Full Length Research Paper

# Acaricidal activity of tropical plant extracts against citrus mites and their effect on predator and citrus plants

# Edy Syahputra<sup>a</sup> and Otto Endarto<sup>b</sup>

<sup>a</sup>Fakultas Pertanian, Universitas Tanjungpura, JI A Yani, Pontianak 78124, Indonesia
 <sup>b</sup>Balai Penelitian Tanaman Jeruk dan Buah Subtropika, Batu-Jawa Timur, Indonesia
 \*Corresponding Author E-mail: e\_sitorus\_2000@yahoo.com, HP +628121996086

#### ABSTRACT

The objectives of this study were to evaluate the acaricidal activity of 30 tropical plants extracts against citrus mite and their effect on predator and citrus plants. The citrus rust mite *Phyllocoptruta oleivora* and the citrus red mite *Panonichus citri* were used as mite test, while *Harmonia axyridis* was as predator test. Plant materials were extracted with ethanol and water separately using infusion methods. Bioassays were conducted by leaf-residual feeding method. The results showed that some new resources of botanical acaricide screened from 30 plants species. Not all of ethanol extracts screened showed very strong or strong acaricidal activity. Aqueous seed extract of *J. curcas* (Euphorbiaceae) exhibited the strong acaricidal activity against *P. oleivora* with LC<sub>50</sub> of 0.8%, followed by the aqueous seeds extract of *M. elengi* (Sapotaceae), *P. pinnata* (Sapindaceae), and the fruit extract of *Br. javanica* (Simaroubaceae) with LC<sub>50</sub> of 1.06%, 1.29%, and 1.45%, respectively. All of the aqueous extracts assayed could not cause the death of the predator *H. axyridis*. Aqueous seed extracts of *J. curcas* and *M. elengi* at a concentration of 5% could not cause phytotoxicity symptoms on the citrus leaves of *Citrus sinensis*. Further studies are required to identify active compounds in those active extract.

Keywords: Botanical acaricides, citrus mite, tropical plant extracts.

## INTRODUCTION

A number of plant pests may attack citrus plants such as citrus rust mite *Phyllocoptruta oleivora* (Ashmead) (Acari: Eriophydae) and citrus red mite *Panonychus citri* (McGregor) (Acari: Tetranychidae) (Puspitarini *et al.*, 2012; Bergh, 2000). Attack of this mite could cause the fruit peel of citrus mottle (Yang *et al.*, 1994), and may reduce the quality, appearance, even the price of the fruit. Therefore the presence of mite on citrus plant needs to be controlled (Childers *et al.*, 1996).

The information of mites control on citrus by using the tropical plant extracts as botanical acaricide is still limited. The sources of botanical acaricide from the tropical plants, especially in West Borneo, Indonesia, are explored. It was initially initiated from a number of

studies of utilization of active plant extract for controlling citrus mites on citrus plantation in Indonesia.

In addition to empower the local tropical plant, the general purpose of this study is to find out the plant candidates as new source of botanical acaricide that can be used as an alternative citrus mite control. The research includes the study of ethnobotany of plant utilization as a source bioactive, the screening of the acaricidal activity of ethanol plant extract against citrus rust mite *P. oleivora* and citrus red mite *Pa. citri* and the evaluation the lethal effects of aqueous extract of active plant against citrus rust mite *P. oleivora*. The research also studies the toxicity of the aqueous extract of active plant against their predator *Harmonia axyridis* Pallas

(Coleoptera: Coccinellidae) and the phytotoxicity of aqueous extract of active plant on citrus plant (*Citrus sinensis* Osbeck).

#### MATERIALS AND METHODS

#### Mites and Predator Test

For the test mites, two species of mites that attack the citrus plants, namely citrus rust mite *P. oleivora* and citrus red mite *Pa. citri* were used, while *H. axyridis* was as the predator. Both mites and predator tests were obtained from a laboratory colony maintained at the Laboratory of Pesticides, Faculty of Agriculture, Tanjungpura University, Pontianak, Indonesia. The colony was maintained under ambient conditions (25-33°C, 65-85% RH, ca. 12 L: 12 D regime).

#### Source of Plant Materials

Leaves, barks, flowers, and seeds (if available) of 30 plants species were used as the sources of extracts taken randomly and based on information of biological activity on ethnobotanical study from some different regions in Indonesia. Plant materials received kept in cool-dried condition. Plant species were identified by a botanist at the National Herbarium in Bogor, Indonesia.

