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PREVALENCE OF NEONICOTINOID RESISTANCE IN COTTON APHID *APHIS GOSSYPII* (GLOVER)

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ABSTRACT

Bioassays were conducted against neonicotinoids viz., Acetamiprid, Clothianidin, Dinotefuran, Imidacloprid, Thiacloprid and Thiamethoxam using the field populations of cotton aphids collected from ten locations of Tamil Nadu. Perambalur population of aphids were found to be resistant to all six neonicotinoids with LC_{50} values of 0.884, 1.718, 1.535 and 0.521 ppm for acetamiprid, imidacloprid, thiamethoxam and thiacloprid, respectively. Aphids of study locations showed resistance to thiamethoxam with LC_{50} values in range of 0.034 to 2.184 ppm. No resistance in respect of clothianidin and dinotefuran was found across all locations with resistant ratios of 3.00 and 4.56-folds. Hence, clothianidin and dinotefuran could be promoted against aphids with their recommendation in rotation to protect their efficiency.

Key words: Neonicotinoids, acetamiprid, imidacloprid, thiamethoxam, thiacloprid, clothianidin, dinotefuran, *Aphis gossypii*, leaf-dip bioassay, LC_{50}

Cotton is an important commercial crop in India and is extensively used in textile industry. India has produced 370 lakh bales of cotton from an area of 124.44 lakh ha during 2018 (Cotton Advisory Board). The production and productivity of cotton has been challenged by many insect pests including the bollworm complex and sucking pests. With the introduction of *Bt* transgenic cotton in India, the bollworm complex is under check. However, dominance of sucking pests on *Bt* cotton is evident and warrants application of insecticides. Farmers use a wide array of insecticides to manage the insect pests ranging from organophosphates to a number of newer class of chemicals such as neonicotinoids. Continuous use of same insecticides had paved way to develop resistance by insects against these insecticides.

The neonicotinoids viz., acetamiprid, clothianidin, dinotefuran, imidacloprid, thiacloprid and thiamethoxam are handy insecticides used by the farmers to mitigate dominance of sucking pests such as aphids, thrips, whiteflies and leafhoppers on cotton (Bass et al., 2015). Insecticide resistance is the major influencing factor for pest management under agro ecosystems. In recent years, many insects such as aphids, whiteflies, planthoppers and also some coleopteran, lepidopteran and dipteran insect species have developed resistance to neonicotinoids worldwide (Bass et al., 2015). The cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), is a cosmopolitan, polyphagous insect and attacks numerous crops including cotton, vegetables, fruits and flowers. Aphids suck the plant sap by their piercing and sucking mouthparts. In addition, it also transmits several viral diseases. Due to its short life cycle and high reproductive capability, *A. gossypii* has high potential to develop resistance against insecticides (Shi, 2012). There are three major detoxification enzymes involved in the development of resistance against insecticides viz., cytochrome P450 monooxygenases, carboxylesterases and glutathione S-transferases (Taniai et al., 2003). *A. gossypii* has developed resistance against neonicotinoids in other countries with continuous usage of these insecticides (Bass et al., 2015). In India, neonicotinoids have been used for nearly three decades and there is often control failure of aphids. It is important to assess the resistance levels to design future steps for sustainable insect pest management. Hence, a study was conducted to determine the resistance levels of *A. gossypii* population collected from ten different locations of Tamil Nadu against six neonicotinoid insecticides.

MATERIALS AND METHODS

Field populations of cotton aphid, *A. gossypii* were collected from ten locations of Tamil Nadu viz., Aruppukottai, Coimbatore, Dindigul, Madurai, Manamadurai, Perambalur, Srivilliputtur, Tirunelveli, Trichy and Tuticorin. The collected insects were reared on ARBH 1401 cotton plants in greenhouse conditions. The susceptible populations were maintained for calibrating discriminate doses without exposing them to pesticides. The cotton seeds were sown in pots in staggered manner at weekly intervals. Aphid populations were continuously reared on potted plants with replacement of plants every four weeks in order to keep cultures for further generations.

