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Evaluation of Developmental and Reproductive Fitness of Laboratory Selected Thiamethoxam Resistance in Black Legume Aphid *Aphis craccivora* Koch.

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Abstract

Thiamethoxam is a neonicotinoid group of insecticide which are selective agonists of the insect nicotinic acetylcholine receptor (nAChR), a pentameric cys-loop ligand-gated ion channel located in the central nervous system of insects. They provide farmers with invaluable, highly effective tools against some of the world's most destructive crop pests. Black legume aphids, *Aphis craccivora* is one of the devastating polyphagous pests in agroecosystem and to manage their population, farmers are still relying on application of synthetic insecticides like Thiamethoxam. To evaluate the risk of resistance development, selection of *A. craccivora* was done to characterize Thiamethoxam resistance along with evaluation of developmental and reproductive fitness cost of the resistance. About 86.19 folds of resistance were developed after selecting for 24 generations. In the first twelve generations steady development of resistance was noticed followed by development become stiffer till 16th generation and more or less stable till 24th generations. Relative fitness of the selected resistant strain of aphids was decreased with longer nymphal duration and adult longevity. The mean nymphal duration was increased from 4.35±1.02 days (in F_0) to 7.9±0.57 days (in F_{24}). The fecundity rate was significantly less in resistant population (16.0±5.34) as compared to the susceptible strain (54.71±7.63) whereas, oviposition periods were significantly longer (12.49±1.44 days) in resistant population as compare to susceptible strain (9.50±1.22 days).

Keywords: Thiamethoxam, resistance, fitness cost, aphids, fecundity, nachr

1. Introduction

Aphids are small, soft bodied insects belonging to the Family Aphididae, Order Hemiptera. Around 4000 species of aphids are known to occur worldwide and 400 species are reported from India (Sullivan, 2008). Black legume aphid *Aphis craccivora* Koch. (Aphididae: Homoptera) also called as cowpea aphid, groundnut aphid is a highly polyphagous pest attack about 50 crops in 19 different plant families (Radha, 2013; Keating et al., 2015). This insect is involved in transmission of several viral diseases e.g. rosette virus in groundnut, poty virus in sunflower (Singh et al., 2005), alfalfa leaf curl virus (Ryckebusch et al., 2020) etc. It also causes significant damage to green gram and black gram foliage and pods along with other related legumes (Swaminathan et al., 2012). The yield losses in cowpea can reach or exceed 50% in the case of high and uncontrolled

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aphid infestation or in the case of virus infection even at low population (Obopile and Ositile, 2010). It also may cause up to 100 per cent yield loss in different varieties of country bean, barbate, black gram and mung bean.

A large number of insecticides have been evaluated and recommended from time to time for their control (Sharma and Singh, 1993). The indiscriminate and large-scale use of synthetic chemical insecticides to control aphids has resulted in the development of insecticide resistance (Han and Li, 2004). The extensive use of neonicotinoids against aphids has resulted in the development of resistance in aphids (Srigiriraju, 2008). Although many insect species are still successfully controlled by neonicotinoids, their popularity has imposed a mounting selection pressure for resistance, and in several species resistance has now reached levels that compromise the efficacy of these insecticides (Bass et al., 2015). A total of about 100 aphid species have successfully exploited agro-ecosystems to become economically significant pests, with about twenty of them developing at least one recognised with insecticide resistance mechanism. (Simon, 2008; Van Emden and Harrington, 2007). Insecticide resistance is one of the most serious problems that entomologists face in crop defence around the world, affecting the quantity and quality of the finished product and causing economic disasters in some cases.

Insecticide resistance mutations are widely assumed to carry fitness costs. However, studies to measure such costs are rarely performed on genetically related strains (Ffrench-Constant and Bass, 2017). Fitness costs are suggested to be a consequence of trade-offs in energy between traits underlying insecticide resistance and fitness-related traits such as reproduction, development time and adult body size. Such study in insecticide-resistant insect strainsare carried out to see if any fitness-related characteristics, such as reproduction, development time, and adult body size, have changed (Fenton et al., 2010). Fitness costs have vital role in sustainable utilization of pesticides because they restrain the progression of resistance in agro ecosystems. This effect is prominent when fitness costs are partly dominant (Raymond et al., 2007).

