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RESISTANCE AGAINST SPINOSAD IN A LAB-REARING *Plutella xylostella* POPULATION AND ITS IMPACT ON FITNESS COST

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ABSTRACT

Plutella xylostella commonly known as diamondback moth (DBM), is a polyphagus pest and has the ability to confer resistance and cross resistance to a number of insecticides. This experiment investigated the resistance development in DBM against spinosad and its implication on the fitness cost of the DBM population. A field strain of diamondback moth was collected from an organic farm in Semenyih, Selangor, Malaysia. Selection of DBM with spinosad, a spinosyn class of insecticide within 15 generations resulted in 42.81-fold resistance ratio (490.63 ppm at 15th generation (G15) compared to 11.5 ppm of parent (P) generation). Survival rate of 1st and 2nd instars was significantly higher in susceptible (SS) strain than in spinosad –selected (Spi-Sel) strain. Immatures of Spi-Sel strain took significantly shorter time to develop. Spi-Sel strain had high fitness cost i.e., low fitness (relative fitness = 0.25) in the absence of spinosad. In the absence of insecticide exposure, spinosad resistance accompanied by high fitness cost had adverse effect on the development and biological performance of the resistant strain. The results obtained provide a current understanding in resistance of spinosad after approximately 20 years of its introduction in the agriculture market and therefore, provides a new insight in resistance study of the insecticides with novel mode of action.

Keywords Diamondback moth, fitness cost, resistance, selection, Spinosad

INTRODUCTION

Diamondback moth (DBM); *Plutella xylostella* (Lepidoptera: Plutellidae) is one of the most damaging pests of crucifers. Due to its tolerance to a wide ecological condition, it is distributed all over the world from North America, Europe and Southeast Asia to New Zealand (Knodel and Ganehiarachchi, 2008). Its biology and availability of host crops throughout year have known to be an advantage in accelerating its resistance development to various groups of insecticides (Shakeel *et al*., 2017). Its short life cycle and rapid generation turnover contributed to high selection pressure in their populations, therefore spurring the process of resistance development (Cheema *et al*., 2011).

Due to its outstanding pest status in crucifer crops, an increasing cost was observed in managing DBM in the agriculture field. Previously, cost in managing DBM on a worldwide basis was estimated to be US$ 1 billion dollar per annum (Talekar and Shelton, 1993), whereas Zalucki *et al*. (2012) estimated the cost increased to US$ 4 - 5 billion annually.

Spinosad is a natural, broad-spectrum insecticide derived from *Saccharopolyspora spinosa* (Actinomycetales: Pseudonocardiaceae) (a soil actinomycete) which was firstly introduced in Japan in 1997. Its active ingredients comprised of two macrocyclic lactones (spinosyn A and D). It has a unique mode of action which primarily acts on nicotinic acetylcholine receptor, and has secondary target; the gamma aminobutyric acid (GABA) receptor) (Scott, 2008). Interaction with spinosad causes neuromuscular fatigue, leading to tremors, paralysis and ultimately death.

It has been a very promising chemistry back in the earlier times of introduction since it has strong insecticidal activity against many pest species including DBM (*Plutella xylostella*) (Zhao *et al*., 2006), fruit fly (*Bactrocera oleae*) (Kakani *et al*., 2010), common cutworm (*Spodoptera litura*) (Wang *et al*., 2013), beet armyworm (*Spodoptera exigua*) (Ishtiaq and Saleem, 2011) and cotton bollworm (*Helicoverpa armigera)* (Gill and Dhawan, 2006). However, very shortly after its discovery, a high level of its resistance was detected in several DBM populations at California (Zhao *et al*., 2002), Malaysia (Sayyed *et al*., 2004) and Georgia (Zhao *et al*., 2006).

