Current status of insecticide resistance in *Aphis gossypii* and *Aphis spiraecola* (Hemiptera: Aphididae) under central Indian conditions in citrus

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Current status of insecticide resistance in *Aphis gossypii* and *Aphis spiraecola* (Hemiptera: Aphididae) under central Indian conditions in citrus

A. George¹, C. N. Rao¹ and S. Rahangadale¹

**Abstract:** Most commonly followed practice to manage sucking insect pests during flushing season in citrus by citrus growers is foliar application of insecticides. Leaf dip bioassays for insecticides, viz., acephate 75 SP, dimethoate 30 EC, quinalphos 25 EC, chlorpyriphos 20 EC, spinosad 45 SC, imidacloprid 17.8 SL and thiamethoxam 25 WG, were conducted to assess the susceptibility of field collected *Aphis gossypii* and *Aphis spiraecola* (Hemiptera–Aphididae) adults. In general, the resistance ratio (RR) values indicated that the current levels in aphid population from major citrus belts of Maharashtra are in very low resistance category. Among seven insecticides tested on adult aphids collected from Nagpur, Amravati and Wardha districts during 2013–2015, neonicotinoid group proved the most effective in causing mortality as indicated by the lower LC₅₀ values (0.01–0.04 ppm for imidacloprid; 0.03–0.05 ppm for thiamethoxam). Among the three locations, Amravati population has registered comparatively higher RR values indicating less susceptibility to insecticides. RR values calculated based on the base population indicated that the current resistance levels are between no and very low levels (1 < RR < 10).

**ABOUT THE AUTHORS**

Anjitha George, PhD (Entomology), is currently working as scientist involved in research focused on management of insect pests in citrus-growing belts of India. She is currently working on management of fruit sucking moths which pose serious economic losses due to fruit drop.

C.N. Rao, PhD (Entomology), is also working at ICAR-Central Citrus Research Institute as principal scientist and has wide knowledge and experience in the field of integrated pest management, biological control and insecticides.

Sandeep Rahangdale, who worked as senior research fellow, has technical knowledge in biochemical mechanisms of insecticide resistance.

**PUBLIC INTEREST STATEMENT**

We have tried to collect adult aphid populations from major citrus growing belts of central India to study the current status of insecticide resistance to the commonly used insecticides by the citrus growers. Frequency of insecticide sprays using these molecules was intense that there were discrepancies about the utility of particular group of insecticides. In this context, less response or less effectiveness of any molecule may be attributed to either insecticide resistance development or usage of spurious chemistries. Hence, laboratory bioassays were conducted on field population of aphids to find out the reason for the same. It was observed that among the three locations, Amravati location had registered comparatively higher resistance values indicating less susceptibility to insecticides. Resistance ratio (RR) values calculated based on the base population indicated that the current insecticide resistance levels were between no and very low levels (1 < RR < 10).
1. Introduction

In citrus, around 25 aphid species have been reported globally (Viggiani, 1988), of which predominant 4 species include Aphis gossypii Glover, Toxoptera citricida (Kirkaldy), Toxoptera aurantii (Boyer de Fonscolombe) and Aphis spiraecola Patch (Komazaki, Sakagami, & Korenaga, 1979). Aphis gossypii (Hemiptera – Aphididae) commonly known as cotton or melon aphid is one of the major sucking insect pests of citrus apart from being a vector for citrus tristeza virus (CTV) throughout the world (Blackman & Eastop, 1985). Spirea aphid, Aphis spiraecola Patch, is a moderately polyphagous species with citrus and apple as major crop hosts (Blackman & Eastop, 2000). It also transmits a wide range of viruses, including CTV, citrus psorosis virus, cucumber mosaic virus, papaya ringspot virus and so on (Blackman & Eastop, 2000). Its role as a major vector of CTV has been described in Spain (Pena Martinez, Villegas Jimenez, Lomeli Flores, & Trejo Loyo, 2004), Florida (Powell et al., 2005) and India (Naidu, 1980).

Direct damage is through feeding which can kill the host, apart from reduced productivity before plant dies (Andrews & Kitten, 1989; Cartwright, 1992). Adults and nymphs of aphids suck the sap from tender leaves and shoots resulting in devitalization of the plants. Affected leaves get deformed and finally the growth of young shoots is reduced. Blossoms and newly set fruits are also attacked. Also, heavy aphid populations at bloom time have been associated with reduced fruit set.

Aphid population peaks in January–February and June–July on citrus orchards in India and is kept at low level by hot weather and increased activity of the natural enemies such as lady bird beetle, syrphids and lacewing larvae. Aphid incidence on pre-bearing Citrus reticulata Blanco in central India coincides with new flush of monsoon flowering (June–July) and continues till September. In October, the incidence increases once again continues till December and later enters into overwintering. In general, the most favorable period for aphid colonization on citrus plants is from November to February months (Chavan & Singh, 2005).

Mostly asexual reproduction with either alate or apterous females is observed in A. gossypii but exhibits an anholocyclic life cycle in warmer environments and either a heteroecious or autoecious holocyclic life cycle in cooler areas (Slosser, Pinchak, & Rummel, 1989; Zhang & Zhong, 1990). Aphids with heteroecious life cycle migrate from a primary host to a secondary host during spring and return to a primary host in the fall for egg laying. The aphid has the ability to become resistant to many pesticides which is a growing concern due to its adverse environmental impacts. In citrus, Aphis craccivora, A. gossypii, A. fabae, A. nerii, Acrhythosiphon pisum (Harris), Myzus persicae (Sulzer) and T. citricida are found to transmit both mild and severe strains of tristeza virus (Sharma, 1989). A. gossypii transmits this virus semi-persistently, remaining infectious for over 24 h (Bar-Joseph, Marcus, & Lee, 1989). Different strains of A. gossypii did not differ in their ability to transmit citrus tristeza, but different strains of the virus differed in their transmission rates by this aphid (Raccach, Loenenstein, & Singer, 1980). The aphid acquires the virus more easily from some citrus cultivars than from others. The acquisition period can be 5 min but was more efficient at periods of 30 min–24 h. Thus, it is observed that A. gossypii was able to transmit the virus to certain cultivars more efficiently than to others (Roistacher & Bar-Joseph, 1984).

In the past, this aphid has been controlled with a wide array of insecticides. The growing concern over the use of pesticides is a major theme due to environmental contamination and the economic impact of pesticide resistance. In view of large infestation due to this pest complex and the huge losses caused by them, this must be kept below economic threshold level (ETL). Currently, aerial
application of conventional aphicides like dimethoate, methyl demeton and newly introduced acetamiprid and imidacloprid (17.8 SL) from the neonicotinoid group was found unable to provide satisfactory control of *A. gossypii* on cotton. *A. spiraecola* has become resistant to a number of insecticides, including pirimicarb (Benfatto, Gafa, & Giudice, 1970; Hong, Han, & Boo, 2003). Song, Oh and Motoyama (1995) investigated the mechanism of tolerance to organophosphorus insecticides, after resistance to this group of insecticides was found in Korea. Several reports indicate that the cotton aphid has developed a high resistance to several commonly used insecticides in many agricultural areas, including organophosphorus, carbamates, pyrethroids and neonicotinoids (Wang, Guo, Xia, Wang, & Lu, 2007; Wang, Liu, Yu, Jiang, & Yi, 2002). Aphids being a polyphagous pest, our aim was to assess the current insecticide resistance levels in *A. gossypii* and *A. spiraecola* population from major citrus belts in central India.