# Preparation and Screening of Acaricidal Activity of Ethanol Extract

Plant materials were ground separately with a blender and sieved with 1 mm mesh sieves. Then a known amount of particular ground materials were extracted with ethanol by an infusion method. Extraction was conducted in an erlenmeyer flask and stirred for 24 hours. Each extract was filtered and the marc was washed with ethanol three times until the filtrate was colorless. The filtrates were pooled, and then the solvent was evaporated in a rotary evaporator at temperature of 55-60°C and pressures of 580-600 mmHg. The extract was kept (< 4°C) until used.

The ethanol extracts were screened against secondinstar of mite using a leaf-residu feeding method. Initially, each extract was screened at a concentration of 0.5%(w/v). A certain amount of each extract was mixed with an emulsifier polioxietilen alkylaryl ether and ethanol, and then the mixture was dilluted with water to a desired volume. The final concentrations of emulsifier and etanol were 0.1% and 1%, respectively. Water containing the emulsifier and ethanol at the same concentration served as a control solution.

Portions of citrus leaves were dipped in particular extract emulsions for ca. 10 seconds and air-dried. Control leaves were dipped in the control solution. The upper surface of both treated and controlling leaves were given arena (Puspitarini et al., 2011). The arena is a movement boundary made of layers of moist tissue which has 3 holes. Each hole is 1 cm x 1 cm in size and 1 cm spacing between holes. Treated or controlling leaves portions were placed singly in glass petri dishes (9 cm diameter) lined with moist sponge. 10 second-instar of mite (within 4 hours after moulthing) were placed into each dish. A total of 30 mites (10 mites/dish) were used in the treatment with each extract or control. The test mites were fed treated leaves for 4 days, and then fed untreated leaves. The number of the dead mite was recorded daily and stopped when the mortality of mites in the control had reached 20%.

Mite mortality in the treatment was corrected with control mortality using Abbott's formula (Abbott, 1925). Based on the mite mortality, the activity of each ethanol extract was arbitrarily classified into the following categories (i) very strong activity: mortality (m) 100%; (ii) strong: 85% < m < 99%; (iii) fairly strong: 60% < m < 85%; and (iv) sufficiently strong: m < 60%. Furthermore, plant materials that their ethanol extract showing a very strong or strong acaricidal activity and were available in the laboratory, the screening was continued using aquaeous extract.

# Preparation and Screening of Acaricidal Activity of Aqueous Extract

The preparation of aqueous extract is a practical approach for particularly Indonesian farmers. Plant materials extracted were selected from plant materials that their ethanol extract grouping in very strong and strong acaricidal activity based screening result of acaricidal activity of ethanol extract. Plant materials extracted were selected from plant seeds only, thus further assaying focused on seven plants species seeds extract, seed of M. elengi, P. pinnata, B. asiatica, A. indica, J. curcas, and also fruits of Br. javanica and Piper sp. Aqueous extracts were prepared at a concentration of 5% (w/v). Powder of the plant seeds and fruits were blended throughly in distilled water containing 0.1% of emulsifiers Besmor (polioxietilen alkylaryl ether) and 1% of etahanol with a blender. Each extract was then filtered through fine muslin cloth and the filtrates were ready to be used. Similar methods of screening of ethanol extracts were used in this screening.

# Bioassays of Active Aqueous Seeds Extracts against *P. oleivora*

Active aqueous seed extracts were assayed only against second instar of P. oleivora mite. The selection of P. oleivora mite was based on the results of field surveys which showed that P. oleivora population were more dominant than Pa. citri mite. These bioassays were to explore the LC<sub>50</sub> values of each active aqueous seed extracts. Preparation and treatment procedure were similar with screening test of aqueous extract as explained above, but in these tests each treatment was replicated three times. Aqueous extracts were tested at six concentration levels within ranges of concentrations which were expected to cause >0 and <100% mortality as determined in preliminary tests. The number of dead instar from the second to imago was recorded daily and the data were analyzed by the probit method (Finney, 1971) via PROC PROBIT of the SAS Package (SAS Institute, 1990).