Development of resistance in insects is usually accompanied by fitness cost (Gao *et al*., 2014). During insecticide application, resistant insects are more fit than susceptible ones. However, this advantage will be diminished in the absence of insecticide application. In such condition, the resistant population with high fitness cost will lose its advantages in term of survival rate, duration in completing a life cycle and reproductive performance (Ribeiro *et al*., 2014). Therefore, investigating the fitness cost in a resistant population will help in managing the resistance problem. Previous studies have recorded the adverse impact of the resistance development towards the fitness cost in the selected population (Sayyed *et al*., 2008) (Tang *et al*., 1997) (Cao and Han, 2006) (Sun *et al*., 2012).

Despite the ability of DBM to confer quick resistance to vast types of insecticides, knowledge and information regarding the aspect were limited in Malaysia, although insecticide practices are vigorously applied in the farms. Therefore, this study was initiated and conducted to examine the current resistance status of this pest in the country. Since spinosad is one of the products that specifically targets the DBM, it was chosen as the selected insecticide in this study. Moreover, it is crucial to examine the effectiveness of this insecticide after 20 years of introduction in the industry. The knowledge obtained from the study will act as insight to current efficacy status of the insecticide in controlling DBM, thus will add in as recent guideline for resistance monitoring program. This study aimed to investigate the development of the spinosad resistance development in the population that does not have any pre-exposure history of spinosad, therefore, a DBM population that originated from an organic farm was used. The findings obtained will be useful in future studies of spinosad resistance in conventional farms. Consequently, we examined the fitness cost experienced by the resistant DBM in the absence of spinosad. It is hypothesized that DBM strain in this study will develop resistance to spinosad after several generations and resistance will be accompanied by fitness cost. The goal of this study was to provide a current knowledge of DBM resistance towards DBM and incorporate the finding for a better resistance monitoring program.

MATERIALS AND METHODS

Collection and rearing of *Plutella xylostella*: This study was conducted in Faculty of Agriculture, Universiti Putra Malaysia, Selangor, Malaysia. The DBM were collected from an organic farm located in Semenyih, Selangor (2°56'23.3"N 101°53'52.3"E) where there was no record of exposure of any chemical that may influence the resistance selection process.

Rearing procedure followed the method proposed by Qian *et al*. (2008). The DBM collected from the field was labelled as susceptible (SS) strain. It was reared in glasshouse at 25 °C temperature and 60% relative humidity. The collected larvae and pupae were released in rectangular cages each containing a pot of *Brassica rapa* var. *chinensis* (white pak choy) as host plant. The host plant was observed daily and replaced whenever necessary. Apart from serving as food source to the larva, the host plant also functioned as the oviposition site for the female adult during eggs laying. The adults of the DBM were fed the food source by means of cotton wool soaked in 10% honey solution. Once dried, the cotton wool was changed with a new one to continuously provide food source for the adults.

Resistance selection: Resistance selection was conducted by feeding the DBM with spinosad–sprayed host plant. Resistance selection began at 12th generation (G12) of SS strain by feeding the larvae with spinosad-sprayed host plant. Selection was started at the particular generation due to time taken by the DBM to establish its population beforehand. The strain that has been selected with spinosad was labelled as spinosad-selected (Spi-Sel) strain. The G12 of SS strain was acknowledged as Parent (P) generation of Spi-Sel strain thereafter.

Leaf-dip bioassay method: Leaf-dip bioassay method was conducted following procedure of Sayyed *et al.* (2008) to determine the spinosad resistance development in DBM. Bioassays were conducted on third instar of the Spi-Sel strain. Five concentrations of spinosad (10, 15, 25, 35 and 50 ppm) were prepared together with distilled water that served as control. The 5% surfactant Triton X - 100 was added as emulsifier and mixed into the control and spinosad solutions. Four replicates were used for each concentration. Round-cut cabbage leaves were dipped in the solutions for 10 seconds. They were placed on the corrugated aluminium foil to allow air dry for approximately 2 hours.

