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Effect of Various Plant Extracts and Organic Emulsifiers on Acaricidal Activity of Two-spotted Spider Mite (*Tetranychus urticae*)

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The objective of this research was to determine acaricidal activity (AA) on the two-spotted spider mite (*Tetranychus urticae*; TSSM) in persimmon trees by (1) using plant extracts (PEs) and different extraction methods (water, boiling water, fermentation, and ethanol) from various plant parts (leaves, stems, fruits, and roots) in 46 species from 28 families, (2) using mixers of various PEs and organic emulsifiers (OEs), and (3) using selected PEs and OEs. Boiling water extracts of *Chrysanthemum zawadskii* and *Mentha arvensis* all parts above ground, and *Rehmannia glutinosa* and *Coptis japonica* roots among 46 plant species from 28 families were found to be more effective on AA of TSSM, and TSSM was 57–75% controlled by 10% of the PEs in a laboratory test. The order of AA on TSSM by OEs at 5% concentration was powder soap (100%; PS) > natural emulsifier-B (98%; NEB) > loess sulphur (89%; LS) > natural emulsifier-A (51%; NEA) > brown rice vinegar (45%; BRV). In the treatment by PEs alone, TSSM was 60.4% and 44.0% controlled by 5% extracts of *Chrysanthemum zawadskii* and *Rehmannia glutinosa*, respectively, in an organically produced persimmon tree field. However, in the combination treatments by PEs and OEs, TSSM was 55.5–77.9% controlled by *Chrysanthemum zawadskii* extract (5%) + NEB (1%), *Rehmannia glutinosa* extract (5%) + NEB (1%), *Chrysanthemum zawadskii* extract (5%) + PS (1%), and *Rehmannia glutinosa* extract (5%) + PS (1%) when compared with non-treated controls. Persimmon leaf injuries did not show after treatments by 10% *Chrysanthemum zawadskii* and *Rehmannia glutinosa* extracts, and 5% NEB and PS. Thus, the PEs and OEs may be used for controlling TSSM in organically produced crop fields.

Key Words: emulsifiers, persimmon tree, plant extract, *Tetranychus urticae*, two-spotted spider mite

Abbreviations: AA – acaricidal activity, BRV – brown rice vinegar, DAT – days after treatment, LS – loess sulphur, NEA – natural emulsifier-A, NEB – natural emulsifier-B, OE – organic emulsifier, PE – plant extract, PS – powder soap, TSSM – two-spotted spider mite

INTRODUCTION

The two-spotted spider mite (*Tetranychus urticae*; TSSM) is a pest all over the world. It damages various crops such as persimmon trees, pear fruit trees, soybean, cotton, cucumber, and tomato. The damaged crop leaves are initially discolored before turning white, thereafter changing gradually to brown, and finally the damaged leaves fall from the plants (Croft et al. 1987; Hall and Ferree 1975; Ho 2000; McNab and Jerier 1993; Song et al. 1995; Stacey et al. 1985; Takafuji et al. 2000; Wilson et al. 1991).

Pesticides have been used for a long time to control pests such as TSSM. However, recent studies have shown that ecosystem destruction, environmental pollution, and resistant pests have resulted from abuse or repeated use of pesticides (Cho et al. 1995; Song et al. 1995). Thus, in order to overcome problems caused by pesticides, many studies have focused on plant extracts (PEs) and organic emulsifiers (OEs) that do not significantly affect the environment and show low toxicity to natural enemies and wide spectrums of pest control (Choi et al. 2006; Hwang et al. 2009; Kim et al. 2009; Kwak et al. 2012; Park et al. 2008; Park et al. 2012).