Biological characteristics evaluation of insecticide resistant populations can be very useful in formulating the insecticide resistance management strategies (Campanhola et al*.,* 1991). Insecticide resistance contributed to fitness decline in *P. xylostella* (Cao and Han, 2006), *Nilaparvata lugens* (Liu and Han, 2006), *S. exigua* (Jia et al., 2009), *Bemisia tabaci* (Feng et al., 2009), *H. armigera* (Wang et al., 2010), *S. litura* (Abbas et al., 2014; Zaka et al., 2014) and *P. solenopsis* (Afzal et al., 2015). In the present work we have tried to evaluate the Thiamethoxam resistance risk in *A. craccivora* by resistance selection and fitness analysis through studies of developmental and reproductive characteristics.

2. Materials and Methods

2.1. Collection and rearing of Aphis craccivora

The present study was carried out at Department of Entomology, College of Agriculture, OUAT, Bhubaneswar, Odisha, India during 2017-2019 under laboratory set of conditions (28±2°C; 75-80%). Field strain of *A. craccivora* were collected from research field of OUAT, Bhubaneswar (20.2647° N, 85.8141° E) during July, 2017 without being exposed to any insecticide previously and were reared in laboratory till January 2018.

Aphids were reared in well ventilated cages and the insects were kept on cowpea seedlings grown in plastic pots (15 cm diameter). This strain was reared continuously without being exposed to any insecticides throughout the study. Apterous adult aphids from this strain were used for bioassay, establishing a toxicity base line for Thiamethoxam and as a reference strain in studies of resistance ratio (RR). A commercial formulation of Thiamethoxam (Actara; *Syngenta*) was used in this experiment

2.2. Bioassay and resistance selection

Baseline toxicity and further toxicity level in selected generations to Thiamethoxam were determined using Leaf dip method of bioassay according to IRAC (method No. 019). About 6 to 7 concentrations of Thiamethoxam with 3 to 4 replications were used. Results were expressed as percentage mortalities, further corrected using Abbott's formula (1925).

For resistance selection, the lethal concentration (LC_{60}) of Thiamethoxam (based on the baseline data) was used for the first-generation selection. Adult apterous aphids were released on untreated cowpea plants 24 hr prior to the insecticide spraying. And the neonates from the surviving adults were continued to be reared on the same treated plants till the next generation. A new LC_{50} was used based on the progress in resistance in generations. Folds of resistance were calculated by dividing the LC_{50} of the selected generation by the LC_{50} of the susceptible strain. Selected aphids F_n were the populations selected for *n* generations.

2.3. Biological parameters study

Different biological parameters of different selected generations (parent strain, F_2, F_4, F_6, F_8, F_{10}, F_{12}, F_{14}, F_{16}, F_{18}, F_{20}, F_{22} and F_{24}) of *A. craccivora* were studied at different times under laboratory conditions (28±2°C; 75-80%). The 2nd or 3rd opened fresh leaves of cowpea leaves at the peak vegetative stage were used for studying the biology of aphids. Fresh leaves were taken in glass petri plates of 9 cm diameter lined with moist blotting paper.

The adult apterous aphids were kept in foliage to get freshly laid first instar nymphs. Subsequently by the help of camel hair brush the first instar nymphs of aphids were released on excised cowpea leaves at the rate of one nymph per petri plate. The developmental characters of aphids were studied under binocular microscope (MAGNUS Stereoscopic binocular microscope Model MS 24 Alpha with objective (2x

& 4x) and eyepiece 10x (F.N.22) having with in-built Light Stand (Incident: 6V15W Lamp/Transmitted:5W Fluorescence Lamp). Observations were taken at every 12 hours duration, on moulting, change in instars, nymphal durations, adult longevity (pre oviposition, oviposition and post oviposition periods) and fecundity. Fresh leaves were provided as and when required. The moisture content of the blotting papers was also maintained accordingly. A total of 20 replications per selected generation were used in the study. Statistical comparisons were done using Student's t-test (p<0.05) to test for significant differences in nymphal durations, adult longevity (pre oviposition, oviposition and post oviposition periods) and fecundity.

3. Results and Discussion

The data regarding numphal durations and nymphal developmental periods were summarized in Table 1. And the data regarding adult longevity and fecundity of different selected generations were tabulated in Table 2.