2. Material and methods

2.1. Collection of field populations of aphids

The field populations of *A. gossypii* and *A. spiraecola* were collected from three districts of Vidarba region, viz., Nagpur (21°22'27"N, 78 89'E), Amravati (21°30'14"N 78 13'E) and Wardha (21°26'13"N 78 56'E) of Maharashtra. The heavily infested shoots of *C. reticulata* along with aphid colonies were collected and carried to laboratory and used on the same day for bioassay.

2.2. Multiplication of susceptible population of cotton aphids

*A. gossypii*- and *A. spiraecola*-infested Nagpur mandarin twigs were collected from the young Nagpur mandarin orchards for the establishment of susceptible culture, where test insect population was not previously exposed to any insecticides. The collected aphid populations were released on *C. reticulata* budlings maintained in the screen house entomology at ICAR-Central Citrus Research Institute (21°14N, 79°08E) for further multiplication. Aphid populations from a single parthenogenetic female (apterous forms) thus reared for four generations were used as susceptible strain for comparison of insecticide resistance development in the field populations of aphids.

2.3. Insecticides

Bioassays were conducted using commercial formulations of insecticides, viz., of acephate 75 SP (Starthane; Swal Co. Ltd.), dimethoate 30 EC (Rogor; Insecticides India Ltd., Wadi, Maharashtra), quinalphos 25 EC (Ekalux; Syngenta India Limited, Chinchbhuwan, Maharashtra), chlorpyriphos 20 EC (Hilban; Hindustan Insecticides Limited, Abids, Hyderabad), spinosad 45 SC (Spintor; Dow Agrosciences India Private Limited, Mumbai), imidacloprid 17.8 SL (Confidor; Bayer Crop Science Ltd., Indore, Madhya Pradesh) and thiamethoxam 25 WG (Actara; Syngenta Crop Protection Inc., Chinchbhuwan, Maharashtra).

2.4. Insecticide bioassay

The bioassay technique with leaf dip assay on agar beds as prescribed by Insecticide Resistance Action Committee (IRAC no.19) was followed in the present investigation. Preliminary range fixing tests were done based on the base population to fix the dose range which caused 20%–80% mortality. Tender *C. reticulata* leaf disks (40 mm diameter) were dipped in five different doses of each test insecticides in geometric progression prepared through serial dilution with double distilled water along with a control (leaves dipped in double-distilled water); replicated four times (40 aphids/replication). After air drying for 1 h, leaf disks were placed in 5-cm plastic petri dishes lined with solidified agar (3–5 ml of 1.5% agar). Petri dishes were sealed with parafilm and kept in an environmental chamber (Remi Instruments Limited, New Delhi) at 24°C ± 2°C and 65% ± 5% RH (Relative Humidity). Mortality observations on aphids were taken 24 h after treatment (HAT) and moribund thrips confirmed with the help of a Weldon water color brush no. 2.

2.5. Protein estimation

For protein estimation by Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951), 0.2 ml of bovine serum albumin (BSA) was used as a working standard in five test tubes and made up to
1 ml using distilled water, while test tube with 1 ml distilled water served as blank. To this mixture, 4.5 ml of Reagent I (48 ml of 2% Na₂CO₃ in 0.1N NaOH, 1 ml of 1% NaK tartrate in H₂O, 1 ml 0.5% CuSO₄, 5 H₂O in H₂O) was added and incubated for 10 min. After incubation, 0.5 ml of Reagent II (1 part folin-phenol [2N]:1 part water) was added and incubated for 30 min. The absorbance at 660 nm was measured, and the amount of protein present in the given sample from the plotted standard graph was estimated.

2.6. Glutathione S-transferase assay
Glutathione S-transferase (GST) activity was measured using 1-chloro-2,4-dinitrobenzene (CDNB) (Sigma Aldrich, Mumbai) as the substrate (Tiwari, Pelz-Stelinski, Mann, & Stelinski, 2011). Six aliquots of the enzyme solution (10 μl), 2 μl of 200 mM CDNB and 188 μl of 10.35 mM glutathione (GSH) in phosphate buffer (0.1M; pH 7.5; pH 7.5) were pipetted into 96-well microplate. Changes in absorbance were measured continuously for 1 min at 340 nm, and 25°C was used to determine GST activity. Control wells consisted of 2 μl of CDNB, 188 μl of GSH and 10 μl of phosphate buffer (0.1 M; pH 7.5; pH 7.5). Changes in absorbance per minute were converted into micromole of CDNB conjugated per minute per milligram of protein using the extinction coefficient of the resulting S-(2,4-dinitrophenyl)-GSH: ε 340 nm = 9.6/nM/cm.

The enzyme activity was calculated as follows: CDNB-GSH conjugate formed in micromoles per minute per milligram of protein = (ABS (increase in 5 min) × 3 × 1000)/(9.6 × 5 × protein in milligram)

where 9.6 is the difference in the millimolar extinction coefficient between CDNB-GSH conjugate and CDNB.

2.7. Acetylcholinesterase assay
For insensitive acetylcholinesterase (AChE) enzyme assay (Brogdon, Hobbs, St Jean, Jacques, & Charles, 1988), a total of six replicates of homogenate (50 ml) were filled in the 96 wells for homogenization in potassium phosphate buffer; centrifuged at 14,000 rpm for 10 min at 4°C. A 50 ml of reaction mixture containing 10% acetone buffer solution of 2.6 mM acetylthiocholine iodide (ACTHI), 0.3 mM of 5,5′-dithio-bis-(2-nitrobenzoic acid) (DTNB) and 0.1% propoxur inhibitor was added into each well. As positive control, a 50 ml of reaction mixture without inhibitor was used. The reaction was incubated at room temperature (28°C) for 30 min, followed by the measurement of optical density at 412 nm. The contents of the sample cuvette were replaced with enzyme sample, and the increase in absorbance in the sample cuvette was recorded at 412 nm for 30 min against the blank.

AChE activity in mole per minute per milliliter of enzyme = (ΔE × 1000 × 3.0)/(1.36 × 10^4 × 0.10)

where ΔE is change in absorbance per minute, 1.0 is the total volume of reaction mixture (ml), 0.1 is the volume of enzyme (ml), 1000 is the factor to obtain mole, and 1.36 × 10^4 is the molar extinction coefficient of the chromophore at 412 nm.

2.8. Data analysis
Abbott’s (1925) formula was used for correction of mortality data, followed by Probit analysis (Finney, 1971) using POLO-PC (LeOra Software, 1987), with a type 1 error rate of α = 0.05. Pair-wise comparison with LC₅₀ values was made at the 1% significance level where individual 95% confidence interval (CI) for two treatments did not overlap, and a chi-square goodness-of-fit test was used to test the significance of the Probit regression. Resistance ratio (RR) was calculated as a measure of resistance in the test population compared to that of the LD₅₀ of the susceptible population (LC₅₀ in the case of our study). RRs were considered significantly different from 1 (P < 0.05) when their 95% CIs did not include 1. Levels of resistance (RR) were categorized as 1: no resistance, 2–10: very low resistance, 11–20: low resistance, 21–50: moderate resistance, 51–100: high resistance and >100: very high resistance (Ahmad, Arif, & Ahmad, 2009; Kaufman, Nunez, Mann, Christopher, & Scharfa, 2010; Robertson, Russell, Priesler, & Savin, 2007).
3. Results
Our present investigation updates the current insecticide resistance levels for Aphis sp. populations to commonly used insecticides to verify the reasons for reduced effectiveness of insecticides from three geographically separated aphid populations during 2013–2015 (Tables 1-6).

