

# INSECTICIDAL JOINT ACTION OF TERNARY EXTRACT MIXTURES OF FOUR SPECIES OF TROPICAL PLANTS AGAINST *Plutella xylostella* AND *Crocidolomia pavonana*

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## ABSTRACT

**Insecticidal joint action of ternary extract mixtures of four species of tropical plants against *Plutella xylostella* and *Crocidolomia pavonana*.** The diamondback moth (DBM), *Plutella xylostella*, and the cabbage head caterpillar (CHC), *Crocidolomia pavonana*, were two main pests of cruciferous vegetable crops. Among the viable alternative ingredients that could be utilized to control those two pests were eco-friendly botanical insecticides. This study was conducted to evaluate the joint action of ternary extract mixtures of four tropical plant species, i.e. *Piper aduncum* (Pa), *Piper retrofractum* (Pr), *Sapindus rarak* (Sr), and *Tephrosia vogelii* (Tv), against DBM and CHC. Results of leaf-residue feeding bioassays showed that based on co-toxicity ratio at both LC<sub>50</sub> and LC<sub>95</sub> levels, ternary mixtures of Pa extract with Sr and Tv extracts at concentration ratios of 1:5:1, 1:5:2, and 2:5:1 indicated synergistic joint action on DBM larvae. A mixture of Pa, Sr and Tv extracts at a ratio of 2:5:1 was also synergistic to CHC at both LC<sub>50</sub> and LC<sub>95</sub> levels. This mixture at the 1:5:1 ratio was synergistic on CHC at the LC<sub>50</sub> level but antagonistic at the LC<sub>95</sub> level whereas at the 1:5:2 ratio was antagonistic to CHC at both LC<sub>50</sub> and LC<sub>95</sub> levels. Furthermore, ternary mixtures of Pr extract with Sr and Tv extracts at the three concentration ratios were synergistic to CHC. Thus, ternary mixtures of Pa or Pr extract with Sr and Tv extracts at appropriate concentration ratios are potential alternatives for the control of DBM and CHC.

**Key words:** botanical insecticides, cabbage pests, joint action, ternary mixtures, tropical plants

## INTRODUCTION

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), and the cabbage head caterpillar (CHC), *Crocidolomia pavonana* (F.) (Lepidoptera: Crambidae), were two major pests of cruciferous vegetable crops (Sastrosiswojo & Setiawati, 1993; Durairaj *et al.*, 2016). Normally DBM could be adequately suppressed by its natural enemies complex when chemical control was not intensively undertaken (Sastrosiswojo & Sastrodihardjo, 1986; Sarfraz *et al.*, 2005; Furlong *et al.*, 2013; Philips *et al.*, 2014), but CHC had no effective natural enemies (Sastrosiswojo & Setiawati, 1992). Consequently, crucifer growers in Indonesia often use older, broad-spectrum and toxic insecticides to control crucifer pests, especially against CHC, due to limited knowledge of farmers on effective non-chemical control methods (Sastrosiswojo & Setiawati, 1992; Rauf *et al.*, 2004; Shepard *et al.*, 2009; Grzywacz *et al.*, 2010). Intensive use of chemical insecticides on crucifers would badly interfere with the activity of pest natural

enemies and impede the implementation of crucifer integrated pest management (Shepard *et al.*, 2009). In addition, intensive use of synthetic insecticides could cause various undesirable side effects including pest resistance and resurgence, killing of non-target organisms including pest natural enemies, food web contamination, residue hazards, and health risks to pesticide applicators (Aktar *et al.*, 2009; Wyckhuys *et al.*, 2020). Thus, it was necessary to develop alternative control agents that are effective against crucifer pests but relatively safe for non-target organisms including pest natural enemies. In this regard, botanical insecticides are worthwhile to be evaluated.

Extracts of three plant species, i.e. spiked pepper (*Piper aduncum*), Javanese long pepper (*Piper retrofractum*), and fish-poison bean (*Tephrosia vogelii*), were reported possessing strong insecticidal activity against DBM and CHC (Zarkani *et al.*, 2009; Zarkani *et al.*, 2010; Chenta & Prijono, 2014; Nailufar & Prijono, 2017). In addition, soapberry (*Sapindus rarak*) extract was reported exhibiting moderate insecticidal activity against CHC (Syahroni & Prijono,

2013; Mediana & Prijono, 2014). One of the attempts to enhance the insecticidal activity of botanical insecticides is by using mixed plant extracts. Binary mixtures of *P. aduncum* and *T. vogelii* as well as *P. retrofractum* and *T. vogelii* extracts were reported to have strong and synergistic insecticidal activity against CHC (Nurfajrina & Prijono, 2015; Nailufar & Prijono, 2017), while those of *P. aduncum* and *S. rarak* (Syahroni & Prijono, 2013) as well as *T. vogelii* and *S. rarak* (Irawan, 2012) had moderate but synergistic insecticidal activity against CHC.

