Resistance to imidacloprid in different field populations of *Aphis gossypii* Glover (Hem.: Aphididae) in South of Iran

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Abstract

The cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), is a key cucurbits pest in Iran and is managed with repeated insecticide applications. Reports of insecticide control failures have recently increased, particularly with imidacloprid. To quantify resistance to imidacloprid in cotton aphid, seven populations were collected from 7 different places in South of Iran (Shiraz, Jahrom, Saadatshahr, Marvdasht, Kavar, Sadra1 and Sadra2, all in Fars province). To estimate the response of 5 days old *A. gossypii* populations to imidacloprid, leaf dip bioassays were performed in the laboratory. Lethal concentrations at 50% (LC_{50}) values were estimated by probit analysis and used to calculate the resistance ratios (RR). The bioassay results showed significant discrepancy in susceptibility to imidacloprid among the populations. The lowest and highest LD_{50} were estimated for Shiraz population with 37.09 and Sadra1 with 636.80 μg mL^{-1} respectively. The highest levels of resistance to imidacloprid were detected for Sadra1 (RR=17.17 fold). In the other populations some levels of resistance were detected. In Jahrom, Kavar, Marvdasht and SaadatShahr populations the RRs were from 3.85 to 7.11. As emphasized by the slope of responses and after a comparison of RRs with other studies it is supposed that resistant populations appear in first stages of development and have the ability to become more resistant with age.

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Introduction

The cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), is a worldwide insect pest on cotton and many field crops and vegetables (Kim *et al.*, 1986). It is a cosmopolitan, polyphagous species widely distributed in tropical, subtropical and temperate regions (Kresting *et al.*, 1999). In Iran in addition to cotton, it is the major pest of Cucurbitaceae, especially on cucumber (Khanjani, 2005). *A. gossypii* causes direct damage through sucking nutrients from the plant and indirect damage through contamination with honeydew and by vectoring viral pathogens (Ebert & Cartwright, 1997). Due to its short life cycle and high reproductive capability, *A. gossypii* has a high potential for resistance development to insecticides (Shi, 2012). The first documented evidence of insecticide resistance in this species dates back to 1964 when it was resistant to demeton in cotton crops in China (Ghong *et al.*, 1964). In the following years, the cotton aphid has developed a high resistance to numerous commonly used insecticides in many agricultural areas, including organophosphorus, carbamates, pyrethroids and neonicotinoids (Gubran *et al.*, 1992; Furk & Hines, 1993; Hollingsworth *et al.*, 1994; Martin & Workman, 1997; Wang *et al.*, 2002; Ahmad *et al.*, 2003; El-Kady, 2007; Wang *et al.*, 2007; Herron & Wilson, 2011).

The most extensively used neonicotinoid is imidacloprid that introduced in 1991 (Karunker *et al.*, 2009). Although resistance to neonicotinoids was slow to develop, several insect pests including *A. gossypii* have been shown to possess a potential for resistance development (Shi *et al.*, 2011). Fars province is located in the southern part of Iran, which is in arid and semi-arid region. Fars province has an area around 122,607 km^2 (7.5% of Iran's total area). It is situated between 27°30' and 31°42' Northern latitude and 50°30' and 55°36' Eastern longitude. Fars is one of the most important provinces in agricultural production (Fars Comprehensive Agricultural, http://www.fcadb.ir/default.en.htm). Neonicotinoids have widely been used in Iran to control pests. In the past few years, imidacloprid has been a major neonicotinoid insecticide to control piercing-sucking pests, as well as *A. gossypii* as a major pest of cucurbits in South of Iran. Therefore, potential of resistance to imidacloprid is of concern especially in greenhouses where insecticide selection pressures are generally most intense. The purpose of this study was to estimate resistance of different popultions of *A. gossypii* to imidacloprid in South of Iran (Fars province).

Materials and methods

Insects

Shiraz population of *A. gossypii* was collected from *Althaea officinalis* L. (Malvaceae). Other six populations were collected from cucurbit host plants located in Jahrom, Saadatshahr, Marvdasht, Kavar and two different places in Sadra [one population on cucumber, *Cucumis sativus* L., (Sadra1) and another on the calabash (*Lagenaria siceraria* (Mol.) Standl.) (Sadra2)] in South of Iran in 2012-2013 (Figure 1). The collected populations, except Shiraz and Sadra2, had a history of previous exposure to pesticides including imidacloprid. The populations were routinely reared in separate net-covered cages, 70×50×40 cm, under greenhouse conditions at 28 to 18°C, 65±5 relative humidity (RH) and 16:8 light:dark (L:D) photoperiod on cucumber plants, *Cucumis sativus* L. cv. Negin (gynoecious) (Cucurbitaceae) which were individually sown in pots (18 cm diameter, 17 cm height) containing 2 parts sand and 1 part decomposed litter. The plants in the cages were replaced every 2 weeks with new ones in order to keep colonies alive.