3.1. Dimethoate
Three aphid populations of A. gossypii and A. spiraecola were tested against dimethoate. Resistance values for A. gossypii were 2.4-, 3.2- and 2.2-fold and 2.2-, 2.8- and 2.6-fold in A. spiraecola from Nagpur, Amravati and Wardha, respectively, during 2015. The insecticide treatments on field population were significantly different over three years in all the locations for A. gossypii and in 2015 for A. spiraecola in Nagpur and 2014–2015 in Amravati and Wardha districts as indicated by the non-overlap of 95% CI. Among the three locations, RR values were highest in Amravati, that is, 1.0–3.2 for A. gossypii and 1.0–2.8 for A. spiraecola.

3.2. Acephate
Among the three A. gossypii field populations tested against acephate, higher LC\textsubscript{50} value of 0.32, 0.34 and 0.28 ppm was observed in aphid population in 2015 from Nagpur, Amravati and Wardha, respectively, all the three population being significantly different from the susceptible population (due to non-overlap of 95% CI). Highest RR of 4.2 was observed in A. gossypii population from Warud region of Amravati district among the three field-collected ones in 2015. In the case of A. spiraecola, LC\textsubscript{50} value of Wardha population was on higher side, that is, 0.32 ppm when compared to Nagpur population (0.24 ppm) in 2015.

3.3. Quinalphos and chlorpyriphos
We included quinalphos 25 EC and chlorpyriphos 20 EC at a later stage in this study as they are locally available and preferred by nursery growers in Vidarbha region. RR values were in general in very low range as to 2.6, 2.3, 2.2 in Nagpur, Amravati and Wardha, respectively, with all the three population being significantly different from the susceptible population (due to non-overlap of 95% CI). However, the LC\textsubscript{50} values significantly differed in both quinalphos and chlorpyriphos treatments over the years as indicated by the non-overlap of 95% CI. But RR values were on higher side for A. spiraecola, that is, 2.3–2.5 and 2.5–2.8 for quinalphos and chlorpyriphos, respectively, from the three locations under monitoring.

3.4. Spinosad
Aphid adults collected from Nagpur were found to be relatively susceptible as indicated by close LC\textsubscript{50} values of 0.10–0.11 ppm, and current resistance in A. gossypii is only 1.2-fold when compared to susceptible culture. Among the three locations, Wardha population recorded RR value of 1.7 in 2015, and the insecticide effect was significantly different over field and susceptible strain as indicated by the non-overlap of CI in 2014 and 2015. There was no significant difference in RR values (1.4-fold) from the three locations in the case of A. spiraecola.

3.5. Imidacloprid and thiamethoxam
At present, we found that insecticide resistances in A. gossypii and A. spiraecola to neonicotinoids are in very low levels (RR < 10). In the case of imidacloprid, aphid population has registered higher values when compared to thiamethoxam from Nagpur and Amravati locations, but in Wardha, RR values to both insecticides are on higher side, indicating an increasing trend of resistance in the target pest.

3.6. Biochemical assays for citrus aphids
To confirm the underlying mechanism of insecticide resistance in aphid adult population from three locations, biochemical assays were also performed during 2015–2016 on A. gossypii and A. spiraecola. High levels of GST (0.195 \text{μmol/min/mg}) in A. gossypii population were collected from Amravati area followed by Nagpur population (0.175 \text{μmol/min/mg}) and susceptible population (0.109 \text{μmol/min/mg}). Similarly, protein level was higher in Amravati population (33.2 mg/ml) as
Table 1. Susceptibility of field populations of *A. gossypii* adults to insecticides from Nagpur district

| Insecticide (Aphis gossypii) | Location     | Year | No. of test insects | LC<sub>50</sub> ml/l | 95% FL | LC<sub>90</sub> ml/l | Slope ± SE | χ²   | df | Resistance factor |
|-------------------------------|--------------|------|---------------------|----------------------|--------|----------------------|------------|------|----|-------------------|
| Dimethoate 30 EC              | Susceptible  | 2013 | 360                 | 0.05                 | 0.04-0.05 | 0.07           | 7.398 ± 1.654 | 2.62 | 3  | 1.0               |
|                              | NO           | 2013 | 360                 | 0.08                 | 0.07-0.09*  | 0.26           | 2.607 ± 0.394 | 0.86 | 3  | 1.6               |
|                              | NO           | 2014 | 360                 | 0.12                 | 0.11-0.14*  | 0.30           | 3.307 ± 0.269 | 3.47 | 3  | 2.4               |
| Acephate 75% SP               | Susceptible  | 2013 | 360                 | 0.08                 | 0.06-0.10  | 0.48           | 1.662 ± 0.281 | 2.31 | 3  | 1.0               |
|                              | NO           | 2013 | 360                 | 0.25                 | 0.21-0.29*  | 0.72           | 2.854 ± 0.519 | 0.24 | 3  | 3.1               |
|                              | NO           | 2014 | 360                 | 0.31                 | 0.27-0.34*  | 0.83           | 3.005 ± 0.375 | 0.49 | 3  | 3.9               |
|                              | NO           | 2015 | 360                 | 0.32                 | 0.25-0.45*  | 1.74           | 1.753 ± 0.281 | 3.59 | 3  | 4.0               |
| Quinalphos 25% EC             | Susceptible  | 2013 | 360                 | 0.09                 | 0.05-0.12  | 0.26           | 2.890 ± 0.337 | 5.07 | 3  | 1.0               |
|                              | NO           | 2014 | 360                 | 0.23                 | 0.16-0.44*  | 1.32           | 1.685 ± 0.294 | 3.42 | 3  | 2.5               |
|                              | NO           | 2015 | 360                 | 0.24                 | 0.21-0.31*  | 1.40           | 1.705 ± 0.239 | 1.91 | 3  | 2.6               |
| Chlorpyriphos 20% EC          | Susceptible  | 2014 | 360                 | 0.07                 | 0.05-0.08  | 0.36           | 1.812 ± 0.235 | 0.58 | 3  | 1.0               |
|                              | NO           | 2014 | 360                 | 0.14                 | 0.11-0.18*  | 0.57           | 2.167 ± 0.229 | 4.33 | 3  | 2.0               |
|                              | NO           | 2015 | 360                 | 0.16                 | 0.13-0.23*  | 1.53           | 1.334 ± 0.215 | 1.53 | 3  | 2.2               |
| Spinosad 45% EC               | Susceptible  | 2013 | 360                 | 0.09                 | 0.07-0.10  | 0.20           | 3.689 ± 0.683 | 0.83 | 3  | 1.0               |
|                              | NO           | 2013 | 360                 | 0.10                 | 0.08-0.12  | 0.21           | 4.312 ± 0.600 | 3.95 | 3  | 1.1               |
|                              | NO           | 2014 | 360                 | 0.10                 | 0.09-0.11  | 0.30           | 2.922 ± 0.403 | 1.62 | 3  | 1.1               |
|                              | NO           | 2015 | 360                 | 0.11                 | 0.09-0.13  | 0.21           | 4.726 ± 0.497 | 4.64 | 3  | 1.2               |
| Imidacloprid 17.80 SL         | Susceptible  | 2013 | 360                 | 0.01                 | 0.005-0.017 | 0.07           | 1.592 ± 0.415 | 0.47 | 3  | 1.0               |
|                              | NO           | 2013 | 360                 | 0.02                 | 0.01-0.03  | 0.28           | 1.143 ± 0.151 | 3.90 | 3  | 2.0               |
|                              | NO           | 2014 | 360                 | 0.03                 | 0.02-0.03  | 0.12           | 2.203 ± 0.256 | 2.94 | 3  | 3.0               |
|                              | NO           | 2015 | 360                 | 0.04                 | 0.03-0.06*  | 0.95           | 0.947 ± 0.277 | 0.69 | 3  | 4.0               |