Seventy kinds of natural PEs have been used as alternatives to pesticides. The representative PEs and their active ingredients are *Sophora flavescens* Ait (matrine and oxymatrine), *Azadirachta indica* A. Juss. (L.) (neem oil and azadirachtin), *Chrysanthemum cinerariaefolium* (Trev.) Vis. (pyrethrin), Derris (rotenone), tobacco (nicotine), and tea (saponin). Eighty percent of the alternatives are derived from PEs (Korea Eco-friendly Farming Products Association 2012). Pyrethroid compounds contained in *Chrysanthemum cinerariaefolium* (Trev.) Vis. among the plant-derived extracts were most commonly used for producing insecticides, and currently these products are sold as insecticides (Korea Eco-friendly Farming Products Association, 2012; Taiz and Zeiger 1998). In addition, *Melia azedarach* L., a deciduous broad-leaved arboreous tree grown in the Chonnam coastal regions, provides plant leaves, bark, and fruit extracts that have avoidance, feeding inhibition, and insecticidal activity to various pests, and insecticidal active ingredients extracted from the plant parts are limonoids (Carpinella et al. 2002). Moreover, these extracts have been used in some organic rice cultivation (Kim et al. 2010; Korea Eco-friendly Farming Products Association 2012; Taiz and Zeiger 1998). In experiments on acaricidal activity (AA) of TSSM by methanol and hexane extracts of 35 medicinal plant species from 25 families, results showed 56.8%, 47.8%, 47.7%, and 47.7% AA in *Torreya nucifera* fruit and seeds of *Daphne genkwa, Xanthium strumarium*, and *Pharbitis nil* extracts, respectively (Lee et al. 2011). In addition, AA of TSSM by methanol extract with plant parts of 21 plant species collected from Africa showed big differences in terms of plant parts and plant species. Among these extracts, methanol extracts of *Celosia trigyna* and *Combretum micronthum* all parts above ground, *Combretum glutinotum* leaves, *Prosopis chinensis* leaves and fruits showed higher acaricidal activities on TSSM (Hiremath et al. 1995).

In Korea, a total of 1,363 eco-friendly agricultural materials are registered. The number of PEs among the items is 40 (RDA National Academy of Agricultural Science 2008). The plant-derived extracts with boreudoaek, soapy water, a mixture of lime sulfur, sulfur, ethyl alcohol, and paraffin are used in organic cultivation (RDA National Academy of Agricultural Science 2008). However, agricultural organic materials for controlling diseases and insects in organically produced crops have not been as effective as conventional methods (pesticides) and a number of the agricultural organic materials registered are still needed to determine controlling effects on various crop diseases and insects. Moreover, studies on agricultural organic materials for control of TSSM in persimmon trees are very limited.

The objective of this research was to determine controlling effects on AA of TSSM by (1) using PEs from different extraction methods (water, boiling water, fermentation, and ethanol) from various plant parts (leaves, stems, fruits, and roots) in 46 species from 28 families, (2) using mixers of various PEs and OEs, and (3) using selected PEs and OEs in persimmon trees.

MATERIALS AND METHODS

Plant Materials

Forty-six plant species from 28 families were used for determining AA of TSSM in this study. The plant species were selected after considering potential controlling effects on TSSM by a literature review (ISU Extension and Outreach, Organic Agriculture 2015; Korean Organic Agriculture Material Center 2016; Prince Edward Island 2016) and a survey of the farms where plant species were to be collected. Some species of these plants were collected directly in the fields; other plant species were purchased from the Chonnam Hanyaknonghyup Corporation. The collected plant species were identified by an expert, Dr. Kang Byoung-Man (Jeollanamdo Development Institute of Traditional Korean Medicine) for plant identification. Specific information on the plant species is given in Table 1.

Acaricidal Activity of Two-spotted Spider Mite by Plant Parts and Extraction Methods in Various Plant Species

Leaves, stems, roots, and barks of 46 plant species shown in Table 1 were dried and ground. The extraction methods used for this study were water, boiling water, ethanol, and fermentation, as described in a previous study by Jang et al. (2016). Thirty PSSM adult females were inoculated on a kidney bean leaf disc (diameter 4 cm) and then sprayed with plant extracts at 5% concentration with a hand sprayer. AA was investigated at 1, 3, and 5 d after treatment.

Table 1. Plant species used in this study.

