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Residual contact vial bioassay for the on-site detection of acaricide resistance in the two-spotted spider mite

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ABSTRACT

To develop a less technique-dependent bioassay technique that can be conveniently used by practitioners or farmers in the field for the monitoring of acaricide resistance of the two-spotted spider mite, *Tetranychus urticae* Koch, a residual contact vial (RCV) method was established using a 5-ml glass vial coated with acaricides. The RCV bioassay procedures were optimized by using abamectin and tebufenpyrad, two widely used acaricides. The diagnostic concentrations causing 100% mortality within 8 h post-treatment in a susceptible strain of *T. urticae* was set at 30 and 60 ppm for abamectin and tebufenpyrad, respectively. The vial-coated pesticides were stable at least one year when stored at −20 °C as determined by HPLC. There was no significant difference in the bioassay results in repeated RCV bioassay by three different experimenters, indicating its high reproducibility and reliability. RCV-based resistance monitoring of 15 field populations of mites revealed that abamectin resistance begins to spread but tebufenpyrad resistance is already prevalent in Korea. The RCV diagnostic kit, when used by practitioners or farmers on site, should provide crucial information for the selection of most suitable acaricides for different field populations of *T. urticae*.

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Introduction

Early detection of pest resistance to insecticides and acaricides is a crucial element of the resistance management system to suppress or delay resistance development. This is particularly true for the two-spotted spider mite, *Tetranychus urticae* Koch, that has a great potential of resistance development to many kinds of acaricides (Stumpf et al., 2001; Suh et al., 2006; Koh et al., 2009), due to its short life cycle and high biotic potential in conjunction with arrenotokous reproduction (Saito et al., 1983).

Several bioassay methods for the resistance detection of mites have been developed. The slide dip (SD) method, in which mites immobilized on a microscopic slide with two layers of adhesive tape are immersed in the test toxicant solution for a few seconds, has been widely used to test the acute effects of acaricides on both herbivorous and predatory mites (Dittrich, 1962). This topical method usually provides less variable results but can significantly underestimate field resistance compared with other residual methods. In addition, unlike other methods, SD is not suitable for selection experiments because the surviving mites affixed to adhesive tape cannot be recovered from the tape. The leaf dip (LD) method, in which young bean seedlings infested with mites are dipped in the test solution, requires less manual manipulation compared to SD method but results are more variable (Dittrich, 1962). A whole-plant-residual (WPR) bioassay method was developed and shown to be reliable in estimating the dicofol resistance of spider mites in cotton fields (Dennehy et al., 1983). However, it required whole plants and more extensive handling, limiting its practicability as a routinely applicable resistance monitoring tool. Later, a residual contact bioassay method using tight-fitting plastic petri dishes, referred as a practitioner-assessable (PA) bioassay, was developed to facilitate the routine evaluation of susceptibility changes by practitioner and determined to produce reliable results as other assay methods (Dennehy et al., 1987). All these bioassay methods require certain degree of handling techniques and results are often affected by handling skills.

In an attempt to develop a less technique-dependent bioassay that can be mass produced and conveniently used by practitioners or farmers in the fields, the PA bioassay was modified to establish a

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bioassay method using acaricide-coated 5-ml glass vials, dubbed as residual contact vial (RCV) bioassay, and evaluated its performance.

Materials and methods

Acaricide susceptible mite strain

An acaricide-susceptible mite strain (PyriF) was obtained from Sumitomo Chemical (Osaka, Japan) and has been maintained in the laboratory without exposure to any acaricides. A PTF strain, moderately resistant to abamectin but susceptible to tebufenpyrad, was collected from a field site (Pyungtaek, Korea) and maintained in the laboratory without selection. An abamectin-resistant (AbaR) strain was obtained from a green house (Suwon, Korea) and maintained under constant selection with LC_{70} dose of abamectin. All mite strains have been reared on kidney bean plants (2-wk old, *Phaseolus vulgaris* variety *humilis*) under the conditions of 25 ± 1 °C and 55 ± 5% relative humidity (RH) and a photoperiod of 16:8 (L:D) h.

RCV bioassay and determination of diagnostic doses

The procedures for RCV bioassay were as follows. Technical grade of abamectin (8% avermectin B1a + avermectin B1b, Chemservice, PA, USA) and tebufenpyrad (98.5%, Dr. Ehrenstorfer Gmbh, Augsburg, Germany) were dissolved in acetone to make 10,000 ppm stock, serially diluted to appropriate concentrations (3.73, 7.5, 15, 30, and 60 ppm), and a 100 µl aliquot was transferred into a 5-ml transparent glass vial (Taeshin Bioscience, Guri, Republic of Korea). The vial containing acaricide solution was horizontally rotated using a rolling wave rotator (Eberbach, Ann Arbor, MI) for ca. 1 h under a fume hood until acetone is completely evaporated. Fifteen female mites were collected with an aspirator that was fabricated for this study, and directly transferred into an acaricide-coated vial. The collection chamber of the aspirator was made of a piece of transparent silicon tubing (7.0 mm o.d./5.0 mm i.d. × 350 mm), with its bottom part closed with a clamp when collecting mites. To transfer collected mites, the bottom part of the collection chamber was placed into the coated vial, opened and tapped gently. The acaricide-coated vials were closed with cap and held at 25 ± 1 °C, 55 ± 5% RH and a photoperiod of 16:8 (L:D) h. Mortality of mites was examined under a portable convex lens (×10) at various time points after infestation. Mites showing immobility or irregular trembling were considered as dead. RCV bioassay was conducted with three replications. LC_{50} and LC_{99} were calculated by using PoloPlus program (LeOra software Company, Petaluma, CA) (Robertson et al., 2007).

