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Research Article

RESISTANCE TO INSECTICIDES IN DIFFERENT FIELDS POPULATION OF COTTON APHID, *Aphis gossypii* Glover

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Abstract: The resistance to insecticides was assayed for field populations of cotton aphid collected from Bharuch district, Gujarat by leaf dip bio-assay at Main Cotton Research Station, Navsari Agricultural University, Surat during 2019-20. The result indicated that LC_{50} values for acetamiprid 20 SP, thiamethoxam 25 WG, flonicamid 50 WG, imidacloprid 17.8 SL and profenofos 50 EC ranged from 0.001 to 0.003, 0.002 to 0.003, 0.003 to 0.005, 0.002 to 0.005 and 0.025 to 0.049 per cent and LC_{90} values ranged from 0.009 to 0.025, 0.008 to 0.017, 0.015 to 0.023, 0.009 to 0.045 and 0.089 to 0.247 per cent, across the locations respectively. The two insecticides tested *viz*., acetamiprid 20 SP and imidacloprid 17.8 SL were less toxic to across all locations to aphid recording higher LC_{50} and LC_{90} values. The relative toxicity among the insecticides revealed that flonicamid 50 WG, profenofos 50 EC and thiamethoxam 25 WG were highly toxic to aphid population across the locations showed that resistance to these insecticides not much developed against aphid. Jambusar populations were more susceptible to tested insecticides as compared to other locations. The comparison between the LC_{90} values obtained with field recommended rate showed the lowest relative resistance ratio for flonicamid (1.00 to 1.53- fold), profenofos (0.89 to 2.47- fold) and thiamethoxam (1.60 to 3.40- fold) whereas higher ratios for imidacloprid (2.02 to 10.11- fold) and acetamiprid (4.50 to 12.50- fold). There was large variation between the LC_{90} and recommended rate of imidacloprid 17.8 SL and acetamiprid 20 SP across locations showing evolving of low to moderate resistance in aphid population.

Keywords: *Aphid, Aphis gossypii, Bioassay, Cotton, Resistance, Insecticides*
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Introduction

Cotton is the 'King of fibre' popularly known as "White gold", an important cash crop in India. India occupies first place in area and second in production on global basis after China. Gujarat is the largest cotton producing state with 80.96 lakh bales of the total production of the country from approximately 22.57 lakh hectares with 610 kg/ha productivity during 2021-22 [1]. The introduction of *Bt* cotton for commercial cultivation in India during 2002 has become boon to the cotton growing farmers and protected the crops from bollworms damage and saved the seed cotton yield losses. *Bt* cotton provide effective management of bollworm complex but nowadays sucking pests *viz*., aphid, *Aphis gossypii* Glover; leafhopper, *Amrasca biguttula biguttula* Ishida; thrips, *Thrips tabaci* Lindeman and whitefly, *Bemisia tabaci* Gennadius are the major importance in Gujarat [2]. *A. gossypii* is an important sucking pest of *Bt* cotton. It is small, yellow to dark green, adaptable, easily spread, parthenogenesis reproduction, polyphagous and ability to cause serious damage. The economic importance of aphids summarized as; removal of plant sap causes wilting and curling of the leaves, by the toxic action of their salivary secretions, causing galls on leaves, stems or roots, honeydew excretion favours the secondary growth of fungus and young shoots, as plant virus vectors, causing many diseases of plants [3]. Cotton aphid population is increasing in last ten years in worldwide in different crops. The cotton aphid, *A. gossypii*, is one of the most economically important pests in agriculture and has developed different levels of resistance to broad-spectrum insecticides, including organophosphates, pyrethroids, carbamate and neonicotinoids [4]. *A. gossypii* has a high potential for resistance development to insecticides [5]. The first documented evidence of insecticide resistance in *A. gossypii* dates to 1964 when it was resistant to methyl-o-demeton in cotton crops in China [6]. As per the report of Arthropod Pesticide Resistance Database, *A. gossypii* documented total 281 cases of resistance to different insecticides in different crops including cotton [7]. Recently many reports evidenced development of insecticidal resistance in cotton aphid and leafhopper [8].