Upon drying, one cabbage leaf was placed in a round 70 mm petri dish and subsequently 10 larvae were placed in each petri dish. The larva was firstly starved for two hours prior to be feeding on spinosad-soaked leaves. The dishes were sealed with parafilm to prevent larvae from escaping and were maintained at 25 °C at 11: 13 (L: D) hour photoperiod. Mortality of the larvae was recorded after 48 hour of exposure and was confirmed when there is no movement of larvae when disturbed with tips of tissue. Bioassay on Spi–Sel strain was conducted from 3rd generation (G3) of the strain until it become resistance. Unless the population was not sufficient, bioassay was conducted in each generation.

Data analysis of resistance selection study: The mortality of DBM was analysed through probit analysis (Finney, 1971) using POLOPLUS software (LeOra Software Co., Petaluma, CA, USA). The LC_{50}, 95% fiducial limit (FL) and the slope (±SE) of the log-dose probit line was obtained from the analysis. The significance of LC_{50} among the generations of DBM was determined when the 95% fiducial limit values were not overlapped with each other.

Resistance ratio (RR) of DBM to spinosad was calculated by dividing the LC_{50} of the *n* generation with LC_{50} of the parent (P) generation. The resistance level was classified following the classification of Ahmad *et al.* (2007); susceptible (RR = 1), low resistance (RR = 2-10), moderate resistance (RR = 11-30), high resistance (RR = 31-100) and very high resistance (RR = > 100).

Fitness comparison: Fitness comparison between SS and Spi-Sel strain were measured using several biological parameters proposed by Abbas *et al.* (2012). The SS strain was reared in parallel with Spi-Sel strain in laboratory. Prior to experiment, Spi-Sel strain was reared free from spinosad for two generations to minimize the effects of the insecticide. A total of 150 neonates for each strain were used in the experiment. In order to obtain the neonate, newly emerged male and female adults were pooled in plastic cups, letting them to mate and start to oviposit. Later, the oviposited eggs were observed daily until it hatched. Upon hatching, the neonates were collected within 24 hour and was placed singly on pak choy seedling. The seedling was stored in a closed, small plastic container, letting the neonate to grow, and whenever necessary, the pak choy seedlings was replaced with a new one.

Immature: The development of neonate at all four larval instars and pupa were observed daily. Number of mortalities, duration of development and weight of pupa was documented. During 4th instar, the sexes were determined according to description suggested by Liu and Tabashnik (1997). At the end of 4th instar stage, each of the larvae were placed in a petri dish single, lined with aluminium foil inside the dish and let it pupate. Subsequently pupae were weighed using four-decimal weighing scale.

Adult: Within 24 hour of emergence, two male adults and one female adult were grouped together in a plastic cup to form a family Cao and Han (2006). The plastic cups were labelled by date of family formed. The DBM were allowed to mate and once the female has laid eggs, the population was transferred to another plastic cup. It was purposed for easier daily egg calculation process in assessing egg hatchability and female fecundity. These steps were repeated daily until there were no more eggs laid by the female. Number of neonates hatched was also documented daily.

Data on emergence rate of healthy adult, female ratio, and longevity of the adults were recorded. Subsequently, the reproductive rate (R_0) and relative fitness were determined for each strain (Abbas *et al.*, 2012).

The net reproductive rate (R_0) was calculated as follows:

$$\frac{\mathrm{Nn}+1}{\mathrm{Nn}}$$

N_n = Total neonate in parent generation

N_{n+1} = Total neonate in the next generation

Relative fitness of the Spi-Sel strain was calculated as follows:

$$\frac{R_0 \text{of the resistant strain}}{R_0 \text{ of the susceptible strain}}$$

Data analysis of fitness cost study: Biological parameters data for fitness comparison study was analysed using SAS software version 9.4 (SAS Institute, North Carolina, US). When the parameters in both strains were not normally distributed (Shapiro-Wilk, $p < 0.05$), consequently, the non-parametric test Wilcoxon Mann-Whitney (at $p < 0.05$) was employed to examine the mean differences of the biological parameters between both strains. The Spearman's correlation coefficient (R) was conducted using PROC CORR to measure the correlation between parameters to investigate whether the parameters have influence on each other.