# Effect of Aqueous Extracts against Predator H. axyridis

This test using selected aqueous seed extracts are mostly used as a botanical acaricide. Preparation and concentration were similar to screening test of aqueous extracts as explained above. The extract preparations were tested by spraying method. Second-instar of larvae of predators (10 hours after moulthing) were used for the test. A total of 10 larvae of predators were removed to the leaves of citrus plants by using a soft brush. Then the citrus leaves and larvae of predators were sprayed with extract preparation using handsprayer (20 ml ca.). To keep the larvae of predators remain in the treatment of leafs, the leaves were given a well-ventilated plastic cage (8 cm diameter, 20 cm height). The larvae of predators were fed with aphid. The treatment was repeated 3 times. Mortality of larvae observated daily until the death of larvae control reached 20%. Larvae mortality in the treatment was corrected with control mortality using Abbott's formula (Abbott, 1925).

## Effect of Aqueous Extracts on Citrus Plant

Preparation and concentration were similar to screening test of aqueous extracts as explained above. These extracts preparations were done by spraying method using handsprayer (ca. 1.5 l). Extracts preparations and controls were separately sprayed on citrus leaves. The test was repeated 3 times. The symptoms of fitotoxicity were observed at 3 and 7 days after spraying. Observations were carried out by measuring the citrus leaves that showed the necrotic tissue with compare patches area/initial leaf area x 100%.

### RESULTS AND DISCUSSION

#### Screening of Acaricidal Activity of Ethanol Extracts

Since treated with 30 preparations of ethanol extract on this acaricidal screening test, mortality of *P. oleivora* and *Pa. citri* varies considerably enough (Table 1). High mortality of mites was showed by each preparation indicating the strength of acaracidal activity of each preparation. Very strong acaricidal activity of ethanol extract against *P. oleivora* was showed by the 6 preparations, extract of seeds of *M. elengi*, *P. pinnata*, *B. asiatica*, *A. indica*, extracts of fruits of *Br. javanica* and

Emparu. Strong acaricidal activity was showed by extracts of leaves of A. indica, fruit of Piper sp., seed of J. curcas, fruit peel and leaves of P. graciliflorum, leaves of the buah tuba as well. The rest, preparations showed a relatively strong and less acaricidal activity against P. oleivora. In contrast, the amount of preparations that showed very strong and strong activity against Pa. citri less than *P. oleivora*. Preparation with very strong activity against Pa. citri was only showed by extract of seed of M. elengi, whereas strong activity was showed by the extracts of seed of P. pinnata, and fruit of Br. javanica. Seeds of M. elengi, P. pinnata, B. asiatica, A. indica, and J. curcas, and fruits of Br. javanica and Piper sp were used for screening of aqueous extract for they have very strong and strong acaricidal activity of ethanol extracts, the availability of the plant materials in the laboratory, and high chance of extracts to be used in the field. Various factors could influence the extract preparations in causing the death of the target organism, such as the kinds and the amount of active ingredient of extract, techniques of application, species of mite, age and stage of development, as well as environmental factors. Those factors are function of toxicity in the cause of the death of mites.

# Screening of Bioassays of Acaricidal Activity of Aqueous Extracts

The seven kinds of aqueous extracts tested showed different acaricidal activity between against *P. oleivora* and *Pa. citri* (Table 2). The pattern of mortality of mite in screening the aqueous extract was similar to the pattern of mortality of mite in screening the ethanol extract. This

No	Plant Family	Species	Parts of plants	Mortality ± S			
NU	Flant Failing	Species		F	ra		
1	Sapotaceae	Mimusops elengi	Sd	100	±	0	
2			Lv	26.4	±	16.4	
3		Achras zapota	Sd	60.8	±	26.7	
4	Sapindaceae	Pometia pinnata	Sd	100	±	0	
5		Nephelium cuspidatum	Bk	28.7	±	16.3	
6	Lecythidaceae	Barringtonia asiatica	Sd	100	±	0	
7			Lv	18.1	±	13.3	
8		B. sarcostachys	Bk	5.5	±	2.5	
9	Meliaceaea	Azadirachta indica	Sd	100	±	0	
10			FI	78.6	±	8.7	
11			Lv	92.9	±	10.1	
12			Tw	35.8	±	8.7	
13	Simaroubaceae	Brucea javanica	Fr	100	±	0	
14		-	Bk	73.9	±	18.7	
15	Piperaceae	<i>Piper</i> sp.	Sd	95.5	±	5.3	
16		Piper bettle	Lv	23.1	±	22	
17	Menispermaceae	Tinospora crispa	St	77.2	±	16.5	
18	Caesalpiniaceae	Casia tora	Lv	66.5	±	25	
19	Euphorbiaceae	Jatropha curcas	Sd	96.4	±	5.0	
20	Crassulaceae	Kalanchoe pinnata	Lv	22.2	±	14.5	
21	Rutaceae	Evodia swafiolens	Lv	52.9	±	13.1	
22	Achantaceae	Pseuderanthemum graciliflorum	Ff	93.1	±	8.4	
23			Lv	91.7	±	5.8	
24	Dioscoreaceae	Dioscorea hispida	Tb	21.6	±	18.2	
	Unidentifie	ed plants (local name)					
25		Sempayan	Bk	20.9	±	18.4	
26		Tuba empliau	Bk	40.6	±	21.5	
27		Buah tuba	Fr	21.6	±	18.2	
28			Lv	91.7	±	5.8	
29		Emparu	Fr	100	±	0	
30			Bk	59.2	±	28.9	

