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Toxicity evaluation of botanical insecticides and their mixture against the spiraling whitefly, *Aleurodicus dispersus*

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Abstract: 【Background】*Aleurodicus dispersus* Russell, a destructive invasive pest was first found in Hainan, China, in April 2006. Botanical insecticides have long been suggested as attractive alternatives to synthetic ones for pest management because of their high potency, efficacy and more eco-friendly nature. 【Method】The spraying and film contact methods were used to measure the toxicity of nine botanical insecticides and their mixture against *A. dispersus*. 【Result】The maximum mortality of adults was recorded for pyrethrum and rotenone with LC_{50} at 24 h being 2.56 mg · L^{-1} and 34.15 mg · L^{-1}, respectively. These were followed by azadirachtin (24 h LC_{50} being 158.36 mg · L^{-1}) and bitter melon leaf extracts (24 h LC_{50} being 311.02 mg · L^{-1}). Pyrethrum showed significant insecticidal action to the eggs and nymphs of *A. dispersus* with 24 h LC_{50} being 77.39 mg · L^{-1} and 61.42 mg · L^{-1}, respectively. Bioassay results indicated that mixed azadirachtin or rotenone with pyrethrum (1∶1) exhibited a significant synergism against *A. dispersus*, and their cotoxicity coefficient (*CTC*) were 193.11 and 224.35, respectively. 【Conclusion and significance】Several of the examined botanical pesticides showed potential to control *A. dispersus*. The mixture of azadirachtin or rotenone with pyrethrum could not only have synergism but also delay the resistance of *A. dispersus*.

Key words: pyrethrum; azadirachtin; rotenone; *Aleurodicus dispersus*; insecticidal activity

植物性杀虫剂及其混剂对螺旋粉虱的毒力测定

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摘要:【背景】螺旋粉虱是新入侵海南省的严重为害经济作物及园林苗木的害虫。目前,植物源杀虫剂因具有高效和环境友好等特性而被广泛用于害虫防治中。【方法】采用喷雾法和药膜接触法分别测定了9种植物性杀虫剂对螺旋粉虱的毒力。【结果】在供试的9种植物性杀虫剂中,除虫菊素和鱼藤酮对螺旋粉虱成虫的毒力最强,24 h 的 LC_{50} 分别为2.56和34.15 mg · L^{-1};印楝素和苦瓜叶提取物的毒力次之,LC_{50} 分别为158.36和311.02 mg · L^{-1}。除虫菊素对螺旋粉虱若虫和卵也有一定的触杀作用,LC_{50} 分别为61.42和77.39 mg · L^{-1}。将印楝素和鱼藤酮分别与除虫菊素以1∶1的比例混合,对螺旋粉虱成虫的毒力表现出明显的增效作用,其共毒系数(*CTC*)分别为193.11和224.35。【结论与意义】除虫菊素、鱼藤酮、印楝素和苦瓜叶提取物对螺旋粉虱均具有较强的毒性;印楝素或鱼藤酮与除虫菊素(1∶1)的混合物不仅能增强触杀效果,而且能延缓害虫抗药性的产生。

关键词: 除虫菊素; 印楝素; 鱼藤酮; 螺旋粉虱; 杀虫活性

Introduction

The spiraling whitefly (*Aleurodicus dispersus* Russell) is a destructive invasive pest. This is native to the Caribbean islands and Central America (Russell, 1965), but has spread very fast in African and Asian countries besides several Pacific islands and poses serious problem (Martin & Lucas, 1984; Neuenschwander, 1994). *A. dispersus* is a highly polyphagous pest (Lambkin, 1999; Srinivasa, 2000). Its extensive host range covers 182 plants belonging to 146 genera and 57 families in Hainan (Yu, 2011). The major host plants of economic concern are *Psidium guajava* L., *Carica papaya* L., *Musa sapientum* L., *Annona squamosa* L., *Euphoria longana* Lam, *Solanum melongena*

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L., *Phaseolus vulgaris* L., and *Manihot glaziovii* Muell., in addition to several species of avenue trees such as *Pterocarpus indicus* Willd., *Terminalia catappa* L., *Canna indica* L., and *Cercis chinensis* Bunee in the urban environment (Han *et al*., 2008). Both adults and nymphs of *A. dispersus* suck sap from the leaf surfaces and can cause leaf wilting and dropping. Honeydew and wax excreted by the nymphs and adults on leaf surfaces can decrease the photosynthetic capacity of the host plants (Ramani *et al*., 2002).

