Crop Protection 81 (2016) 163–167

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Resistance monitoring and enzyme activity in three field populations of cowpea aphid (*Aphis craccivora*) from Egypt

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ARTICLE INFO

Article history:
Received 10 July 2015
Received in revised form
18 December 2015
Accepted 20 December 2015
Available online xxx

Keywords:
Aphis craccivora
Resistance
Enzyme activity
Carboxylesterase
Monitoring

ABSTRACT

The cowpea aphid (*Aphis craccivora*) is one of the most important sucking insect pests attacking certain legumes in Egypt particularly faba bean, cowpea and pea. In this study we monitored the resistance level of three field populations of *A. craccivora* to seven insecticides belonging to three different chemical classes (organophosphates, carbamates and neonicotinoids). The three populations were collected from three governorates in Egypt namely Dakahlia, Qalyobia and Beni Suef. Diagnostic concentrations (LC_{90} values for susceptible strain) for each insecticide were established using a leaf dipping technique. Resistance monitoring showed that the field population from Dakahlia was highly resistant to all the tested insecticides. In a similar manner, the population from Qalyobia was also resistant to all insecticides except for fenitrothion to which only moderate resistance was observed. The field population from Beni Suef exhibited a lower level of resistance to all the seven tested insecticides.

Biochemical assay showed that esterase activity in these three field populations was generally higher as the enzyme activity ratio ranged from 4.3 to 7.8 fold more than that for the susceptible strain. The activity of the other measured detoxifying enzymes (glutathione -S- transferase and mixed function oxidases) was moderate in the populations from Qalyobia and Dakahlia. Nevertheless, the enzyme activity in *A. craccivora* collected from Beni Suef was variable and differed slightly from the activity measured in the susceptible strain. Monitoring insecticide resistance among the three aphid populations was a proactive approach to detect any shift in insecticide efficiency. The possible occurrence of resistance in the cowpea aphid to the tested insecticides may be due to the higher activity of carboxylesterases. Further studies on the resistance mechanism to these insecticides are needed to provide insights in how to manage and delay the onset of the resistance and thus prolong the performance of insecticides against *A. craccivora*.

1. Introduction

The cowpea aphid (*Aphis craccivora*) has become a serious insect pest in Egypt on a variety of legumes such as faba bean, cowpea and pea (El-Ghareeb et al., 2002). *A. craccivora* causes major yield losses not only by sucking plant sap but also by transmission of two major viruses; faba bean necrotic yellows virus and bean leaf roll virus (Laamari et al., 2008). Moreover, while feeding; *A. craccivora* can inject a powerful toxin into the plant causing stunting and killing the plant (Anonymous, 2015).

Current management of the aphid basically relies on chemical control using insecticides such as organophosphates, carbamates, pyrethroids, and neonicotinoids (Jackal and Daoust, 1986; Shetlar, 2001). Over time, however, extensive and recurrent use of these insecticides has selected for resistance to the applied insecticides and eventually failure in aphid control. Globally, insecticide resistance in over 20 aphid species has been documented (Georghiou, 1990).

On the other hand, due to the great difficulty and large investments associated with the development of new insecticides, there is always a need to sustain and prolong the efficacy of current and even future developed active ingredients. Early and rapid detection and monitoring of the occurrence of resistance before it appears has become crucial to identify the cause of the resistance. Determination of the activity of the enzymes that are known to be responsible for conferring the resistance is one of the factors that

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http://dx.doi.org/10.1016/j.cropro.2015.12.015
0261-2194/

helps in the early detection of resistance that may aid in the insect control program.

Therefore, this work aimed to assess the potential resistance of three field populations of *A. craccivora*, collected from three governorates in Egypt, against certain organophosphorus, carbamate and neonicotinoid insecticides. In addition, activity of the known insecticide metabolizing enzymes was determined to examine the possible role of these enzymes in the occurrence of resistance in the cowpea aphid.

2. Materials and methods

2.1. Insecticides tested

Insecticide tested in the present work is shown in Table 1.

2.2. Test insect

2.2.1. Laboratory susceptible strain

The laboratory susceptible strain (S) of the cowpea aphid was obtained from Plant Protection Research Institute, Cairo–Egypt, and kept ever since away from any insecticide contamination under laboratory conditions (22 ± 2 °C, 70 ± 5% R.H. and 12:12 light/dark photoperiod). Aphids were reared on metallic stands in chambers and the insects were kept on faba bean seedlings grown in plastic pots (15 cm diameter). The pots with faba bean seedlings were maintained in another chamber without exposure to any insecticides until needed. This strain was used for bioassay, establishing a toxicity baseline as a standard reference strain in the biochemical studies.

