest. attacks leguminous crops in Egypt. It causes harm through directly sapsucking or through indirectly viral diseases transmission. Aphid control mainly rely on the use of synthetic insecticides. Sulfoxaflor, a fourth-generation neonicotinoid, used to manage sap-sucking pests which had developed resistance to other insecticide. But, risk assessment of sulfoxaflor resistance in the cowpea aphid has not been studied before. So, the leaf-dip bioassay method was used to predict sulfoxaflor resistance, cross resistance and resistance stability in *A. craccivora*. Sulfoxaflor resistant strain of the cowpea aphid was obtained by selecting the field strain for 17 generations. The sulfoxaflor-selected strain (SFX- SEL strain) showed a 125.39-fold. Realized heritability ( $h^2$ ) of resistance was calculated to be 0.19. According to predicted rates of sulfoxaflor resistance indicated that, if  $h^2 = 0.19$  with is fifty percent at each generation, then a tenfold increase in  $LC_{50}$  would be anticipated in 12.29 generations. The obtained results showed increased levels of cross-resistance to flupyradifurone (18.16-fold), and pymetrozine (14.19-fold). Oppositely, the R-strain did not show cross-resistance to pirimicarb (3.64-fold), carbosulfan (3.5-fold), malathion (3.41-fold), dinotefuran (3.39-fold), -cyhalothrin (1.22-fold), and fipronil (1.12-fold), respectively. Fortunately, resistance to sulfoxaflor was reversed around control strain throughout 20 generations without exposure to any insecticide. Our findings revealed the cowpea aphid's ability to evolve sulfoxaflor resistance under continual selection pressure. The retreat of sulfoxaflor makes *A. craccivora* compatible with techniques for managing resistance such as non-cross-resistant insecticides rotation.

**Keywords:** *Aphis craccivora*, resistance, cross resistance, revers, sulfoxaflor

### Introduction

In Egypt, leguminous crops are important nutrient crops. These crops infested by insect pests in both field and stores. In field, the cowpea aphid, *A. craccivora* infests seriously these crops (El-Ghareeb *et al.*, 2002). Globally, *A. craccivora* regarded as a major crop pest infesting approximately 50 crops from 19 botanical families causing significant yield losses (Hulle *et al.*, 2020; Radha, 2013). The pest affects their hosts, especially in the early growing season, either directly by sucking sap or indirectly through viral transmission such as (FBNYV) faba necrotic yellow virus and (BLRV) been leaf roll virus (Blackman and Eastop, 2006; Laamari *et al.*, 2008). The aphids infest many parts of the hosts such as leaves, pods, and other aerial tissues. So, chemical control is required to limit aphid injury. Up to the 1990s, conventional insecticides were mainly used for aphid control. These insecticides have been widely used, which has led to a rise in resistance.

Neonicotinoid pesticides were introduced as a result, and they quickly rose to the top of the list for controlling aphids. (Foster *et al.*, 2003). As a result, numerous aphid species have been found to be resistant to neonicotinoids. (Bass *et al.*, 2015). Furthermore, the lack of specificity and adverse effects on beneficial insects led to neonicotinoids restriction in the European Union (Siviter and Muth, 2020). In order to effectively manage *A. craccivora*, it is necessary to introduce pesticides with novel modes of action and safe environmental profiles.

Sulfoxaflor is a fourth-generation neonicotinoid insecticide belongs to the sulfoximine class. It was introduced to combat a broad range of sap-sucking insect pests (Babcock *et al.*, 2011). Compared with other nAChR-acting insecticides, sulfoxaflor interactions with nicotinic acetylcholine receptors (nAChRs) differently (Wang *et al.*, 2016; Watson *et al.*, 2017). This interprets the non-cross resistance between sulfoxaflor and these

insecticides. As consequently, sulfoxaflor may be a valuable tool for resistant management of sap-sucking insects (Longhurst *et al.*, 2013; W. Wang *et al.*, 2017).

Several reports proved the potential of *A. craccivora* to develop resistance to various insecticides. So, resistance risk assessment a certain insecticide prior to resistance resurgence in field is crucial to late resistance or avoiding (Keiding, 1986). To predict resistance to certain insecticide, data from selection experiments can be applied to quantitative analysis genetic to estimate resistance heritability (Jutsum *et al.*, 1998). Realized heritability ( $h^2$ ) is a measure that quantifies the degree to which a specific characteristic is pushed across a population.  $h^2$  is the genetic variation to overall phenotypic variation ratio. It offers a useful tool for forecasting future evolution of resistance in responses to selection. (Tabashnik, 1992). As a result, assessing resistance risk before resistance emerges can provide valuable information to maintain susceptibility and sustain the efficacy of an insecticide.