A. dispersus was first found in Hainan, China, in April 2006 (Yu, 2011; Yu *et al*., 2007). Within a short period of time, the species has spread to the whole island, and damage to both economic crops and street trees have been documented (Han *et al*., 2008; Zheng *et al*., 2008).

Some synthetic insecticides are proven effective in spiraling whitefly control (Kambrekar *et al*., 2003; Lin *et al*., 2007; Liu *et al*., 2007). However, they are hazardous to human health because of the pest's widespread distribution and its presence in densely populated areas. In addition, indiscriminate use of synthetic insecticides kills beneficial insects and leads to the development of pest resistance to synthetic insecticides. Thus, there is an urgent need for alternative approaches to pest management that can completely or partially replace current chemically-based pest management practices (Dubey & Sundararaj, 2004). Botanical insecticides can contribute towards this goal as attractive alternatives to synthetic chemical insecticides for pest management because of their high potency, efficacy and more eco-friendly nature (Isman, 2006). With this in mind, nine botanically-based insecticide products and their mixtures were evaluated for the suppression of *A. dispersus*. We found several promising alternatives, and identified a combination with a synergistic effect, holding out promise for a more enviornmentally friendly control of this invasive species.

Materials and methods

Insects

The various developental stages of *A. dispersus* used in assays were obtained from a natural population of *A. dispersus* in *Terminalia catappa* L. trees growing on the grounds of the Environment and Plant Protection Institute, Chinese Academy of Tropical Agriculture, Danzhou, Hainan Province, where they were grown free from any insecticides. *A. dispersus* adults, at least 24 h old, were collected randomly from populations of several hundred individuals reared on *T. catappa* for use in the bioassays.

Botanical insecticides

Technical grade of Matrine [98% active ingredient (AI)], Stemonine (2% AI), Sanguinarine (15% AI) and Celangulin (6% AI), were purchased from Shanxi Fangsheng Biological Development Co. Ltd., China. Osthole technical (98% AI) was purchased from Nanjing Zelang Medical Technology Co. Ltd., China. Rotenone (40% AI) and pyrethrum (50% AI) were obtained from Yunnan Nanbao Plant Chemicals Co. Ltd., China. Azadirachtin (20% AI) was purchased from Luxi Light Neem Industry Development Co. Ltd., China.

The chloroform extract of bitter melon (*Momordica charantia* L., BM) leaves were obtained and isolated following Abo & Matsuda (2000). Fresh leaves for extraction were collected from mature *M. charantia* (after the fruiting stage). The leaves were cut into pieces and extracted with methanol at room temperature (29 ~ 31 ℃, 24 h). The extract was filtered and subsequently concentrated to dryness under reduced pressure with a rotary evaporator. The methanol extract was dissolved in water and successively partitioned with hexane and chloroform. A portion of the chloroform extract was dried under reduced pressure.

Bioassays of botanical insecticides

All bioassays were conducted in the laboratory at 30 ± 1 ℃ and 16 h : 8 h (L : D) regime.

Activity of botanical insecticides to adults of A. dispersus by spraying

A 10 mL sample of each test solution (concentration 500 $mg \cdot L^{-1}$ with 50% acetone in water) was prepared. Bioassays were carried out on freshly cut leaves of young *T. catappa*, which had not been subjected to any prior chemical treatment. Single leaves were placed onto moistened filter paper in Petri dishes (9 cm diameter), with the undersides of the leaves facing upward. After anaesthetizing the *A. dispersus* adults (> 24 h old) on the *T. catappa* with ether, thirty anaesthetized adults of *A. dispersus* were gently introduced onto each leaf in the Petri dishes with a fine brush. When most of the insects had recovered and resumed activity, dead and weak insects were removed before starting the exposure. One mL of the 500 mg · L^{-1} test solution was sprayed onto each leaf, by means of a spray tower (Potter, Burkard Company). The solvent was quickly removed by air-drying and the lid of Petri dish was covered by a plastic wrap. Control dishes were treated similarly with 50% acetone in water. Each test was replicated three times. The treated adults were examined for mortality at 12 h intervals after treatment. Adults that did not show any responses when probed with a small brush were considered dead. In the case of pyrethrum, rotenone, azadirachtin and BM extracts, *A. dispersus* adults were examined for mortality at 24 h after treatment of five concentrations gradient, respectively.