To determine diagnostic doses of abamectin and tebufenpyrad, PyriF strain was exposed to vials coated a series of concentrations of acaricides, and the mortality was evaluated at an interval of 2 h for 12 h period (Fig. 2).

Evaluation of temperature effects on toxicities

Temperatures effects on toxicities were determined under the following temperature conditions: 20, 25, 30 and 35 °C. Fifteen females of PyriF strain were transferred to a vial pre-coated with acaricides (30 ppm abamectin or 60 ppm tebufenpyrad) as described above. The experiment was conducted three replications and its mortalities were observed for 12 h with 2-h interval.

Reproducibility test

To test reproducibility and reliability of RCV bioassay, three different experimenters conducted bioassay independently using pre-coated vials from mite capture to mortality evaluation after being instructed on how to use the kit. Three different mite strains (PyriF, PTF and AbaR) were chosen for the test as they showed differential levels of resistance to test acaricides (PyriF, susceptible to both acaricides; PTF, moderately resistant to abamectin but susceptible to tebufenpyrad; AbaR, resistant to both acaricides). Statistical analysis was conducted by the analysis of variance using Proc ANOVA in SAS (SAS Institute, 2002), and the means were separated by Tukey's test.

RCV bioassay for field populations of T. urticae

Field mite populations were collected from 10 sampling locations (Table 1) selected from the major greenhouse regions. Mites were collected from the canopy of various host plants. At least 5 leaves infested with *T. urticae* were collected from each site and were brought to the laboratory. Then only adult female mites were transferred to kidney bean plants (2-wk old) and maintained separately in the rearing rooms as described above. Additional field populations were collected from four different locations (GC, SW2, GS, YC and YJ; Table 1) and directly used for on-site bioassay. Fifteen female mites per vial were used for RCV bioassay with three replications as described above.

Residue analysis of acaricide-coated vial

Vials were coated with 30 ppm abamectin or 60 ppm tebufenpyrad as described above, wrapped with aluminum foil, and stored in a commercial freezer (−20 °C). Vials were retrieved at the points of 0, 2, 6 and 12 months post-storage and extracted with 500 µl of acetone two times. The combined extracts of tebufenpyrad or abamectin were evaporated under the stream of nitrogen gas.

Tebufenpyrad extract was dissolved in 1 ml acetonitrile and 20 µl aliquot was analyzed by HPLC (HP1100) using Luna-C18 column (5 µm, 4.6 × 250 mm) (Phenomenex Inc., Torrance, CA, USA). Tebufenpyrad sample was eluted with isocretic solvent system of acetonitrile:water (70:30, v/v) at a flow rate of 1.0 ml/min for 20 min and detected with UV 260 nm.

Abamectin extract was dissolved 100 µL *N*-methylimidazole solution [*N*-methylimidazole:acetonitrile (1:1, v:v)], to which 100 µl TFA solution [trifluoroacetic anhydride:acetonitrile (1:2, v:v)] for the fluorescence derivatization of abamectin. Following fluorescence derivatization for 1 min, acetonitrile was added to the solution to the final volume of 1 ml, and 20 µl aliquot was analyzed by HPLC (HP1100) using Eclipse XDB-C8 column (5 Luna-C18 column (5 µm, 4.6 × 250 mm) (Agilent Technologies Inc., Santa Clara, CA, USA). Abamectin sample was eluted isocretically with mobile phase of acetonitrile:water (95:5, v/v) at a flow rate of 2.0 ml/min for 15 min and detected with a fluorescence detector (365 nm λEx vs. 475 nm λEm). Statistical analysis was conducted by the analysis of variance using Proc ANOVA in SAS (SAS Institute, 2002), and the means were separated by Tukey's test.

Table 1
Collection site and host of *T. urticae* field populations used for RCV bioassay.

Population	Collection site	Host	Remarks
AD1	Pungsan-myeon, Andong-si, Gyeongbuk	Strawberry	"
AD2	Pungsan-myeon, Andong-si, Gyeongbuk	Strawberry	"
CA	Mockcheon-eup, Cheonan-si, Chungnam	Paprika	"
N1	Sangweol-myeon, Nonsan-si, Chungnam	Strawberry	"
N2	Sangweol-myeon, Nonsan-si, Chungnam	Strawberry	"
N3	Yeonsan-myeon, Nonsan-si, Chungnam	Strawberry	"
N4	Boojeok-myeon, Nonsan-si, Chungnam	Strawberry	"
PTF	Jinwi-myeon, Pyeongtaek-si, Gyeonggi-do	Rose	"
SW1	Jangan-gu, Suwon-si, Gyeonggi-do	Strawberry	"
SW2	Jangan-gu, Suwon-si, Gyeonggi-do	Strawberry	"
TA	Taean-eup, Taean-gun, Chungnam	Rose	"
GC	Daesan-myeon, Gochang-gun, Jeonbuk	Water melon	On-site bioassay
GS	Gyeongsan-si, Gyeongbuk	Melon	"
YC	Yeongcheon-si, Gyeongbuk	Apple tree	"
YJ	Geumsa-myeon, Yeoju-gun, Gyeonggi-	Melon	"

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