Keeping this in view, the present investigation was undertaken on resistance to insecticides build up in different field population of aphid in Bharuch district.

Materials and Methods

Collection, transport, multiplication of aphid for bio-assay

Cotton aphid, *A. gossypii* was chosen as the test insect for the experiment. The population of *A. gossypii* was collected from five different location; Amod (21°52'2" N; 72°55'14" W), Bharuch (21°45'30" N; 72°55'59" W), Jambusar (22°04'53" N; 72°45'13" W), Netrang (21°37'26" N; 73°16'53" W) and Valia (21°34'21" N; 73°10'5" W) of Bharuch district which was not treated with insecticides for more than fifteen days. The samplings for field populations of aphid were carried out during the September to October, 2019 when there was sufficient population of aphid from the farmers' fields of five locations. For collecting the samples, infested cotton leaves with aphid colonies at reasonable population pressure (50 to 150/leaf) were plucked and collected in the special plastic bucket (26 cm×30 cm) having 40 mesh wire net fitted window at the whole central periphery to allow air circulation and the mouth of the bucket covered with muslin cloth and tied with rubber band. Such fifteen buckets full of infested aphid collected from one location were brought to the laboratory for further experimentation. The collected aphid samples of each location were reared separately under field caged condition on hybrid, G.Cot.Hy.8 BG II for three generations at Main Cotton Research Station, Surat. One additional plot of similar size was also sown with hybrid, for rearing and maintaining susceptible population under unsprayed condition. The established populations after three generations of each location were utilized for bioassay.

Test insecticides and preparation of insecticidal solution

The commonly used five insecticides, acetamiprid 20 SP, thiamethoxam 25 WG, flonicamid 50 WG, imidacloprid 17.8 SL and profenofos 50 EC were used with eight concentrations in distilled water with three repetitions for bioassay against aphid [Table-1].

Table-1 Insecticides used for cotton aphid, A. gossypii bio-assay

SN	Insecticides	Field dose/ha in 500 litre			Concentration used for bio-assay (%)
		a.i./ ha	Formulation (g or ml)	Field use conc.	
1	Acetamiprid 20 SP	10	50	0.002	0.016, 0.008, 0.004, 0.002, 0.001, 0.0005, 0.00025, 0.00
2	Thiamethoxam 25 WG	25	100	0.005	0.04, 0.02, 0.01, 0.005, 0.0025, 0.00125, 0.000625, 0.00
3	Flonicamid 50 WG	75	150	0.015	0.12, 0.06, 0.03, 0.015, 0.0075, 0.00375, 0.001875, 0.00
4	Imidacloprid 17.8 SL	25	125	0.00445	0.0356, 0.0178, 0.0089, 0.00445, 0.002225, 0.0011125, 0.00055625, 0.00
5	Profenofos 50 EC	500	1000	0.1	0.8, 0.4, 0.2, 0.1, 0.05, 0.025, 0.0125, 0.00

Table-2 Relative resistance ratio based on LC_{50} of insecticides against aphid populations at different locations

SN	Locations	Acetamiprid 20 SP		Thiamethoxam 25 WG		Flonicamid 50 WG		Imidacloprid 17.8 SL		Profenofos 50 EC	
		LC_{50}	RR	LC_{50}	RR	LC_{50}	RR	LC_{50}	RR	LC_{50}	RR
1	Amod	0.002	2.00	0.002	1.00	0.005	1.67	0.003	1.50	0.040	1.60
2	Bharuch	0.002	2.00	0.002	1.00	0.004	1.33	0.002	1.00	0.034	1.36
3	Jambusar	0.001	1.00	0.002	1.00	0.003	1.00	0.002	1.00	0.025	1.00
4	Netrang	0.003	3.00	0.003	1.50	0.005	1.67	0.004	2.00	0.049	1.96
5	Valia	0.003	3.00	0.003	1.50	0.005	1.67	0.005	2.50	0.044	1.76