RESULTS

Selection of a field collected diamondback moth with Spinosad: The continuous selection of susceptible (SS) DBM strain in the laboratory to produce a spinosad-selected (Spi-Sel) strain was conducted for 15 consecutive generations. The LC_{50} value from Parent (P) until G15 ranged from 11.46 – 490.63 ppm (Table 1). At G3 and G4, the resistance ratio resolved around value of 1, indicating no resistance has been developed in the generations. At G5, low resistance began to develop in the population (RR = 3.59), of which the rate of resistance increased gradually until G13. Between G13 and G15, a huge increment in resistance was observed, summarizing the resistance ratio (RR) of 42.81-fold from P until G15. Significant difference in resistance ratio was only detected between the last two generations (based on non-overlapping fiducial limit; 95% FL), while no such observation has been made in other generations. The concentration-mortality curves estimated in each generation fitted to the probit model (χ^2 not significant, $P > 0.05$). **Fitness comparison:** The data on life history traits of SS and Spi-Sel strain in spinosad-free environment are summarized in Table 2. SS and Spi-Sel strain each represented by 19 and 14 families, respectively. In the 1st and 2nd instar, the immature of SS strain had higher survival rate than in Spi-Sel strain. In 1st instar, SS had 12.66% higher survival rate compared to Spi-Sel while in 2nd instar the survival rate was 17.15% higher than Spi-Sel strain. Survivorship rate was similar between 3rd and 4th in stars of the strains ($p > 0.05$), of which 3rd and 4th in stars of the SS strain had 1.43% and 1.27% higher survival rate than SS, respectively. Likewise, higher pupation rate with no significant difference ($p > 0.05$) was observed in SS strain with 1.27% more larva succeeded to pupate compared to its counterpart.

Table 1: Selection history of spinosad-resistant (Spi-Sel) diamondback moth strain.

Generation	n^a	LC$_{50}$ (ppm) (95%FL)	Fit of probit line			RR^b
			Slope ± SE	χ^2	df	
Parent	240	11.46 (3.51 - 16.90)	1.19 ± 0.37	1.52	3	-
G3	240	12.59 (9.03 - 15.50)	2.41 ± 0.42	2.06	3	1.10
G4	240	14.37 (0.50 - 23.72)	0.81 ± 0.36	1.96	3	1.25
G5	240	41.14 (30.68 - 76.18)	1.54 ± 0.39	1.07	3	3.59
G7	240	79.67(49.51 - 361.50)	1.47 ± 0.42	1.21	3	6.95
G8	200	103.65 (n.a)	1.29 ± 0.68	1.45	3	9.04
G10	180	108.46(87.04 - 204.23)	2.64 ± 0.77	2.04	2	9.46
G13	240	125.42(112.46 - 144.26)	3.86 ± 0.89	2.09	3	10.94
G15	240	490.63(160.10 - n.a)	0.90 ± 0.44	2.69	3	42.81

Table 2: Comparison of biotic fitness between SS and Spi-Sel strain of DBM.

Life History Traits	**Strain**	
	Susceptible (SS)	Spinosad-Resistant (Spi-Sel)
Neonate no.	150	150
Larva		
Survival Rate (%)		
1st instar	97.33 ± 1.32*	84.67 ± 2.95*
2nd instar	95.89 ± 1.65*	78.74 ± 3.65*
3rd instar	98.57 ± 1.01	100.00 ± 5.45
4th instar	97.10 ± 5.63	95.83 ± 2.05
Duration (day)		
1st instar	3.09 ± 0.44*	2.87 ± 0.06*
2nd instar	2.18 ± 0.06*	1.74 ± 0.08*
3rd instar	2.13 ± 0.07 *	1.37 ± 0.10*
4th instar	2.08 ± 0.09*	1.37 ± 0.10*
Pupa		
Pupation Rate (%)	97.10 ± 5.63	95.83 ± 2.05
Weight (mg/pest)		
Male	5.19 ± 0.13	5.00 ± 0.15
Female	4.61 ± 0.10	5.23 ± 0.17
Duration (day)		
Male	5.22 ± 0.14*	4.68 ± 0.26*
Female	4.74 ± 0.19*	4.21 ± 0.35*
Adult		
Duration (day)		
Male	5.43 ± 0.39	6.11 ± 0.59
Female	6.47 ± 0.38	5.78 ± 0.53
Next generation neonate	786	146
Net Reproductive Rate	3.93	0.97
Relative Fitness	1.00	0.25