Log-probit regression equations were established for pyrethrum, rotenone, azadirachtin and BM extracts and their 24 h median lethal concentrations (LC_{50}) were calculated, respectively.

Activity of botanical insecticides applied as dry films to adults

Bioassays were carried out on freshly cut leaves of young *T. catappa* which had not been subjected to any chemical treatment. The leaves were placed individually on moistened filter paper in Petri dishes (9 cm diameter) with the underside of the leaf facing upward. One mL of the 500 mg · L^{-1} test solution was sprayed onto each leaf using a spray tower, and the solvent was quickly removed by air-drying. Thirty adults of *A. dispersus* (> 24 h old) were anaesthetized and gently introduced onto the treated leaves in the Petri dishes. When most of the adults were awake and active, inactive individuals were eliminated, and the Petri dish lid was closed. Controls were treated similarly with 50% acetone in water. Each test was replicated three times. Insects were examined for mortality at 12 h intervals after treatment. Adults that did not show any activity when probed with a small brush were considered dead. Mortalities were compared statistically.

Activity of pyrethrum to the eggs and nymphs of A. dispersus by spraying

Ten mL samples of pyrethrum dissolved in 50% acetone in water were diluted to concentrations of 12.5, 25, 50, 100, 200 and 400 mg · L^{-1}. The assays were performed on fresh *P. guajava* leaves heavily infested with a natural population of eggs and various stages of nymphs of *A. dispersus*. The leaves were placed individually on moistened filter paper in Petri dishes (9 cm diameter) with the eggs and nymphs on the undersides of the leaves facing upward. The number of eggs and nymphs in each Petri dish were counted with a stereo dissecting microscope. A 1 mL aliquot of the 500 mg · L^{-1} test solution was applied to the eggs and nymphs on the leaf in each Petri dish using a spray tower. The solvent was quickly removed by air-drying and the lid of Petri dish was then covered. Control leaves were treated similarly with 50% acetone in water. Each test was replicated six times. The eggs and nymphs of *A. dispersus* were examined for mortality after five days. Eggs that exhibited shrinkage and darkening were considered dead. Nymphs that showed activity when probed with a small brush were considered to be survivors.

Tests with mixed extracts

The synergistic effect of the mixtures of azadirachtin or rotenone with pyrethrum on adults of *A. dispersus* were determined by the co-toxicity coefficient (*CTC*) method in the laboratory (Sun & Johnson, 1960; Zhang *et al.*, 2008). Five concentrations of two different mixtures were applied by a spray tower to treat thirty *A. dispersus* adults incubated in Petri dishes. The control was distilled water. Each treatment was replicated five times. The number of mortality was recorded at 24 h after treatment. *CTC* of the mixtures were calculated as (Zhang *et al.*, 2008):

$$CTC = [(1/LC_{50M})/(P_A/LC_{50A} + P_B/LC_{50B})] \times 100\%$$

Where: LC_{50A}, LC_{50B} and LC_{50M} mean 50% lethal concentration of pesticide A, B and mixture, respectively. P_A and P_B mean percentage of pesticide A and B in the active constituent of mixture respectively. When *CTC* is 100, it indicates a probability of similar action. With $CTC \gg 100$, a synergistic action can be assumed, while at $CTC \ll 100$ indicates antagonism.

Statistical analysis

Mean values were given with the standard error (*SE*). Differences in mortalities among treatments were analyzed by one-way ANOVA of arcsine square root transformed values, using Duncan's Multiple Range Test for post-hoc comparison of means. LC_{50} values of the tested botanical insecticides and confidence limits were calculated for *A. dispersus* with Log dosage-mortality probit regression equations. All statistical analyses were performed using SAS 9.0 software (SAS Institute Inc. 2005).