2.2.2. Field populations

Three field populations of *A. craccivora* were collected from faba bean fields, at Qalyobia, Dakahlia and Beni Suef governorates. These aphids were tested in all experiments directly without any further rearing.

2.2.3. Bioassay tests

The toxicity of the aforementioned tested insecticides against the laboratory susceptible strain of *A. craccivora* adults was assessed (Table 1).

The leaf-dipping method described by Moores et al. (1996) was used with slight modifications. A series of concentrations of each commercial insecticide was prepared in aqueous solution. Fresh faba bean leaves were dipped into these solutions for 10 s, and allowed to dry on a paper towel. Leaves were then placed upside down on an agar bed in small Petri dishes (60 mm diameter). Ten apterous adults of *A. craccivora* were placed on the treated leaf surface, while leaves dipped in water served as controls. Three replicate batches of aphids (i.e. 30 insects) were used per insecticide concentration. Petri dishes containing aphids were kept in the rearing chamber until mortality was scored after 24 h. Adults failing to exhibit coordinated forward movement when probed with a soft camel hair brush were considered dead. Percentage mortality was corrected by Abbott formula (Abbott, 1925). Toxicity values (LC_{50}) were calculated by probit analysis using Ldp-line software.

2.2.4. The resistance percentage for field populations

The diagnostic dose (LC_{90} of laboratory strain) technique was selected to measure the insecticide susceptibility among field populations. The resistance percentage was calculated based on the formula of Roush and Luttrell (1989).

$$\text{Resistance percentage} = 100 - (M_r \times 100)/M_s$$

where:

M_r = % mortality at discriminating concentration in the field population.

M_s = % mortality (constant) at discriminating concentration in the laboratory strainDiscriminating dose = LC_{90} for laboratory strain

2.3. Detoxifying enzymes activity

2.3.1. Total esterase activity

Esterase activity was assayed with α-naphthyl acetate (α-NA), as a substrate according to Van Asperen (1962) with the modification of Cao et al. (2008). Fifty adults from each strain were homogenized in 500 μL of ice-cold phosphate buffer (0.1 M, pH 7.0). The homogenates were centrifuged at 12,000 g for 15 min at 4 °C and the supernatants were transferred to new tubes. 50 μl enzyme solution was incubated with 50 μl α − naphthyl acetate (30 mM) for 15 min at 30 °C. The reaction was stopped by adding 50 μl of stop solution (two parts of 1% Fast Blue RR and five parts of 5% sodium dodecyl sulfate). The absorbance was measured at 600 nm for the hydrolysis of α-NA at UV/Vis spectrophotometer (V-530). Mean levels of total esterase activity cited were based on protein content and α − naphthol standard curves.

2.3.2. Glutathione S- transferase (GST) activity

GST activity was assayed as described by Habing et al. (1974). Ten adults from each strain were homogenized in 200 μL of ice-cold phosphate buffer (0.1 M, pH 6.5). The homogenates were centrifuged at 12,000 g for 15 min at 4 °C and the supernatants were transferred to new tubes. The reaction solution contained 100 μl supernatant, 10 μl 1- chloro, 2,4- dinitrobenzene (30 mM), and 10 μl GSH (50 mM). Enzyme activity was determined by continuously monitoring of the change in absorbance at 430 nm for three min at 25 °C with a UV/Vis spectrophotometer (V-530).

Table 1
The tested insecticides.

Common name	Trade name	Chemical group	Manufacturer
Pirimicarb	Aphox 50% DG	Carbamate	Syngenta
Carbosulfan	Marshel 25% WP	Carbamate	FMC
Fenitrothion	Sumithion 50% EC	Organophosphorus	Sumitomo
Chlorpyriphos-methyl	Reldan 50% EC	Organophosphorus	Dow Agro Sciences
Malathion	Malson 57% EC	Organophosphorus	Ficom Organics
Thiamethoxam	Actra 25%WG	Neonicotinoids	Syngenta
Acetamiprid	Mospilan 20%SP	Neonicotinoids	Nippon Soda