Synchronization

In order to obtain synchronous cohorts of the experimental aphids, 100 apterous adults were placed on 9 cm diameter cucumber leaf discs. Each leaf disc was set upside down on a layer (4-5 mm) of 0.9% agar into a 9 cm diameter plastic petri dish with a screened hole (3 cm diameter) in its lid for ventilation. Damped cotton wool was placed around petiole of each leaf. Each petri dish was sealed with parafilm. After 24 h, first instar nymphs were removed from each Petri dish and placed on new leaf discs as described above (200 nymphs were placed on a leaf disc). The aphids were reared in incubator 25±1°C, 65% RH and 16:8 (L:D) photoperiod for 5 days. Five days old aphids were used in all experiments.

Figure 1. Locations for collecting *Aphis gossypii* in Fars province, Iran. 1, Jahrom; 2, Shiraz; 3, Sadra1; 4, Sadra2; 5, Marvdasht; 6, Saadatshahr; 7, Arsanjan.

Insecticides and chemicals

Imidacloprid (95% technical grade) used in all bioassays was obtained from Moshkfam Fars Co., Iran. Triton X-100, acetone and agar media were purchased from subagency of Merck Company (Mannheim, Germany) in Iran.

Leaf-dip bioassay

To prepare 100 mL of 1000 μg mL^{-1} stock solution, technical insecticide was dissolved in 4 mL acetone. This solvent was diluted in aqueous solutions of Triton X-100 (0.5 g L^{-1}) up to 100 mL. Stock solutions were made immediately before use. The solvent used to make serial dilutions (five concentrations) was acetone 4% in triton X-100 (0.5 g L^{-1}).

Toxicity assays were conducted according to the standard *leaf dipped method* recommended by Food and Agriculture Organisation (FAO) Method No. 10a (FAO, 1980). Cucumber leaf discs (55 mm diameter) were dipped in insecticide solutions for 10 s and allowed to dry on paper towel. Then they were placed upside down on an agar bed (9 g L^{-1}) in 55 mm Petri-dishes with a screened hole (15 mm diameter) in its lid for ventilation. Leaves dipped in the solvent, which was used to make serial dilutions, served as controls. Five days old apterous adults *A. gossypii* were placed on the treated leaf surface. Each Petri dish was sealed with parafilm. Petri-dishes containing aphids were kept in incubator at 25±1°C, 65% RH and 16:8 (L:D) photoperiod. Initially, on each population, bracketing test was done to determine doses that produce satisfactory range (10-90% mortality). Aphids were examined for mortality at 48 h. Insects were considered alive if they showed any sign of movement after multiple prodding with a fine-haired paintbrush. Usually aphids that died also turned black. Each bioassay test used four replicates of five concentrations each.

Statistical analysis

LC_{50} was determined using probit analysis with the PC-software Polo-Plus Ver. 2 (LeOra Software, Berkeley, CA, USA). Both tests of parallelism and of different intercept were carried out. LC_{50} values were considered significantly different when the number 1 was not in respective 95% confidence limits of LC_{50} ratio of the two compared populations (Robertson *et al.*, 2007).

Results

LC_{50} values for imidacloprid in seven populations of *A. gossypii* calculated from probit analysis are given in Table 1. Significant differences ($P<0.05$) were observed among the LC_{50} values of the populations. The lowest (37.09 μg mL^{-1}) and highest (636.80 μg mL^{-1}) LC_{50} values were determined in the Shiraz and Sadra1 populations, respectively. Among the collected populations, the highest levels of resistance to imidacloprid were detected for Sadra1 (RR=17.17 fold). Also in other populations, some levels of resistance were detected. In Jahrom, Kavar, Marvdasht and SaadatShahr populations the RRs were from 3.82 to 7.11 (Table 1). According to LC_{50} values, populations can be divided in four groups shown in the table by different letters. LC_{50} values among Jahrom, Kavar, Marvdasht, among Kavar, Marvdasht, SaadatShahr and among Sadra2 and Shiraz were not significantly different to each other.