Cross-resistance defined as resistance to an insecticide induces the emergence of resistance to another insecticide which was not used previously against the pest (Yorulmaz *et al.*, 2015). Insecticides with different modes of action, cross-resistance may be as a result of a shared mechanism or in connection with related independent genetic components (Afzal *et al.*, 2015). Therefore, it may be due to the presence of iso-enzyme. It influences various types of insecticide (Ahmad *et al.*, 2007). Studies on cross-resistance is crucial for effective control and contribute in a better rotation of insecticides for pest control (Stumpf and Nauen, 2001).

Investigating resistance stability may help to manage insecticide resistance and preserve insecticides efficacy (Shah *et al.*, 2015). Resistance reversion can be attributed to fitness costs of resistance such as negative impacts on life table parameters, fecundity, reproduction, and several biotic variables (Ninsin and Tanaka, 2005). So, resistance stability may be a prerequisite for resistance management programs (Tabashnik, 1990). Reverse of resistance occurs rapidly in cases of high fitness cost and incomplete resistance (Basit *et al.*, 2011; Carrière and Tabashnik, 2001).

## Material and Methods

### 2.1. Insect

The cow aphid, (*A. craccivora*) have been obtained from fields of faba bean in Egypt, Sharqia Governorate, in October 2021. Two aphid strains (S-strain and R-strain) were separated and reared on faba bean seedlings (*Vicia fabae*) with consistent lab conditions [ $22 \pm 2^\circ\text{C}$ ,  $70 \pm 5\%$  relative humidity and 12:12 dark-light- photoperiods]. Faba bean seedlings

were set up into plastic pots with diameter (15cm). Following the germination of faba bean seeds, they were continuously supplied until the necessary aphids were obtained. Aphids were raised in chambers atop metallic stands, and the insects were fed faba bean seedlings developed in pots made of plastic. The pots containing faba bean seedlings had been kept in another location with no insecticides till they were needed. To get the (SFX-SEL strain), the strain was continuously selected with sulfoxaflor for 17 generations.

### 2.2. Insecticide

The commercial formulation of the following insecticides was used for bioassays. The tested insecticides were: sulfoxaflor (Transform, 50% WG, Corteva Agriscience company, the country), flupyradifurone (Sevanto 20% SL, Bayer company, the country), dinotefuran (Oshin 20% SG, Shuora company, the country), fipronil (Tepiki, 50% WG, Starchem company, the country), pirimicarb (Aphox 50% DG, Syngenta company, the country), carbosulfan (Marshal 20% EC, Delta chemicals company, the country), malathion (Malathion 57% EC, Kafr elzayat Pesticides company, the country), lambda-cyhalothrin (Icon 2.5% EC, CAM company, the country), pymetrozine (Chess 50% WG, Syngenta company, the country).

### 2.3. Bioassays

Bioassays of insecticides were conducted by leaf-dipping bioassay technique by Moores *et al.* (1996). Nine insecticide concentrations were prepared with tap water as serial concentrations. Each concentration was replicated three times for each bioassay. Broad bean leaves were submerged in the required pesticide solution about 20 s, after drying on paper towel, Petri dish (60 mm diameter) were set lying down over an agar layer. For all replicate, ten adults had been transferred onto a treated leaf. The control had been submerged leaves in water. All bioassay experiments developed in laboratory condition and mortality was recorded 24 h after exposure. Aphid failed to exhibit ordinary forward movement when touched with a soft camel hair were considered dead.

### 2.4. Selection of resistance strain

*A. craccivora* originated from Sharkia governorate and reared on faba bean seedlings. The selection treatment depended on sulfoxaflor doses that resulted in 25-40% mortality. The strain was continuously selected with sulfoxaflor for 16 generations. The dipping method was used to select in accordance with Guo *et al.*, (1996). Faba bean seedlings were immersed in the required concentration for 20 s after being infested with adults of aphids about 24 hours prior to treatment. After that, they left to dry and then placed in the raising room. The live aphids have been transferred to fresh plants and preserved till adults of the subsequent generation were utilized in the bioassay.

## 2.5. Realized heritability ( $h^2$ ) estimation

The realized heritability ( $h^2$ ) value of sulfoxaflor resistance was analyzed according to (Tabashnik, 1992) by the following equation.

$$h^2 = R/S$$

Where R represents response to selection (R) and calculated as.

$$R = (\log(\text{final LC}_{50}) - \log(\text{initial LC}_{50})) / n$$

Where, the final  $\text{LC}_{50}$  represents the  $\text{LC}_{50}$  of selected strain after number of generations, while the initial  $\text{LC}_{50}$  represents the  $\text{LC}_{50}$  of the parental generation before to selection.