Results

Toxicity of botanical insecticides to adults of *A. dispersus* by spraying

Mortalities of *A. dispersus* adults on *T. catappa* leaves, sprayed with different botanical insecticides under laboratory conditions showed significant differences (Table 1). The maximum adult mortality was recorded for pyrethrum and rotenone (mortality of 98.33% and 90.98% at 12 h and 100% and 98.89% at 24 h, respectively. Celangulin, azadirachtin and BM extracts were also relatively effective with mortality at 36 h between 58.8% and 71.5%. There were no significant differences between control and matrine, sanguinarine, osthole and stemonine (Table 1).

Table 1　Toxicity of the nine tested botanical insecticides to adults of *A. dispersus* by spraying

Insecticide name[a]	Mortality of adults (%)[b] at		
	12 h after treatment	24 h after treatment	36 h after treatment
Pyrethrum	98.33 ± 2.89a	100a	100a
Rotenone	90.98 ± 2.10a	98.89 ± 1.92a	98.89 ± 1.92a
Celangulin	63.83 ± 24.08b	71.52 ± 13.11b	71.52 ± 13.11b
Azadirachtin	61.80 ± 17.65b	63.10 ± 2.06b	63.10 ± 2.06b
BM extracts	55.48 ± 4.37b	57.49 ± 3.23b	58.82 ± 5.08b
Matrine	4.20 ± 1.27c	25.48 ± 12.46c	28.15 ± 15.13c
Sanguinarine	12.52 ± 2.85c	22.20 ± 7.99c	23.28 ± 6.9c
Osthole	11.79 ± 7.36c	18.03 ± 12.11c	19.15 ± 10.60c
Stemonine	3.44 ± 3.00c	17.75 ± 10.33c	19.08 ± 8.55c
Control	3.33 ± 5.77c	11.60 ± 1.53c	11.60 ± 1.53c

[a] The concentration was 500 mg · L^{-1}. [b] Mean ± *SE* in the same column followed by different letters indicate significant difference at 0.05 level (Duncan's multiple range test).

Based on the Log-probit regression equations (Table 2) *A. dispersus* adults were the most sensitive to pyrethrum and the least sensitive to BM extracts. LC_{50} of pyrethrum was 1.56 mg · L^{-1}, which was significantly lower than that of rotenone, azadirachtin and BM extracts.

Table 2　Comparative toxicity of nine botanical insecticides to *A. dispersus* adults after 24 h

Insecticide name	Log-probit regression equation[a] (correlation coefficient)	LC_{50} (95% confidence interval) (mg · L^{-1})	Cotoxicity coefficient (*CTC*) (%)
Pyrethrum	$Y = 4.32 + 3.53X$ ($r = 0.98$)	1.56 (1.39 ~ 1.76)	
Totenone	$Y = 2.26 + 1.79X$ ($r = 0.93$)	34.15 (25.00 ~ 46.64)	
Azadirachtin	$Y = 0.43 + 2.07X$ ($r = 0.91$)	158.97 (114.47 ~ 220.77)	
BM extracts	$Y = 0.43 + 1.82X$ ($r = 0.95$)	311.02 (254.58 ~ 379.99)	
Pyrethrum: azadirachtin (1:1)	$Y = 4.48 + 2.55X$ ($r = 0.96$)	1.60 (1.39 ~ 1.84)	193.11
Pyrethrum: rotenone (1:1)	$Y = 4.50 + 4.00X$ ($r = 0.93$)	1.33 (1.23 ~ 1.45)	224.35

[a] *Y* is the logarithm of the treatment concentration (mg · L^{-1}), and *X* is the mortality converted to probit.

The effects of sprays of azadirachtin or rotenone mixed with pyrethrum on adult *A. dispersus*

The effects of two different mixtures on the adults of *A. dispersus* were similarly synergistic. Bioassay results revealed that the synergism was significant, and their *CTC* were 193.11 and 224.35, respectively (Table 2).