* Indicate a significant difference between strains (Wilcoxon Mann-Whitney, $p < 0.05$)

Higher survival rate in the immature stages of SS did not translate into shorter time taken for the immature in their growth. A significantly shorter time was observed in all four instars of Spi-Sel strain ($p < 0.05$). All stages of the SS instars needed one day extra to complete the stage before pupation than Spi - Sel strain. Similarly, the time taken by male and female pupa of Spi - Sel strain to complete the stage before adult emergence was distinctly shorter compared to SS strain ($p < 0.05$). On the other hand, while male pupal weight did not differ significantly ($p > 0.05$) between the strains, female pupa was significantly heavier in Spi - Sel than in SS strain ($p < 0.05$). The female of the latter strain exhibited about 1% reduction in the weight compared to its counterpart. Adult male of Spi - Sel has longer life than of SS and the duration was vice versa in their females. However, no significant differences detected in both strains in term of adult longevity. Spi - Sel strain scored low fitness value (0.25) compared to the SS strain, an indicative of low fitness and high fitness cost of the former strain. The discrepancy of the fitness between the strains was mainly associated with the 5 - fold difference of the neonate number produced in the next generation.

The reproductive potential in female of the DBM represented by female percentage, number of eggs produced per female and percentage of egg hatchability was shown in Figure 1. While SS strain possessed higher female percentage and fecundity, higher percentage of egg hatchability was documented in Spi - Sel strain. A huge reduction of 32.46% and 45.75% were found respectively in female percentage and fecundity of Spi - Sel strain to the SS strain. Meanwhile, percentage of the egg hatchability was quite similar between both strains with only 9.10% difference. All the parameters differed significantly between the strains ($p < 0.05$).

Relationship of the parameters: Table 3 depicted correlation values of the parameters calculated using Spearman's rho (R) correlation coefficient. Among all parameters analysed, weight of female and male pupa ($r = 0.71$, $p = 0.0001$), number of eggs laid (fecundity) and percentage of eggs hatched ($r = 0.71$, $p = 0.0001$) had the strongest and highly significant relationship to each other. In addition, male pupal weight also possessed higher positive and highly significant influence on fecundity and percentage of egg hatchability (Table 3, $r = 0.66$, $p = 0.0001$ and $r = 0.44$, $p = 0.000$, respectively), compared to female pupal weight.