S refers to selection differential (S) and calculated as:

$$S = i \sigma p$$

Where i is the selection intensity and estimated with the formula.

$$i = 1.583 - 0.0193336p + 0.0000428p^2 + 3.65194/p$$

P is the average percent survivals of the selected strain

$\sigma p$  is the phenotypic standard deviation and calculated as:

$$\sigma p = \frac{1}{\text{Mean Slope}}$$

Mean slope represents the average of the slopes of respective generations.

According to  $\text{LC}_{50}$  values, the total number of generations necessary to exceed the ten-fold resistance was estimated as:

$$G = R^{-1} = (h^2 S)^{-1}$$

## 2.6. Cross-resistance

Cross-resistance among sulfoxaflor and other eight insecticides was assessed on Sul-UNSEL and Sul-SEL strains of *A. craccivora*. The insecticides used were assessed on the adults using leaf dip bioassay method as described previously. The tested insecticides belong to different classes including neonicotinoid, organophosphorus, carbamate, Pyrethroids, Pyridine azomethine derivatives and Phenylpyrazol insecticides.

## 2.7. Sulfoxaflor resistance stability

Sul-SEL strain was reared without exposure to sulfoxaflor for twenty generations (G17-G37) in a laboratory to investigate the stability of resistance to sulfoxaflor. The bioassay was then performed every 4 generations starting with G21. The resistance ratio (RR) of Sul-SEL was obtained by comparing  $\text{LC}_{50}$  of dimethoate in Sul-SEL with  $\text{LC}_{50}$  of sulfoxaflor in Lab Population, Unsel Pop. Sulfoxaflor's rate of decrease (DR) in  $\text{LC}_{50}$  value was determined as (Tabashnik *et al.*, 1994):

$$DR = \frac{(\log \text{ final LC}_{50} - \log \text{ initial LC}_{50})}{n}$$

Where 'n' is the number of generations reared without sulfoxaflor selections.

## 2.8. Data analysis

Mortality data were corrected in relation to control mortality by Abbott's formula (Abbott, 1925). Then the data were analyzed by probit analysis (Finney 1971) throughout Probit-MS Chart program (Chi, 2020). Resistance ratios (RR) have been calculated by divided on the  $\text{LC}_{50}$  value of Sul-SEL strain by those of the Unsel-strain susceptible. The cross-resistance ratios (CR) have been calculated by divided on the  $\text{LC}_{50}$  value of every insecticide for Sul-SEL strain by the same insecticide for Unsel-strain. Insecticide resistance levels were classified using the following criteria: susceptibility (resistance ratio (RR) < 5), resistance moderate (RR is 5:10), and high resistance (RR > 10) (Mazzarri and Georgiou, 1995).

## Result

### Selection for sulfoxaflor resistance dividing

Before selecting sulfoxaflor resistance selection,  $\text{LC}_{50}$  value of the parents was 2.42 (2.00 – 2.91)  $\text{mg L}^{-1}$ . Selection process was achieved every generation to produce Mortality ranged from 25-40%. Data in Table 1 revealed that, after selection for 17 generations, the final  $\text{LC}_{50}$  recorded 57.68 (37.95 - 87.55). So, the resistance ratio of sulfoxaflor was increased gradually from 5.26-fold to parent strain to 125.39-fold after selection for 17 generations.

**Table 1.** Development of resistance in *A. Craccivora* exposed to laboratory selection pressure with sulfoxaflor.

Generations	$\text{LC}_{50}$ $\text{mg. L}^{-1}$ (95% FL)	Fit of probit line			RR*	RR**
		Slope $\pm$ S.E.	$\chi^2$	df		
SS-Strain	0.46(0.41 - 0.53)	0.95 $\pm$ 0.12	0.48	6	0.998	-
G <sub>0</sub> (Parent)	2.42(2.00 – 2.91)	1.12 $\pm$ 0.14	0.86	5	0.973	5.26
G <sub>1</sub>	3.88(2.96 – 5.03)	1.21 $\pm$ 0.18	1.22	4	0.874	8.43
G <sub>2</sub>	4.18(3.58 – 4.87)	1.04 $\pm$ 0.12	0.80	6	0.992	9.08
G <sub>3</sub>	7.35(5.51 - 9.78)	1.06 $\pm$ 0.14	2.01	5	0.847	9.45
G <sub>4</sub>	7.41(6.39 - 8.57)	1.37 $\pm$ 0.15	0.81	5	0.976	16.10
G <sub>5</sub>	10.06(7.86 - 12.85)	1.01 $\pm$ 0.12	2.02	6	0.917	21.86
G <sub>6</sub>	11.95(10.99 - 13.01)	1.15 $\pm$ 0.14	0.19	5	0.999	25.97