(Continued)
Table 1. (Continued)

| Insecticide (Aphis gossypii) | Location | Year | No. of test insects | LC₅₀ ml/l | 95% FL | LC₉₀ ml/l | Slope ± SE | χ² | df | Resistance factor |
|-----------------------------|----------|------|---------------------|-----------|--------|-----------|------------|----|----|------------------|
| Thiamethoxam 25 WG          | Susceptible | 2013  | 360                 | 0.03      | 0.02-0.04 | 0.08      | 3.338 ± 0.838 | 1.12 | 3  | 1.0              |
| NO                          | 2013      | 360   | 0.04                | 0.02-0.05 | 0.08    | 3.917 ± 0.602 | 5.07 | 3  | 1.3              |
| NO                          | 2014      | 360   | 0.04                | 0.03-0.04 | 0.27    | 1.541 ± 0.285 | 0.62 | 3  | 1.3              |
| NO                          | 2015      | 360   | 0.04                | 0.03-0.04 | 0.19    | 1.900 ± 0.360 | 0.40 | 3  | 1.3              |

Resistance ratio (RR) is calculated as LC₅₀ of field resistant population/LC₅₀ of susceptible population.

*Significant difference of 95% CL between field and susceptible population (non-overlap of 95% CI).

NOTE: NO-Nagpur district.
Table 2. Susceptibility of field populations of *A. gossypii* adults to insecticides from Amravati district

| Insecticide (*Aphis gossypii*) | Location | Year | No. of test insects | LC$_{50}$ ml/l | 95% FL LC$_{50}$ ml/l | LC$_{90}$ ml/l | Slope ± SE | $\chi^2$ | df | Resistance factor |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Dimethoate 30 EC | Susceptible | 2013 | 360 | 0.05 | 0.04-0.05 | 0.07 | 7.398 ± 1.654 | 2.62 | 3 | 1.0 |
| | AWO | 2013 | 540 | 0.11 | 0.07-0.14* | 0.29 | 3.156 ± 0.956 | 0.30 | 3 | 2.2 |
| | AWO | 2014 | 540 | 0.15 | 0.13-0.17* | 0.29 | 4.467 ± 0.924 | 2.69 | 3 | 3.0 |
| | AWO | 2015 | 540 | 0.16 | 0.13-0.20* | 0.41 | 3.163 ± 0.823 | 0.87 | 3 | 3.2 |
| Acephate 75% SP | Susceptible | 2013 | 360 | 0.08 | 0.06-0.10 | 0.48 | 1.662 ± 0.281 | 2.31 | 3 | 1.0 |
| | AWO | 2013 | 540 | 0.27 | 0.19-0.33* | 0.66 | 3.320 ± 0.881 | 2.32 | 3 | 3.3 |
| | AWO | 2014 | 540 | 0.33 | 0.30-0.36* | 0.25 | 4.259 ± 0.558 | 1.52 | 3 | 4.1 |
| | AWO | 2015 | 540 | 0.34 | 0.29-0.39* | 0.81 | 3.402 ± 0.625 | 0.07 | 3 | 4.2 |
| Quinalphos 25% EC | Susceptible | 2013 | 360 | 0.09 | 0.05-0.12 | 0.26 | 2.890 ± 0.337 | 5.07 | 3 | 1.0 |
| | AWO | 2014 | 540 | 0.20 | 0.16-0.27* | 1.62 | 1.437 ± 0.260 | 1.72 | 3 | 2.2 |
| | AWO | 2015 | 540 | 0.21 | 0.18-0.27* | 0.47 | 3.676 ± 1.188 | 1.80 | 3 | 2.3 |
| Chlorpyriphos 20% EC | Susceptible | 2014 | 360 | 0.07 | 0.05-0.08 | 0.36 | 1.812 ± 0.235 | 0.58 | 3 | 1.0 |
| | AWO | 2014 | 540 | 0.15 | 0.11-0.20 | 1.51 | 1.279 ± 0.203 | 0.83 | 3 | 2.1 |
| | AWO | 2015 | 540 | 0.16 | 0.11-0.26 | 1.16 | 1.487 ± 0.377 | 0.15 | 3 | 2.2 |
| Spinosad 45% EC | Susceptible | 2013 | 360 | 0.09 | 0.07-0.10 | 0.20 | 3.689 ± 0.683 | 0.83 | 3 | 1.0 |
| | AWO | 2014 | 540 | 0.11 | 0.09-0.12 | 0.25 | 3.519 ± 0.634 | 2.06 | 3 | 1.2 |
| | AWO | 2013 | 540 | 0.12 | 0.09-0.14 | 0.21 | 5.59 ± 1.438 | 0.67 | 3 | 1.3 |
| | AWO | 2015 | 540 | 0.12 | 0.11-0.13* | 0.24 | 4.634 ± 0.878 | 0.66 | 3 | 1.3 |
| Imidacloprid 17.80 SL | Susceptible | 2013 | 360 | 0.01 | 0.005-0.017 | 0.07 | 1.592 ± 0.415 | 0.47 | 3 | 1.0 |
| | AWO | 2014 | 540 | 0.03 | 0.02-0.04 | 0.08 | 3.118 ± 0.530 | 2.99 | 3 | 3.0 |
| | AWO | 2013 | 540 | 0.04 | 0.03-0.06 | 0.11 | 3.078 ± 1.083 | 0.83 | 3 | 4.0 |
| | AWO | 2015 | 540 | 0.04 | 0.03-0.05* | 0.14 | 2.379 ± 0.522 | 0.48 | 3 | 4.0 |

(Continued)
| Insecticide (Aphis gossypii) | Location | Year | No. of test insects | LC$_{50}$ ml/l | LC$_{90}$ ml/l | Slope ± SE | $\chi^2$ | df | Resistance factor |
|-----------------------------|----------|------|---------------------|---------------|---------------|------------|---------|----|------------------|
| Thiamethoxam 25 WG         | Susceptible | 2013 | 360                 | 0.03          | 0.02-0.04     | 0.08       | 3.338 ± 0.838 | 1.12 | 3                | 1.0 |
|                             | AWO      | 2014 | 540                 | 0.03          | 0.03-0.04     | 0.15       | 2.154 ± 0.405 | 0.74 | 3                | 1.0 |
|                             | AWO      | 2013 | 540                 | 0.04          | 0.03-0.06     | 0.11       | 3.034 ± 0.986 | 0.54 | 3                | 1.3 |
|                             | AWO      | 2015 | 540                 | 0.04          | 0.03-0.05     | 0.09       | 3.531 ± 0.648 | 3.0  | 3                | 1.3 |

Resistance ratio (RR) is calculated as LC$_{50}$ of field resistant population/LC$_{90}$ of susceptible population.

