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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 5(10): 1074-1076, 2002

Selection of a Discriminating Concentration (DC) for Propargite-resistance Detection in *Tetranychus urticae* (Koch)

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Abstract: A petri dish residue-potter tower method was used to determine the concentration which discriminate between the susceptible and propargite-resistant strains of *Tetranychus urticae*. A concentration 0.04% ai (active ingredient) of propargite was selected as the discriminating concentration. This discriminating concentration could be used in propargite resistance detection and subsequent monitoring. The estimate of dominance for F1 hybrid females was -0.08 and -0.07 for R ♂ X S ♀ and S ♂ X R ♀, respectively. These values indicate that propargite resistance in *T. urticae* has no substantial dominance. The response of the reciprocal F1 cross hybrids to propargite was, therefore, intermediate and no effort was made to select a concentration for discrimination between the hybrids and either of the strains.

Key words: Discriminating concentration, propargite, pesticide resistance, monitoring, *Tetranychus urticae*

Introduction

The traditional approach involves a multiple concentration test with 4-6 concentrations that would produce 5-95% mortality in a test strain. Resistance is then expressed as ratio of the LC_{50} or LC_{90} of the test strain to that of a susceptible strain (e.g., Tabashnik *et al.*, 1987; Halliday and Burnham, 1990; Rowland *et al.*, 1991). Among the several disadvantages of this technique, the most important one is that it requires a large number (typically several hundred) of test individuals (Roush and Miller, 1986). Other more recent techniques include biochemical (Hemingway, 1986), immunological tests (Brown and Brogdon, 1987) and DNA probes (Keiding, 1986). These techniques are of limited use at present.

The most widely used method is the discriminating or diagnostic concentration test. This involves a comparison of mortalities of test and susceptible strains at a single concentration and needs fewer test organisms than the multiple concentration technique. After comparing the two techniques Roush and Miller (1986) concluded that the discriminating concentration test was rapid, efficient and accurate.

The discriminating concentration (DC) test has been widely used because of its simplicity and rapidity (Gunning *et al.*, 1984; Guillet *et al.*, 1985; Graves *et al.*, 1988; Croft *et al.*, 1989). A DC is often selected arbitrarily, for example the LC_{99} or two or three times the LC_{99}, however, the use of a higher concentration could result in underestimation of the resistance level because more resistant individuals are likely to be killed at much higher concentrations. Dennehy *et al.* (1983) in a study of the relevance of two bioassay methods to detect the resistance in spider mites showed that slide dip assays of dicofol-resistant spider mites, a dose 2-3-folds greater than the susceptible LC_{99} would have killed > 98% of resistant strains. Halliday and Burnham (1990) recommended using a concentration producing 94-99% mortality of the susceptible strain as the DC. McCutchen *et al.* (1989) suggested selecting a concentration between LC_{80} and LC_{90} and the resistance percentage be calculated by considering the observed mortality of a susceptible strain to that particular concentration. Kabir (1991) also confirmed that data for observed mortality of the reference or susceptible strain must be used to determine a DC and establish criteria for determining resistance levels.

The reported research work has been conducted during 1994-95 at Lincoln University, New Zealand, with the objective to determine a discriminating concentration (DC) which will discriminate between the susceptible and propargite-resistant strains of *Tetranychus urticae*.

Materials and Methods

Sources of the susceptible and propargite-resistant strains of *T. urticae*: A susceptible strain of two spotted spider mites (*T. urticae*) was collected from wild hosts from the Lincoln University, Organic Production area. No pesticide had been applied in this area for approximately 20 years. A resistant strain of *T. urticae* was air-freighted from an Auckland (New Zealand) glasshouse where there had been intensive use of miticides. Both strains were reared on French dwarf bean (*Phaseolus vulgaris*, cv. 'Tendergreen') in separate controlled temperature (CT) rooms at 21 ± 3 °C, 60 ± 15 % RH and a 16L:8D photoperiod.

Bioassay technique: A Petri dish residue-potter tower method outlined by Kabir (1991) was used to determine the discriminating concentration. Clear plastic petri dishes (Falcon 1006 petri dish, Dickinson and Co., Cockeysville, MB 21030 USA) measuring 50 mm dia and 9 mm depth with tight fitting lids were used in this bioassay. Prior to every treatment, the petri dishes were cleaned with 100% ethanol to remove any surface residues. Individual petri dishes (internal surfaces of bases and lids) were sprayed with 2 ml of propargite suspension of the required concentration under the potter tower at 10 psi pressure. A 10 seconds period was allowed for the spray deposits to settle. Following treatment, petri dishes were left uncovered for 30 minutes to air dry and then small agar plugs (1.5 % w/v) were placed on the inner surfaces of lids to maintain humidity inside the dishes. Twenty adult females were then transferred to each petri dish. The prepared petri dishes were then placed in covered plastic trays in a CT room.

Mortality was assessed after 24 hours and mites were scored as dead, moribund or alive. Mites that could walk at least one body length after a gentle probe with a fine brush were scored alive; those which could move their legs but could not walk (as described above) were scored as moribund and those which showed no movements at all were scored as dead. For analysis moribund mites were considered as dead.