<b>G7</b>	12.01(9.37 - 15.36)	1.20±0.15	1.83	5	0.872	26.10	4.96
<b>G8</b>	12.84(10.28 - 16.06)	1.08±0.12	1.83	6	0.934	27.91	5.30
<b>G9</b>	12.95(11.48 - 14.60)	1.04±0.12	0.50	6	0.997	28.15	5.35
<b>G10</b>	16.00(15.11-16.93)	0.90±0.13	0.06	5	0.999	34.78	6.61
<b>G11</b>	18.99(15.34 - 23.52)	1.35±0.15	1.68	5	0.891	41.28	7.84
<b>G12</b>	22.86(21.13 - 24.72)	1.78±0.25	0.10	3	0.991	49.69	9.44
<b>G13</b>	27.95(24.39- 32.03)	1.09±0.14	0.47	5	0.993	60.76	11.54
<b>G14</b>	29.55(22.52 -38.66)	1.43±0.18	1.70	4	0.790	64.23	12.21
<b>G15</b>	34.05(29.89-38.77)	1.54±0.19	0.47	4	0.976	74.02	14.07
<b>G16</b>	50.03(44.20 -56.63)	1.09±0.17	0.24	4	0.993	108.76	20.67
<b>G17</b>	57.68(37.95 -87.55)	1.36±0.15	6.60	5	0.252	125.39	23.83

RR\* Resistance ratio = LC<sub>50</sub> of tested generation / LC<sub>50</sub> of susceptible strain

RR\*\* Resistance ratio = LC<sub>50</sub> of tested generation / LC<sub>50</sub> of parent generation (F0)

### Realized heritability ( $h^2$ ) estimation

Estimated  $h^2$  of sulfoxaflor resistance in *A. craccivora* (G<sub>0</sub>-G<sub>17</sub>) was 0.19. Response to selection

was 0.08 and selection differential was 0.428 (Table 2). Higher  $h^2$  (0.19) suggested that more genetic variation.

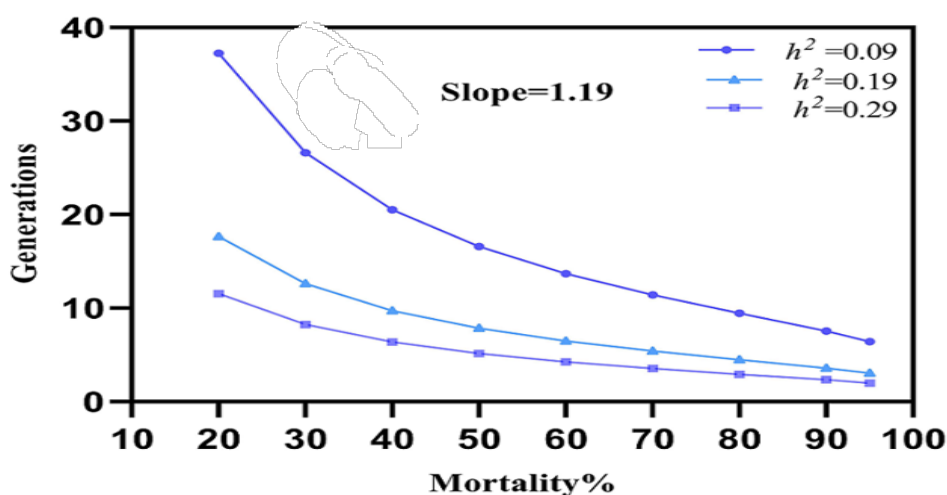
**Table 2.** Estimation of realized heritability ( $h^2$ ) of sulfoxaflor resistance in *Aphis craccivora*

generations	N	Estimate of mean response per generation				Estimate of mean selection differential per generation				
		Log of initial LC <sub>50</sub>	Log of final LC <sub>50</sub>	R <sup>a</sup>	p <sup>a</sup>	i <sup>b</sup>	Mean slope	σ <sup>c</sup>	S <sup>d</sup>	$h^2$
(G <sub>0</sub> -G <sub>17</sub> )	17	0.38	1.76	0.08	68.66	0.51	1.19	0.84	0.428	0.19