RR: Resistance Ratio and LC: Lethal Concentration, Note: Relative resistance ratio was estimated considering lowest LC_{50} as susceptible population at respective locations for each insecticides with other remaining locations

Table-3 Relative resistance ratio based on LC_{90} of insecticides at recommended concentration against aphid populations at different locations

SN	Locations	Acetamiprid 20 SP		Thiamethoxam 25 WG		Flonicamid 50 WG		Imidacloprid 17.8 SL		Profenofos 50 EC	
		LC_{90}	RR	LC_{90}	RR	LC_{90}	RR	LC_{90}	RR	LC_{90}	RR
1	Amod	0.017	8.50	0.009	1.80	0.020	1.33	0.018	4.04	0.203	2.03
2	Bharuch	0.015	7.50	0.010	2.00	0.021	1.40	0.017	3.82	0.182	1.82
3	Jambusar	0.009	4.50	0.008	1.60	0.015	1.00	0.009	2.02	0.089	0.89
4	Netrang	0.025	12.50	0.015	3.00	0.023	1.53	0.039	8.76	0.247	2.47
5	Valia	0.021	10.50	0.017	3.40	0.020	1.33	0.045	10.11	0.203	2.03

RR: Resistance Ratio and LC: Lethal Concentration
Note: Relative resistance ratio was estimated considering LC_{90} values of respective locations with recommended conc. of insecticides

The concentrations for each test insecticide rendering mortality between 20 to 80 per cent considered for bio-assays based on pilot scale testing. Insecticide solutions with graded concentration especially in geometrical progression with three lower and higher field recommended doses to get better responses along with no exposure were prepared by serial dilution technique and properly labelled.

Bio-assay for *A. gossypii* to insecticides

The agar beds in petri dishes were prepared and used under bioassay technique followed in the present investigation as recommended by the Insecticide Resistance Action Committee (IRAC) for monitoring of insecticide resistance in aphid [9]. In around two months, the established populations of aphid of one location on hybrid were brought to the laboratory by plucking infested leaves in the plastic buckets from the plot. About fifty apterous cotton aphids were released per healthy leaf of cotton in petri dishes with the help of pointed camel hairbrush. The cut end of the petioles was wrapped immediately with cotton swab moisten with 10 per cent sucrose solution and sealed with parafilm. Amongst selected, three leaves were dipped for 30 second in insecticidal solutions of each concentration for each of the insecticide. A control was run which were sprayed with distilled water. After dipping, each leaf was allowed to naturally shade dry for fifteen minutes under fan and placed individually in the petri dishes (9 cm diameter). Such 24 petri dish, each containing 50 aphids were used for bioassay for single insecticide of one location. For one location, total of 120 sets comprising of five insecticides were kept for observations. Observations on mortality of aphid were recorded at 24 hours interval up to 72 hours after exposure to different test concentrations under laboratory. For flonicamid 50 WG, additionally the mortality counts were recorded up to 120 hours after exposure as per the IRAC method. At every 24 hours, the numbers of dead aphid at the bottom of the petri dish were counted. The aphid which was not unable to right themselves within ten seconds once turned on their back was considered dead. In the event of doubt, the suspected individuals were gently touched using fine camel hair brush and mortality was recorded. At the end of 72 hours, the number of live and dead aphid was counted and the data so obtained for each concentrations including control were subjected to LDP analysis through Polo Leora software. The setup of bioassay was maintained separately for each location. The mortality data of each treatment were corrected with respect to control mortality as per formula for bioassay [10].