3.1. Thiamethoxam resistance selection

The LC_{50} value of Thiamethoxam (baseline toxicity) to adult apterous *A. craccivora* was found to be 2.62 ppm. From F_2 generation to F_{12} generation the increase in resistance folds (1.37 folds to 28 folds) were steady. Also the LC_{50} for values of different generation from F_2 to F_{12} increased (3.61 ppm, 7.11 ppm, 23.49 ppm, 31.32 ppm, 47.08 ppm, 48.76 ppm and 75.92 ppm) (Table 1). The slope value gradually increased from F_2 generation to F_{12} generation (1.6± 0.22 to 2.59± 0.41) making the probit graph stiffer.

Table 1: Development of resistance to thiamethoxam in *A. craccivora* under laboratory conditions selected for 24 generations

Selected generations (Fn)	LC_{50} (ppm)	Fiducial limits (ppm)		Regression coefficient (Slope) b±SE	Regression Equation	Resistance ratio (RR)
		Lower limit	Upper limit			
F_0	2.62	1.92	3.44	1.60±0.22	Y=1.60X+4.32	-
F_2	3.61	2.54	4.69	1.65±0.28	Y=1.65X +4.07	1.37
F_4	7.11	5.49	8.83	2.04±0.27	Y=2.04X+3.35	2.71
F_6	23.49	19.03	28.66	2.19±0.30	Y=2.19X+2.09	8.96
F_8	31.32	24.10	38.72	2.14±0.28	Y=2.14X+1.85	11.95
F_{10}	47.08	37.43	56.97	2.34±0.32	Y=2.34X+1.25	17.96
F_{12}	48.76	38.24	59.87	2.12±0.30	Y=2.12X+1.46	18.61
F_{14}	75.92	63.05	89.28	2.59±0.41	Y=2.59X+0.19	28.97
F_{16}	181.73	157.13	209.41	3.08±0.63	Y=3.08X-1.83	69.36
F_{18}	196.58	174.04	216.87	4.18±0.78	Y=4.18X-4.63	75.02
F_{20}	199.57	177.08	220.45	4.14±0.78	Y=4.14X-4.41	76.17
F_{22}	207.21	189.24	225.75	4.99±0.83	Y=4.99X-6.24	79.08
F_{24}	225.83	204.45	244.41	5.20±0.98	Y=5.20X-7.36	86.19

n: Number of generations selected

From 14th to 16th generation there was sharp increase in resistance fold from 28.97 folds to 69.36 folds. Then again from F_{18} to F_{24} the resistance level became more or less stable varying from 75.02 folds to 86.19 folds. At F_{24} the LC_{50} value was 225.83 ppm with slope value 5.2± 0.98.

3.2. Influence of resistance on the developmental characteristics

The overall nymphal duration increased towards the resistance acquisition in different selected generations of aphids (Table 2). In the susceptible strain the nymphal duration was 4.35±1.02 days and the same value at F_{24} was 7.9±0.57 days (significantly different). The duration of 1st instar nymph (varied from 1.28±0.26 to 1.50±0.34 days) did not differ significantly among different generations. The duration of 2nd instar in F_{24} was 1.59±0.28 days which was significantly different that of the F_0. The duration of both 3rd and 4th instar of F_{24} also differed significantly that of the susceptible strain.

3.3. Influence of resistance on the reproductive characteristics

Thiamethoxam resistance clearly affected the oviposition period, adult longevity and fecundity of aphids (Table 3). Both pre oviposition and post oviposition periods increased as the resistance to Thiamethoxam was acquired. The mean oviposition period increased from 7.78±1.40 days (F_0) to 8.89±1.27 days (F_{24}). Adult longevity also significantly increased from 9.50±1.22 days (in F_0) to 12.49±1.44 days (in F_{24}). The F_{24} generation had significant less fecundity (16.0±5.34) as compared to the susceptible strain (54.71±7.63).