### Projected rate of resistance to sulfoxaflor

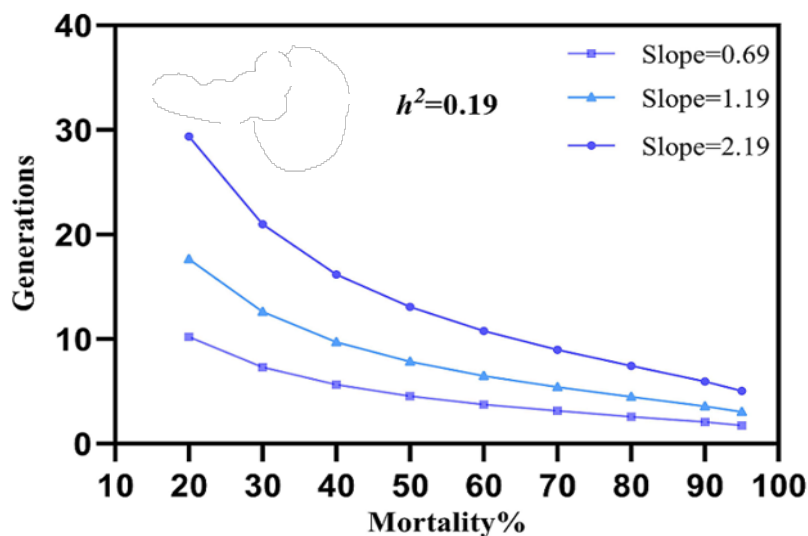
Projected rate of sulfoxaflor resistance in *A. craccivora* has been investigated. Number of generations is directly proportional to  $h^2$  value. In case of varying  $h^2$  values and slope instant, while  $h^2 = 0.19$  and slope = 1.19 (mean slope to Sul-SEL generations), 17.64, 7.84, and 3.56 generations are needed for a 10-fold increase in the LC<sub>20</sub>, LC<sub>50</sub>, and

LC<sub>90</sub>, respectively. On the other hand, if  $h^2$  was 0.29, the same event would happen in 11.55, 5.14, and 2.33 generations, respectively, at the same value slope and selection intensity. A tenfold increase in resistance was needed after 37.24, 16.56, and 7.53 generations when  $h^2$  was set to 0.09, respectively. (Fig.1).



The expected resistance development rate is inversely proportional with the slope. Assume, for example, that  $h^2 = 0.19$  (heritability of sulfoxaflor resistance evaluated in this study) then slope increased to 2.19, then a tenfold increase in the LC<sub>50</sub> 29.37, 13.06, and 5.94 generations are need for a 10-

fold increase in the LC<sub>20</sub>, LC<sub>50</sub> LC<sub>90</sub>, respectively. While,  $h^2$  with the same value, if the slope = 0.69, then it 10.22, 4.55, and 2.06 generations required for a tenfold increase in the LC<sub>20</sub>, LC<sub>50</sub> LC<sub>90</sub>, respectively (Fig.2).



### Cross-resistance study

Results illustrated in Table 3 showed obvious cross-resistance to flupyradifurone and pymetrozine with resistance ratio of 18.168 and 14.19-fold, respectively. While the other tested insecticides

showed low resistance and exhibited resistance ratio values of (1.12-fold) to fipronil, (1.22-fold) to cyhalothrin, (3.39-fold) to dinotefuran, (3.41) to malathion, (3.5-fold) to carbosulfan and (3.64-fold) to Pirimicarb.

**Table 3.** Cross-resistance evaluation to certain insecticides with sulfoxaflor resistance of *Aphis craccivora*

Strains	Insecticides	LC <sub>50</sub> (mgL <sup>-1</sup> ) 95%CI	Fit of probit line				RR
			Slope ± S. E	χ <sup>2</sup>	df	P	
Unsel-Pop	Flupyradifurone	0.21(0.10 - 0.45)	1.22±0.18	4.3324	3	0.227	1.00
	Fipronil	27.88(21.85 - 35.74)	1.01± 0.12	0.6838	4	0.953	1.00
	Pymetrozine	0.86(0.66 - 1.10)	1.07 ±0.14	1.5482	5	0.907	1.00
	Dinotefuran	3.99(3.16 - 5.06)	1.23±0.15	0.9924	4	0.910	1.00
	Malathion	1.88(0.64-4.80)	0.94±0.17	8.0052	4	0.091	1.00
	Pirimicarb	0.15(0.15-0.16)	1.27±0.18	6.778	4	0.148	1.00
	λ -cyhalothrin	0.65(0.55-0.79)	0.95±0,14	0.6880	5	0.983	1.00
	Carbosulfan	0.009(0.0007-0.001)	1.31±0.18	0.3719	4	0.984	1.00
Sul- Pop	Flupyradifurone	3.89(3.45-4.37)	0.94±0.11	0.3997	6	0.998	18.17
	Fipronil	31.31(24.75-39.62)	0.98±0.14	1.1994	5	0.944	1.12
	Pymetrozine	12.21(7.08 -20.82)	1.18±0.15	8.228	5	0.144	14.19
	Dinotefuran	13.54(10.01 - 18.27)	1.02 ± 0.14	2.1011	5	0.834	3.39
	Malathion	6.43(5.36 - 7.70)	1.49±0.17	1.261	5	0.938	3.41
	Pirimicarb	0.56(0.42 -0.74)	1.25 ± 0.18	1.537	4	0.820	3.64
	λ -cyhalothrin	0.80(0.69 -0.92)	1.28 ± 0.18	0.4035	4	0.982	1.22
	Carbosulfan	0.028(0.025-0.031)	1.37 ± 0.18	0.2058	4	0.995	3.5