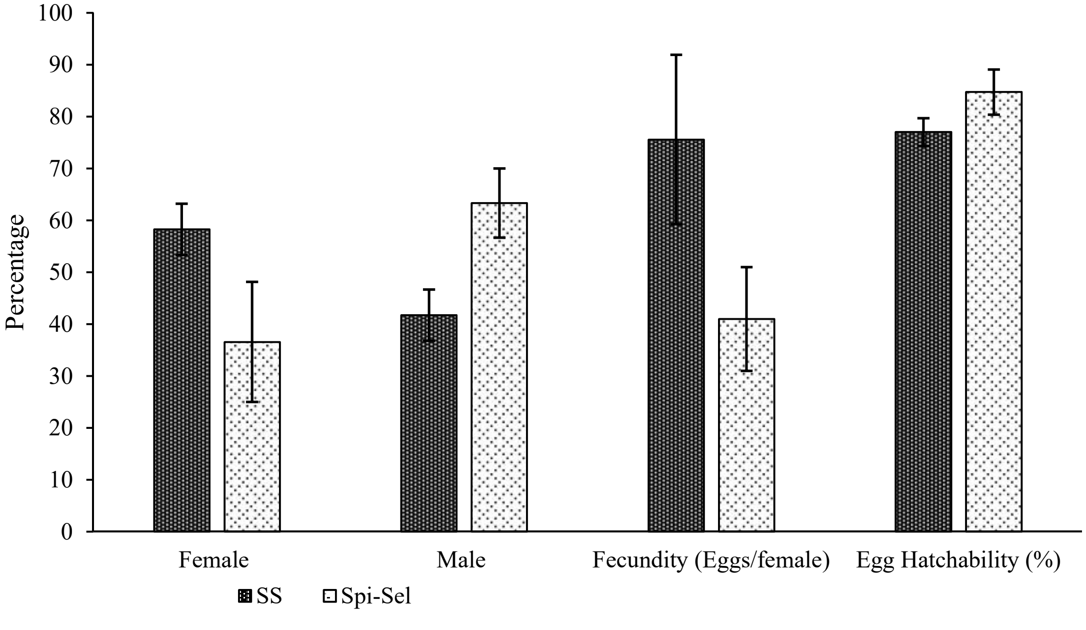

Figure 1: Percentage of female and male, fecundity and egg hatchability in SS and Spi-Sel strain. All the parameters differed significantly different between both strains (Mann – Whitney; $p < 0.05$).

Table 3: Spearman's rho correlation coefficient between parameters measured in the fitness study

	SRate1	**SRate2**	**SRate3**	**SRate4**	**DDay1**	**DDay2**	**DDay3**	**DDay4**	**PuFDu**	**PMaD**	**AdMD**	**AdFD**	**FePWe**	**MaPW**	**NoEgg**	**Egghat**
SRate1	1	-	0.31[c]	-	0.30[c]	0.42[d]	-	-	-	-	-	-	0.18[a]	0.19[a]	-	-
SRate2		1	0.29[c]	0.19[a]	-	-	-	-	-	0.20[a]	-	-	0.25[b]	-	0.24[b]	0.22[b]
SRate3			1	0.60[d]	0.46[d]	-	-0.32[d]	-0.22[c]	0.25[c]	0.32[d]	-	0.24[b]	-	-	0.26[b]	-
SRate4				1	0.41[d]	-	-0.36[d]	-0.41[d]	0.27[c]	0.29[c]	0.27[b]	0.32[d]	-	-	-	-
DDay1					1	-	-0.25[b]	-0.27[b]	0.23[b]	-	-	0.18[a]	0.21[a]	-	-	-
DDay2						1	0.27[b]	0.21[b]	-	-	-	-0.19[a]	-	-	-	0.23[b]
DDay3							1	0.47[d]	-0.22[b]	-0.19[a]	-	-	-	-	-	-
DDay4								1	-0.22[b]	-	-0.29[c]	-0.33[d]	-	-	-	-
PuFDu									1	0.52[d]	0.36[d]	0.41[d]	-0.45[d]	-0.42[d]	-0.30[c]	-0.19[a]
PuMD										1	0.31[c]	0.32[d]	-0.32[d]	-0.29[c]	-0.21[a]	-
AdMD											1	0.64[d]	-0.37[d]	-0.46[d]	-0.62[d]	-0.49[d]
AdFD												1	-0.27[c]	-0.39[d]	-0.53[d]	-0.55[d]
FePWe													1	0.71[d]	0.59[d]	0.32[d]
MaPW														1	0.66[d]	0.44[d]
NoEgg															1	0.71[d]
Egghat																1

[a, b, c, d] denote significant levels (p-value) at 0.05, 0.01,0.001 and 0.0001 respectively

SRate (1 – 4) = Survival Rate of instar 1 – 4, respectively
DDay (1 – 4) = Development Day of instar 1 – 4, respectively
PuFDu = Duration of Female Pupa
PuMD = Duration of Male Pupa
AdMD = Duration of Male Pupa

