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Effect of synergist to breakdown the neonicotinoid resistance in cotton aphid, *Aphis gossypii* (Glover)

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Abstract
Cotton aphid, *Aphis gossypii* is an important sucking pest on cotton and other crops. This insect has developed resistance against insecticides, and neonicotinoids have been widely used for its control for a longer period. *A. gossypii* has shown resistance against imidacloprid and other neonicotinoids. Synergists along with insecticides are useful to break the insecticide resistance in insects. Laboratory and field evaluation was conducted to determine the efficiency of synthetic synergists and plant products as a synergist along with neonicotinoids against *A. gossypii* in cotton at the Agricultural College and Research Institute, Madurai, Tamil Nadu. From laboratory evaluation of synergists, piperonyl butoxide (PBO) and rosemary oil (RO) were the best synergist for acetamiprid, imidacloprid and thiamethoxam with synergism ratio of PBO ranged from 1.27-1.47, 1.16-5.5 and 1.54-4.66 and RO ranged from 0.90-1.04, 1.02-1.12 and 1.02-1.17, respectively. Thiamethoxam + PBO was the most effective treatment with the highest population reduction of aphids (88.22% and 100%), followed by imidacloprid + PBO with 87.17 and 99.74 per cent reduction and acetamiprid + PBO with 86.13 and 97.11 per cent reduction after first and second sprays, respectively over untreated check. Among the plant products tested, thiamethoxam + RO was found most effective after second spray in reducing aphid population with 95.39 % followed by imidacloprid + rosemary oil (91.60%) and acetamiprid + RO (89.45%). Neonicotinoid resistance, if reported, shall be managed using synthetic PBO and or the plant based RO in the field.

Keywords: Synergist, piperonyl butoxide, rosemary oil, neonicotinoids, *Aphis gossypii*

Introduction

Cotton, *Gossypium* spp. is an important commercial crop in India and is extensively used as a raw material in textile industry. India has produced 370 lakh bales of cotton from an area of 124.29 lakh ha with an yield of 506 kg/ha. In Tamil Nadu, cotton was grown in an area of 1.85 lakh hectares with a total production of 5.60 lakh bales and with an average yield of 505.41 kg/ha (Cotton Advisory Board, 2018). The production and productivity of cotton is hampered by many ways and one of the most important factor is the scourge by insect pests. The cotton is devastated by numerous insect pests causing rigorous reduction in the production (Bennett *et al.*, 2004). The sucking insects *viz.*, aphids (*Aphis gossypii* Glover), leafhopper (*Amrasca biguttula biguttula* Ishida), whitefly (*Bemisia tabaci* Gennadius) and thrips (*Thrips tabaci* Lindeman) damage the cotton crop in the early stages and cause both qualitative and quantitative losses (Dhaliwal *et al.*, 2006) [9], while the bollworm complex causes significant damage to the crop in the later stages (Dhawan *et al.*, 1988) [10]. The cotton aphid, *A. gossypii* (Aphididae: Hemiptera) is a serious pest on cotton by sucking sap from the underside of leaves and also by transmission of plant diseases. Aphids also defecate honeydew on the plant surface over which sooty mould develops (Kim *et al.*, 1986) [15], which interferes with the photosynthetic process of leaves. Due to extensive and frequent use of insecticides to manage the pest, it has developed a high level of resistance to numerous commonly used insecticides including organophosphates, organochlorines, carbamates, pyrethroids and neonicotinoids (Herron and Wilson, 2011) [12]. In India, with the introduction of *Bt* cotton in the beginning of the 21st century, imidacloprid and other neonicotinoids are being continuously sprayed to manage the sucking pests (Udikeri *et al.*, 2009) [23]. Synergists are compounds that are either negligibly toxic or non-toxic to insects when applied on independently, but when used in combination with an insecticide, enhance the efficacy of that insecticide (Casida, 1970) [7]. The PBO (Piperonyl butoxide), and DEM (Diethyl maleate) are inhibitors of MFO (Mixed function oxidase) and GST (Glutathione S-transferase), respectively (Abdallah *et al.*, 2016) [2]. Also the both DEF (S, S, S-tributyl phosphorotrithioate) and TPP (Triphenyl phosphate) are inhibitors of carboxylesterases (Wu *et al.*, 2004) [24]. In aphids, neonicotinoid resistance is enzymatically supported with the increased expression of MFO, GST and esterases (Seyedebrahimi *et al.*, 2015).