Corrected mortality (%): [(T-C)/(100-C)] X 100

Where, T= Per cent mortality in treatment; C= Per cent mortality in the control

Estimation of LC_{50} for susceptibility of insecticide to aphid

The value of median lethal concentration (LC_{50}) for each insecticide was worked out using probit analysis [11] and by computer software Polo Leora software provided earlier under TMC project by ICAR-CICR, Nagpur. Similarly, LC_{90} values of these insecticides against the collected population of cotton aphid were calculated. The LC_{50} and LC_{90} values of each insecticide so obtained through bioassay studies on aphid population collected from different locations were compared.

To find out the resistance build up in aphid population

The relative resistance ratio between cotton aphid populations collected from five different locations was determined for each insecticide using the formula as under whereas insecticide resistance levels were described using RFs [12] as follows: susceptibility (RF=1), decreased susceptibility (RF= between 3-5), low resistance (RF= between 5-10), moderate resistance (RF= between 10-40), high resistance (RF= between 40-160) and very high resistance (RF>160).

Relative Resistance Ratio : LC_{50} of aphid population (Location under study)/LC_{50} of relatively susceptible location aphid population

Results and Discussion

Resistance ratios are useful to monitor the evolution of insecticide resistance in a given field population. Resistance ratio usually estimated by dividing the LC_{50} of field population by the LC_{50} of a susceptible strain. In the absence of baseline susceptible data of tested insecticides against aphid infesting cotton, resistance ratios were estimated from the variability in LC_{50} of respective insecticides in the field population collected from five locations of Bharuch district in Gujarat. The data on the LC_{50} of five different insecticides *viz*., acetamiprid 20 SP, thiamethoxam 25 WG, flonicamid 50 WG, imidacloprid 17.8 SL and profenofos 50 EC against aphid population of five different locations *viz*., Amod, Bharuch, Jambusar, Netrang and Valia are presented in [Table-2] and the population of specific location for each insecticide showing lowest LC_{50} was considered susceptible population and used for comparing and working out the relative resistance ratio across locations for respective insecticides.

The data on the relative resistance ratio of acetamiprid to five different population of *A. gossypii* are presented in the [Table-2]. The results indicated that there has been marked difference in relative resistance ratio among the different location population. Aphid population of Jambusar location showed lowest LC_{50} (0.001%) and the relative resistance ratio with susceptible population of Jambusar showed 2.00- fold (Bharuch and Amod) and 3.00-fold (Netrang and Valia) increased in LC_{50} against aphid population. For thiamethoxam, the aphid population of Jambusar, Bharuch and Amod location showed lowest LC_{50} (0.002%) and the relative resistance ratio with susceptible population showed 1.50- fold to Netrang and Valia population against aphid. Similarly, the aphid population of each of the locations *viz*., Bharuch showed 1.33- fold whereas Amod, Netrang and Valia showed 1.67- fold increase in LC_{50} value of flonicamid against susceptible population of Jambusar (LC_{50}=0.003%). The LC_{50} of imidacloprid for five different population of *A. gossypii* revealed that there was a lowest LC_{50} (0.002%) in aphid population of Bharuch and Jambusar and the relative resistance ratio at LC_{50} calculated against susceptible population was found to be increase by 1.50- fold in Amod, 2.00- fold in Netrang and 2.50- fold in Valia aphid population. For profenofos, aphid population of Jambusar location showed lowest LC_{50} (0.025%) and the relative resistance ratio with susceptible population of Jambusar showed 1.36, 1.60, 1.76 and 1.96 folds increases in LC_{50} in Bharuch, Amod, Valia and Netrang populations, respectively. The present study revealed that the relative resistance ratio for acetamiprid, thiamethoxam, flonicamid, imidacloprid and profenofos varied from 2.00 (Amod and Bharuch) to 3.00 (Netrang and Valia); 1.50 (Netrang and Valia); 1.33 (Bharuch) to 1.67 (Amod, Netrang and Valia); 1.50 (Amod) to 2.50 (Valia) and 1.36 (Bharuch) to 1.96 (Netrang) fold to susceptible population of Jambusar locations having LC_{50} values for acetamiprid, thiamethoxam, flonicamid, imidacloprid and profenofos as 0.001, 0.002, 0.003, 0.002 and 0.025 per cent, respectively. Thus, in the present study, moderate level of resistance was found in field population of cotton aphid against conventional neonicotinoids, acetamiprid 20 SP, imidacloprid 17.8 SL and low level to thiamethoxam 25 WG.