FePWe = Weight of Female Pupa
MaPW = Weight of Male Pupa
NoEgg = Number of Eggs Laid
Egghat = Percentage of Egg Hatchability
AdFD = Duration of Adult Female

The initial stage in DBM development played a significant contribution in its later life stage as pictured by the impact of 1st and 2nd survival rate instar on pupal weight, fecundity and percentage of egg hatchability. For instance, a parallel relationship was recorded between 2nd instar survival rate with number of eggs produced per female (Table 3, $r = 0.24$, $p = 0.01$) and the percentage of egg hatchability (Table 3, $r = 0.22$, $p = 0.01$). In addition, a positive relationship existed between duration of 2nd instar development and percentage of egg hatchability. In contrast, the duration in the pupa and adult stage were mostly conversely related to each other as well as to fecundity and percentage of egg hatchability.

DISCUSSION

The 42.81-fold RR within 15 generations of selection implying moderate resistance development in the DBM population in this study. Despite originated from an organic farm which received zero exposure of chemical insecticides, the LC_{50} value measured in Parent (P) generation reflected the possibility of the presence of resistance genes in the population. The potential to develop high resistance to spinosad was documented in DBM and other lepidopteran pests (Stanley *et al*., 2009; Fauziah *et al*., 2012; Reyes *et al*., 2012). The ability of insects in developing resistance is varied depending on the insect species evaluated, its genetic background (Alphey *et al*., 2011), selection intensity (Shad *et al*., 2010) and history of insecticides application (Attique *et al*., 2006).

Finding in the present study revealed a relatively delayed resistance development in DBM compared to previous findings. For comparison, selection of DBM to spinosad for 12 generations in Pakistan revealed 283-fold and 1983-fold of resistance to unselected strain and susceptible strain, respectively (Sayyed *et al*., 2008). Furthermore, a field-collected *Spodoptera exigua* had 345.5-fold of RR to spinosad after five generations of the selection (Wang *et al*., 2006), while *S. litura* exhibited 3921-fold of RR only in 11 generations after the selection (Rehan and Freed, 2014).

High resistance recorded in the above studies was primarily contributed by pre-exposure of spinosad and other insecticides in the field as most of the populations were collected from the conventional farms prior to selection in the laboratory. The continuous exposure of spinosad to the population suggests a pre-existence of stable resistant alleles in the population, thus contributed to a high resistance when selected in the laboratory (Brown, 1958). On the contrary, the SS strain in the present finding was initially collected from the organic farm and this might explain the moderate resistance development in Spi-Sel strain. Moreover, slow resistance development observed in the early 13 generations hypothesized a low initial frequency of resistance alleles in the population prior to spinosad exposure. The initial frequency of resistance alleles is characterized by the pre-exposure history the population to the insecticides in the field (Liu *et al*., 2008).

In numerous previous studies i.e., Abbas *et al*. (2012); Dangal and Huang (2015) and Oftadeh *et al*. (2015), duration of the development in the immature stages was conversely related to the fitness of the insect, i.e., shorter development time indicated better fitness while the weight of the female of the insect is parallel to the fecundity, i.e., heavier female produced a greater number of eggs. The delayed development in the immature of SS strain can be hypothesized to accomplice with advantages in accumulating resources needed in the next stages. In particular, the precursors of the adult tissue of the holometabolous insects e.g., Lepidoptera begin to develop in the larval stage and maturing to the reproductive stage during metamorphosis (Hemming, 2003), whereas larval tissue was hystolised during pupation (Joraŝik and Honek, 2007). On a different note, the aforementioned study suggested that it is adaptive for the females to emerge earlier than the males because females typically need to feed and gather energy for egg maturation before mating. Meanwhile as for the males, prolonged immature stages are necessary as this could increase their survivorship until reproductive age. This opinion elaborates the prolonged male pupa duration and shorter female pupa duration of both strains in the current study. Further, extended duration of larva in DBM will benefit insect pest management program by DBM functioning as host to the natural enemies such as *Cotesia plutella* and *Diadegma insulare* (Kwon *et al*., 2006; Sarfraz *et al*., 2005). Thus, the longer larval duration in SS strain in this study can be manipulated in the field to amplify the beneficial insects' population, hence mitigating the resistance development in an agricultural area.