The present investigation was undertaken to evaluate the effect of synergist with three neonicotinoids against cotton aphid, *A. gossypii* both under laboratory and field conditions.

Materials and Methods

Chemicals and insecticides

Piperonyl butoxide (PBO, 90%), Diethyl maleate (DEM, 97%), Triphenyl phosphate (TPP) were purchased from Sigma-Aldrich. Plant products were Rosemary oil and Lemongrass oil, which were resourced from Bawa Trading Company, Madurai. Insecticides used in this study are shown in Table 1.

Laboratory assay

Collection of *A. gossypii*

Ten field populations of cotton aphid, *A. gossypii* were collected from different locations of Tamil Nadu *viz*., Aruppukottai, Coimbatore, Dindigul, Madurai, Manamadurai, Perambalur, Srivilliputtur, Tirunelveli, Trichy and Tuticorin for assessing the levels of resistance by applying discriminating dose. The same population was used for the current study.

Culturing of *A. gossypii*

The collected aphids were cultured in the greenhouse of insectary, Agricultural College and Research Institute, Madurai on plants of cotton (cultivar ARBH 1401). The plants were grown on coco pith and soil medium with proper fertilizing and watering. The susceptible aphid populations were maintained without exposing them to pesticides for calibrating discriminate doses. The cotton seeds were sown in the pots in a staggered manner at weekly intervals. The populations were continuously reared on potted plants and these plants were replaced every four weeks with new ones in order to keep the cultures for further generations. The individual plants were covered with 100-micron mesh cloth separately in order to prevent the damage of insects other than the test insects.

Synergism bioassay

In laboratory experiment, the synthetic synergists and plant products were tested along with neonicotinoids to check their ability to increase susceptibility of resistant aphid populations using leaf-dip bioassay. The toxicity of synergist to *A. gossypii* populations from ten different locations was assessed using IRAC method No. 019 recommended by Insecticide Resistance Action Committee (IRAC, 2009). The lethal concentration of each insecticide solution was prepared and the synergist was diluted in to five concentrations with distilled water. Fresh cotton leaves with petiole were dipped in insecticide solutions for 10 seconds and allowed to dry on filter paper. The leaves dipped in distilled water served as an untreated control. After drying the petiolated leaves, synergist was sprayed on insecticide dipped leaves and petiole of leaves was inserted into the mouth of glass vial filled with water (the petiole should reach the base of the vial) and the vial mouth was sealed with cotton. Forty *A. gossypii* nymphs from the collected population were transferred using camel hair brush on the top of the leaf surfaces (adaxial). After releasing the aphids, the mortality of population was recorded after 74 h and each test was replicated thrice. Finally, the LC_{50} and LC_{95} values were computed for the different populations to make comparison among the populations. The effective synergists were used in further field testing.

Field evaluation of synergist

The field experiment was conducted at the Agricultural College and Research Institute, Madurai (Latitude: 9.92’ N, Longitude: 78.11’ E) during January 2019 using cotton cultivar (TCH1819) to evaluate the interaction among three neonicotinoids and synergists against the cotton aphid, *A. gossypii* to break down the resistance of the aphid against these insecticides. The experiment was conducted in a randomized block design (RBD) with a plot size of 5 x 4 m replicated three times. The crop was maintained properly by adopting standard agronomic practices except plant protection measures. The details of the treatments are listed in Table 2. Two sprays were given with a Knapsack sprayer® at the recommended doses. First and second sprays were given at 40 and 55 days after sowing (DAS). The assessment of *A. gossypii* populations was done on 3, 5 and 7 days after treatment (DAT). The observations on the population of aphid were made on three leaves / plant, one each from top, middle and bottom strata in five randomly selected tagged plants in each treatment.