Six commercial formulations of neonicotinoids were used (acetamiprid 20 SP, clothianidin 50 WDG, dinotefuran 20 SG, imidacloprid 17.8 SL, thiacloprid 21.7 SC and thiamethoxam 25 WG). The toxicity of neonicotinoids to *A. gossypii* populations from ten different locations was assessed using Insecticide Resistance Action Committee (IRAC) method No. 019 with slight modification to monitor the resistant levels. The six commercial formulations of neonicotinoid were diluted at five to six concentrations with distilled water. Untreated petiolated cotton leaves were dipped in insecticide solutions for ten seconds and allowed to dry on filter paper. The leaves dipped in distilled water served as an untreated control. After drying the leaves, petiole of leaves was inserted into the mouth of glass vial filled with water (petiole reaching the base of the vial) and the vial mouth was sealed with cotton. Forty third instar nymphs from the collected population were transferred on top of the leaf surfaces using camel hair brush. Mortality of aphids was recorded after 72 h and each test was carried out with three replications. The LC_{50} values were computed for different populations to make comparisons among the populations. The corrected mortality was worked out using Abbott's formula (Abbott, 1925). The median lethal concentrations (LC_{50}) for insecticides used were determined manually by Finney's probit analysis (Regupathy and Dhamu, 2001). Resistant ratio (RR) was computed with LC_{50} of resistant population / LC_{50} of susceptible population.

RESULTS AND DISCUSSION

Resistance levels of ten field populations of cotton aphid, *A. gossypii* collected from different locations of Tamil Nadu to neonicotinoids viz., acetamiprid, clothianidin, dinotefuran, imidacloprid, thiamethoxam and thiacloprid using discriminating dose were assessed. Aphid populations showed different degrees of resistance to insecticides (Table 1).

Acetamiprid: LC_{50} values of acetamiprid varied from 0.085 to 0.884 ppm for aphids from ten different locations. LC_{50} was the lowest in Aruppukottai and the highest in Perambalur. Perambalur population had the highest resistance ratio of 68-fold over susceptible population. Perambalur (0.884 ppm), Tuticorin (0.807 ppm), Madurai (0.639 ppm), Dindigul (0.611 ppm) and Manamadurai (0.409 ppm) populations had significantly higher LC_{50} than the susceptible population (0.013 ppm) with resistance ratios of 68.0, 62.0, 49.1, 47.0 and 31.4-fold, respectively. Aphid population collected from Aruppukottai (0.085 ppm), Trichy (0.108 ppm), Tirunelveli (0.131 ppm), Srivilliputtur (0.176 ppm) and Coimbatore (0.359 ppm) had LC_{50} values on par with susceptible population indicating that these populations did not develop resistance to acetamiprid. Koo et al. (2014) stated that Yeoju-A (YJ-A) strain had developed resistance against acetamiprid with the LC_{50} value of 0.52 ppm. Herron and Wilson (2011) found that maximum resistance of acetamiprid against *A. gossypii* was 9-fold in 2007-2008 and 6.4-fold in 2008-2009. Fouad et al. (2016) reported that the susceptible strain of *Aphis craccivora* developed resistance against acetamiprid with 0.369 ppm.

Clothianidin: Aruppukottai population was highly susceptible to clothianidin as indicated from the lowest LC_{50} (0.012 ppm; RR-3.0). A high level of resistance was noticed in Perambalur population with LC_{50} of 0.311 ppm (RR-77.7), followed by Tuticorin (0.242 ppm; RR-60.5) and Madurai (0.169 ppm; RR-42.25). Similar results were obtained by Herron and Wilson (2011) observed that different strains of *A. gossypii* Kat S, Nar and Ewer with LC_{50} values 0.0074, 0.013 and 0.0024 respectively, and resistance ratio was 6-fold.

Dinotefuran: Perambalur population showed highest resistance (0.834 ppm) followed by Tuticorin (0.619 ppm) and Madurai (0.491 ppm) populations. The maximum resistance ratio was 52.13-fold for Perambalur population and minimum of 4.56-fold for Aruppukottai population. Mokbel and Mohamed, (2009) indicated increasing resistance ratios with laboratory selection of *A. craccivora* against dinotefuran with 6.62, 14.34, 42.70 and 148.80-fold after 5th, 10th, 15th and 20th generations, respectively.