In insects, neonicotinoid insecticides act as agonists at the postsynaptic nAChR with much higher affinity (nanomolar level) than that of nicotine (micromolar level). The high-affinity binding site is conserved in neonicotinoid sensitivity and specificity (structure activity relationships) across a broad range of insects. The first neonicotinoid launched was imidacloprid in 1991, followed by nitenpyram and

Table 2: Nymphal durations of different selected generations of *A. craccivora*

Generations	I instar (days)	II instar (days)	III instar (days)	IV instar (days)	Nymph dur. (days)
F_0	1.28a±0.26	1.00a±0.40	1.07a±0.44	1.00a±0.40	4.35a±1.02
F_2	1.21a±0.26	1.07a±0.34	1.00a±0.40	1.00a±0.28	4.28a±0.95
F_4	1.28a±0.26	1.07a±0.34	1.00a±0.40	1.00a±0.28	4.35a±0.95
F_6	1.28a±0.26	1.14a±0.47	1.00a±0.40	1.07a±0.44	4.49a±1.15
F_8	1.35a±0.24	1.21a±0.39	1.07a±0.18	1.85b±0.55	5.48b±1.00
F_{10}	1.35a±0.24	1.28ab±0.26	1.42ab±0.44	2.78c±0.26	6.83c±0.62
F_{12}	1.42a±0.18	1.35ab±0.24	1.57b±0.44	2.8c±0.34	7.14c±0.69
F_{14}	1.42a±0.34	1.42b±0.24	1.67b±0.37	2.85c±0.34	7.36c±0.74
F_{16}	1.49a±0.18	1.49b±0.34	1.69b±0.26	2.86c±0.26	7.53c±0.24
F_{18}	1.49a±0.34	1.52b±0.26	1.71b±0.34	2.89c±0.55	7.61c±0.93
F_{20}	1.50a±0.23	1.57b±0.26	1.75b± 0.25	2.89c±0.50	7.71c±0.74
F_{22}	1.51a±0.37	1.59b±0.24	1.82b± 0.34	2.9c±0.34	7.82c±0.95
F_{24}	1.50a±0.34	1.59b±0.28	1.83b± 0.44	2.98c±0.26	7.9c±0.57
Mean	1.39	1.33	1.43	2.22	6.37
SD	0.1	0.21	0.34	0.88	1.52
SEm±	0.02	0.07	0.11	0.32	0.52
CD (p=0.05)	0.07	0.21	0.35	0.98	1.58

Means within a column followed by the same letters are not significantly different at p<0.05

Table 3: Adult Longevity and Fecundity of different selected generations of *A. craccivora*

Generations	Pre oviposition (days)	Oviposition (days)	Post oviposition (days)	Adult longevity (days)	Fecundity
F_0	0.57a±0.18	7.78a±1.40	1.14a±0.24	9.50a±1.22	54.71a±7.63
F_2	0.57a±0.18	7.85a±1.28	1.21a±0.26	9.64a±1.21	53.57ab±8.22
F_4	0.57a±0.18	7.85a±1.28	1.21a±0.26	9.71a±1.21	52.00ab±8.22
F_6	0.64a±0.24	7.92a±1.30	1.21a±0.26	9.85a±1.14	45.14bc±9.59
F_8	0.64a±0.24	8.28a±1.28	1.21a±0.26	10.14a±1.28	38.85c±5.69
F_{10}	0.64a±0.24	8.35a±1.24	1.28a±0.26	10.28a±1.4	22.71d±7.04
F_{12}	0.85a±1.17	8.57a±1.17	1.42a±0.44	10.85ab±1.57	19.71d±5.70
F_{14}	1.28b±0.56	8.71ab±1.07	1.92b±0.34	11.92b±1.20	18.85d±5.66
F_{16}	1.34b±0.56	8.8b±1.23	1.95b±0.44	12.09b±1.30	18.5d±5.89
F_{18}	1.4b±0.24	8.9b±1.30	1.97b±0.34	12.27b±1.43	17.4d±5.38
F_{20}	1.5b± 0.24	8.8b ±1.27	1.97b±0.35	12.27b±1.45	17.2d±5.76
F_{22}	1.5b ± 0.56	8.9b±1.28	2.00b±0.36	12.4b±1.43	16.9d±5.89
F_{24}	1.6b±0.34	8.89b±1.27	2.00b±0.37	12.49b±1.44	16.0d±5.34
Mean	1.00	8.43	1.57	11.03	30.12
SD	0.43	0.45	0.38	1.23	15.98
SEm±	0.08	0.13	0.09	0.28	5.52
CD (p=0.05)	0.26	0.38	0.27	0.85	16.55