*Significant difference of 95% CL between field and susceptible population (non-overlap of 95% CI).

NOTE: AWO- warud in amravati district.
| Insecticide (Aphis gossypii) | Location | Year | No. of test insects | LC50 ml/l | 95% FL | LC90 ml/l | Slope ± SE | χ² | df | Resistance factor |
|-----------------------------|----------|------|---------------------|-----------|--------|-----------|-----------|-----|----|--------------------|
| Dimethoate 30 EC            | Susceptible | 2013 | 360 | 0.05 | 0.04-0.05 | 0.07 | 7.398 ± 1.654 | 2.62 | 3 | 1.0                |
|                             | WO       | 2013 | 360 | 0.09 | 0.05-0.12 | 0.30 | 2.582 ± 0.788 | 0.32 | 3 | 1.8                |
|                             | WO       | 2014 | 360 | 0.10 | 0.08-0.13* | 0.47 | 2.00 ± 0.488 | 0.47 | 3 | 2.0                |
|                             | WO       | 2015 | 360 | 0.11 | 0.09-0.13* | 0.31 | 2.902 ± 0.569 | 0.68 | 3 | 2.2                |
| Acephate 75% SP             | Susceptible | 2013 | 360 | 0.08 | 0.06-0.10 | 0.48 | 1.662 ± 0.281 | 2.31 | 3 | 1.0                |
|                             | WO       | 2013 | 360 | 0.23 | 0.14-0.29* | 0.76 | 2.471 ± 0.736 | 0.37 | 3 | 2.9                |
|                             | WO       | 2014 | 360 | 0.23 | 0.18-0.27* | 0.81 | 2.582 ± 0.496 | 1.45 | 3 | 2.9                |
|                             | WO       | 2015 | 360 | 0.28 | 0.19-0.36* | 0.60 | 3.918 ± 0.618 | 0.60 | 3 | 3.5                |
| Quinidphos 25% EC           | Susceptible | 2013 | 360 | 0.09 | 0.05-0.12 | 0.26 | 2.890 ± 0.337 | 5.07 | 3 | 1.0                |
|                             | WO       | 2014 | 360 | 0.19 | 0.15-0.24* | 0.96 | 1.830 ± 0.339 | 0.26 | 3 | 2.1                |
|                             | WO       | 2015 | 360 | 0.20 | 0.16-0.28* | 0.87 | 2.035 ± 0.511 | 1.26 | 3 | 2.2                |
| Chlorpyriphos 20% EC        | Susceptible | 2014 | 360 | 0.07 | 0.05-0.08 | 0.36 | 1.812 ± 0.235 | 0.58 | 3 | 1.0                |
|                             | WO       | 2014 | 360 | 0.14 | 0.11-0.18 | 0.96 | 1.541 ± 0.234 | 1.16 | 3 | 2.0                |
|                             | WO       | 2015 | 360 | 0.16 | 0.11-0.27 | 1.18 | 1.491 ± 0.384 | 0.90 | 3 | 2.3                |
| Spinosad 45% EC             | Susceptible | 2013 | 360 | 0.09 | 0.07-0.10 | 0.20 | 3.689 ± 0.683 | 0.83 | 3 | 1.0                |
|                             | WO       | 2013 | 360 | 0.12 | 0.09-0.14 | 0.21 | 5.534 ± 1.558 | 1.41 | 3 | 1.3                |
|                             | WO       | 2014 | 360 | 0.13 | 0.12-0.15* | 0.26 | 4.507 ± 0.884 | 1.51 | 3 | 1.4                |
|                             | WO       | 2015 | 360 | 0.15 | 0.13-0.21* | 0.49 | 2.65 ± 0.803 | 0.36 | 3 | 1.7                |
| Imidaclorpid 17.80 SL       | Susceptible | 2013 | 360 | 0.01 | 0.005-0.017 | 0.07 | 1.592 ± 0.415 | 0.47 | 3 | 1.0                |
|                             | WO       | 2013 | 360 | 0.03 | 0.02-0.05 | 0.16 | 1.907 ± 0.654 | 0.03 | 3 | 3.0                |
|                             | WO       | 2014 | 360 | 0.03 | 0.02-0.03 | 0.08 | 3.411 ± 0.545 | 1.24 | 3 | 3.0                |
|                             | WO       | 2015 | 360 | 0.03 | 0.02-0.03 | 0.09 | 2.953 ± 0.525 | 1.74 | 3 | 3.0                |

(Continued)
| Insecticide (Aphis gossypii) | Location | Year | No. of test insects | LC<sub>50</sub> ml/l | 95% FL | LC<sub>90</sub> ml/l | Slope ± SE | χ<sup>2</sup> | df | Resistance factor |
|----------------------------|----------|------|---------------------|----------------------|-------|-------------------|-----------|--------|-----|------------------|
| Thiamethoxam 25 WG         | Susceptible | 2013 | 360                | 0.03                 | 0.02–0.04 | 0.08 | 3.338 ± 0.838 | 1.12 | 3   | 1.0              |
| WO                         | 2013     | 360  | 0.03                | 0.02–0.04            | 0.09 | 3.196 ± 0.840 | 1.00     | 3      | 3.0            |
| WO                         | 2014     | 360  | 0.03                | 0.03–0.04            | 0.09 | 3.094 ± 0.551 | 1.47     | 3      | 3.0            |
| WO                         | 2015     | 360  | 0.04                | 0.03–0.04            | 0.10 | 0.03 ± 0.04 | 0.46     | 3      | 4.0            |

Resistance ratio (RR) is calculated as LC<sub>50</sub> of field resistant population/LC<sub>50</sub> of susceptible population.

*Significant difference of 95% CL between field and susceptible population (non-overlap of 95% CI).