Statistical analysis

Lab experiments

The per cent corrected mortality was worked using Abbott’s formula (Abbott, 1925). The median lethal concentrations (LC_{50} and LC_{95}) for insecticides used were determined by Finney’s probit analysis (Regupathy and Dhamu, 2001) [20].

$$\text{Per cent corrected mortality} = \frac{\text{(Per cent test mortality)} - \text{(Per cent control mortality)}}{\text{(100 – Per cent mortality)}}$$

Resistance ratio (RR) and Synergism ratio (SR) was calculated by using formula,

RR = LC_{50} of resistant population /LC_{50} of susceptible population

SR = LC_{50} of insecticide /LC_{50} of insecticide + synergist

Data were analyzed by employing ANOVA and Duncan’s (1995) Multiple Range Test (DMRT) using SPSS (version 15) was applied for comparing the treatment means of LC_{50}.

Field experiments

The data was subjected to statistical analysis after square root transformation of the data. While the significance of difference between the treatment values was compared by LSD at 5 per cent probability. The per cent reduction over untreated check in pest population was calculated by using the following formula (Abbott, 1925).

$$\text{Per cent reduction} = \frac{C - T}{C}$$

Where,

C - Aphid population in control

T - Aphid population in treatment

Results and Discussion

The results of laboratory studies. The LC_{50} (ppm) values of acetamiprid varied from 0.085 to 0.884 ppm for the field collected aphids from ten different locations. The acetamiprid when combined with synergists (PBO, TPP, DEM, RO and LO) resulted in increased toxicity and lowered the LC_{50} which ranged from 0.010-0.636, 0.010-0.670, 0.012-767, 0.012-0.777 and 0.002-0.878, respectively for the synergists (Table 3). For imidacloprid alone the LC_{50} recorded had ranged from 0.011 to 1.718 for the ten different populations of *A. gossypii*. When imidacloprid combined with the synergists PBO, TPP, DEM, RO and LO resulted in 0.002-1.394, 0.006-1.556, 0.008-1.652, 0.010-1.674 and 0.010-1.696, respectively for the synergists (Table 4). The LC_{50} values of thiamethoxam alone ranged from 0.013-2.184 for the ten different populations of *A. gossypii* collected from the cotton field. The synergists PBO, TPP, DEM, RO and LO increased the toxicity of thiamethoxam and the LC_{50} ranged from 0.008-1.422, 0.006-1.646, 0.010-1.869, 0.012-1.919 and 0.011-1.997, respectively (Table 5).

Among the synergists evaluated in the laboratory bioassay, the synthetic synergist, PBO and the plant product, rosemary oil were found to be promising and were selected for field testing along with the neonicotinoid insecticides *viz*., acetamiprid, imidacloprid and thiamethoxam. The synergists PBO, DEM and DEF are known inhibitors of MFO, GST and esterases, enzymes associated with insecticide resistance development in insects, respectively. The specific activity of detoxifying enzymes *viz.*, GST, MFO and carboxylesterases (CarE) were high in resistance populations collected from Perambalur followed by Tuticorin in the present study (data not shown in this paper) and was with 17.01 and 7.62-fold increase over susceptible population for GST, 1003.55 and 651.85 for MFO, 2.64 and 2.48 for CarE, respectively. As there was more MFO expression noticed in the resistant populations in the present study, we used PBO for the field testing. Seyedebrahimi *et al*. (2015) reported that imidacloprid was approximately six times more toxic to *A. gossypii* in the presence of PBO than in the absence of PBO (SR = 5.82). The DEM didn't display any synergism with imidacloprid in resistant population of *A. gossypii* as it is known to be associated with the inhibition of GST enzyme (SR = 1.18). In thiamethoxam resistant populations of *A. craccivora* (Abdallah *et al*., 2016) [2], synergists DEF, PBO and DEM effectively increased thiamethoxam toxicity by registering the ratios of the synergism of 5.58, 2.09 and 2.18 as a result of inhibition of esterases, MFO and GST, respectively. However, Wu *et al*. (2018) [25] found that the MFO activity of thiamethoxam resistant population of *A. gossypii* was inhibited by PBO with synergism ratio of 3.00-fold.

