한국농약과학회지 제5권 제4호(2001)
The Korean Journal of Pesticide Science
Vol. 5, No. 4, pp.27~32(2001)

Toxicity of various fruit tree extracts to five agricultural and four stored-product anthropod pests

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Abstract : Methanol extracts from 43 materials of 17 fruit trees were tested for their insecticidal activities toward five agricultural and four stored-product anthropod pests. Efficacy varied with insect species, plant species, and tissue sampled. At a concentration of 2,500 ppm, strong activity was observed with extracts of *Chaenomeles sinensis* and *Punica granatum* seeds against *Myzus persicae* adult females, *Vitis vinifera* seeds against *Nilaparvata lugens* adults, *Citrus junos, Citrus paradisi, Cucumis melo Linne* var. *reticulatus, Diospyros kaki, Malus pumila* var. *dulcissima, Prunus persica,* and *V. vinifera* seeds against *Plutella xylostella, Citrus sinensis* seeds against *Spodoptera litura* larvae, and *Ch. sinensis* and *V. vinifera* against *Tetranychus urticae* adults. Against four stored-product insect pests at 50 ppm, seed extracts of *Ch. sinensis* and *V. vinifera* against *Sitophilus oryzae* adults, and *Ch. sinensis, C. junos, D. kaki,* and *V. vinifera* against *Callosobruchus chinensis* adults gave over 80% mortality. Extracts of all samples exhibited little and no activity against *Lasioderma serricorne* adults and *Plodia interpunctella* larvae.(Received August 23, 2001; accepted December 26, 2001)

Key words : insecticidal activity, fruit tree, stored-product insect, agricultural insect.

Introduction

One of the most important and challenging aspects of pesticide research is the urgent need to develop new and effective methods of controlling various insect pests; these methods should cause no harm to human health and the environment, and they must be accepted as safe by the general public (Brown, 1978; Hayes and Laws, 1991). Natural products, with their tremendous structure diversity, are an important source of new alternatives. Many natural products showing pesticidal activities are isolated every year (Swain, 1977; Wink, 1993). If their properties allow and sufficient quantities can be obtained from natural sources such as plants, these compounds may be used as agricultural chemicals.

Plants may provide an alternative to the currently used insecticides against insect pests, because they constitute a rich source of bioactive chemicals (Swain, 1977; Wink, 1993). Since these are often active against a limited number of pest species, are biodegradable to nontoxic products, and are potentially suitable for use in integrated management program, they could lead to the development of new classes of safer control agents. Much effort has, therefore, been focused on plant materials for potentially useful products as commercial insecticides or as lead compounds (Balandrin *et al.*, 1985; Benner, 1993; Isman, 1995; Hedin *et al.*, 1997). However, little work has been done on the insecticidal activities of various fruit extracts in spite of their excellent nutritional, pharmacological and industrial significance (Gibson *et al.*, 1998; Billson *et al.*, 1999). We have examined the insecticidal activity of fruit extracts against five agricultural and four stored-product anthropod pests.

Materials and Methods

Plant materials and sample preparation. The fruits were purchased from Boeun shop, Kyungdong Market, Seoul (Table 1). The insecticidal activities of methanol extracts of test materials from 17 fruit trees in Actinidiaceae (2), Bromeliaceae (2), Cucurbitaceae (5), Ebenaceae (3), Musaceae (2), Punicaceae (4), Rosaceae (10), Rutaceae (12), and Vitaceae (3) were tested

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against five anthropod pests and four stored-product insect pests. They were dried in an oven at 60℃ for 3 days and finely powdered using a blender (Model : RM 100, F. Kurt Retsch GmbH & Co. KG, Germany). Each sample was extracted twice with 500 ml methanol at room temperature and filtered (Toyo filter paper No. 2, Toyo Roshi, Japan). The combined filtrate was concentrated *in vacuo* at 35℃ using a rotary vacuum evaporator (Model : N-3NW, EYELA, Japan). The yield of methanloic extraction is shown in Table 1.