NOTE: WO-Wardha district.
Table 4. Susceptibility of field populations of *A. spiraecola* adults to insecticides from Nagpur district

| Insecticide (Aphis spiraecola) | Location (Nagpur) | Year | No. of test insects | LC50 ml/l | 95% FL | LC90 ml/l | Slope ± SE | χ² | df | Resistance factor |
|-------------------------------|-------------------|------|---------------------|-----------|--------|-----------|-----------|-----|----|------------------|
| Dimethoate 30 EC              | Susceptible       | 2013 | 240                 | 0.05      | 0.02-0.07 | 1.0       | 2.548 ± 0.776 | 0.32 | 3   | 1.0              |
|                               | NO                | 2013 | 240                 | 0.08      | 0.04-0.10 | 0.24      | 2.686 ± 0.907 | 0.54 | 3   | 1.6              |
|                               |                  | 2014 | 240                 | 0.09      | 0.06-0.11 | 0.61      | 1.566 ± 0.383 | 0.76 | 3   | 1.8              |
|                               |                  | 2015 | 240                 | 0.11      | 0.09-0.14* | 0.31      | 3.000 ± 0.658 | 0.30 | 3   | 2.2              |
| Acephate 75% SP               | Susceptible       | 2013 | 240                 | 0.09      | 0.04-0.14  | 0.43      | 2.001 ± 0.536 | 0.52 | 3   | 1.0              |
|                               | NO                | 2013 | 240                 | 0.10      | 0.04-0.20  | 0.99      | 1.302 ± 0.444 | 0.70 | 3   | 1.1              |
|                               |                  | 2014 | 240                 | 0.23      | 0.18-0.27* | 0.90      | 2.173 ± 0.393 | 0.86 | 3   | 2.6              |
|                               |                  | 2015 | 240                 | 0.24      | 0.20-0.28* | 0.75      | 0.638 ± 0.505 | 0.73 | 3   | 2.7              |
| Quinclorps 25% EC             | Susceptible       | 2013 | 240                 | 0.08      | 0.05-0.11  | 0.45      | 1.796 ± 0.354 | 2.97 | 3   | 1.0              |
|                               | NO                | 2014 | 240                 | 0.18      | 0.15-0.22* | 0.88      | 1.878 ± 0.283 | 2.45 | 3   | 2.3              |
|                               |                  | 2015 | 240                 | 0.20      | 0.15-0.32* | 1.40      | 1.526 ± 0.404 | 1.99 | 3   | 2.5              |
| Chlorpyriphos 20% EC          | Susceptible       | 2013 | 240                 | 0.06      | 0.05-0.07  | 0.30      | 1.934 ± 0.203 | 1.47 | 3   | 1.0              |
|                               | NO                | 2014 | 240                 | 0.16      | 0.13-0.24* | 1.81      | 1.245 ± 0.203 | 0.76 | 3   | 2.6              |
|                               |                  | 2015 | 240                 | 0.17      | 0.13-0.25* | 1.33      | 0.13 ± 0.25  | 1.98 | 3   | 2.8              |
| Spinosad 45% EC               | Susceptible       | 2013 | 240                 | 0.10      | 0.05-0.12  | 0.23      | 3.656 ± 1.216 | 1.26 | 3   | 1.0              |
|                               | NO                | 2013 | 240                 | 0.11      | 0.08-0.13  | 0.24      | 3.985 ± 1.225 | 0.08 | 3   | 1.1              |
|                               |                  | 2014 | 240                 | 0.13      | 0.11-0.15  | 0.28      | 4.038 ± 1.090 | 0.22 | 3   | 1.3              |
| Imidacloprid 17.80 SL         | Susceptible       | 2013 | 240                 | 0.01      | 0.00-0.02  | 0.06      | 2.18 ± 0.770  | 0.67 | 3   | 1.0              |
|                               | NO                | 2013 | 240                 | 0.02      | 0.02-0.03  | 0.09      | 2.485 ± 0.502 | 0.12 | 3   | 2.0              |
|                               |                  | 2014 | 240                 | 0.03      | 0.02-0.04  | 0.10      | 2.732 ± 0.426 | 5.57 | 3   | 3.0              |
| Thiamethoxam 25 WG            | Susceptible       | 2013 | 240                 | 0.03      | 0.02-0.04  | 0.07      | 3.997 ± 0.915 | 0.41 | 3   | 1.0              |
|                               | NO                | 2013 | 240                 | 0.03      | 0.01-0.04  | 0.11      | 2.376 ± 0.779 | 0.56 | 3   | 1.5              |

(Continued)
| Insecticide (Aphis spiraecola) | Location (Nagpur) | Year | No. of test insects | LC$_{50}$ ml/l | 95% FL | LC$_{90}$ ml/l | Slope ± SE | $\chi^2$ | df | Resistance factor |
|-------------------------------|-------------------|------|---------------------|----------------|-------|---------------|------------|--------|----|-----------------|
| NO                            | 2014              | 240  | 0.04                | 0.03–0.05      | 0.23  | 1.682 ± 0.392 | 0.36       | 3      | 2.0 |                 |
| NO                            | 2015              | 240  | 0.04                | 0.03–0.05      | 0.11  | 3.221 ± 0.610 | 0.17       | 3      | 2.0 |                 |

Resistance ratio (RR) is calculated as LC$_{50}$ of field resistant population/LC$_{50}$ of susceptible population.

*Significant difference of 95% CL between field and susceptible population (non-overlap of 95% CI).
Table 5. Susceptibility of field populations of *A. spiraecola* adults to insecticides from Amravati district

| Insecticide (Aphis spiraecola) | Location     | Year | No. of test insects | LC50 ml/l | 95% FL | LC90 ml/l | Slope ± SE | χ² | df | Resistance factor |
|-------------------------------|--------------|------|---------------------|-----------|--------|-----------|------------|----|----|------------------|
| Dimethoate 30 EC              | Susceptible  | 2013 | 240                 | 0.05      | 0.02–0.07 | 1.8       | 2.548 ± 0.776 | 0.32 | 3  | 1.0              |
|                              | AWO          | 2013 | 240                 | 0.11      | 0.07–0.14 | 0.29      | 2.986 ± 0.860 | 0.33 | 3  | 2.2              |
|                              | AWO          | 2014 | 240                 | 0.12      | 0.09–0.15* | 0.50      | 2.066 ± 0.500 | 0.51 | 3  | 2.4              |
|                              | AWO          | 2015 | 240                 | 0.14      | 0.12–0.17* | 0.35      | 3.287 ± 0.700 | 0.27 | 3  | 2.8              |
| Acephate 75% SP               | Susceptible  | 2013 | 240                 | 0.09      | 0.04–0.14 | 0.43      | 2.001 ± 0.536 | 0.52 | 3  | 1.0              |
|                              | AWO          | 2013 | 240                 | 0.26      | 0.18 – 0.33* | 0.67      | 3.182 ± 0.860 | 0.14 | 3  | 2.9              |
|                              | AWO          | 2014 | 240                 | 0.30      | 0.25–0.37* | 0.98      | 2.54 ± 0.518  | 0.76  | 3  | 3.3              |
|                              | AWO          | 2015 | 240                 | 0.31      | 0.26–0.38* | 0.95      | 2.701 ± 0.38  | 0.95  | 3  | 3.4              |
| Quindphos 25% EC              | Susceptible  | 2013 | 240                 | 0.08      | 0.05–0.11 | 0.45      | 1.796 ± 0.354 | 2.97  | 3  | 1.0              |
|                              | AWO          | 2014 | 240                 | 0.21      | 0.15–0.35* | 1.48      | 1.522 ± 0.415 | 2.59  | 3  | 2.6              |
|                              | AWO          | 2015 | 240                 | 0.22      | 0.18–0.29* | 0.65      | 2.727 ± 0.812 | 0.58  | 3  | 2.8              |
| Chlorpyriphos 20% EC         | Susceptible  | 2013 | 240                 | 0.06      | 0.05–0.07 | 0.30      | 1.934 ± 0.203 | 1.47  | 3  | 1.0              |
|                              | AWO          | 2014 | 240                 | 0.14      | 0.09–0.29* | 0.24      | 1.045 ± 0.282 | 1.87  | 3  | 2.3              |
|                              | AWO          | 2015 | 240                 | 0.16      | 0.12–0.26* | 0.85      | 1.805 ± 0.497 | 0.85  | 3  | 2.7              |
| Spinosad 45% EC              | Susceptible  | 2013 | 240                 | 0.10      | 0.05–0.12 | 0.23      | 3.656 ± 1.216 | 1.26  | 3  | 1.0              |
|                              | AWO          | 2013 | 240                 | 0.12      | 0.09–0.14 | 0.23      | 4.479 ± 1.324 | 0.71  | 3  | 1.2              |
|                              | AWO          | 2014 | 240                 | 0.13      | 0.11–0.15 | 0.28      | 4.121 ± 1.059 | 2.75  | 3  | 1.3              |
|                              | AWO          | 2015 | 240                 | 0.14      | 0.12–0.16 | 0.35      | 3.232 ± 0.834 | 0.16  | 3  | 1.4              |
| Imidacloprid 17.80 SL        | Susceptible  | 2013 | 240                 | 0.01      | 0.00–0.02 | 0.06      | 2.18 ± 0.770  | 0.67  | 3  | 1.0              |
|                              | AWO          | 2013 | 240                 | 0.03      | 0.02–0.04 | 0.08      | 3.289 ± 0.908 | 1.84  | 3  | 3.0              |
|                              | AWO          | 2014 | 240                 | 0.03      | 0.02–0.03 | 0.06      | 3.781 ± 0.567 | 3.83  | 3  | 3.0              |
|                              | AWO          | 2015 | 240                 | 0.04      | 0.03–0.05* | 0.16      | 2.094 ± 0.515 | 0.17  | 3  | 4.0              |
Table 5. (Continued)