The test of parallelism emphasized that the hypothesis of parallelism could not be rejected; the slopes of the regression lines of resistant and not resistant populations did not differ significantly ($P>0.05$) (Table 2), whereas the test of equality of intercepts gave significant difference between resistant and not resistant populations ($P<0.05$) (Table 3).

Table 1. Log dose probit-mortality data for imidacloprid susceptible and resistant populations of *A. gossypii* using leaf dip method in Fars province, Iran.

Population	N-d*	N-i°	LC_{50} (LCL-UCL)#	Slope±SE	Chi-square (df)§	RR^
Sadra1	8	856	636.80[a] (494.11-853.86)	0.91±0.07	32.89 (45)	17.17
Jahrom	5	247	263.76[b] (179.75-447.19)	1.24±0.18	6.07 (18)	7.11
Kavar	5	475	203.23[bc] (152.49-271.85)	1.05±0.11	10.49 (18)	5.48
Marvdasht	5	300	186.93[bc] (125.04-281.40)	0.93±0.11	7.93 (18)	5.04
SaadatShahr	8	1078	130.38[c] (102.70-165.09)	0.80±0.05	48.46(58)	3.82
Sadra2	5	240	54.69[d] (35.00-85.15)	0.98±0.11	14.31 (18)	1.47
Shiraz	5	291	37.09[d] (23.44-57.21)	0.98±0.11	10.33 (18)	1

*Number of doses; °number of insects tested without controls; #the lethal concentrations at 50% (LC_{50}) values are expressed as μg mL^{-1}; §values of χ^2, lower than ($P \leq 0.05$) indicate a significant fit between the observed and expected regression lines; ^resistance ratio: LC_{50} of population/LC_{50} of Shiraz population. [a–d]Means within the same rank followed by different letters are significantly different at $P<0.05$. LCL, lower confidence limit at 95%; UCL, upper confidence limit at 95%; SE, standard deviation; df, degree of freedom; RR, resistance ratio.

Table 2. Parallelism hypothesis for the probit lines.

Population	Chi-square	Degrees of freedom	Tail probability
Jahrom	3.07	1	0.08
Kavar	1.16	1	0.28
Marvdasht	0.01	1	0.92
SaadatShahr	1.47	1	0.23
Sadra2	0.29	1	0.59
Shiraz	0.30	1	0.58

Table 3. Equality hypothesis for the probit lines.

Population	Chi-square	Degrees of freedom	Tail probability
Jahrom	7.76	2	0.02
Kavar	33.70	2	0.00
Marvdasht	26.43	2	0.00
SaadatShahr	90.34	2	0.00
Sadra2	82.03	2	0.00
Shiraz	114	2	0.00

Discussion

With the exception of Shiraz population, reared under mentioned greenhouse conditions since 2012-2014, all the other populations were bioassayed by leaf dip method after 3 weeks of rearing in greenhouse. Since studies on the resistance dynamics showed that imidacloprid resistance is not stable and declines quickly when selection pressure is suspended (Wen *et al.*, 2009), so maintaining the populations for a long time may decrease the level of resistance to imidacloprid. The differences in LC_{50} seemed related to the history of imidacloprid applications or to genetic variation of populations.

Despite the use of leaf dip method for testing imidacloprid effects by different researchers (Wang *et al.*, 2002; Nauen & Elbert, 2003; Li & Han, 2004; Shi *et al.*, 2012), some problems arised in the application of this method. Sometime it happened that mortality at high doses was lower than at low doses. Generally, aphids reared on leaf discs and treated with higher concentrations accumulated more amount of insecticide, but sometimes this did not happen. One reason probably was bound to the antifeedant effect of imidacloprid (Nauen *et al.*, 1998). Two ways of entrance (*via* ingestion and direct contact) using the leaf dip method is another possible reason. At low doses, aphids begin to feed and pesticide enters into their body *via* ingestion in addition to direct contact, but at high doses, contact effect of toxin causes prostration and the aphids never have the ability to feed on leaf. In this manner imidacloprid enters into the body only *via* direct contact and, being this pesticide more toxic orally than by contact (Suchail *et al.*, 2000), at higher doses the aphids displayed intense symptoms of poisoning but they did not die. Therefore, leaf dip bioassays were done with many replications and high numbers of aphid.

Another problem with this method was the preparation of high concentrations of imidacloprid technical grade in aqueous solutions. The technical grade imidacloprid is not soluble in water, so the preparation of solutions in concentrations above 2000 μg mL^{-1} require an addition of acetone above 4 percent, but high amounts of acetone damage leaf disks and do not let the leaf to get wet.