Determination of the discriminating concentration (DC): To determine an appropriate DC, firstly 1070 and 1410 adult females of the susceptible and propargite-resistant strains, respectively, were exposed to a wide range (0.0013-2.0 % ai) of propargite concentrations over time. As a result, concentrations between 0.0013-0.04% ai for the susceptible strain were selected for final assessment. Another 480 mites of the susceptible strain were then bioassayed and concentration-mortality data were subjected to Probit analysis (Finney, 1977). For the resistant strain, 400 adult females were exposed to concentrations between 0.005-2.0 % ai and for each of the reciprocal F1 hybrids (R♂ X S♀ and S♂ X R♀) 400 adult females were exposed to concentrations between 0.01-0.66 % ai of propargite. In a control group, 80 females were tested for each bioassay.

Fresh suspensions were made each time and control groups were treated with water only. To avoid using very young or old females only 3 or 4-days old females were used in the bioassay. Lethal concentrations (LC_{50}, LC_{90} LC_{95} and LC_{99}) of propargite for the

susceptible, propargite-resistant strains and reciprocal *F1* cross hybrids were estimated using the POLO computer programme (Robertson *et al.*, 1980).

As selection of the DC is an arbitrary decision, both susceptible and propargite-resistant strains were exposed to LC_{99} of the susceptible strain, 1.3 X LC_{99}, 1.6 X LC_{99} and 2.6 X LC_{99} (0.03, 0.04, 0.05 and 0.08% ai of propargite, respectively) and the observed mortality was recorded. For 0.03% ai, 20 replicates were used consisting of 20 adult females/replicate, whereas, for the rest of the remaining concentrations, 15 replicates were used. Control groups were treated with water only using 4 replicates consisting of 20 adult females/replicate. Observed mortalities were corrected using Abbott's formula (Abbott, 1925). Resistance ratios were calculated by dividing the LC_{50} of test strain by LC_{50} of the susceptible strain (Herne, 1971 and Osman *et al.*, 1991).

Degree of dominance (D): The degree of dominance for F1 females was estimated by the following formula:

$$D = \frac{2X_b - X_a - X_c}{X_a - X_c}$$

Where, X_a = logarithm to the base 10 (log_{10}) of the LC_{50} of the resistant colony, X_b = log_{10} of the LC_{50} of the heterozygous colony (F1 females) and X_c = log_{10} of the LC_{50} of the susceptible colony (Stone, 1968). This will result in a value of -1 if the resistance is fully recessive, a value of 0 if there is no dominance and a value of +1 if the resistance is fully dominant.

Results and Discussion

The estimated LC_{50}, LC_{90}, LC_{95} and LC_{99} values for propargite with the susceptible and propargite-resistant strains and reciprocal F1 cross hybrids of *T. urticae* using Petri dish residue-Potter tower method are shown in Table 1. The LC_{50} values of the susceptible and resistant strains and F1 hybrids (R♂ X S♀ and S♂ X R♀) are 0.006 % ai (60 ppm), 0.403 % ai (4030 ppm), 0.041 % ai (410 ppm) and 0.042 % ai (420 ppm) for propargite representing a 67.2-, 6.8- and 7.0-fold resistance for the propargite-resistant strain and F1 hybrids, respectively, when their LC_{50} values were compared with the susceptible strain. Dennehy *et al.* (1987), using a residual (cell) bioassay, found the LC_{50} values for propargite with susceptible and dicofol-resistant strains of *T. urticae* collected from cotton to be 24 and 635 ppm, respectively. This represents a 26-folds resistance, whereas, in their study of field-collected mites, LC_{50} values for propargite were 27-1123 ppm representing a 42-folds resistance. Estrada and Sanches (1990) found that *T. urticae* collected from carnation had a propargite LC_{50} value of 1464.18 ppm and Cheng and Pan (1994) showed that *T. urticae* collected from cotton had LC_{50} value of 8104.01 ppm for propargite using glass slide dipping method. The LC_{50} values measured by different bioassay techniques cannot be directly compared, however, the LC_{50} values of the strains used in this study are within the range described in literature.

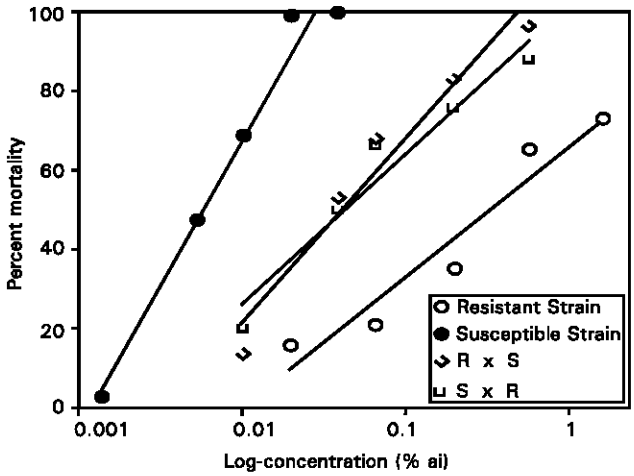

Fig. 1: Concentration-mortality regression lines of susceptible and resistant strains and F1 reciprocal cross hybrids of *Tetranychus urticae* tested with propargite