Imidacloprid: Perambalur populations of *A. gossypii* showed high resistance *A. gossypii* to

Table 1. Acute toxicity of neonicotinoids to *A. gossypii* (from 10 locations of Tamil Nadu

Insecticide	Population	Slope ± SE	X^2	LC_{50} (95% CL)*	RR
Acetamiprid	Aruppukottai	0.433 ± 0.427	0.31	0.085 (0.012-0.584)^{de}	6.54
	Coimbatore	0.496 ± 0.385	0.21	0.359 (0.063-2.042)^{abcde}	27.62
	Dindigul	0.510 ± 0.382	0.31	0.611 (0.109-3.424)^{abcd}	47.00
	Madurai	0.491 ± 0.395	0.67	0.639 (0.108-3.799)^{abc}	49.15
	Manamadurai	0.467 ± 0.408	0.27	0.409 (0.065-2.571)^{abcd}	31.46
	Perambalur	0.517 ± 0.383	0.61	0.884 (0.157-4.984)^{a}	68.00
	Srivilliputtur	0.491 ± 0.383	0.11	0.176 (0.031-0.990)^{bcde}	13.54
	Tirunelveli	0.449 ± 0.414	0.31	0.131 (0.020-0.847)^{bcde}	10.08
	Trichy	0.446 ± 0.415	0.24	0.108 (0.017-0.708)^{cde}	8.31
	Tuticorin	0.518 ± 0.381	0.56	0.807 (0.144-4.509)^{ab}	62.08
	Susceptible	0.359 ± 0.513	0.54	0.013 (0.001-0.131)^{e}	-
Clothianidin	Aruppukottai	0.499 ± 0.382	0.48	0.012 (0.002-0.069)^{a}	3.00
	Coimbatore	0.412 ± 0.447	0.63	0.092 (0.012-0.688)^{a}	23.00
	Dindigul	0.598 ± 0.322	0.60	0.132 (0.031-0.562)^{a}	33.00
	Madurai	0.619 ± 0.313	0.65	0.169 (0.041-0.695)^{a}	42.25
	Manamadurai	0.526 ± 0.359	0.57	0.125 (0.025-0.630)^{a}	31.25
	Perambalur	0.697 ± 0.289	0.98	0.311 (0.085-1.145)^{a}	77.75
	Srivilliputtur	0.408 ± 0.451	0.65	0.055 (0.007-0.417)^{a}	13.75
	Tirunelveli	0.451 ± 0.412	0.78	0.030 (0.005-0.192)^{a}	7.50
	Trichy	0.522 ± 0.363	0.45	0.021 (0.002-0.062)^{a}	5.25
	Tuticorin	0.708 ± 0.283	0.89	0.242 (0.068-0.868)^{a}	60.50
	Susceptible	0.448 ± 0.432	0.69	0.004 (0.001-0.028)^{a}	-
Dinotefuran	Aruppukottai	0.445 ± 0.415	0.87	0.073 (0.011-0.473)^{a}	4.56
	Coimbatore	0.539 ± 0.355	0.79	0.290 (0.058-1.437)^{a}	18.13
	Dindigul	0.493 ± 0.384	0.85	0.310 (0.055-1.758)^{a}	19.38
	Madurai	0.488 ± 0.392	0.83	0.491 (0.084-2.883)^{a}	30.69
	Manamadurai	0.500 ± 0.382	0.82	0.400 (0.071-2.241)^{a}	25.00
	Perambalur	0.503 ± 0.388	0.72	0.834 (0.144-4.810)^{a}	52.13
	Srivilliputtur	0.534 ± 0.356	0.83	0.223 (0.045-1.112)^{a}	13.94
	Tirunelveli	0.540 ± 0.351	0.82	0.187 (0.038-0.914)^{a}	11.69
	Trichy	0.508 ± 0.369	0.67	0.123 (0.023-0.651)^{a}	7.69
	Tuticorin	0.487 ± 0.395	0.73	0.619 (0.104-3.681)^{a}	38.69
	Susceptible	0.392 ± 0.471	0.80	0.016 (0.002-0.135)^{a}	-
Imidacloprid	Aruppukottai	0.509 ± 0.358	0.83	0.047 (0.009-0.237)^{b}	4.27
	Coimbatore	0.438 ± 0.410	0.84	0.033 (0.015-0.055)^{ab}	3.00
	Dindigul	0.482 ± 0.375	0.77	0.241 (0.044-1.309)^{ab}	21.91
	Madurai	0.517 ± 0.353	0.55	0.406 (0.082-1.997)^{ab}	36.91
	Manamadurai	0.462 ± 0.390	0.82	0.178 (0.031-1.033)^{ab}	16.18
	Perambalur	0.665 ± 0.302	0.47	1.718 (0.440-6.715)^{a}	156.18
	Srivilliputtur	0.425 ± 0.423	0.81	0.111 (0.016-0.748)^{ab}	10.09
	Tirunelveli	0.424 ± 0.423	0.81	0.119 (0.018-0.805)^{ab}	10.82
	Trichy	0.453 ± 0.398	0.87	0.102 (0.017-0.615)^{b}	9.27
	Tuticorin	0.553 ± 0.348	0.66	1.556 (0.323-7.495)^{ab}	141.45
	Susceptible	0.571 ± 0.338	0.99	0.011 (0.002-0.053)^{b}	-