Different resistance in field populations of cotton aphid has been reported between 2.21 and 97 (El-Kady, 2007; Li & Han, 2007; Wang *et al.*, 2007; Zhang *et al.*, 2014). Shi *et al.* (2012) and Zhang *et al.* (2014) found levels of resistance from intermediate to low (Shi *et al.*, 2012; Zhang *et al.*, 2014), comparing with few highly resistant field populations belonging to *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae), *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) and *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) (Shi *et al.*, 2012).

As shown in Tables 2 and 3, the regression line of resistant (Sadra1) population is parallel but not equal with other populations, that is their slopes are not significantly different but their intercepts differ significantly. The slope of a probit regression reflects the quality of enzymes involved in detoxification. Thus, parallel lines with different intercepts should indicate that populations have qualitatively identical, but quantitatively different levels of detoxification enzymes (Robertson *et al.*, 2007).

It was observed that the slope of susceptible population was lower than the one of resistant populations, it was explained by the higher number of heterozygotes in the susceptible population (Alizadeh *et al.*, 2011), but in the present research slopes between susceptible and resistant populations were not significantly different (Tables 1 and 2), indicating homozygosis in resistant population did not increase. Presumably if selection pressure should continue, the RR value and homogeneity would increase (Prabhaker *et al.*, 1997) reaching higher levels of resistance in the future. The observed slopes and a comparison of RRs with other studies suggest that resistant populations appear in the first stages of treatment and have the ability to become more resistant.

Imidacloprid resistance dynamics after selection of several generations of cotton aphid with the pressure of this pesticide was intensively studied. It was emphasized that RR increased to 3.6 after 12 generations (Wang *et al.*, 2002), to 25.03 after 25 times (Li & Han, 2007) and to 66.49 after 60 generations of selection (Shi, 2012). For future studies it is suggested to set resistant population under regular selection pressure with imidacloprid to find out how the RR and slopes change and to analyze if the population is capable to become more resistant.

It was observed that the leaf dip method gives imidacloprid LC_{50} values rather variable in relation to different experimental conditions. These differences are due to host species, geographical variation in aphid populations, previous exposure and duration of exposure to insecticide, as well as the type of insecticide used (*i.e.*, technical material or formulation) (Amini Jam *et al.*, 2014). Results are also related to the age of the insects tested, the incubation time and the decision of researcher, in establishing which aphid is dead and which is alive. The LC_{50} observed in the present research (Table 1) were in the range of the ones reported from different places in Iran at almost the same conditions, for example 125 and 209 μg mL^{-1} were observed in Torbat Jam (Tabacian *et al.*, 2011), 285 μg mL^{-1} in Karaj (Gerami & Heidari, 2013) and 90.1 μg mL^{-1} (Amini Jam *et al.*, 2014). Imidacloprid LC_{50} values observed in Iranian *A. gossypii* populations are higher in comparison with the ones observed from other locations in almost the same conditions. The LC_{50} of susceptible strains of this pest in China was 0.35 and for resistant strain after selection of 60 generations with the pressure was 23.27 μg mL^{-1} (Shi *et al.*, 2012). In Akola, India, the LC_{50} after 72 h is 0.036 μg mL^{-1} (Awasthi *et al.*, 2013) and it is 1.2 μg mL^{-1} in an insecticide susceptible laboratory strain of the cotton aphid maintained on cotton in Germany (Nauen & Elbert, 2003). In another work in China the LC_{50} was 5 μg mL^{-1} after one generation and 13 μg mL^{-1} after over 60 generations feeding on Bt cotton (Hong *et al.*, 2006). So this comparison confirms a weaker toxicity of this neonicotinoid for collected populations of *A. gossypii* in Iran with the other locations. The difference is so high that the condition of experiment by itself is not the sole determining factor. More researches are essential for confirming this idea. Certainly different factors can affect on susceptibility of natural populations of an insect to an insecticide. An investigation between different populations of this pest from different countries is needed to understand the reason of the variation of LC_{50}. A possible answer can be the presence of endosymbiotic bacteria in aphids, which could influence the resistance to imidacloprid. It was emphasized that *M. persicae* without endosymbiotic had higher susceptibility to this insecticide (Nauen *et al.*, 1998). The present study suggests that resistance situation of cotton aphid to imidacloprid in Iran is more alarming in field populations, possibly due to indiscriminate use of this insecticide in the field. Resistance management tactics are needed to enhance the efficacy of insecticide.

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