Rosemary oil showed high level of synergistic activity to monocrotophos, quinalphos, carbosulfan and fenvalerate against *Plutella xylostella* (Manoharan *et al*., 2008) [17]. Rosemary oil also exhibited significantly moderate level of synergism with malathion against stored product weevils *Sitophilus oryzae* and *Tribolium castaneum* (Ragavendra *et al*., 2017).

The results of the field experiment conducted to study the effect of synergist with neonicotinoids on the population of *A. gossypii* is presented in Table 6. The results revealed that after the first spray, thiamethoxam + PBO exhibited high level of suppression of *A. gossypii* and was significantly superior over all the other treatments by registering the lowest mean populations of aphids (0.90 number / 3 leaves). The next best treatments were imidacloprid + PBO with a mean population of 0.98 aphids/ 3 leaves. These were followed by acetamiprid + PBO with 1.06 aphids / 3 leaves. These results were in concomitant with Abdallah *et al*. (2016) [2] who found that the black legume aphid, *Aphis craccivora* developed resistance against thiamethoxam with LC_{50} of 3.70 ppm, however when combined with PBO the LC_{50} value decreased to 1.76 ppm. The green peach aphid, *Myzus persicae* moderately resistant clone (5191A) registered a LC_{50} of 31.1 and 19.7 ppm for imidacloprid and thiamethoxam, respectively and PBO synergized the effect of these insecticides with reduced LC_{50} of 1.55 and 1.06, respectively for the same clone of aphid (Bass *et al*. 2011). In yet another study, Chen *et al*. (2015) reported the synergistic effect of PBO with imidacloprid against resistance strain (RF_{75}) of *A. gossypii* where in the LC_{50} of 25.41 ppm reported for imidacloprid got decreased to 7.28 ppm combined. Similarly, in resistant population of *A. gossypii* significant difference between LC_{50} values of imidacloprid (LC_{50} of 673.04 ppm) and PBO + imidacloprid (LC_{50} of 115.01 ppm) was reported (Seyedebrahimi *et al*., 2015). These results revealed that imidacloprid toxicity against resistant population of *A. gossypii* could be enhanced with the presence of PBO.

The results further revealed that treatment of thiamethoxam + rosemary oil showed a mean number of 2.11 aphids/ 3 leaves followed by imidacloprid + rosemary oil (2.69 aphids / 3 leaves), acetamiprid + rosemary oil (3.02 aphids / 3 leaves), thiamethoxam (3.94aphids / 3 leaves), imidacloprid (4.48aphids / 3 leaves), acetamiprid (4.67aphids / 3 leaves), PBO (4.94 aphids / 3 leaves) and rosemary oil (5.26 aphids / 3 leaves). The highest population was recorded in the untreated plots with 7.64 aphids per three leaves.

In case of the second spray, the same trend was followed, where in the least population of aphid was registered in thiamethoxam + PBO with the highest reduction of aphid population was recorded (88.22 and 100% aphid reduction) after first and second sprays respectively, which had significantly differed from all other treatments and was followed by imidacloprid + PBO (0.04 aphids / 3 leaves) with 87.17 and 99.74 per cent reduction and acetamiprid + PBO (0.44 aphids / 3 leaves) with 86.13 and 97.11 per cent reduction after first and second spray, respectively (Table 6).