| Insecticide (*Aphis spiraecola*) | Location | Year | No. of test insects | LC$_{50}$ ml/l | 95% FL | LC$_{90}$ ml/l | Slope ± SE | $\chi^2$ | df | Resistance factor |
|---------------------------------|----------|------|---------------------|----------------|--------|----------------|------------|--------|----|-----------------|
| Thiamethoxam 25 WG              | Susceptible | 2013 | 240                 | 0.03           | 0.02-0.04 | 0.07           | 3.997 ± 0.915 | 0.41   | 3  | 1.0             |
|                                | AWO      | 2013 | 240                 | 0.04           | 0.02-0.06 | 0.13           | 2.639 ± 0.898 | 0.55   | 3  | 2.0             |
|                                | AWO      | 2014 | 240                 | 0.04           | 0.03-0.05 | 0.17           | 2.011 ± 0.503 | 1.02   | 3  | 2.0             |
|                                | AWO      | 2015 | 240                 | 0.05           | 0.04-0.06 | 0.14           | 2.711 ± 0.841 | 1.11   | 3  | 2.5             |

Resistance ratio (RR) is calculated as LC$_{50}$ of field resistant population/LC$_{50}$ of susceptible population.

*Significant difference of 95% CL between field and susceptible population (non-overlap of 95% CI).
Table 6. Susceptibility of field populations of *A. spiraecola* adults to insecticides from Wardha district

| Insecticide (Aphis spiraecola) | Location | Year | No. of test insects | LC50 ml/l | 95% FL | LC90 ml/l | Slope ± SE | χ² | df | Resistance factor |
|-------------------------------|----------|------|---------------------|-----------|--------|-----------|------------|------|----|------------------|
| Dimethoate 30 EC              | Susceptible | 2013  | 240                | 0.05      | 0.02-0.07 | 1.8       | 2.548 ± 0.776 | 0.32 | 3   | 1.0              |
|                              | WO       | 2013  | 240                | 0.09      | 0.06-0.11 | 0.24      | 3.013 ± 0.815 | 0.59 | 3   | 1.8              |
|                              | WO       | 2014  | 240                | 0.12      | 0.09-0.17*| 0.64      | 1.804 ± 0.494 | 0.64 | 3   | 2.4              |
|                              | WO       | 2015  | 240                | 0.13      | 0.09-0.19*| 0.75      | 1.686 ± 0.503 | 0.29 | 3   | 2.6              |
| Acephate 75% SP               | Susceptible | 2013  | 240                | 0.09      | 0.04-0.14 | 0.43      | 2.001 ± 0.536 | 0.52 | 3   | 1.0              |
|                              | WO       | 2013  | 240                | 0.29      | 0.20-0.37*| 0.73      | 3.243 ± 0.983 | 0.94 | 3   | 3.2              |
|                              | WO       | 2014  | 240                | 0.30      | 0.25-0.35*| 0.94      | 2.592 ± 0.592 | 0.18 | 3   | 3.3              |
|                              | WO       | 2015  | 240                | 0.32      | 0.23-0.45*| 0.85      | 3.003 ± 0.556 | 3.38 | 3   | 3.6              |
| Quinalphos 25% EC             | Susceptible | 2013  | 240                | 0.08      | 0.05-0.11 | 0.65      | 1.796 ± 0.354 | 2.97 | 3   | 1.0              |
|                              | WO       | 2014  | 240                | 0.17      | 0.13-0.22*| 0.92      | 1.750 ± 0.402 | 0.64 | 3   | 2.1              |
|                              | WO       | 2015  | 240                | 0.18      | 0.14-0.23*| 0.73      | 2.141 ± 0.541 | 2.22 | 3   | 2.3              |
| Chlorpyriphos 20% EC          | Susceptible | 2013  | 240                | 0.06      | 0.05-0.07 | 0.30      | 1.934 ± 0.203 | 1.47 | 3   | 1.0              |
|                              | WO       | 2014  | 240                | 0.14      | 0.10-0.20*| 1.32      | 1.316 ± 0.244 | 2.01 | 3   | 2.3              |
|                              | WO       | 2015  | 240                | 0.15      | 0.11-0.23*| 0.88      | 1.685 ± 0.460 | 1.40 | 3   | 2.5              |
| Spinosad 45% EC               | Susceptible | 2013  | 240                | 0.10      | 0.05-0.12 | 0.23      | 3.656 ± 1.216 | 1.26 | 3   | 1.0              |
|                              | WO       | 2013  | 240                | 0.13      | 0.10-0.15 | 0.25      | 4.862 ± 1.497 | 0.82 | 3   | 1.3              |
|                              | WO       | 2014  | 240                | 0.13      | 0.11-0.14 | 0.33      | 3.128 ± 0.783 | 0.52 | 3   | 1.3              |
|                              | WO       | 2015  | 240                | 0.14      | 0.12-0.15 | 0.27      | 4.360 ± 0.925 | 1.18 | 3   | 1.4              |
| Imidacloprid 17.80 SL         | Susceptible | 2013  | 240                | 0.01      | 0.00-0.02 | 0.06      | 2.18 ± 0.770  | 0.67 | 3   | 1.0              |
|                              | WO       | 2013  | 240                | 0.04      | 0.03-0.06*| 0.13      | 2.720 ± 0.892 | 0.07 | 3   | 4.0              |
|                              | WO       | 2014  | 240                | 0.04      | 0.03-0.05*| 0.19      | 1.895 ± 0.443 | 0.28 | 3   | 4.0              |
|                              | WO       | 2015  | 240                | 0.04      | 0.03-0.04*| 0.09      | 3.510 ± 0.635 | 0.05 | 3   | 4.0              |

Resistance ratio (RR) is calculated as LC50 of field resistant population/LC50 of susceptible population.