Table 1. Mortality of Phyllocoptruta oleivora and Panonychus citri in screening test of acaricidal activity of ethanol extract<sup>a</sup>

<sup>a</sup> Ethanol extracts screened at a concentration of 0.5%
<sup>b</sup> Mortality calculated at 4 days after treatment and corrected by Abbott (1925), SD = Standard Deviation (-) not screened because the extracts were not enough Sd: Seed; Fr: Fruit; Bk: Bark; Ff: Fruit peel; Lv: Leaves; Fl: Flowers; Tw: twig; St: stolon; Tb: Tube

No	Plant spesies	Mortality ± SD (%) <sup>b</sup>						
NO		P. ole	ivora	Pa	Pa. citri			
1	M. elengi	100	±	0	100	±	0	
2	P. pinnata	100	±	0	30	±	4,7	
3	B. asiatica	100	±	0	56,7	±	4,7	
4	A. indica	96,7	±	4,7	23,3	±	18,9	
5	Br. javanica	100	±	0	56,7	±	4,7	
6	<i>Piper</i> sp.	100	±	0	50	±	9,4	
7	J. curcas	100	±	0	33,3	±	0	

Tabel 2. Mortality of Phyllocoptruta oleivora and Panonychus citri in screening of aqueous extracts

<sup>a</sup> Aqueous extract was tested at a concentration of 5%

<sup>b</sup>Mortality calculated 4 days after treatment, the percentage mortality was corrected by Abbott (1925) SD = Standard Deviation

evidence indicates that the active ingredients in seven plants seeds are both soluble in ethanol and dissolved in water. In general, more aqueous extracts that showed very strong acaricidal activity against *P. oleivora* than *Pa. citri.* Particularly, acaricidal activity against *P. oleivora*, from seven aqueous extracts tested, six of them showed very strong (mortality 100%) and one showed strong (96.7%). Against *Pa. citri,* on the other hand, only one extract showed very strong acaricidal activity (100%) and the rest showed lower activity.

The hight of acaricidal activity was showed by all aqueous extracts against *P. oleivora* indicates that the active compounds in the seed at concentration tested in that preparation can be suspended properly. In contrast to *Pa. citri*, all of the aqueous extract except *M. elengi* showed lower activity. Thus, the differences activities among that extracts were not due to differences in kinds or amount of active ingredients in the extract. Different with other aqueous extracts, extract *M. elengi* showed a very strong activity against both *P. oleivora* and *Pa. citri*. This activity could be due to the same active compounds or other, or both of them.

Other aqueous extracts look selective on *P. oleivora*. This selectivity may occur due to differences in the structure of external and internal morphology of both mites. They may cause differences in the processes of penetration and metabolism of active substance in the body of mites. Similar to the botanical insecticides, some examples of botanical acaricide have specific targets, so only could kill the certain mites. Even though there are also examples of the botanical acaricide that could kill amount of mites. To ensure the cause of the difference, it is necessary to study beyond this test.

Because of its active of aqueous extract of *M. elengi* against both mites it is expected that the application of these preparations in the field can control both pest mites

at once. A number of aqueous extract preparations to control the pests have been reported. Extract preparation can be prepared in a simple preparation through using of the aqueous extract. Therefore, other aspects of study related to the use of aqueous extract directly in the field need to be done.