Family	Plant Species	Plant Part
Apiaceae	*Angelica gigas* Nakai.	Root
	Torilis japonica (Houtt.) DC.	Root
	Angelica dahurica (Fisch.er) Bentham et Hook.er F.	Root
Apocynaceae	*Nerium indicum* Mill.	Leaf, Stem
Araceae	*Acorus gramineus* Sol.	All parts above ground
Chenopodiaceae	*Chenopodium album* L. var. *centrorubrum* Makino	All parts above ground
Caeomposit	*Cirsium japonicum var. maackii* (Maxim.) Matsum.	Root
	Polymnia sonchifolia Poepp. & Endl	Leaf, Root
	Artemisia princeps Pamp.Hara	All parts above ground
	Chrysanthemum zawadskii Herb. var. *latilobum* (Maxim.) Kitamura.	All parts above ground
	Xanthium strumarium L.	Fruit
	Eclipta prostrata L.	All parts above ground
	Conyza canadensis L.	All parts above ground
	Helianthus tuberosus L.	Bulb, Leaf
	Lactuca indica var. *laciniata*	All parts above ground
	Erigeron annuus (L.) Pers.	All parts above ground
	Petasites japonicus (Siebold & Zucc.) Maxim.	Leaf, Stem
	Taraxacum platycapum Dahlst	All parts above ground
Cucurbitaceae	*Trichosanthes kirilowii*	Stem, Fruit, Root
Cupressaceae	*Thuja orientalis* L.	Leaf, Stem
Cyperaceae	*Cyperus rotundus* L.	Fruit
Euphorbiaceae	*Vernicia fordii* (Hemsl.) Airy Shaw	Leaf, Stem
	Ricinus communis L.	All parts above ground
Geraniaceae	*Geranium thunbergii* Siebold & Zucc.	All parts above ground
Labiatae	*Salvia miltiorrhiza* Bunge	Root
	Leonurus japonicus Houtt.	All parts above ground
	Mentha arvensis L.	All parts above ground
Lauraceae	*Cinnamomum cassia* Blume	Bark
Leguminosae	*Sophora flavescens* Ait	Root
	Astragalus mongholicus Bunge	Root
Meliaceae	*Melia azedarach* L.	Fruit
Papaveraceae	*Chelidonium majus* L. var. *asiaticum* Ohwi	All parts above ground
Pittosporaceae	*Pittosporum tobira* (Thunb.) W.T. Aiton	Leaf, Stem
Plantaginaceae	*Plantago asiatica* L.	All parts above ground
Polygonaceae	*Rheum rhabarbarum* L.	Root
	Rumex crispus L.	All parts above ground
Portulacaceae	*Portulaca oleracea* L.	All parts above ground
Ranunculaceae	*Coptis japonica* (Thunb.) Makino	Root
Saururaceae	*Houttuynia cordata* Thunb.	All parts above ground
Scrophulariaceae	*Rehmannia glutinosa* var. *purpurea* (Makino) Makino & Nemoto	Root
Solanaceae	*Nicotiana tabacum* L.	All parts above ground
Stemonaceae	*Stemona japonica* (Bl.) Miq.	Root
Styracaceae	*Styrax japonicus* Siebold & Zucc.	Leaf, Stem
Theaceae	*Camellia japonica* L.	Leaf, Stem
Umbelliferae	*Cnidium officinale* Makino	Root
Zingiberaceae	*Curcuma longa* L.	Root

Acaricidal Activity of Two-spotted Spider Mite by Selected Plant Extracts

Four PEs, boiling water extracts of *Chrysanthemum zawadskii* and *Mentha arvensis* all parts above ground, and *Rehmannia glutinosa* and *Coptis japonica* roots that showed higher AA on TSSM were selected from Table 1 for further study. AA on TSSM was evaluated after applying PE treatments at 0%, 3%, 5%, and 10% concentrations. The experiment on AA was carried out in the same way as described in the above section.

Acaricidal Activity of Two-spotted Spider Mite by Organic Emulsifiers

To determine AA on TSSM by various OEs, we used NEA or NEB, LS, BRV, and PS. Manufacturing and experimental methods on the OEs were used as described in the previous study (Jang et al. 2015). In addition, selected PEs (boiling water extracts of *C. zawadskii, M. arvensis, R. glutinosa,* and *C. japonica*) at 5% concentration and OEs at 1% concentration alone or with combination were used for investigating the AA of TSSM. The experiment was carried out in the same way as described in the above section. The PEs and OEs used in this study are approved for use in organic farming by the Korea Eco-friendly Farming Products Association (2012).

Controlling Effect of Two-spotted Spider Mite in Organic Cultivation

The selected PEs, boiling water extracts of

Chrysanthemum zawadskii and *Rehmannia glutinosa* at 5% concentration combination with OEs, NEB and PS at 1% concentration were sprayed on leaves of persimmon trees (15 yr old, cv. Charang, three trees per replication). Fifty TSSM adult females were inoculated on trees grown in organic cultivation fields in Suncheon, South Korea (latitude 34° 57′ and longitude 127° 29′) and the controlling effect was investigated at 5 d after treatment (DAT). Damage of persimmon tree leaves by PEs and OEs was evaluated as described in the previous study by Jang et al. (2016).

Statistical Analysis

All experiments were conducted two or three times with three replicates for each treatment. Data were analyzed using analysis of variance (ANOVA) procedure in the Statistical Analysis Systems (SAS 2000) software. Means were separated using Duncan's multiple range test ($P = 0.05$).