Concentration-mortality regression lines for reciprocal F1 cross hybrid females (R♂ X S♀ and S♂ X R♀) were intermediate between the lines for the susceptible and propargite-resistant strains (Fig. 1). The estimate of dominance for F1 was -0.08 and -0.07 for R♂ X S♀ and S♂ X R♀, respectively. These values indicate that propargite resistance in *T. urticae* has no substantial dominance. Keena and Granett (1990) also observed that reciprocal F1 hybrids of *T. urticae* were intermediate in response to propargite and found similar values as estimates of dominance (-0.07 and -0.02 for R♂ X S♀ and S♂ X R♀, respectively). As the response of the

Table 1: The estimated lethal concentrations of propargite for susceptible, propargite-resistant strains and reciprocal *F1* cross hybrids of *Tetranychus urticae* Koch using the Petri dish residue-Potter tower method

Strain/ Hybrid	LC_{50}[a] (95 % CI)	LC_{90} (95 % CI)	LC_{95} (95 % CI)	LC_{99} (95 % CI)	Slope	SE	χ^2	df
Resistant	0.403 (0.233-0.705)	9.451 (3.756-53.22)	23.113 (5.67-772.97)	123.710 (18.67-159.31)	0.94	0.12	3.54	3
Susceptible	0.006 (0.004-0.008)	0.015 (0.011-0.025)	0.019 (0.013-0.071)	0.031 (0.017-0.233)	3.18	0.39	7.04	3
R ♂ X S ♀	0.041 (0.029-0.054)	0.304 (0.214-0.488)	0.537 (0.352-0.976)	1.563 (0.877-3.675)	1.47	0.16	2.36	3
S ♂ X R ♀	0.042 (0.026-0.062)	0.681 (0.406-1.471)	1.498 (0.789-4.047)	6.584 (2.676-27.763)	1.06	0.13	2.69	3

[a]LC values are expressed as percentage active ingredient. Values in parentheses indicate 95% confidence interval

Table 2: The observed mortalities of susceptible and resistant strains of *Tetranychus urticae* Koch using the Petri dish residue-Potter tower method

Concentration (a.i.%)	Numbers tested	Observed mortality (%) Susceptible strain (± SE)	Resistant strain (± SE)
0.03	400	87.10 (1.18)	13.95 (1.88)
0.04	300	96.14 (1.09)	10.18 (1.09)
0.05	300	96.84 (1.00)	10.88 (1.40)
0.08	300	99.30 (0.48)	16.84 (1.94)

hybrids was intermediate no effort was made to select a DC which would distinguish the hybrids from either of the strains.

The χ^2 test for goodness-of-fit showed that the concentration-mortality data for the susceptible and propargite-resistant strains and reciprocal F1 cross hybrids were adequately described by the Probit model (Table 1). The likelihood ratio test of equality (the slopes and intercepts are the same) for both the strains and reciprocal F1 cross hybrids showed that the hypothesis of equality was rejected ($\chi^2 = 436.53$; df = 3; $P < 0.001$) and thus the lines were clearly significantly different.

The observed mortalities of both the strains exposed to 0.03, 0.04, 0.05 and 0.08% ai of propargite are given in Table 2. The concentration of 0.04% ai (400 ppm) propargite which was 1.3 fold larger than LC_{99} for the susceptible strain was selected as DC. The other concentrations caused either lower percentage mortality of the susceptible strain or higher percentage mortality of the propargite-resistant strain and, therefore, were not selected as a DC.

Keena and Granett (1990) used 316 ppm of propargite to measure *T. urticae* field resistance frequencies in almonds. Kabir *et al.* (1991) used a concentration of 0.105% ai (1050 ppm) of propargite as a discriminating concentration to monitor propargite resistance in field populations of *T. urticae*. Dennehy *et al.* (1987) used 100 ppm and 1000 ppm of dicofol which resulted in 99 and 99.9 % mortality, respectively, of the susceptible strain and used both the concentrations to assess susceptibility of field-collected mites. The selected DC should provide a compromise between allowing few susceptible survivors yet does not risk killing as many resistant individuals as a higher dose might (Roush and Miller, 1986). Dennehy *et al.* (1983) showed that in slide dip assays of dicofol-resistant spider mites, a dose 2-3-folds greater than the susceptible LC_{99} would have killed > 98 % of the resistant strain. In this study, a concentration of 2.6 X LC_{99} (0.08 % ai of propargite) of the susceptible strain caused about 17 % mortality in the resistant strain and was therefore, not selected as the DC. The selected DC of 0.04 % ai of propargite caused 96.14 % and 10.18 % mortalities in the susceptible and propargite-resistant strains, respectively, therefore, any test strain showing mortality less than 96.14% would be suspected to have some resistance.

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