Table 1. The yield of methanol extracts from various samples

Scientific Name	Family Name	Part	Yield[a] (%)
Actinidia arguta	Actinidiaceae	Fruit	2.8
Actinidia arguta	Actinidiaceae	Seed	5.1
Ananas bracteatus	Bromeliaceae	Fruit	5.9
Ananas bracteatus	Bromeliaceae	Leaf	4.3
Chaenomeles sinensis	Rosaceae	Fruit	2.3
Chaenomeles sinensis	Rosaceae	Peel	2.9
Chaenomeles sinensis	Rosaceae	Seed	3.4
Citrus junos	Rutaceae	Fruit	9.6
Citrus junos	Rutaceae	Peel	4.3
Citrus junos	Rutaceae	Seed	5.3
Citrus paradisi	Rutaceae	Fruit	5.3
Citrus paradisi	Rutaceae	Peel	2.8
Citrus paradisi	Rutaceae	Seed	6.2
Citrus sinensis	Rutaceae	Fruit	2.6
Citrus sinensis	Rutaceae	Peel	5.1
Citrus sinensis	Rutaceae	Seed	3.5
Citrus unshiu	Rutaceae	Fruit	5.2
Citrus unshiu	Rutaceae	Peel	4.5
Citrus unshiu	Rutaceae	Leaf	4.9
Cucumis melo var. *makuwa*	Cucurbitaceae	Fruit	7.7
Cucumis melo var. *makuwa*	Cucurbitaceae	Peel	2.9
Cucumis melo var. *makuwa*	Cucurbitaceae	Seed	4.6
Cucumis melo var. *reticulatus*	Cucurbitaceae	Fruit	6.5
Cucumis melo var. *reticulatus*	Cucurbitaceae	Seed	7.4
Diospyros kaki	Ebenaceae	Fruit	4.2
Diospyros kaki	Ebenaceae	Seed	5.1
Diospyros kaki	Ebenaceae	Leaf	2.9
Fragaria ananassa	Rosaceae	Fruit	7.7
Malus pumila var. *dulcissima*	Rosaceae	Fruit	5.8
Malus pumila var. *dulcissima*	Rosaceae	Seed	6.2
Musa acuminata	Musaceae	Fruit	4.2
Musa acuminata	Musaceae	Peel	7.1
Prunus persica	Rosaceae	Fruit	5.9
Prunus persica	Rosaceae	Seed	3.9
Punica granatum	Punicaceae	Fruit	2.5
Punica granatum	Punicaceae	Peel	3.2
Punica granatum	Punicaceae	Seed	3.0
Punica granatum	Punicaceae	Leaf	5.7
Pyrus ussuriensis var. *macrostipes*	Rosaceae	Fruit	4.2
Pyrus ussuriensis var. *macrostipes*	Rosaceae	Seed	3.6
Vitis vinifera	Vitaceae	Fruit	3.1
Vitis vinifera	Vitaceae	Peel	4.1
Vitis vinifera	Vitaceae	Seed	2.9

[a](Dried weight of methanol extract/dried weight of the sample fruit)×100.

Insects. The brown planthopper (*Nilaparvata lugens* Stål) was reared on rice plant (*Oryza sativa* L.) seedlings (7~10 days after germination) in acrylic cages (26×30×20 cm). The green peach aphid (*Myzus persicae* Sulzer) and the diamondback moth (*Plutella xylostella* L.) were reared on tobacco plant (*Nicotiana tabacum* L.) and beet (*Rhaphanus sativus* L.) seedlings (5~6 days after germination), respectively, in acrylic cages (26×30×20 cm). The tobacco cutworm (*Spodoptera litura* F.) was reared on artificial diet (Lee et al., 2000) in plastic container (24×16×8 cm), and the two-spotted spider mite (*Tetranychus urticae* Koch) was reared on kidney bean (*Phaseolus vulgaris* var. *humilis*). They have been maintained in the laboratory without exposure to any insecticide at 24 ± 2℃ and 50~60 relative humidity (RH) under 16:8 h light:dark cycle.

Four stored-product insect pests were used in this study. Laboratory-reared strains of the rice weevil (*Sitophilus oryzae* L.) and the adzuki bean weevil (*Callosobruchus chinensis* L.) were reared on rice grain and red bean, respectively, in plastic containers (26×30×20 cm) at 28±1℃ and 50~60% RH under 16 : 8 h light : dark cycle. Laboratory-reared strain of the indian meal moth (*Plodia interpunctella* Hübner) were reared on peanut in plastic containers (26×30×20 cm) at 28 ± 1℃ and 50~60% RH under 16 : 8 h light : dark cycle. A susceptible strain of the cigarette beetle (*Lasioderma serricorne* F.) was reared in 0.5-liter masonry rearing jars containing 150 g of sterilized diet (wheatfeed/yeast, 95 : 5, wt/wt) at 28±1℃ and 70~75% RH under 12 : 12 h light:dark cycle.