This study was conducted to assess joint action of ternary extract mixtures containing *P. aduncum*, *S. rarak* and *T. vogelii* extracts at concentration ratios of 1:5:1, 1:5:2, and 2:5:1 against DBM and CHC. The other ternary extract mixtures containing *P. retrofractum*, *S. rarak* and *T. vogelii* extracts at the same concentration ratios were tested against CHC only.

## MATERIALS AND METHODS

**Research Site.** This study was conducted at the Laboratory of Insect Physiology and Toxicology, Department of Plant Protection, Bogor Agricultural University, Bogor, Indonesia, from September 2018 to July 2019.

**Procurement of Insecticidal Plants.** Spiked pepper (*P. aduncum*) fruits, were taken from bushes at Dramaga campus of Bogor Agricultural University, Javanese long pepper (*P. retrofractum*) fruits were obtained from the Spice and Medicinal Plants Research Institute in Bogor; fish-poison bean (*T. vogelii*) leaves were procured from Bina Sarana Bakti organic farm at Cisarua District, Bogor Regency and soapberry (*S. rarak*) fruits were purchased from a local market in Bogor, West Java, Indonesia. Spiked pepper fruits, Javanese long pepper fruits and fish-poison bean leaves were cut to small pieces and air-dried for one week in the laboratory before extraction. Soapberry fruits were directly sliced prior to extraction.

**Cultivation of Broccoli Plants.** Pesticide-free broccoli leaves were used for feeding the test insects and as treatment substrates. Those leaves were taken from potted broccoli 'Super Royal Green' of at least 2 months after sowing. Broccoli plants were cultivated following Abizar & Prijono (2010).

**Rearing of Test Insects.** DBM and CHC used as the test insects in this study were offsprings of DBM and CHC adults collected from the teaching farm of the

Faculty of Agriculture, Bogor Agricultural University at Pacet District, Cianjur Regency, West Java (S 6°46'6.7", E 107°2'57.6", 1100 m asl). DBM and CHC were reared in the laboratory according to the method described by Prijono *et al.* (2019) and Prijono & Hassan (1992), respectively. Briefly, DBM larvae and CHC were fed pesticide-free broccoli leaves and their adults were fed 10% honey solution in a cotton swab.

**Extraction of Plant Materials.** Extraction of four test plant materials was done using immersion method (Houghton & Raman, 1998). Cut pieces of spiked pepper fruits, Javanese long pepper fruits and fish-poison bean leaves were extracted with ethyl acetate (Nurfajrina & Prijono, 2015) and slices of soapberry fruits were extracted with methanol (Syahroni & Prijono, 2013). Solvent in the extracts was evaporated to dryness using a rotary evaporator, then all extracts obtained were kept in refrigerator (ca. 4 °C) until used for bioassays.

**Method of Toxicity Testing.** Extracts of four plant species were tested singly and as ternary mixtures against DBM and CHC using a leaf-residue feeding method. Each extract was tested at five concentration levels that were expected to give insect mortality between 15% and 95% as determined in preliminary tests. Each test extract was mixed thoroughly with methanol (solvent) and Tween 80 (emulsifier) and then diluted with distilled water to the desired volume. Final concentrations of methanol and Tween 80 in each extract dilution were 1% and 0.2%, respectively. Distilled water containing 1% methanol and 0.2% Tween 80 was used as a control solution.

Bioassays procedures against DBM followed Prijono *et al.* (2019) and those against CHC followed Nailufar & Prijono (2017). Fifty third-instar DBM larvae and 60 second-instar CHC, in groups of 10 each, were used for each concentration level and control. Test larvae were fed treated broccoli leaves for 72 h and then kept on untreated leaves for an additional 24 h. The number of dead larvae was counted daily until 96 h after treatment (HAT) and cumulative larval mortality data at 96 HAT were analyzed with probit method using PoloPlus (Robertson *et al.*, 2003).