Resistance Ratio = LC<sub>50</sub> of resistance strain /LC<sub>50</sub> of susceptible strain

### Stability of resistance to sulfoxaflor

To investigated stability of sulfoxaflor resistance in *A. craccivora*, further rearing of Sul-SEL strain without exposure to the tested insecticide. A significant decrease in sulfoxaflor LC<sub>50</sub> was noticed when the selection pressure removed for 20 generations (G17-G37). Decline resistance (DR) rate of sulfoxaflor resistance was -0.064. Over 20

generations of rearing the Sul-SEL strain without exposure to any insecticide, resistance ratio for sulfoxaflor significantly declined from 125.39-fold (G17) till 62.67-fold (G21). Continuous rearing without exposure to insecticides led to further decrease in resistance ratio to reach to susceptible strain level in (G35). This implies that resistance to sulfoxaflor remained unstable in *A. craccivora*. (Table 4).

**Table 4.** Sulfoxaflor resistance reversion in resistance strain of *Aphis craccivora*

Generations	LC <sub>50</sub> mg. L <sup>-1</sup> (95% FL)	Fit of probit line				RR*	RR**	DR
		Slope ± S.E.	χ <sup>2</sup>	df	P			
SS-Strain	0.46(0.41 - 0.53)	0.95±0.12	0.48	6	0.998	-	-	-
G <sub>0</sub> (Parent)	2.42(2.00 - 2.91)	1.12±0.14	0.86	5	0.973	5.26	-	-
G17	57.68(37.95 -87.55)	1.36±0.15	6.60	5	0.252	125.39	23.83	-
G21	28.83(21.25 - 39.39)	1.14±0.15	2.368	5	0.796	62.67	11.91	-
G25	24.74(22.54- 27.16)	1.23±0.15	0.2728	5	0.998	53.78	10.22	-
G29	8.88(4.51 - 16.83)	1.10±0.14	9.6534	5	0.085	19.30	3.66	-
G33	4.26(3.42 - 5.29)	1.69 ±0.20	1.5140	4	0.824	9.26	1.76	-
G37	2.94(1.95- 4.37)	1.37±0.16	5.731	5	0.333	6.39	1.21	-
								0.064

RR\* Resistance Ratio = LC<sub>50</sub> of tested generation/LC<sub>50</sub> of susceptible strain

RR\*\* Resistance Ratio = LC<sub>50</sub> of tested generation/LC<sub>50</sub> of parent generation (F<sub>0</sub>)

DR = Log final LC<sub>50</sub> - Log initial LC<sub>50</sub> / n

## Discussion

Sulfoxaflor is a fourth-generation neonicotinoid insecticide used to control many sucking insects that have developed resistance to other insecticide groups (Wang et al., 2018). High efficacy made it preferred alternative for controlling of *A. gossypii* (Babcock et al., 2011; Zhu et al., 2011). In this current study, the possibility of *A. craccivora* to develop resistance among sulfoxaflor was investigated through laboratory selection experiment. The selected strain showed 125.39-fold of resistance after continuous selection for 17 generations. Aphid potential to develop resistance to sulfoxaflor has been proved in several aphid species either in laboratory or in the field. In laboratory, obvious levels of resistance to sulfoxaflor was obtained as a result to continuous selection in the green peach aphid, *Myzus persicae* (Pym et al., 2022; Wang et al., 2018). Similarly, *A. gossypii* resistance to sulfoxaflor was obtained via gradual selection with increased concentrations (Wang et al., 2021). In field, moderately to highly resistant to sulfoxaflor was detected in several populations of *S. miscanthi*, *R. padi*, and *M. dirhodum* (Li et al., 2021). In addition, (Wang et al., 2021) reported that, six *Aphis gossypii* field populations showed resistance values ranged from 24.81-75.89-fold. In the present work, sulfoxaflor resistance development was relatively slow compared with other species and pesticides (Ejaz et al., 2017). This phenomenon was noticed previously with imidacloprid in *Bemisia tabacci* (Prabhaker et al., 1997), and in *Nilaparvata lugens* (Zewen et al., 2003). It can interpret due to the parthenogenesis nature of *A. craccivora* which provided limited genetic variation (Hartley et al., 2006). So, rather than recombination between alleles articulation genetic variation, mutations and posttranscriptional modifications would provide the genetic variations (Bass et al., 2014).