Bioassay. In relation to the search for new bioactive substances against agricultural insect pests, we have established a bioassay method suitable for rapid mass-screening of synthetic organic compounds or plant-derived extracts for insecticidal activities with reproducible results, using only a minute quantity of compounds. Test materials were tested at a concentration of 5,000 and 2,500 ppm for insect pests. If a fruit extract exhibited activity, titration studies were performed. Test samples were suspended in distilled water with Triton X-100 added at the rate of 0.1 mℓ/L.

Spray method was used for the bioassay of *N. lugens*. Twenty female adults were transferred onto a test tube (3×20 cm) containing five 'Chucheong' rice seedlings wrapped with cotton and 20 mℓ water. Spray of test materials was done with a glass spray unit connected to a forced air supply (Pacific Chemical Co., Ltd., Seoul).

Leaf-dipping method was used for aphid, lepidopteran larvae, and mite. Chinese cabbage (*Brassica oleracea* var. *capitata* L.) leaves for 3rd larvae of each *P. xylostella* and *S. litura*, kidney bean leaves for *T. urticae*, and tobacco leaves for *M. persicae* females from each plant species grown in glasshouse were collected, and disks (5.5 cm diameter) were punctured from each leaf. Three leaf disks were dipped in each test solution for 30 sec. After evaporation in a fume hood for 2 hr, 20 larvae of *P. xylostella* and *S. litura* and 20 *M. persicae* females and 30 *T. urticae* adults were placed separately onto the treated and control leaf disks in petri dishes (6×1.5 cm).

Table 2. Toxicity of the extracts derived from fruit trees to five insect pests at 5,000 ppm

Sample Name	Activity[a]				
	BPH[b]	GPA	DBM	TCW	TSSM
Ch. sinensis (S)[c]	++++	-	++	-	++++
C. junos (S)	-	-	++++	-	++
C. paradisi (P)	-	++	-	-	++
C. paradisi (S)	-	-	++++	-	-
C. sinensis (S)	-	-	-	++++	-
C. unshiu (P)	-	++	-	-	-
C. melo var. *reticulatus* (F)	-	-	-	-	++
C. melo var. *reticulatus* (S)	-	-	++++	-	-
D. kaki (S)	-	-	++++	-	-
M. pumila var. *dulcissima* (S)	-	-	++++	-	-
P. persica (S)	-	-	++++	-	-
P. granatum (S)	++++	-	++	-	-
V. vinifera (S)	-	++++	++++	-	++++

[a]++++, >90%; +++, 80~90%; ++, 61~80%; +, 40~60%; and -, <40%. [b]BPH, *Nilaparvata lugens*; GPA, *Myzus persicae*; DBM, *Plutella xylostella*; TCW, *Spodoptera litura* and TSSM, *Tetranychus urticae*. [c]F, Fruit; P, Peel; S, Seed.

The insecticidal activity of the test materials to stored-product insect pests used was determined by the direct contact application. Appropriate rates of each test compound dissolved in 100 μL of methanol were applied to filter papers (Whatman No. 2; 5.5 cm dia.). Controls received 100 μL of methanol. After drying in a fume hood for 2 min, each paper was placed in the bottom of a petri dish (5.5 dia.×1.2 cm), and then 20 adults of *S. oryzae*, *C. chinensis*, and *L. serricorne* were placed separately in each petri dish and covered with a lid.

Treated and control insects were held under the same conditions described earlier. Mortalities were determined 24 hr after treatment. Test insects were considered dead if appendages did not move when prodded with a camel's hair brush. All treatments were replicated three times. The insecticidal activities were classified as follows: the very strong activity ++++, mortality >90%; strong +++, mortality 81~90%; moderate ++, mortality 61~80%; weak +, mortality 40~60%; and little or no activity -, mortality <40%.