Mixtures of spiked pepper, fish-poison bean and soapberry extracts were tested against DBM and CHC at three concentration ratios, i.e. 1:5:1, 2:5:1, and 1:5:2 (w/w). Mixed extracts of Javanese long pepper, fish-poison bean and soapberry at the same concentration ratios were tested against CHC only. Each extract mixture was tested at five and six concentration levels

against DBM and CHC, respectively, which were expected to give mortality of test insects between 15% and 95%. Ternary extract mixtures were prepared by mixing suspension of the three component extracts at appropriate proportions. The method of treatment and observation in mixed extract tests were the same as in the single extract tests. Cumulative larval mortality data were analyzed with probit method as in the single extract tests.

The type of joint action of each ternary extract mixture was determined based on independent action hypothesis by calculating co-toxicity ratio at  $LC_x$  level ( $x = 50$  or  $95$ ). The co-toxicity ratio (CTR) at the  $LC_x$  level was calculated as follows (Robertson & Smith, 1984):

$$CTR = \text{Expected } LC_x \div \text{Observed } LC_x$$

where observed  $LC_x$  was obtained from the result of probit analysis while expected  $LC_x$  was calculated based on the following estimation (extended from Robertson & Smith, 1984):

$$P_E = m + \left\{ (1-m) \left[ P_1 + (1-P_1)P_2 + (1-P_1)P_3 - (1-P_1)P_2P_3 \right] \right\}$$

where  $P_E$  was the expected proportional mortality due to  $LC_x$ ,  $m$  is the proportional mortality in the control group, and  $P_1$ ,  $P_2$ , and  $P_3$  were the predicted proportional mortality due to concentration of extract 1, extract 2, and extract 3 in the mixture, respectively, and estimated from the probit regression lines for extract 1, extract 2, and extract 3 separately.

The type of joint action of the test extract mixtures was categorized as follows (the reciprocal of combination index described by Chou & Talalay [1984]): (1) if  $CTR > 1.0$ , then synergistic joint action was

indicated; (2) if  $CTR = 1.0$ , then the mixture exhibited additive joint action; (3) if  $CTR < 1.0$ , then the mixture had an antagonistic joint action.

## RESULTS AND DISCUSSION

Based on the comparison of  $LC_{50}$  of single extracts against DBM, spiked pepper (Pa) extract was about 1.45 and 9.9 times more toxic than fish-poison bean (Tv) and soapberry (Sr) extract, respectively (Table 1). The spiked pepper extract was about as toxic as Tv extract against CHC while Sr extract was about 9.7 and 10.4 times less toxic than Pa and Tv extract, respectively (Table 2). In another set of bioassays, fish-poison bean extract was about 2.6 and 23.1 times more toxic than Javanese long pepper (Pr) and Sr extract, respectively, against CHC (Table 3).

Mixed extracts of Pa, Sr and Tv at the three concentration ratios (1:5:1, 1:5:2, and 2:5:1) exhibited synergistic joint action against DBM at both  $LC_{50}$  and  $LC_{95}$  levels (Table 4). On the other hand, only at the concentration ratio of 2:5:1 that the extract mixture of Pa, Sr and Tv was synergistic against CHC at both  $LC_{50}$  and  $LC_{95}$  levels. This mixture at the 1:5:1 ratio was synergistic on CHC at the  $LC_{50}$  level but antagonistic at the  $LC_{95}$  level whereas at the 1:5:2 ratio was antagonistic to CHC at both  $LC_{50}$  and  $LC_{95}$  levels (Table 4). Furthermore, mixed extracts of Pr, Sr and Tv at the three concentration ratios (1:5:1, 1:5:2, and 2:5:1) were synergistic against CHC at both  $LC_{50}$  and  $LC_{95}$  levels (Table 5).

The results of this study showed that  $LC_{95}$  of Pa and Tv extract was less than 0.5% indicating that these extracts had strong insecticidal activity against DBM. On the other hand, Sr extract exhibited rather weak

Table 1. Toxicity of single and mixed extracts of *P. aduncum*, *S. rarak*, and *T. vogelii* on *P. xylostella* larvae at 96 h after treatment