### Toxicity of Aqueous Seed Extracts against P. oleivora

The results of toxicity testing of aqueous seed extracts against *P. oleivora* is presented in Table 3. The whole aqueous seed extracts showed  $LC_{50}$  values are below 1.5%. Aqueous seed extracts of *J. curcas* possessed the strongest toxicity against *P. oleivora* with  $LC_{50}$  of 0.8%. These toxicity values are not different from the toxicity of aqueous seed extracts of *A. indica* with  $LC_{50}$  of 0.89%. Both of toxicity of aqueous seed extracts are different compared with the toxicity of three other aqueous seed extracts, *M. elengi, P. pinnata,* and *Br. javanica.* The toxicity among last of three aqueous seed extracts mentioned is not significant. Each extract showed  $LC_{50}$  values of 1.06%, 1.29%, and 1.45%, respectively.

From all toxicity of seed extracts were reported in this study, only seed extract of *A. indica* that its acaricidal properties have been reported. Information of other acaricidal properties from seed extract of *M. elengi* (Sapotaceaea), *P. pinnata* (Sapindaceae), *J. curcas* (Euphorbiaceae), and fruit extracts of *Br. javanica* (Simaroubaceae) as introdused from this study are the first report. Previous studies on active plants have reported activities in insecticide, fungicide, molluscucide, and other biological activities. Activity of *M. elengi* (Sapotaceae) is now known as a fungicide and bactericide. Satish *et al.* (2008) reported that an

Aqueous seed extract	a ± SE		b ± SE			LC <sub>50</sub> (CI 95%)(%)		
A. indica	0.07	±	0.12	1.56	±	0.38	0.89	(0.57-1.32)
M. elengi	-0.06	±	0.13	2.44	±	0.51	1.06	(0.79-1.38)
P. pinnata	-0.29	±	0.15	2.70	±	0.45	1.29	(0.99-1.59)
Br. javanica	-0.38	±	0.17	2.40	±	0.49	1.45	(1.05-1.87)
J. curcas	0.11	±	0.13	1.23	±	0.35	0.80	(0.29-1.24)

**Tabel 3.** The parameters of concentration-mortality relationship (LC<sub>50</sub>) of five kinds of aqueous seed extracts against *Phyllocoptruta oleivora*<sup>a</sup>

<sup>a</sup> A number of larvae instars II treated and control, 1050 and 90, repectively

a = intercept of the regression line, b = slope of the regression line,

SE = Standard Error, CI = Confidential limits

No	Aqueos Extracts	Plant part	Mortality (%) <sup>b</sup> ± SD
1	A. indica	Seed	0
2	M. elengi	Seed	0
3	P. pinnata	Seed	0
4	Br. javanica	Fruit	0
5	J. curcas	Seed	0

Tabel 4. Effect of active aqueos extracts on larvae predator Harmonia axyridis<sup>a</sup>

<sup>a</sup> Aqueous extracts were tested at a concentration of 5%

<sup>b</sup> Mortality calculated at 4 days after treatment

SD = Standard Deviation

aqueous extract of seed of *M. elengi* inhibited the growth of the mycelia Aspergillus niger. Hazra et al. (2008) isolated two kinds of pentahydroxy flavonoid compounds from the seed of *M. elengi* that serves as a bactericide. Lavaud et al. (2008) isolated six kinds of saponin compounds from the seeds of *M. elengi*, *M. hexandra*, and M. manilkara. Ethanol extract of seed of P. pinnata (Sapindaceae) showed insecticidal activity against C. pavonana with LC<sub>50</sub> value of 0.16%. Sapindus rarak, other Sapindaceae, is known possessing biological activity as an insecticide. Various extracts of seeds and leaves of plants J. curcas (Euphorbiaceae) showed antimolluscs, anti-insect, and anti-fungal. The preparation of Euphorbiaceae seeds were reported having active against some insects and mites. Biological activities of Simaroubaceae plants from around the world have been reported useful, such as for medicine, insecticides, herbicides, and amoebicide. Brucein, which is isolated from fruit of Br. javanica, is one example of the active compounds that possess antimalarial activity (Kim et al., 2004). Quassin from Quassia amara, another species of Simaroubaceae, has been reported active as an insecticide, its compound active against larvae of mosquitoes and lice. Q. amara, besides active as drugs and insecticides, along with Q. undulata their extract were also active as a fungicide and bactericide

(Ajaiyeoba et al., 2003). Considering the strong of acaricidal activity from that aqueous seed extracts, further studies are required to isolate and to identify the active compounds. The active compound of azadirachtin has been isolated from the plant A. indica (Schmutterer & Rembold, 1995). These active compounds have a broadrange of insect pests including mite target, and act as an insecticide and acaricide. Seed plant of A. indica has been reported to be used as control mite of Pa. citri. Also various trademarks of botanical acaricide with azadirachtin as active compounds also have been marketed (Mansour, 1993).