Results and Discussion

At 5,000 ppm, the insecticidal activities of the test samples against five agricultural insect pests are shown (Table 2). Responses varied with insect species, plant species, and tissue sampled. Methanol extracts from seeds of *Chaenomeles sinensis* and *Punica granatum* gave over 90% mortality (++++) against *N. lugens* adults, whereas no activity was observed in the extracts of the other samples. In a test with *M. persicae* adults, over 90% mortality was produced from extract of *Vitis vinifera* seed, but peel extracts of *Citrus paradisi* and *Citrus unshiu* revealed a moderate insecticidal activity (++). Against *P. xylostella* larvae, extracts from seeds of *Citrus junos*, *C. paradisi*, *Cucumis melo* var. *reticulatus*, *Diospyros kaki*, *Malus pumila* var. *dulcissima*, *Prunus persica*, and *V. vinifera* showed very strong activity and seed extracts of *Ch. sinensis* and *P. granatum* exhibited moderate insecticidal activity. Seed extract of *Citrus sinensis* exhibited very strong activity against *S. litura* larvae, but no activity was observed in other 42 sample extracts (data not shown). With *T. urticae* adults, very strong acaricidal activity (over 90% mortality) was observed with extracts of *Ch. sinensis* and *V. vinifera*, whereas moderate activity was produced from extracts of *C. junos* seed, *C. paradisi* peel, and *C. melo* var. *reticulatus* fruit.

When treated with 2,500 ppm, seed extracts of *Ch. sinensis* and *P. granatum* gave over 80% mortality on *N. lugens* (Table 3). Extract of *V. vinifera* seed revealed very strong activity gave over 90% mortality against *M. persicae*. For *P. xylostella*, strong insecticidal activity over 80% mortality were observed in seed extracts of *C. junos*, *C. paradisi*, *C. melo* var. *reticulatus*, *D. kaki*, *M. pumila* var. *dulcissima*, *P. persica*, and *V. vinifera* at 2,500 ppm. Seed extracts of *C. sinensis* exhibited strong insecticidal activity against *S. litura*. With *T. urticae* at 2,500 ppm, strong activity gave over 80% mortality was observed in seed extracts of *Ch. sinensis* and *V. vinifera*.

Table 3. Toxicity of the extracts derived from fruit trees to five insect pests at 2,500 ppm

Sample Name	Activity[a)]				
	BPH[b)]	GPA	DBM	TCW	TSSM
Ch. sinensis (S)[c)]	+++	-	++	-	+++
C. junos (S)	-	-	+++	-	+
C. paradisi (P)	-	+	-	-	+
C. paradisi (S)	-	-	+++	-	-
C. sinensis (S)	-	-	-	+++	-
C. unshiu (P)	-	+	-	-	-
C. melo var. *reticulatus* (F)	-	-	-	-	+
C. melo var. *reticulatus* (S)	-	-	+++	-	-
D. kaki (S)	-	-	++++	-	-
M. pumila var. *dulcissima* (S)	-	-	+++	-	-
P. persica (S)	-	-	+++	-	-
P. granatum (S)	+++	-	++	-	-
V. vinifera (S)	-	++++	+++	-	+++

[a)]++++, >90%; +++, 80~90%; ++, 61~80%; +, 40~60%; and -, <40%. [b)]BPH, Nilaparvata lugens; GPA, Myzus persicae; DBM, Plutella xylostella; TCW, Spodoptera litura and TSSM, Tetranychus urticae. [c)]F, Fruit; P, Peel; S, Seed.

Table 4. Insecticidal activity of the extracts derived from fruit trees to stored-product insect pests using direct contact application at 50 ppm

Sample Name	Activity[a]			
	S. oryzae	*C. chinensis*	*L. serricorne*	*P. interpunctella*
Ch. sinensis (S)[b]	++++	++++	-	+
C. junos (S)	-	++++	-	-
D. kaki (S)	-	+++	-	-
P. granatum (S)	-	++++	-	-
V. vinifera (S)	++++	++++	-	+

[a]++++, >90%; +++, 80-90%; ++, 61~80%; +, 40~60%; and -, <40%. [b]F, Fruit; P, Peel; S, Seed.