The data on the LC_{90} values of five different insecticides *obtained* with field recommended rate of *viz*., acetamiprid 20 SP, thiamethoxam 25 WG, flonicamid 50 WG, imidacloprid 17.8 SL and profenofos 50 EC against aphid population of five different locations *viz*., Amod, Bharuch, Jambusar, Netrang and Valia as well as relative resistance ratio across locations for respective insecticides are presented in [Table-3]. Among the insecticides, lowest ratio for flonicamid (1.00 to 1.53- fold), profenofos (0.89 to 2.47- fold), thiamethoxam (1.60 to 3.40- fold), imidacloprid (2.02 to 10.11- fold) and acetamiprid (4.50 to 12.50-fold). There was large difference between the LC_{90} and recommended rate of acetamiprid and imidacloprid showing evolving of resistance in aphid populations. Little tolerance to thiamethoxam was also noticed at two out of five locations. The scale of resistance factor or ratio for aphid population collected from across the location showed high susceptibility to flonicamid and profenofos whereas thiamethoxam showed slightly decreased susceptibility. The imidacloprid had developed low resistance while acetamiprid showed moderate resistance to aphid [Table-3].

The result on the development of resistance in cotton aphids to tested insecticides in the present investigation is in accordance with the earlier findings. The resistance factor 1.00 and 1.80- fold were reported to profenophos against MR98 and Navacelles strain, respectively [13]. The R-imidacloprid strain of *A. gossypii* recorded 4.7- fold resistance whereas, 8.1- fold resistance after 16 consecutive generations of selection of cotton aphid to imidacloprid [14-15]. *A. gossypii* developed 3.4 -folds of resistance to acetamiprid [16]. Insecticide resistance determined in cotton aphid to imidacloprid and reported that 1.82 to 32.55- fold resistance to different strain [17]. Analogously, studies on dose-response assay indicated that *A. gossypii* developed strong resistance to imidacloprid and acetamiprid with resistance ratio of 17 to 97 [18]. *A. gossypii* develop moderate resistance (RF= 3.7 to 6.8) to profenofos and monocrotophos [19]. The low resistance develops against profenofos to cotton aphid with resistance factor ranged from 0.30 to 7.90- fold at LC_{50} and 0.50 to 21.00- fold at LC_{90} [20]. *A. gossypii* confirmed acetamiprid resistance at 6.4 folds and further increase up to 22-fold whereas thiamethoxam had 22-fold resistance ratio and suggest that to reduce neonicotinoid selection to prevent or slow any increase in neonicotinoid resistance [21]. The different levels of cross-resistance were noted between imidacloprid and tested neonicotinoid insecticides (no cross-resistance: dinotefuran, thiamethoxam and clothianidin; a 3.68-5.79-fold cross resistance: acetamiprid, nitenpyram and thiacloprid) [5]. The clones of cotton aphids were 1.6-fold less susceptible to imidacloprid than clones of melon aphids [22]. The cotton aphid susceptibility to commercial insecticides and found high levels of resistance to thiamethoxam and the resistance ratio ranged from 0.9 to 562.6 at 48 hrs and 0.9 to 29.1 at 72 hrs [23]. However, *A. gossypii* would require 30.2 to 38.1 generations to obtain 100-fold resistance to imidacloprid under breeding pressure of imidacloprid with 80-90 per cent mortality for each generation of selection [24]. The regional susceptibility to different insecticides against cotton aphid and showed that despite regional differences, the maximum resistance ratio developed in acetamiprid and imidacloprid was 185 to 2600 and 4.3 to 1542, respectively while in case of flonicamid had resistance level ranged from 1 to 6- fold in four strains whereas two strain developed 56 to 206- fold resistance. Most of the population showed low resistance ratios to the pyrethroids compared with the neonicotinoids [25]. Low mortality of *A. gossypii* when tested with thiamethoxam 7.10 to 42.30 per cent and resistance ratio compared with susceptible population ranged from 43 to 253 while for imidacloprid resistance ratio ranged from 43 to 253 [26]. The Osmanabad population of aphids registered 17.39- fold resistance to imidacloprid and it was higher than other field population of aphid. The resistance ratio varied greatly among the population *viz*., imidacloprid 17.8 SL (1.97 to 3.12), imidacloprid 70 WG (4.36 to 6.00), acetamiprid 20 SP (7.61 to 11.76), thiamethoxam 25 WG (1.93 to 3.27) and clothianidin 50 WG (2.06 to 3.20) [27]. Highest levels of resistance to imidacloprid were detected for Sadral RR= 17.17 folds and Jahrom, Kavar, Marvdasht and Sadatshahr resistance ratio were ranged from 3.85 to 7.11 folds [28]. *A. gossypii* population from Yavatmal district recorded highest resistance against acetamiprid (1.05 to 1.48- fold) as compared to Buldana, Wardha, Akola and Amravati districts [29]. Resistance to thiamethoxam in field strain with resistance ratio between 49 to 85 and correlated it with potential field control failure [30]. Resistance factor for thiamethoxam was 5.70 to 65.70 and for imidacloprid were 54.6 to 206.5 from Korkuyu and Kurkculer region, respectively [31]. The probable mechanism for development of resistance in aphids to imidacloprid was also discussed earlier by many scientists. One point mutation was found in the beta1 subunit loop D region of the nicotinic acetylcholine receptor (nAChR) of the imidacloprid resistance strain. They observed R81T point mutation in field populations collected from five regions. The mutation on nAChR perform important role in the neonicotinoid resistance to aphid and make pest control become more difficult [25]. Thus, these results endorse the results of the present findings.