RESULTS AND DISCUSSION

Acaricidal Activity of Two-spotted Spider Mite by Plant Parts and Extraction Methods in Various Plant Species

AA of TSSM was investigated in water, boiling water, ethanol, and fermentation extracts of leaves, stems, and barks of 46 species (data not shown). Plant species, plant parts, and extraction methods showing over 50% AA on TSSM in PEs at 5% concentration were boiling water extracts of *C. zawadskii* and *M. arvensis* all parts above ground, and *R. glutinosa* and *C. japonica* roots. However, other PEs showed below 50% AA on TSSM, as in the reported insecticidal activity of *Melia azedarach* L. and *Nicotiana tabacum* L. extracts (Kim et al. 2010; Korea Eco-friendly Farming Products Association 2012). On the other hand, the PEs used in our study showed only below 10% AA on TSSM. This result indicates that AA on TSSM was different in terms of plant species, plant parts such as leaves and stems, and extraction methods. For example, Hiremath et al. (1995) and Lee et al. (2011) reported that AA on TSSM varied depending on plant parts used for extraction. This difference is due to different physiologically active ingredients of plant parts (Singh et al. 2010). Moreover, AA on TSSM may appear different by the country of origin or extraction methods of samples in the same plant parts.

Acaricidal Activity of Two-spotted Spider Mite by Selected Plant Extracts

The selected four PEs (boiling water extracts of *Chrysanthemum zawadskii* and *Mentha arvensis* all parts above ground, and *Rehmannia glutinosa* and *Coptis japonica* roots) from experiments in Table 1 were sprayed with various concentrations for determining AA of TSSM (Table 2). The order of AA on TSSM by PEs at 10% concentration was *Chrysanthemum zawadskii* (75%) > *Coptis japonica* (68%) > *Mentha arvensis* (61%) > *Rehmannia glutinosa* (57%).

Acaricidal Activity of Two-spotted Spider Mite by Organic Emulsifiers

NEA or NEB, LS, BRV, and PS at 0%, 0.5%, 1%, and 5%, respectively, were treated to determine AA of TSSM (Table 3). The order of AA on TSSM by PEs at 5% concentration was PS (100%) > NEB (98%) > LS (89%) > NEA (51%) > BRV (45%). Soap and oil were first used among agricultural materials for insect

Table 2. Acaricidal activity of selected plant extracts against *Tetranychus urticae* in a laboratory test.

Plant Species	Plant Part	Extract Conc. (%)	Acaricidal Activity (%)		
			1 DAT	3 DAT	5 DAT
Control			0.0^{e*}	0.0^{e}	0.0^{h}
		3	16.9^{c}	38.2^{c}	40.4^{f}
CZ	All parts above ground	5	27.0^{b}	48.9^{b}	62.5^{c}
		10	34.8^{a}	50.5^{ab}	75.0^{a}
		3	15.7^{c}	21.3^{d}	36.3^{f}
MA	All parts above ground	5	17.0^{c}	36.7^{c}	55.4^{e}
		10	23.5^{b}	39.3^{c}	61.4^{cd}
		3	6.7^{d}	16.9^{d}	23.5^{g}
RG	Root	5	1.9^{d}	21.5^{d}	56.1^{de}
		10	15.7^{c}	34.9^{c}	56.8^{de}
		3	14.6^{c}	17.9^{d}	24.7^{g}
CJ	Root	5	32.9^{a}	55.2^{a}	61.4^{cd}
		10	33.7^{a}	55.0^{a}	68.2^{b}

DAT – days after treatment, CZ – *Chrysanthemum zawadskii*, MA – *Mentha arvensis*, RG – *Rehmannia glutinosa*, CJ – *Coptis japonica*
*Means within a column followed by the same letters are not significantly different at 5% level (Duncan's Multiple Range Test).

Table 3. Effect of various emulsifiers on acaricidal activity against *Tetranychus urticae* in laboratory test.