The next effective treatments were thiamethoxam + RO (0.71 aphids / 3 leaves), imidacloprid + RO (1.29 aphids / 3 leaves), acetamiprid + RO (1.62 aphids / 3 leaves), thiamethoxam (2.54 aphids / 3 leaves), imidacloprid (3.08 aphids / 3 leaves), acetamiprid (3.27 aphids / 3 leaves), PBO (3.54 aphids / 3 leaves) and rosemary oil (4.65 aphids / 3 leaves) with 95.39, 91.60, 89.45, 83.44, 79.92, 78.68, 76.91 and 69.67 per cent reduction of aphid population respectively. The highest population was recorded in the untreated plots (15.32 aphids / 3 leaves) (Table 6).

The neonicotinoid insecticides share the same target site and it may be common that a population of insect resistant to one neonicotinoid shall resist other neonicotinoids. The imidacloprid (neonicotinoid) resistant strain of *A. gossypii* showed resistance to acetamiprid and thiacloprid (Koo *et al*., 2014) [16]. A colony of *Bemisia tabaci* resistant to acetamiprid showed increased resistance to thiamethoxam (Horowitz *et al*., 2004). However, a colony of thiamethoxam resistant (100 fold) strain of *B. tabaci* did not show resistance to acetamiprid and imidacloprid, while another colony which had a 500-fold resistance to thiamethoxam showed a slight-resistance (4 fold) to the other neonicotinoids (Ishaaya *et al*., 2005) [14]. Expression levels of esterase were upregulated significantly in the resistant strain compared to the susceptible strain of the cotton aphid (Pan *et al*., 2015) [18] and resistant populations of the tobacco aphid had approximately 2.5 times greater carboxylesterase activity than the susceptible strain (Harlow and Lampert, 1990) [11]. The GST and MFO apparently had little role in conferring resistance in the selected thiamethoxam resistant *A. craccivora* strain (Abdallah *et al*., 2016) [2] and imidacloprid resistant strains of *A. gossypii* (Koo *et al*., 2014) [16]. Interestingly, the acetylcholine esterase (AChE) activity in the *A. craccivora* resistant strain increased significantly and the neonicotinoid compounds caused an increase in AChE activity (Samson-Robert *et al*., 2014).

Conclusion

Our study suggested that the polyphagous aphid, *A. gossypii* had developed resistance to imidacloprid and other neonicotinoid compounds at the field level and needed to be effectively managed using insecticide resistance management strategies. The use of synergists especially PBO and rosemary oil were able to increase the toxicity of the neonicotinoid insecticides.

Table 1: Detailed information on neonicotinoid insecticides tested against cotton aphid, *A. gossypii* to assess insecticide resistance

Chemical name	Formulation	Tradename	Manufacturer
Acetamiprid	20 SP	Lift	Indofil industries Ltd., Mumbai
Imidacloprid	17.8 SL	Confidor	Bayer CropScience Ltd., Maharashtra
Thiamethoxam	25 WG	Dxtar	Nagarjuna Agrichem Ltd., Hyderabad

Table 2: Detailed information on treatments to evaluate three neonicotinoids and two synergists tested against cotton aphid, *A. gossypii* to assess insecticide resistance in the field

Treatments	Dose (lit^{-1})
Acetamiprid20 SP alone	0.2 g
Acetamiprid20 SP + Piperonyl butoxide (PBO)	0.2 g + 2%
Acetamiprid20 SP + Rosemary oil	0.2 g + 2%
Imidacloprid17.8 SL alone	0.2 ml
Imidacloprid17.8 SL+ Piperonyl butoxide (PBO)	0.2 ml + 2%
Imidacloprid17.8 SL+ Rosemary oil	0.2 ml + 2%
Thiamethoxam25 WG alone	0.2 g
Thiamethoxam25 WG + Piperonyl butoxide (PBO)	0.2 g + 2%
Thiamethoxam25 WG + Rosemary oil	0.2 g + 2%
Piperonyl butoxide	2%
Rosemary oil	2%
Untreated control	-