Thiamethoxam	Aruppukottai	0.464 ± 0.408	0.23	0.034 (0.005-0.217)^{ef}	2.62
	Coimbatore	0.488 ± 0.390	0.41	0.208 (0.036-1.212)^{ef}	16.00
	Dindigul	0.523 ± 0.376	0.07	0.656 (0.120-3.576)^{bcd}	50.46
	Madurai	0.638 ± 0.326	0.12	0.982 (0.226-4.276)^{ab}	75.54
	Manamadurai	0.624 ± 0.330	0.07	0.905 (0.204-4.016)^{abc}	69.62
	Perambalur	0.668 ± 0.322	0.12	1.535 (0.358-6.578)^{ab}	118.08
	Srivilliputtur	0.567 ± 0.351	0.30	0.607 (0.124-2.966)^{cde}	46.69
	Tirunelveli	0.483 ± 0.401	0.49	0.569 (0.093-3.475)^{cdef}	43.77
	Trichy	0.472 ± 0.407	0.44	0.485 (0.077-3.051)^{def}	37.31
	Tuticorin	0.564 ± 0.372	0.29	2.184 (0.407-11.72)^{a}	168.00
	Susceptible	0.445 ± 0.429	0.58	0.013 (0.002-0.090)^{f}	-
Thiacloprid	Aruppukottai	0.393 ± 0.467	0.94	0.028 (0.003-0.227)^{ef}	4.00
	Coimbatore	0.442 ± 0.415	0.90	0.165 (0.025-1.075)^{cdef}	23.57
	Dindigul	0.435 ± 0.423	0.96	0.222 (0.033-1.499)^{abcd}	31.71
	Madurai	0.488 ± 0.381	0.87	0.277 (0.050-1.549)^{abc}	39.57
	Manamadurai	0.426 ± 0.430	0.98	0.171 (0.025-1.195)^{bcde}	24.43
	Perambalur	0.490 ± 0.385	0.69	0.521 (0.092-2.959)^{a}	74.43
	Srivilliputtur	0.402 ± 0.454	0.99	0.100 (0.013-0.775)^{cdef}	14.29
	Tirunelveli	0.592 ± 0.318	0.98	0.093 (0.022-0.393)^{def}	13.29
	Trichy	0.391 ± 0.467	0.93	0.059 (0.007-0.485)^{def}	8.43
	Tuticorin	0.461 ± 0.402	0.73	0.318 (0.052-1.952)^{ab}	45.43
	Susceptible	0.434 ± 0.439	0.95	0.007 (0.001-0.053)^{f}	-

*Mean of three replications; mean followed by same letter not significantly different by DMRT ($p \leq 0.05$); SE: Standard Error, LC: Lethal Concentration, RR: Resistance Ratio