Toxicity of botanical insecticides applied as dry films to adults of *A. dispersus*

When adult *A. dispersus* residing on *T. catappa* leaves were treated with dry films of different botanical insecticides under laboratory conditions (Table 3), pyrethrum caused the highest mortalities througout the experiment, followed by azadirachtin (34.8% mortality at 24 h, increasing to 81.8% at 36 h). Matrine, rotenone and sanguinarine were also effective, but they exhibited significantly lower mortalities than the first two (Table 3). None of the other botanical insecticides caused significantly greater mortality than the control. Rotenone, BM extracts and celangulin, when applied as dry films against adults of *A. dispersus* had reduced effects compared to the effects of spraying.

Toxicity of pyrethrum to the eggs and nymphs of *A. dispersus* by spraying

Log-probit regression equations of pyrethrum to eggs and nymphs of *A. dispersus* were shown in Table 4. LC_{50} of pyrethrum to the eggs and nymphs were 77.39 mg · L^{-1} and 61.42 mg · L^{-1}, respectively. Toxicity of pyrethrum to *A. dispersus* adults was higher than that to nymphs or eggs. The fourth instar nymph was the least sensitive to pyrethrum.

Table 3 Toxicity of the nine tested botanical insecticides applied as dry films to adults of *A. dispersus*

Insecticide name[a)]	Mortality of adults (%)[b)] at 12 h after treatment	24 h after treatment	36 h after treatment
Pyrethrum	98.85 ± 1.99a	100a	100a
Azadirachtin	4.58 ± 1.65bcd	34.78 ± 6.41b	81.83 ± 7.90b
Matrine	4.95 ± 1.21bc	27.84 ± 17.18bc	27.84 ± 17.18c
Rotenone	0e	21.32 ± 13.47bcd	26.25 ± 14.79cd
Sanguinarine	2.34 ± 2.03cde	18.04 ± 9.75cde	22.85 ± 10.84cde
BM extracts	1.04 ± 1.80de	6.60 ± 3.23de	16.57 ± 6.25cdef
Stemonine	5.97 ± 2.32b	9.52 ± 2.54de	12.93 ± 3.82cdef
Celangulin	0.93 ± 1.60de	6.47 ± 3.68de	11.19 ± 4.56def
Osthole	0e	3.82 ± 4.44e	8.27 ± 5.29ef
Control	1.33 ± 2.31de	3.00 ± 2.65e	5.22 ± 1.35f

a) The concentration was 500 $mg \cdot L^{-1}$. b) Mean ± *SE* in the same column followed by different letters indicate significant difference at 0.05 level (Duncan's multiple range test).

Table 4 Toxicity of pyrethrum to eggs and nymphs of *A. dispersus* by spraying

Insect stage	Log-probit regression equation[a)] (correlation coefficient)	LC_{50} (95% confidence interval) ($mg \cdot L^{-1}$)
Eggs	$Y = 0.44 + 2.41X$ ($r = 0.94$)	77.39 (66.78 ~ 89.65)
Nymphs	$Y = 1.36 + 2.04X$ ($r = 0.97$)	61.42 (53.07 ~ 71.08)

a) Y is the logarithm of the treatment concentration ($mg \cdot L^{-1}$), and X is the mortality converted to probit.

Discussion

Chemicals derived from pyrethrum (*Tanacetum cinerariifolium* Trev.), neem (*Azadirachta indica* Juss), and other plant species are traditionally used in many crop pest control operations (Atkinson *et al.*, 2004; Liang *et al.*, 2003). Neem products, tobacco extracts and rosin soap had been found effective against *A. dispersus* in several countries (Dubey & Sundararaj, 2004; Kambrekar *et al.*, 2003; Lü *et al.*, 2009; Singh *et al.*, 2005). Zhong *et al.* (2009) observed that ethanol extracts of *Celosia argentea* L. and *Eupatorium odoratum* L. had high bioactivities against *A. dispersus* with LC_{50} being 753.40 $mg \cdot L^{-1}$ and 999.81 $mg \cdot L^{-1}$, respectively. Our results indicated that pyrethrum, rotenone, azadirachtin, BM extracts, and celangulin at 500 $mg \cdot L^{-1}$ concentration exhibited significant insecticidal action on *A. dispersus* adults with mortality above 57.5%, while matrine, sanguinarine, osthole and stemonine showed little insecticidal action on *A. dispersus* adults by spraying. Among the nine botanical insecticides, the maximum mortality of adults was recorded for pyrethrum, whether applied by spraying or as a dry film (Tables 1, 2 and 3). Acute toxicity of pyrethrum to *A. dispersus* adults was the highest and the most rapid than that to the eggs and nymphs (Tables 2 and 4). This may be releated to the thicker waxy layer covering the insect body at these stages. Consequently, we recommend that pyrethrum should be applied during the peak occurrence of adult *A. dispersus*.