Table 3: Laboratory bioassay on toxicity of synergists with insecticide acetamiprid combinations against cotton aphid, *A. gossypii* populations from different locations of Tamil Nadu

Populations	Acetamiprid		Acetamiprid + PBO		Acetamiprid + TTP		Acetamiprid + DEM		Acetamiprid + RO		Acetaniprid + LO	
	LC_{50} (ppm)	RR (fold)	LC_{50} (ppm)	SR	LC_{50} (ppm)	SR	LC_{50} (ppm)	SR	LC_{50} (ppm)	SR	LC_{50} (ppm)	SR
Aruppukottai	0.085	6.54	0.058	1.47	0.068	1.25	0.076	1.12	0.081	1.05	0.082	1.04
Coimbatore	0.359	27.62	0.282	1.27	0.287	1.25	0.305	1.18	0.313	1.15	0.342	1.05
Dindigul	0.611	47.00	0.380	1.61	0.406	1.50	0.481	1.27	0.520	1.18	0.580	1.05
Madurai	0.639	49.15	0.484	1.32	0.543	1.18	0.583	1.10	0.605	1.06	0.627	1.02
Manamadurai	0.409	31.46	0.320	1.28	0.367	1.11	0.376	1.09	0.382	1.07	0.398	1.03
Perambalur	0.884	68.00	0.636	1.39	0.670	1.32	0.767	1.15	0.777	1.14	0.878	1.01
Srivilliputtur	0.176	13.54	0.136	1.29	0.149	1.18	0.162	1.09	0.167	1.05	0.172	1.02
Tirunelveli	0.131	10.08	0.096	1.36	0.100	1.31	0.115	1.14	0.120	1.09	0.125	1.05
Trichy	0.108	8.31	0.083	1.30	0.101	1.07	0.103	1.05	0.104	1.04	0.107	1.01
Tuticorin	0.807	62.08	0.562	1.44	0.769	1.05	0.772	1.05	0.894	0.90	1.198	0.67
Susceptible	0.013	-	0.010	1.30	0.010	1.30	0.012	1.08	0.012	1.08	0.002	6.50

Table 4: Laboratory bioassay on toxicity of synergists with insecticide imidacloprid combinations against cotton aphid, *A. gossypii* populations from different locations of Tamil Nadu

Populations	Imidacloprid		Imidacloprid + PBO		Imidacloprid + TPP		Imidacloprid + DEM		Imidacloprid + RO		Imidacloprid + LO	
	LC_{50} (ppm)	RR	LC_{50} (ppm)	SR	LC_{50} (ppm)	SR	LC_{50} (ppm)	SR	LC_{50} (ppm)	SR	LC_{50} (ppm)	SR
Aruppukottai	0.047	4.27	0.029	1.62	0.035	1.34	0.043	1.09	0.045	1.04	0.046	1.02
Coimbatore	0.033	3.00	0.014	2.36	0.024	1.38	0.029	1.14	0.030	1.10	0.032	1.03
Dindigul	0.241	21.91	0.207	1.16	0.214	1.13	0.223	1.08	0.230	1.05	0.237	1.02
Madurai	0.406	36.91	0.302	1.34	0.383	1.06	0.388	1.05	0.393	1.03	0.396	1.03
Manamadurai	0.178	16.18	0.119	1.50	0.150	1.19	0.162	1.10	0.169	1.05	0.172	1.03
Perambalur	1.718	156.18	1.394	1.23	1.556	1.10	1.652	1.04	1.674	1.03	1.696	1.01
Srivilliputtur	0.111	10.09	0.031	3.58	0.095	1.17	0.107	1.04	0.109	1.02	0.111	1.00
Tirunelveli	0.119	10.82	0.063	1.89	0.083	1.43	0.103	1.16	0.108	1.10	0.111	1.07
Trichy	0.102	9.27	0.043	2.37	0.080	1.28	0.095	1.07	0.098	1.04	0.100	1.02
Tuticorin	1.556	141.45	1.072	1.45	1.220	1.28	1.355	1.15	1.393	1.12	1.547	1.01
Susceptible	0.011	-	0.002	5.5	0.006	0.00	0.008	0.00	0.010	1.1	0.10	0.00