imidacloprid with LC_{50} of 1.718 ppm and resistance ratio of 156.18-fold over susceptible population. The LC_{50} of populations from Aruppukottai (0.047 ppm), Coimbatore (0.033 ppm), Dindigul (0.241 ppm), Madurai (0.406 ppm), Manamadurai (0.178 ppm), Srivilliputtur (0.111 ppm), Tirunelveli (0.119 ppm), Trichy (0.102 ppm) and Tuticorin (1.556 ppm) were on par with susceptible population (0.011 ppm) with resistant ratio was 4.27, 3.00, 21.91, 36.91, 16.18, 10.09, 10.82, 9.27 and 141.45-folds, respectively. The present findings are in agreement with El-Kady (2007) who reported that all the four strains of *Aphis gossypii* (Damytta, Dakahlia, Qaliobia and Giza) developed resistance against imidacloprid with LC_{50} of 0.089, 1.244, 1.595 and 0.625 ppm, respectively. Among all the four strains, Qaliobia was found to be most resistant against imidacloprid with (32.5-fold), which is comparable with the LC_{50} of 1.805 ppm (Praveen, 2003) and 1.696 ppm (Suganyakanna, 2006). Zhang et al. (2015) investigated that susceptibility of four field populations of *A. gossypii* against imidacloprid and the population from Xuchang was highly resistant with LC_{50} value of 1.03 ppm and the resistant ratio was 1.20. Toxicity of imidacloprid against pea aphid, *Acyrthosiphon pisum* with a LC_{50} of 0.913 µg/ml (Taillebois et al., 2014).

Thiamethoxam: Tuticorin had the highest resistant ratio (168.00-fold) followed by Perambalur (118.08-fold) and Madurai (75.54-fold). The populations of Dindigul (0.656 ppm), Madurai (0.982 ppm), Manamadurai (0.905 ppm), Perambalur (1.535 ppm), Srivilliputtur (0.607 ppm) and Tuticorin (2.184 ppm) had significantly higher LC_{50} than susceptible population (0.013 ppm). The LC_{50} of Aruppukottai (0.034 ppm), Coimbatore (0.208 ppm), Tirunelveli (0.569 ppm) and Trichy (0.485 ppm) were on par with susceptible population. Their resistant ratio was 2.62-fold, 16.00-fold, 43.77-fold and 37.31-fold, respectively. Gore et al. (2013) examined aphid population of Grenada MS^{c} with LC_{50} value of 2.56 ppm at 72 hr after treatment. Taillebois et al. (2014) found that thiamethoxam was most toxic against pea aphid, *A. pisum* with LC_{50} of 0.259 µg/ml after 24 hours. Toxicity of thiamethoxam against susceptible strain of cowpea aphid, *A. craccivora* with LC_{50} of 0.079 ppm (Fouad et al., 2016).

Thiacloprid: The LC_{50} values of thiacloprid from fields of Perambalur (0.521 ppm), Tuticorin (0.318

ppm), Madurai (0.277 ppm), Dindigul (0.222 ppm) and Manamadurai (0.171 ppm) over susceptible population (0.007 ppm) with resistant ratio of 74.43, 45.43, 39.57, 31.71 and 24.43-folds, respectively. The populations of Aruppukottai (0.028 ppm), Coimbatore (0.165 ppm), Srivilliputtur (0.100 ppm), Tirunelveli (0.093 ppm) and Trichy (0.059 ppm) had LC_{50} values on par with susceptible population. Perambalur population was found to be highly resistant against thiacloprid, followed by Tuticorin and Madurai populations. The results of Koo et al. (2014) showed that the LC_{50} value of thiamethoxam against the YJ-B strain of *A. gossypii* was 1.54 ppm and thiacloprid resistant strains with LC_{50} value of 0.35 ppm and 0.18 ppm for the strains BY-B and YJ-A, respectively.

Among the neonicotinoids cotton aphid populations had shown increased level of resistance to imidacloprid and thiamethoxam. Cotton is cultivated consistently grown at the study locations with regular use of imidacloprid and thiamethoxam over a decade of years and farmers use enhanced doses wherever they face decrease is efficiency of the insecticides. Resultant effect of injudicious use of neonicotinoids is the observed higher levels of resistance.

From the *A. gosspii* management perspective, clothianidin was found to be highly effective followed by thiacloprid, dinotefuran, acetamiprid, imidacloprid and thiamethoxam. Perambalur population had developed high resistance to all the neonicotinoids followed by Tuticorin population. All the populations were found highly resistant against thiamethoxam except the Tuticorin population. The Aruppukottai population was highly susceptible to all the select insecticides. To sustain the use of these effective insecticides in cotton ecosystem, research works need to be focused on the resistant management strategies with alternate methods including judicious use of insecticides with applications based on monitoring the pest population, use of synergists to enhance the toxicity of insecticides, alteration of insecticide applications with unrelated mode of action, spraying of insecticide containing two or more compounds with different modes of action.

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