Means within a column followed by the same letters are not significantly different at p<0.05

acetamiprid in 1995, and others such as thiamethoxam in 1998. Thiamethoxam is a new neonicotinoid insecticide belonging to thianicotinyl compounds and is the first example of the second generation of neonicotinoid insecticides. Senn et al. (1998) indicated that dose rates of thiamethoxam between 10 and 200 gm a.i./ha were sufficient for controlling target insect pests, such as aphids, rice hoppers, rice bugs, mealy bugs and some lepidopterous species, under laboratory and field trials. Because of its high efficacy, it has been widely used by the growers and accordingly various studies has indicated the level of resistance in wide range of insect pests to Thiamethoxam. Resistance ratio such as 900-fold a B-type strain of *Bemisia tabaci* (Rauch and Nauen 2003), 19.35 fold in *Aphis gossypii* (Pan et al*.,* 2015), 48.01 fold in *A. craccivora* (Abdallah et al*.,* 2016), 60 fold in *Bemisia tabaci* (Feng et al., 2009) to Thiamethoxam has been reported. Insecticide resistance is associated with fitness costs, such as a reduction in reproductive performance, longer development times and a reduction in body size in several insect species in environments that are free of insecticides (Berticat et al., 2008).

In our study, the resistance acquisition in different generations of aphids increased the nymphal durations. It also resulted in lesser fecundity, longer adult longevity and oviposition periods. Similar findings were reported by Liu and Han (2006). In their study, a laboratory selected imidacloprid resistant strain of *Nilaparvata lugens* (250 fold resistance in 37 generations) had disadvantages in reproduction. The larval survival rate (78%), adult emergence rate (69.6%), copulation rate (64.9%), fecundity (217.9) and hatchability (57.3%) were all significantly lower in resistant population as comparing to that of the susceptible strain that is, survival rate (93.7%), adult emergence rate (92.1%), copulation rate (87.5%), fecundity (491.3) and hatchability (88.2%). According to Belinato and Martins (2016) an organophosphate and insect growth regulator (IGR) resistant population of *Aedes aegypti* had longer developmental time, lower longevity, problems with blood feeding and low reproductive traits. Fitness costs are suggested to be a consequence of trade-offs in energy between traits underlying insecticide resistance and fitness-related traits such as reproduction, development time and adult body size (Fenton et al., 2010). According to Feng et al*.* (2009), a laboratory selected thiamethoxam resistant strain of *Bemisia tabaci* (60 fold resistance in 36 generations) had obvious fitness disadvantages in their development, reproduction and morphology. According to Afzal and Shad (2017) in a spinosad resistant strain of cotton mealybug, *Phenacoccus solenopsis* (282.45 resistance fold in 9 generations) there was increased male and female nymphal duration, pupal duration, emergence rates, male and female generation time with decreased fecundity. The longevity of male and female were significantly increased as compared to the susceptible populations (4.67 to 6.75 days in male and 10.77 to 23.23 days in female). According to Gao et al (2014), survival percentage of 1st instar larva of thiamethoxam resistant western flower thrips, *Frankliniella occidentalis* (15.1 fold of resistance in 55 generations) was significantly lower for the resistant strain (60.2±5.2%) than for the susceptible strain (72.2±6.0%). The percentage of pupation (75.4±2.8%) and fecundity (47.7±1.8 eggs per female) of resistant strain were significantly lower than those of susceptible strain (83.9±2.0% and 54.0±3.2% eggs per female, respectively).

Biological characteristics evaluation of insecticide resistant populations can be very useful in formulating the insecticide resistance management strategies (Campanhola et al*.,* 1991). Often, insecticide resistance may cost significant fitness to the pest population. The biological parameters of insects, for example reduced fecundity and enhanced growth period, may bring changes in relative fitness. The pest survival rate decreases because of resistance, which finally leads to declining in fitness (Roush and McKenzie, 1987; Forrester et al*.,* 1993). In resistant population the reduced fecundity might be the result of less energy expended for reproduction, i.e. part of the metabolic energy may be used in physiological and biochemical defences for insecticides detoxification (Ribeiro et al*.,* 2001).

4. Conclusion

Thiamethoxam resistance development in black legume aphids has significant impact on the mean nymphal duration and adult longevity as both the parameters were increased in due course of acquisition of resistance. Also the reproductive characteristics indicated by mean fecundity value was gradually decreased till 24th generations of selection. Hence, thiamethoxam resistance has strong impact upon the developmental and reproductive characteristics of *Aphis craccivora*. Fitness costs have vital role in sustainable utilization of pesticides because they restrain the progression of resistance in agro ecosystems.

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