Table 5: Laboratory bioassay on toxicity of synergists with insecticide thiamethoxam combinations against cotton aphid, *A. gossypii* populations from different locations of Tamil Nadu

Populations	Thiamethoxam (THM)		THM + PBO		THM + TPP		THM + DEM		THM + RO		THM + LO	
	LC_{50} (ppm)	RR	LC_{50} (ppm)	SR	LC_{50} (ppm)	SR	LC_{50} (ppm)	SR	LC_{50} (ppm)	SR	LC_{50} (ppm)	SR
Aruppukottai	0.034	2.62	0.013	2.62	0.026	1.31	0.029	1.17	0.030	1.13	0.032	1.06
Coimbatore	0.208	16.00	0.112	1.86	0.165	1.26	0.190	1.09	0.196	1.06	0.205	1.01
Dindigul	0.656	50.46	0.408	1.61	0.590	1.11	0.625	1.05	0.643	1.02	0.647	1.01
Madurai	0.982	75.54	0.647	1.52	0.834	1.18	0.952	1.03	0.967	1.02	0.972	1.01
Manamadurai	0.905	69.62	0.456	1.98	0.745	1.21	0.852	1.06	0.890	1.02	0.892	1.01
Perambalur	1.535	118.08	0.652	2.35	0.839	1.83	1.213	1.27	1.397	1.10	1.500	1.02
Srivilliputtur	0.607	46.69	0.355	1.71	0.405	1.50	0.579	1.05	0.585	1.04	0.597	1.02
Tirunelveli	0.569	43.77	0.237	2.40	0.383	1.49	0.501	1.14	0.519	1.10	0.547	1.04
Trichy	0.485	37.31	0.104	4.66	0.340	1.43	0.386	1.26	0.415	1.17	0.463	1.05
Tuticorin	2.184	168.00	1.422	1.54	1.646	1.33	1.869	1.17	1.919	1.14	1.997	1.09
Susceptible	0.013	-	0.008	1.62	0.006	0.00	0.010	0.00	0.012	1.08	0.011	0.00

LC – Lethal concentration, PBO (Piperonyl butoxide), RO (Rosemary oil)
Resistance ratio (RR) = LC_{50} of resistance population/LC_{50} of susceptible population
Synergism ratio (SR) = LC_{50} of insecticide/LC_{50} of insecticide + synergist

Table 6: Field evaluation of efficacy of synergists with neonicotinoid insecticides in combinations on cotton against cotton aphid, *A. gossypii* at Madurai, Tamil Nadu during February-April, 2019