Type of extracts	$b \pm SE^a$	$LC_{50}$ (95% FL) <sup>b</sup> (%)	$LC_{95}$ (95% FL) <sup>b</sup> (%)	$\chi^2$ goodness-of-fit <sup>c</sup>
Single extracts				
<i>P. aduncum</i> (Pa)	11.06 ± 1.41	0.142 (0.128–0.158)	0.200 (0.174–0.287)	4.407 <sup>ns</sup>
<i>S. rarak</i> (Sr)	4.90 ± 0.68	1.406 (1.245–1.539)	3.045 (2.627–3.880)	1.425 <sup>ns</sup>
<i>T. vogelii</i> (Tv)	5.60 ± 0.60	0.206 (0.145–0.312)	0.404 (0.281–2.322)	12.744 <sup>**</sup>
Mixed extracts, Pa:Sr:Tv				
1:5:1	5.62 ± 0.61	0.494 (0.417–0.601)	0.969 (0.745–1.802)	4.987 <sup>ns</sup>
1:5:2	6.16 ± 0.83	0.360 (0.325–0.390)	0.666 (0.591–0.804)	2.944 <sup>ns</sup>
2:5:1	9.18 ± 1.41	0.314 (0.286–0.335)	0.474 (0.434–0.549)	2.230 <sup>ns</sup>

<sup>a</sup> $b$  = slope of the probit regression line, SE = standard error. <sup>b</sup>FL = fiducial limit. <sup>c</sup>\*\* : statistically significant at 0.01 level; ns = not significant.

Table 2. Toxicity of single and mixed extracts of *P. aduncum*, *S. rarak*, and *T. vogelii* on *C. pavonana* larvae at 96 h after treatment

Type of extracts	$b \pm SE^a$	LC <sub>50</sub> (95% FL) <sup>b</sup> (%)	LC <sub>95</sub> (95% FL) <sup>b</sup> (%)	$\chi^2$ goodness-of-fit <sup>c</sup>
Single extracts				
<i>P. aduncum</i> (Pa)	25.18 ± 5.42	0.098 (0.090–0.102)	0.114 (0.111–0.119)	2.206 <sup>ns</sup>
<i>S. rarak</i> (Sr)	4.49 ± 0.87	0.955 (0.376–1.214)	2.219 (1.800–4.624)	3.318 <sup>ns</sup>
<i>T. vogelii</i> (Tv)	3.48 ± 0.77	0.092(0.050–0.118)	0.273 (0.232–0.377)	1.519 <sup>ns</sup>
Mixed extracts, Pa:Sr:Tv				
1:5:1	3.28 ± 0.48	0.510 (0.333–0.633)	1.618 (1.199–3.364)	3.220 <sup>ns</sup>
1:5:2	2.64 ± 0.47	0.424 (0.280–0.530)	1.779 (1.405–2.769)	0.175 <sup>ns</sup>
2:5:1	3.23 ± 0.60	0.050 (0.033–0.061)	0.161 (0.133–0.226)	0.726 <sup>ns</sup>

<sup>a</sup> $b$  = slope of the probit regression line, SE = standard error. <sup>b</sup>FL = fiducial limit. <sup>c</sup>ns = statistically not significant.

Table 3. Toxicity of single and mixed extracts of *P. retrofractum*, *S. rarak*, and *T. vogelii* on *C. pavonana* larvae at 96 h after treatment

Type of extracts	$b \pm SE^a$	LC <sub>50</sub> (95% FL) <sup>b</sup> (%)	LC <sub>95</sub> (95% FL) <sup>b</sup> (%)	$\chi^2$ goodness-of-fit <sup>c</sup>
Single extracts				
<i>P. retrofractum</i> (Pr)	5.98 ± 0.61	0.160 (0.117–0.225)	0.301 (0.217–1.393)	13.478**
<i>S. rarak</i> (Sr)	5.13 ± 0.65	1.412 (1.099–1.642)	2.956 (2.326–5.793)	4.832 <sup>ns</sup>
<i>T. vogelii</i> (Tv)	2.86 ± 0.48	0.061 (0.024–0.084)	0.229 (0.165–0.606)	3.424 <sup>ns</sup>
Mixed extracts, Pa:Sr:Tv				
1:5:1	2.51 ± 0.30	0.219 (0.109–0.371)	0.991 (0.504–30.41)	9.967*
1:5:2	3.87 ± 0.47	0.171 (0.149–0.191)	0.455 (0.380–0.596)	1.330 <sup>ns</sup>
2:5:1	3.72 ± 0.41	0.217 (0.193–0.242)	0.600 (0.495–0.796)	1.273 <sup>ns</sup>

<sup>a</sup> $b$  = slope of the probit regression line, SE = standard error. <sup>b</sup>FL = fiducial limit. <sup>c</sup>Statistically significant at 0.05 (\*) or 0.01 (\*\*) level; ns = not significant.