Toxic effects of test materials on four stored-product insect pests are shown at 50 ppm in Table 4. Responses varied according to insect species, plant species, and tissue sampled. Seed extracts of *Ch. sinensis* and *V. vinifera* caused over 90% mortality against *S. oryzae*. For *C. chinensis* adults, strong activity (over 80% mortality) were produced from seed extracts of *Ch. sinensis, C. junos, D. kaki,* and *V. vinifera,* whereas remaining samples revealed no activity (data not shown). Against *L. serricorne* adults and *Plodia interpunctella* larvae, all test materials exhibited little and no activity (data not shown).

With various fruits belonging to the family Actinidiaceae, Bromeliaceae, Cucurbitaceae, Ebenaceae, Musaceae, Punicaceae, Rosaceae, Rutaceae, and Vitaceae, some of them showed very strong insecticidal activity against the economically important agricultural and stored-product anthropod pests. Insecticidal activity varied with both the fruit species and anthropod pests. In a test with agricultural anthropod pests, *P. xylostella* and *T. urticae* were inhibited more effectively by the application of methanol extracts of various fruits than *M. persicae, N. lugens,* and *S. litura,* and, when tested with four stored-product insects, *S. oryzae* and *C. chinensis* were controlled more effectively by the application of ethanol extracts of various fruits than *L. serricorne* and *P. interpunctella*. Jacobson (1989) pointed out that the most promising botanicals as sources of novel plant-based insecticides at present and in the future are species of the families Meliaceae, Rutaceae, Asteraceae, Annonaceae, Labiatae, and Canellaceae. It has been also reported that Annonaceous plant species can be employed as safe, effective, economical, and environmentally friendly insecticides on the home garden, ornamental, and greenhouse (Hostettman and Potterat, 1997). Various compounds including phenolics, terpenoids, and alkaloids exist in various fruits (Swain, 1977; Wink, 1993). These compounds jointly or independently contribute to generation of biological activities. About 20,000 secondary plant metabolites have been chemically identified so far (Wink, 1993). Since these plant-derived extracts and phytochemicals act in many ways on various types of complex induced by insect pests, and may be applied to the plant in the same way as other agricultural chemicals, they are being considered as potential alternatives for synthetic insecticides (Hostettman and Potterat, 1997; Hedin, 1982), or lead compounds for new classes of synthetic insecticides such as podoblastin produced from *Podophyllum peltatum* (Miyakado, 1986; Hostettman and Potterat, 1997). In our study, these fruits having significant insecticidal activity might give a new clue for managing insect pests and stored-product insect pests in field ecosystem, although their effects on non-target organisms or environment remain unknown.

Acknowledgments

This work was supported by the grant of Post-Doc. Program, Chonbuk National University(2001).

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다양한 과일나무에서 유래된 추출물의 농업해충 및 저장물해충에 대한 살충활성

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요약 : 17과일의 43가지 부위의 메탄올 추출물을 대상으로 5종의 주요 농업해충 및 4종의 저장물 해충에 대한 살충효과를 조사한 효과는 과일류의 종류 및 해충의 종류에 따라 커다란 차이를 보였다. 5,000 ppm 농도에서 80% 이상의 살충효과를 나타내는 10종의 시료를 2,500 ppm에서 살충효과를 검정하였다. 모과와 석류씨가 벼멸구, 포도씨가 복숭아혹진딧물, 유자, 자몽, 메론, 감, 사과, 복숭아, 포도씨가 배추좀나방, 모과씨가 담배거세미나방, 모과와 포도 추출물이 점박이응애에 대하여 80% 이상의 살충효과를 나타냈다. 4종의 저장물해충에 대한 살충효과는 50 ppm의 농도로 처리할 때 모과 및 포도 추출물이 쌀바구미, 모과, 유자, 감 및 포도 추출물이 팥바구미에 대하여 80% 이상의 살충효과를 나타냈다. 그러나 화랑곡나방와 권련벌레에 대하여는 사용된 과일류의 추출물이 활성을 나타내지 않았다. 이상의 결과로부터 복숭아혹진딧물, 벼멸구, 배추좀나방, 담배거세미나방 및 점박이응애에 높은 방제효과를 보인 상기 과일류 추출물들은 농업해충 방제제로서 사용 가능성이 예상되었으며, 또한 쌀바구미와 팥바구미에 강한 살충효과를 보인 추출물은 저장물해충방제에 이용할 수 있을 것으로 기대된다.

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