Conclusion

Cotton aphid collected from five locations leading in cotton area in Bharuch district for resistance to acetamiprid, thiamethoxam, flonicamid, imidacloprid and profenofos by using IRAC leaf dip bio-assay during 2019-20. The relative resistance ratio for acetamiprid 20 SP, thiamethoxam 25 WG, flonicamid 50 WG, imidacloprid 17.8 SL and profenofos 50 EC varied from 2.00 (Amod and Bharuch) to 3.00 (Netrang and Valia), 1.50 (Netrang and Valia), 1.33 (Bharuch) to 1.67 (Amod, Netrang and Valia), 1.50 (Amod) to 2.50 (Valia) and 1.36 (Bharuch) to 1.96 (Netrang)- fold to susceptible populations of Jambusar locations having LC_{50} values for acetamiprid, thiamethoxam, flonicamid, imidacloprid and profenofos as 0.001, 0.002, 0.003, 0.002 and 0.025 per cent, respectively. The comparison between the LC_{90} values obtained with field recommended rate showed the lowest relative resistance ratio for flonicamid (1.00 to 1.53- fold), profenofos (0.89 to 2.47-fold) and thiamethoxam (1.60 to 3.40- fold) whereas higher ratios for imidacloprid (2.02 to 10.11- fold) and acetamiprid (4.50 to 12.50-fold). Due to large variation between the LC_{90} and recommended rate of imidacloprid 17.8 SL and acetamiprid 20 SP across locations showing evolving of low to moderate resistance in aphid populations. The information from this study would be helpful for management of *A. gossypii* on cotton growing areas.

Application of research: The information generated through research helpful for the management of aphid in cotton growing areas.

Research Category: Cotton Research

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Study area / Sample Collection: Bharuch district and Main Cotton Research Station, NAU, Surat

Cultivar / Variety / Breed name: *Bt* Cotton hybrids

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

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