*Significant difference of 95% CI between field and susceptible population (non-overlap of 95% CI).
compared to other populations (14.9–23.9 mg/ml). The AChE activity in *A. gossypii* apterous adults was highest (0.2051 μmol/min/ml) in susceptible population as compared to field populations (0.0130–0.0705 μmol/min/ml) (Figure 1).

*Aphis spiraecola* population collected from Amravati area with moderate levels of insecticide resistance showed high levels of GST (0.270 μmol/min/mg) followed by Wardha population (0.195 μmol/min/mg). Similarly, the protein level was high in Amravati population (28.76 mg/ml) as compared to other populations (14.1–24.3 mg/ml). AChE activity in *A. spiraecola* adults was highest (0.0789 μmol/min/ml) in susceptible population as compared to *Aphis spiraecola* populations from three different locations (0.0088–0.0507 μmol/min/ml) (Figure 2).

### 4. Discussion

The current investigation throws a light into the resistance phenomena of commonly used insecticides by citrus growers against *A. gossypii* and *A. spiraecola*. For sucking insect pest like aphids, chemical control is considered to be the last and effective management option. Nevertheless, in several cases, insecticide treatments may not be effective due to the occurrence of insect resistance to the active chemical ingredients and this pest being polyphagous. Possibilities of aggregate insecticide resistance in this pest are quite certain in this region as they feed on cotton which is a major commercial crop in Vidarbha region. As far as cotton crop is concerned, frequency of insecticide spraying is quite high, and Indian cotton production is heavily associated with the intensive use of hazardous pesticides (EJF, 2007). Aphids live in colonies, and the alate as well as apterous females multiply parthenogenetically and viviparously. In a day, female may give birth to...
8–22 nymphs with an average 12–14 generations per year (Vennila & Biradar, 2007). Multiple insecticidal sprayings per growing season would have resulted in decrease of the efficacy of products used against Aphis sp. Understanding the current insecticide resistance levels in such sucking insect pests will be useful as baseline data to define multiple generation of aphids with different frequencies of resistant individuals, thus aids us to predict future control failures.

The present studies were conducted from 2013 for three consecutive years from different locations in Nagpur, Amravati and Wardha districts in Maharashtra state in India. Bioassay results showed varying degrees of insecticide resistance in field-collected populations of A. gossypii and Aphis spiraeacola. In general, the RR values indicated that the resistance levels in Aphis sp. population in and around major citrus belts of Maharashtra are in the tolerance to very low resistance category, as per Ahmad et al. (2008). Among the insecticides tested against aphid adults, neonicotinoid group proved to be the most effective in causing mortality in the laboratory tests as indicated by the lower LC$_{50}$ values. Nowadays, neonicotinoids are being widely used against sucking insect pests. Several workers have also reported the efficacy of imidacloprid and thiamethoxam against aphids (Akashe, Gud, Shinde, & Deshpande, 2009; Gavkare, Kumar, & Sharma, 2013; Khalequzzaman & Nahar, 2013). Citrus growers in Vidharbha region also cater to usage of neonicotinoid because of its quicker results as confirmed through the questionnaires. At the same time using discriminating dose, aphid strains confirmed acetamiprid, clothianidin and thiamethoxam resistance at 6.4-, 10- and 22-fold resistance in A. gossyppi from Australian cotton (Herron & Wilson, 2010). Cotton is also grown as an intercrop in citrus orchards and may indirectly increase the chance of development of insecticide resistance in citrus as well. At present, spinosad seems to be effective in causing mortality of adult aphids as indexed by the lower RR values. Among organophosphorus group (OP), LC$_{50}$ values in field population from Nagpur are higher for acephate (0.08–0.32 ppm) and quinalphos (0.09–0.24 ppm) followed by chlorpyriphos (0.07–0.16 ppm) and dimethoate (0.05–0.12 ppm). A similar trend was observed in Amravati (Warud) region with resistance in acephate-treated (0.08–0.34 ppm) and quinalphos-treated (0.09–0.21 ppm) aphid population. But among the three locations under study, Warud region (Amravati district) is a commercial citrus belt, and the citrus growers opt for frequent insecticidal applications, which may be one of the reasons for the higher RR values in comparison with other locations. Among the three insecticides tested against A. gossyppi in cotton, viz., acetamiprid 20 SP, methyl demeton 25 EC and dimethoate 30 EC were less toxic to yavatmal, and Amravati populations of recording higher LC$_{50}$ and LC$_{90}$ values (Kulkarni, Khandar, & Madankar, 2017) substantiated our finding. Also, variation in LC$_{50}$ of OP compound, dimethoate in the range of 11.4–464 ppm was observed in A. gossyppi collected from Multan state of Pakistan during 1996–2004 LC$_{50}$ in the range of 5410–13,275 ppm in aphids from Alabama and Texas Province (Kerns & Gaylor, 1992) on cotton has been documented. RR values between susceptible and resistant cotton aphid colonies for chlorpyriphos indicated a significant 4-fold difference in LC$_{50}$ estimates for the two colonies. Spectrophotometric analyses indicated significantly higher carboxylesterase activity in resistant aphids compared with susceptible aphids (O’Brien et al., 1992).

Resistance to insecticides mainly occurs due to changes in insect metabolic enzymes or due to the development of insecticide insensitive target sites in the insect nervous system (Aldridge, 1950). Increased metabolism is often caused by qualitative and/or quantitative change of esterases, GSTs and monoxygenases. Increase of carboxylesterase activity (Cousin et al., 1997), rarely decrease of carboxylesterase activity (Campbell et al., 1997), excessive production of different forms of carboxylesterase (Owusu, Horiike, & Hirano, 1996), overproduction of esterase (Suzuki & Hama, 1998) and qualitative changes in enzyme structure (Brien 1992) are responsible for esterase-mediated metabolic resistance. Higher GST and lower AChE values in Amravati adult aphid population substantiate the chances of development of insecticide resistance. Fournier, Bride, Hoffman and Karch (1992) have reported a GST value of 0.82 ± 0.59 μmol/min/mg and esterase activity of 0.71 ± 0.54 μmol/min/mg in A. gossyppi through biochemical assays. AChE terminates nerve impulse by catalyzing the hydrolysis of the neurotransmitter acetylcholine. Organophosphates and carbamates are among the most commonly used (Aldridge, 1950); however, several insect strains escape poisoning because they possess an altered AChE which is less sensitive to the active metabolite of the organophosphate. Our susceptible
culture indicated maximum AChE which means that is a highly susceptible population. Possibilities of cross-resistance should be further confirmed using molecular techniques for the same population.

5. Conclusion
This study aids in providing a baseline data to be aware of the current insecticide resistance levels in aphids to further support the development of effective resistance management strategies for tackling such sucking insect pests which is also an efficient vector for CTV in the major citrus-growing belts in India.

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Competing Interests
The authors declare no competing interests.

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