Nevertheless, shorter time taken by the Spi-Sel strain to complete its immature stages should not be underestimated. It is important to note that small decreases in development time can lead to a considerable increase of population growth rate depending on the population's density, especially in DBM population which is able to have rapid lifecycle turnover. Lewontin (1965) elucidated that a day of shorter development is possible to boost the intrinsic growth rate and doubling the female fecundity, which in turn capacitate them to quickly gain fitness. On that account, although shorter development time was not associated with fecundity in this study, it is conceivable Spi-Sel may adapt in insecticide-free environment, thus complicating the resistance management program.

In term of fecundity (number of eggs laid per female), females of SS strain scored better fecundity than the resistant females. Therefore, it indicates better fecundity and egg laying ability (Martins *et al*., 2012) in the SS strain. A series of report had described reduction in number of eggs laying by the resistant insect female, including Corrales and Campos (2004), Paris *et al.* (2011), Zaka *et al.* (2014) and Ejaz *et al.* (2017). Since reproduction is resulted from a series of physical, physiological and metabolic factors, later it has an impact on the oviposition (Corrales and Campos, 2004). Studies also have associated and proved the role of pleiotropic effect in determining the fecundity; one of the main direct components in assessing the fitness of the females (Sun *et al*., 2012; Ribeiro *et al*., 2014; Belinato *et al*., 2016).

The provisioning of developing oocytes is usually energetically demanding (Schwenke *et al*., 2016). In the environment free of insecticide exposure, resistant insects with fitness cost need to strive more because the resistance genes lose its advantageous in such environment. This condition reflects the diminishing fecundity of Spi - Sel population compared to SS population in in this study when reared on the spinosad-free seedlings.

Another factor leading to higher reproductive output in SS strain is due to heavier male pupal weight of the strain. Profuse studies recommended the association between female pupal weight and fecundity, however very few studies reported the significance of male pupal weight to the fecundity (Huang *et al*., 2005; Yin *et al*., 2008; Zaka *et al*., 2014). Influence of male pupal weight on the fecundity may have been indirect as the weight of the male could indicate its fitness and later its behaviour in the mating activity. For example, higher mating success was displayed when control males were paired with either control female or indoxacarb - treated female, whereas a reduced mating event was observed when the females paired with the resistant males (Wang *et al*., 2011).

Overall, spinosad resistance affected the fitness of the Spi-Sel strain in the absence of the insecticide specifically to the larval survival rate that was straightforwardly associated with the reproductive output of the adult, implied by the relative fitness value of the strain (0.25). Hackett and Bonsall (2016) suggested that when a fitness cost is associated with resistance alleles, there is an energy imbalance in the resistant insect body to compensate the spinosad in physiological growth and biochemical defence in order to protect themselves from extinction (Wiesch *et al*., 2010). The deficiency in reproductive performance of Spi - Sel could be attributed to higher maintenance costs in the insect body as the insects with higher maintenance costs needs more energy to maximize the energy allocation in the physiological growth and reproductive performance while detoxifying the insecticide at the same time.

Conclusion: Selection of Spi-Sel population with spinosad revealed the moderate level of resistance accompanied with high fitness cost in the strain. The high fitness cost of Spi-Sel in this study would facilitate to withdraw the resistance from the population. Recent findings in the present study can be used as fundamental in resistance study in the near future. The information gather could be manipulated to aid management and monitoring program and shared with authorized bodies and growers. Resistance management program should emphasize on the insecticide rotation as well as controlling the DBM using biological agent in order to ensure the effectiveness of the insecticides used. Additionally, further works need to be done, emphasizing on the characteristic of the resistance in DBM, its potential to be cross resistance with other insecticides and the mechanism that regulate the resistance, which are anticipated to improve the management program.

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