Cross-resistance defined as resistance to another compound because the presence of a shared resistance mechanism (i.e., a specific target site

resistance or metabolic mechanism) (Metcalfe, 1989). Exploring cross-resistance patterns of new insecticides can provide crucial information for insecticide application and resistance management. Our results indicate that Sul-SEL strain of *A. craccivora* showed significant cross-resistance to flupyradifurone, and pymetrozine. While minor cross-resistance to dinotefuran, malathion, pirimicarb, carbosulfan, fipronil and λ-cyhalothrin were observed. Other reports investigated the correlation between sulfoxaflor resistance and cross-resistance to various insecticides. In *Aphis gossypii*, sulfoxaflor resistant strain induced cross-resistance to certain neonicotinoids including flupyradifurone (107.5-fold) imidacloprid (80.8-fold), acetamiprid (19.3-fold), and thiamethoxam (10.0-fold) (Ma et al., 2019). Likewise, a sulfoxaflor-resistant strain of *A. gossypii*, showed cross-resistance ranged from 5.62-fold to 35.90-fold to neonicotinoid, pyrethroid and carbamate insecticides (Wang et al., 2021). Oppositely, little cross-resistance to organophosphates, carbamates, or pyrethroids (Ma et al., 2019). Concerning pymetrozine, although pymetrozine belong to different class, Sul-SEL strain showed obvious cross-resistance. Thus, cross-resistance between various insecticide classes, such as IMI and pymetrozine, can result from metabolic-based resistant throughout a specific P450 (Gorman et al., 2010).

Realized heritability ( $h^2$ ) is a practical tool represents the ratio of genetic to phenotypic variance in a certain in a characteristic. Based on a single genetic variant, exact predictions can be made for the tested characteristics (Brookfield, 2012). So, we can forecast changes in a particular characteristic, like pesticide resistance, by estimating realized heritability through quantitative genetic models. Regarding the development of resistance to a particular insecticide, heredity value directly correlates with resistance rate. (Tabashnik and Mcgaughey, 1994). So, a higher risk of resistance was interpreted into a high achieved heritability

value. A higher  $h^2$  score denotes that characters are more likely to be passed down to future generations (Ijaz and Ali, 2022). In the current work, estimated realized heritability ( $h^2$ ) of sulfoxaflor resistance in *A. craccivora* was 0.19, which suggests a considerable genetic variation in the tested population. Similarly, in the cotton aphid, *Aphis gossypii* Glover, heritability of resistance against acetamiprid was (0.17), Fluxametamide in diamondback moth, *Plutella xylostella* (L.) (0.18), alpha-Cypermethrin in *Musca domestica* were (0.17 and 0.18) for both males and females (Abbas and Hafez, 2023; Mokbel, 2018; Roy et al., 2023). On contrast, realized heritability value in *A. craccivora* to chlorpyrifos-methyl was 0.35 (Mokbel, 2015). The obtained results implied that *A. craccivora* populations has the potential to develop resistance to sulfoxaflor in considerable manner. To visualize the rate of sulfoxaflor resistance development,  $h^2$  and slope values either obtained in the current study or theoretically proposed were used (Figs. 1 and 2). The slope value of the probit line has an inverse relationship with the expected rate of resistance evolution. So, Fig 1. clarified that with slope value increasing heritability will decrease and generation numbers required to exceed 10 -fold generation will increase. Oppositely, the projected rate of resistance evolution is directly proportional to  $h^2$  value. As a result,  $h^2$  value increasing will decrease and generation numbers required to exceed 10 -fold generation. Unlike the laboratory conditions, resistance affected by several factors including insect migration, weather, and application factors practices like pesticide rotation and replacement are crucial in the open-field situation (Ismail et al., 2017; Lai and Su, 2011). Therefore, the actual risk of resistance development in the field could be lower than that occurs in the laboratory (Ijaz and Ali, 2022). The early prediction of sulfoxaflor resistance risk assessment might help to avoid the resurgence of resistance in the field. Practically, our results confirmed that, sulfoxaflor should be used in rotation to manage sulfoxaflor resistance in the cow pea aphid.