Emulsifiers	Extract Conc. (%)	Acaricidal Activity (%)		
		1 DAT	3 DAT	5 DAT
Control		0.0[h*]	0.0[j]	0.0[j]
	0.5	2.2[h]	5.6[i]	6.8[i]
Natural emulsifier-A	1	4.5[gh]	10.1[hi]	10.2[i]
	5	46.0[c]	49.4[c]	51.1[d]
	0.5	20.2[f]	23.6[g]	25.0[g]
Natural emulsifier-B	1	41.5[c]	47.1[cd]	47.7[de]
	5	94.3[a]	95.5[a]	97.7[a]
	0.5	20.2[f]	22.5[g]	23.8[g]
Loess sulfur	1	34.9[d]	41.6[e]	47.7[de]
	5	80.9[b]	83.1[b]	88.6[b]
	0.5	10.1[g]	13.4[h]	13.6[h]
Brown rice vinegar	1	19.0[f]	24.7[g]	29.5[f]
	5	30.3[de]	43.8[de]	45.4[e]
	0.5	28.1[e]	30.3[f]	33.0[f]
Powder soap	1	43.9[c]	51.7[c]	55.7[c]
	5	100.0[a]	100.0[a]	100.0[a]

DAT – day(s) after treatment
[*]Means within a column followed by the same letters are not significantly different at 5% level (Duncan's Multiple Range Test).

control before the development of synthetic pesticides (Puritch 1981). However, agricultural materials such as soap and oil were not used after the development of synthetic pesticides. Recently, several emulsifiers such as insecticidal soap and detergents, which have been found to be less toxic to the environment, show higher insecticidal activities on parasitic bee and *Bemisia tabaci* (Butler et al. 1993; Tremblay et al. 2008). Thus, these emulsifiers are now being used again in organic cultivation. Although powder soap used in our study was different in manufacturing method compared with the above-mentioned insecticidal soap, TSSM was controlled 100% by the PS at 5% concentration, indicating that emulsifiers used in our study can also be used for control of TSSM in organic agriculture.

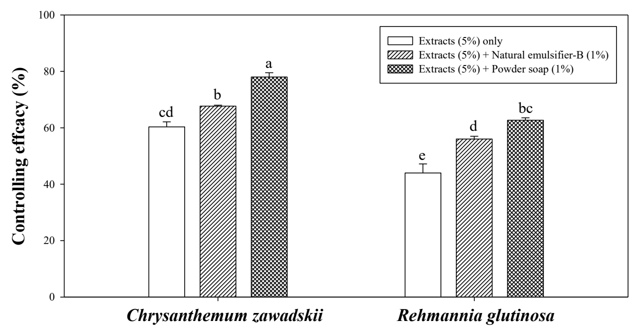

Fig. 1. Effect of plant extracts and emulsifiers mixed in organic field grown persimmon tree (cv. Charang) infected with *Tetranychus urticae*. Parameter was recorded at 5 d after treatment. Means on the bar followed by the same letters are not significantly different at 5% level (Duncan's Multiple Range Test).

Controlling Effect of Two-spotted Spider Mite in Organic Cultivation

TSSM was controlled 60.4% and 44.0% by *C. zawadskii* extract at 5% and *R. glutinosa* extract at 5% alone treatment, respectively, compared with the untreated control in organic cultivation (Fig. 1). TSSM was controlled 67.5% and 55.8% by *C. zawadskii* extract at 5% + NEB at 1% and *R. glutinosa* extract at 5% + NEB at 1%, respectively, compared with the untreated control. In addition, TSSM was controlled 77.9% and 62.9% by *C. zawadskii* extract at 5% + PS at 1% and *R. glutinosa* extract at 5% + powder soap at 1%, respectively, compared with the untreated control.

Synergistic effects appeared as a result of treatments by PE and OE mixtures to control TSSM under organic cultivation field conditions. On the other hand, leaf injuries of persimmon trees by the above PEs and OEs had no significant difference between treated plots and untreated plots (data not shown). These results indicate that the PEs, *Chrysanthemum zawadskii* and *Coptis japonica* at 10% concentration and OEs, NEB, LS, and PS at 5% concentration can be used in organic persimmon tree cultivation because they showed higher controlling effect on TSSM without leaf injury of persimmon.

CONCLUSION

Boiling water extracts of *C. zawadskii* and *M. arvensis* all parts above ground, and *R. glutinosa* and *C. japonica* roots among plant parts and extraction methods in 46 plant species were more effective on AA of TSSM, and TSSM was 57–75% controlled by 10% of the PEs in a laboratory test. The order of AA on TSSM by PEs at 5% concentration was PS (100%) > NEB (98%) > LS (89%) > NEA (51%) > BRV (45%). Synergistic effects by PE and OE mixtures to control TSSM appeared under organic cultivation field conditions. In addition, persimmon leaf injuries did not show after treatments with 10% *C. zawadskii* and *R. glutinosa* extracts, and 5% NEB and PS. Thus, the PEs or OEs may be used for controlling TSSM in organically cultivated crop fields.

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