The second effective botanical insecticide spray was rotenone. Although the toxicity of azadirachtin to *A. dispersus* adults was lower than that of pyrethrum and rotenone, its toxicity when applied as a dry film was higher with a longer duration of toxicity (Table 3). Dubey & Sundararaj (2004) reported that fortnightly and three-weekly applications of neem effectively controlled nymphal populations of *A. dispersus*, causing 62.2% mortality even at 21 days after treatment. Formulatoin does matter: Neemark retained its toxicity (from an initial mortality of 55% to 45% on day 15) better than Neemazal (45% to 25% mortality, Kambrekar *et al.*, 2003). The different results of toxicity tests might be due to the formulation effect and the response of *A. dispersus* to other toxic components of the neem.

The results of this study showed that the synergistic effects of pyrethrum mixed with azadirachtin or rotenone to the adults of *A. dispersus* were significant (Table 2). There are different mechanisms of action on *A. dispersus* between each insecticide. The pyrethrum is to block voltage-gated sodium channels in nerve axons (Xu, 2001). Rotenone is a mitochondrial poison, which blocks the electron transport chain and prevents energy production (Goyal & Srivastava, 1990). In contrast to action mechanisms of pyrethrum and rotenone, azadirachtin has diverse biological effects on insects, demonstrating antifeedant, oviposition deterrent, repellent, insect growth inhibitor, mating disruptor, and toxic properties (Xu, 2001). Mixed use of insecticides with different action mechanisms can enhance the synergism and also delay the development of resistance in *A. dispersus*.

Extracts of *M. charantia* leaves were evaluated not only for oviposition deterrence, antifeedant effect and inhibition of development but also for toxic action against many phytophagous insect pests (Li *et al.*, 2001; Ling *et al.*, 2008, 2009; Mekuria *et al.*, 2005). Our study confirmed that chloroform extracts of *M.*

charantia leaves had insecticidal effect to *A. dispersus* adults. The acetone extracts of fresh leaves of *M. charantia* are toxic to the adults of *Liriomyza sativae* Blanchard, causing up to 50.48% mortality (Li *et al.*, 2001). The methanol extracts of the leaves of *M. charantia* had insecticidal activity on larvae of *Culex quinquefasciatus* Say with LC_{50} of 465.85 mg · L^{-1} (Prabakar & Jebanesan, 2004). Methanol extracts of *M. charantia* have ovicidal action in *Leucoptera coffeella* (Guérin-Mèneville) (Alves *et al.*, 2011). The extracts of *M. charantia* leaves contain a variety of cucurbitane triterpenoid compounds, such as momordicin Ⅰ, momordicin Ⅱ, 23-dihydroxy-3-O-malonycucurbita-5, 24-dien-19-al, (19*S*, 23*E*)-5β, 19-epoxy-19-methoxy-cucurbita-6, 23-diene-3β, and 25-diol, (19*R*, 23*E*)-5β, 19-epoxy-19-methoxycucurbita-6, 23-diene-3β, 25-diol, which deter oviposition of *Liriomyza trifolii* (Burgess) and *L. sativae* adults, and inhibit feeding and development of *Plutella xylostella* L. (Ling *et al.*, 2008, 2009; Mekuria *et al.*, 2005). The study have established the insecticidal potential of *M. charantia* extracts on *A. dispersus*, further research is required to determine the active components and formulate them.

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