Treatments	Population of aphid/leaves*											
	1st Spray						2nd Spray					
	Precount	3 DAS	5 DAS	7 DAS	Grand Mean	% reduction over control	Precount	3 DAS	5 DAS	7 DAS	Grand Mean	% reduction over control
Acetamiprid	6.54 (2.65)	4.82 (2.31)^{g}	4.70 (2.28)^{g}	4.49 (2.23)^{f}	4.67 (2.27)^{h}	38.87	6.26 (2.60)	3.77 (2.07)^{g}	3.25 (1.94)^{fg}	2.78 (1.81)^{g}	3.27 (1.94)^{h}	78.68
Acetamiprid + PBO	5.64 (2.48)	1.21 (1.31)^{a}	1.09 (1.26)^{a}	0.88 (1.17)^{a}	1.06 (1.25)^{b}	86.13	5.84 (2.52)	0.43 (0.96)^{b}	0.00 (0.71)^{bc}	0.90 (1.18)^{c}	0.44 (0.97)^{b}	97.11
Acetamiprid + RO	5.79 (2.51)	3.17 (1.92)^{d}	3.05 (1.88)^{d}	2.84 (1.83)^{d}	3.02 (1.88)^{e}	60.47	6.00 (2.55)	2.12 (1.62)^{e}	1.60 (1.45)^{c}	1.13 (1.28)^{d}	1.62 (1.45)^{e}	89.45
Imidacloprid	6.94 (2.73)	4.63 (2.26)^{f}	4.51 (2.24)^{f}	4.30 (2.19)^{f}	4.48 (2.23)^{g}	41.36	6.17 (2.58)	3.58 (2.02)^{g}	3.06 (1.89)^{e}	2.59 (1.76)^{f}	3.08 (1.89)^{g}	79.92
Imidacloprid + PBO	6.13 (2.57)	1.13 (1.28)^{a}	1.01 (1.23)^{a}	0.80 (1.14)^{a}	0.98 (1.22)^{ab}	87.17	5.18 (2.38)	0.12 (0.79)^{a}	0.00 (0.71)^{a}	0.00 (0.71)^{a}	0.04 (0.73)^{a}	99.74
Imidacloprid + RO	5.88 (2.53)	2.84 (1.83)^{c}	2.72 (1.79)^{c}	2.51 (1.73)^{c}	2.69 (1.79)^{d}	64.79	5.79 (2.51)	1.79 (1.51)^{d}	1.27 (1.33)^{b}	0.80 (1.14)^{c}	1.29 (1.34)^{d}	91.60
Thiamethoxam	6.57 (2.66)	4.09 (2.14)^{e}	3.97 (2.11)^{e}	3.76 (2.06)^{e}	3.94 (2.11)^{f}	48.41	6.07 (2.56)	3.04 (1.88)^{f}	2.52 (1.74)^{d}	2.05 (1.60)^{e}	2.54 (1.74)^{f}	83.44
Thiamethoxam + PBO	6.87 (2.71)	1.05 (1.24)^{a}	0.93 (1.20)^{a}	0.72 (1.10)^{a}	0.90 (1.18)^{a}	88.22	5.05 (2.36)	0.00 (0.71)^{a}	0.00 (0.71)^{a}	0.00 (0.71)^{a}	0.00 (0.71)^{a}	100.00
Thiamethoxam + RO	6.84 (2.71)	2.26 (1.66)^{b}	2.14 (1.62)^{b}	1.93 (1.56)^{b}	2.11 (1.62)^{c}	72.38	5.46 (2.44)	1.21 (1.31)^{c}	0.69 (1.09)^{a}	0.22 (0.85)^{b}	0.71 (1.10)^{c}	95.39
Piperonyl butoxide	6.45 (2.64)	5.09 (2.36)^{h}	4.97 (2.34)^{h}	4.76 (2.29)^{g}	4.94 (2.33)^{i}	35.34	6.29 (2.61)	4.04 (2.13)^{h}	3.52 (2.00)^{h}	3.05 (1.88)^{h}	3.54 (2.01)^{i}	76.91
Rosemary oil	6.64 (2.67)	5.41 (2.43)^{i}	5.29 (2.41)^{i}	5.08 (2.36)^{h}	5.26 (2.40)^{j}	31.15	6.47 (2.64)	5.15 (2.38)^{i}	4.63 (2.26)^{i}	4.16 (2.16)^{i}	4.65 (2.27)^{j}	69.67
Control	6.41 (2.63)	6.58 (2.66)^{j}	7.73 (2.87)^{j}	8.60 (3.02)^{i}	7.64 (2.85)^{k}		10.41 (3.30)	13.54 (3.75)j	15.47 (4.00)^{j}	16.94 (4.18)^{j}	15.32 (3.98)^{k}	
SEd		0.082	0.0792	0.102	0.058			0.132	0.048	0.145	0.068	
CD (0.05)		0.171	0.164	0.212	0.121			0.274	0.099	0.300	0.141	

*Mean of three replications, Figures in parentheses are square root transformed value, Mean followed by same letter do not significantly differ from each other by LSD@0.05 %

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