Resistance stability in the absence of pressure selection is essential in resistance management. In the present study, the decline in sulfoxaflor resistance from 125.39-fold to 6.39-fold after (n) of non-selected generations. The obtained data suggested that sulfoxaflor resistance in *A. craccivora* is unstable. Resistance instability was previously reported in *A. craccivora* with chlorpyrifos-methyl after ten unexposed generations against (Mokbel, 2015). In the peach-potato aphid, *Myzus persicae* (Sulzer), complete loss of resistance to carbamate and organophosphorus insecticides can occur either in a single step or over several generations. also, *Myzus persicae* clone showed instability of sulfoxaflor resistance over time (Pym et al., 2022; Sawicki et al., 1980). Likely, in the

cotton aphid, acetamiprid resistance reversion takes five generations to approach the laboratory strain level (Mokbel, 2018). High fitness costs associated with insecticide resistance represents the main factor leads to recovery of susceptibility (Gassmann et al., 2009). Furthermore, we demonstrate that sulfoxaflor resistance can persist several generations in the laboratory without sulfoxaflor exposure. But in one instance, the Munglinup209 clone shown a marked decline in sulfoxaflor resistance over time (Pym et al., 2022). We show that sulfoxaflor resistance may persist even after months during laboratory cultivation without insecticide exposure (Pym et al., 2022).

In conclusion, the present investigation demonstrated the possibility of sulfoxaflor resistance in *A. craccivora*. However, the sulfoxaflor resistance instability offers the chance to regain *A. craccivora* susceptibility by temporarily ceasing exposure to the insecticide. Fipronil and -cyhalothrin have no cross-resistance with sulfoxaflor, according to cross-resistance research. The amount of cross-resistance was also very low for dinotefuran, malathion, pirimicarb, and carbosulfan. Thus, it can be incorporated into sulfoxaflor resistance management programs either as a replacement in the event of a resistance crisis or through rotation to lessen the likelihood of resistance development.

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## دراسة مخاطر المقاومة، المقاومة المشتركة وثبات المقاومة لمركب السلفوكسافلور لحشرة من البقوليات

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تم عمل ضغط انتخابي للسلالة الحقلية لمن البقوليات بمركب السلفوكسافلور لمدة 17 جيل علي التوالي وقد اكتسبت السلالة مقاومة للمبيد تدريجيا حيث أظهرت النتائج باستخدام المبيد محل الدراسة ما يلي قيمة التركيز النصف المميت  $LC_{50}$  57.68 جزء في المليون حيث ارتفعت نسبة المقاومة من 5.26 ضعف لجيل الإباء الي 21.86 ضعف في الجيل الخامس ، ثم 34.78 ضعف في الجيل العاشر ، ثم 125.39 ضعف في الجيل 17 من الضغط الانتخابي .كما تم دراسة المقاومة المشتركة للسلالة المنتخبة حيث تم قياس استجابة السلالة المنتخبة (المقاومة) بالسلالة المعملية وذلك لعدد 8 مبيدات لمجاميع مختلفة .حيث أوضحت النتائج ان السلالة المنتخبة بالمبيد محل الدراسة أظهرت مقاومة مشتركة لكل من مبيد لفلوبيراديفون و لبيمتروزين بقيمة (RR) 18.168 و 14.19 ضعف علي التوالي .في حين أظهرت السلالة نسبة تحمل بسيط لكل من برميكرب ، الكربوسلفان ، ملاثيون ، داينيتروفيران بقيمة (RR) 3.64، 3.5، 3.41، 3.39 علي التوالي في حين لم يظهر كل من مبيد لمبادا ولفبرونيل أي مقاومة مع المبيد بتسجيل قيم (RR) 1.22، 1.12 علي التوالي .كما تم دراسة ثبات المقاومة بعزل السلالة محل الدراسة (المقاومة) عن التعرض للمبيدات من بعد الجيل 17 الي الجيل 35 دون تعرض لأي أنواع المبيدات و تم قياس قيمة (RR) حيث سجلت الاتي : الجيل 17 (125.39) ضعف في حين الجيل 19 وصلت الي (62.67) ضعف كما سجلت في الجيل 23 (53.78) ضعف. الجيل 27 (19.30) ضعف بينما كانت في الجيل 31 (9.26) ضعف الجيل 35 (6.39) ضعف.