### Effect of Active Aqueos Extracts on Larvae Predator *Harmonia axyridis*

All aqueous extracts tested did not cause the death of the predator *Harmonia axyridis* larvae intar II (Table 4). Activity of botanical acaricide against mites include their predatory insects can be affected by a number of factors, such as species, developmental stages and aging. Certain plant secondary compounds are relatively less toxic or no effect on the character of biological insect parasitoids (Schmutterer, 1997).

No	Aqueous extracts	Plant part	Symptoms (%)on DAT <sup>D</sup> ± SD					
				3		7		
1	A. indica	Seed	0			0		
2	M. elengi	Seed	0			0		
3	Br. javanica	Fruit	8,3	±	1,9	17,5	±9,4	
4	J. curcas	Seed	0			0		

Tabel 5. Phytotoxicity symptoms on citrus plants after spraying aqueous extracts<sup>a</sup>

<sup>a</sup>Aqueous extracts were tested at a concentration of 5%

<sup>b</sup> Phytotoxicity symptoms calculated at 3 and 7 days after treatment (DAT) SD = Standard Deviation

#### Effect of Active Aqueos Extracts on Citrus Plant

The old leaves of citrus plants after spraving with 4 kinds of aqueous extract at concentration tested did not show phytotoxict symptoms. Similarly, spraying the aqueous extract on young leaves did not show phytotoxict symptoms either, except aqueous extract of Br. javanica fruits (Table 5). Aqueous extract of Br. javanica fruits showed phytotoxicity symptoms on the young leaves of citrus by 8.3% at 3 DAT and expand 17.5% at 7 DAT and constant after that. Phytotoxicity of a number of crop plants caused by ethanol fruit extract of Br. javanica has been reported. When an extract of a plant did not cause phytotoxict or could cause phytotoxict but within a plants tolerable limit (plants may regrowth normally), the aqueous extract could be used immediately after preparation. However, if they could cause severe phytotoxict symptoms, components that caused phytotoxict in the extract needs to be separated.

Phytotoxicity symptoms tend to occur in plants treated plants extract, not a pure compound. Azadirachtin and rokaglamida as pure compounds isolated from Meliaceae plants such as *A. indica* and *Aglaia* spp, at concentrations that are active against the target pest do not cause phytotoxict symptoms on some plants. Loke *et al.* (1990) reported that spraying plant seed extract of *A. indica* at a concentration of 0.5% to 4.0% could cause phytotoxict on cabbage, collards, and rice in 4 weeks old.

Considering the high acaricidal activity of aqueous extracts of *Br. javanica* fruits, a study is necessary to eliminate the phytotoxict properties. Utilization of properties of antagonism of a mixing of two different kinds of compounds are an effort to reduce phytotoxict properties. Mixing these compounds are expected to modify the cytotoxic properties from mixtures of compounds without degrading the acaricidal activity (antagonism) or the opposite is expected to exhibit synergism. The properties of the source of botanical acaricidal that need to be searched and developed is the source of an acaricide which is in addition effective against target pests, also has a high rendemen, relatively secure to non-target organisms, and do not poison the plants through their phytotoxict properties. This is a consideration that should be considered in the development of alternative sources of botanical acaricide.

## CONCLUSION

This study revealed some new sources of botanical acaricide among 30 plants extracted from seeds of M. elengi (Sapotaceae), P. pinnata (Sapindaceae), B. asiatica (Lecythidaceae), Br. javanica (Simaroubaceae), J. curcas (Euphorbiaceae) and Piper sp. (Piperaceae). Aqueous extract of J. curcas seeds possessed a strong acaricidal activity against mites P. oleivora with LC50 of 0.8%, followed by seed extract of M. elengi, seed extract of P. pinnata and fruit extracts Br. javanica with LC<sub>50</sub> of 1.06%, 1.29% and 1.45%, respectively. All the aqueous extract did not cause the death of the insect predator H. axyridis instar II. Aqueous extract of seeds J. curcas and M. elengi at a concentration of 5% could not cause phytotoxicity leaves of C. sinensis. Other bioactivity studies and identification of active compounds as acaricide contained in plants extract need to be done in the future.

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