Table 4. Joint effect of *P. aduncum* (Pa), *S. rarak* (Sr), and *T. vogelii* (Tv) extract mixtures on *P. xylostella* and *C. pavonana* larvae at 96 h after treatment

Concentration ratio of Pa, Sr and Tv extract	Expected LC <sub>50</sub> <sup>a</sup> (%)	Co-toxicity ratio at LC <sub>50</sub> <sup>a</sup>	Expected LC <sub>95</sub> <sup>a</sup> (%)	Co-toxicity ratio at LC <sub>95</sub> <sup>a</sup>
<i>P. xylostella</i>				
1:5:1	0.933	1.89	1.295	1.34
1:5:2	0.797	2.21	1.235	1.85
2:5:1	0.567	1.81	0.796	1.68
<i>C. pavonana</i>				
1:5:1	0.588	1.15	0.756	0.47
1:5:2	0.366	0.86	0.792	0.45
2:5:1	0.383	7.66	0.450	2.80

<sup>a</sup>Expected LC<sub>x</sub> and co-toxicity ratio at LC<sub>x</sub> were calculated according to Robertson & Smith (1984) as described in Materials and Methods.

activity with  $LC_{95}$  of about 3%. Prijono (1999) proposed that a plant extract was considered to have strong insecticidal activity if it could give at least 80% insect mortality at concentrations of less than 0.5%. Based on this criteria, only at the concentration ratio of 2:5:1 that the mixture of Pa, Sr and Tv extract had strong insecticidal activity against DBM (Table 1). Like against DBM, Pa and Tv extracts had strong insecticidal activity against CHC and Sr extract indicated rather weak activity. Moreover, only Pa, Sr, and Tv extract mixture at a concentration ratio of 2:5:1 that had strong insecticidal activity against CHC (Table 2). Single Tv and Pr extracts as well as the mixed extract of Pr, Sr and Tv at a concentration ratio of 1:5:2 had strong insecticidal activity against CHC (Table 3).

The strong insecticidal activity of single Pa and Tv extracts against DBM and CHC as well as that of Pr extract against CHC were in agreement with previous reports (Zarkani *et al.*, 2009; Zarkani *et al.*, 2010; Chenta & Prijono, 2014; Nailufar & Prijono, 2017; Prijono *et al.*, 2020). Pr extract had also been reported to have a good contact effect against some sucking pests including papaya mealybug *Paracoccus marginatus* (Asnan *et al.*, 2015), tea mosquito bug *Helopeltis antonii* (Indriati *et al.*, 2015; Rohimatun *et al.*, 2020b), and rice brown planthopper *Nilaparvata lugens* (Nuryanti *et al.*, 2018b). Furthermore, the following binary extract mixtures had been reported to be synergistic against CHC: *P. aduncum* + *S. rarak* (Syahroni & Prijono, 2013), *P. aduncum* + *T. vogelii* (Nurfajrina & Prijono, 2015; Nailufar & Prijono, 2017), *P. retrofractum* + *T. vogelii* (Nurfajrina & Prijono, 2015; Prijono *et al.*, 2020), and *T. vogelii* + *S. rarak* (Irawan, 2012). Recently, Rohimatun *et al.* (2020a) reported that extract mixtures of *P. retrofractum* and *Curcuma xanthorrhiza* at concentration ratios of 4:1, 2:1, 1:1, 1:2, and 1:4 were synergistic against *H. antonii*.

The strong insecticidal activity of Pa fruit extract was mainly attributable to the presence of a phenylpropanoid/lignan compound, dillapiole (Bernard *et al.*, 1995; Hasyim, 2011). At the cellular level, dillapiole

inhibited the activity of cytochrome P450 enzymes in breaking down toxic compounds in living cells resulting in the accumulation of those toxic compounds in the body which might eventually lead to insect death (Bernard *et al.*, 1995).

The main active compounds responsible for the insecticidal activity of Tv leaf extract belong to the rotenoids, especially deguelin, tephrosin, and rotenone (Delfel *et al.*, 1970; Stevenson *et al.*, 2012). Rotenone is a stomach and contact poison against various insect pests (Yu, 2015). It is a cellular respiration poison that acts by inhibiting electron transfer in Complex I of the electron transport chain in the mitochondria (Hollingworth, 2001). This causes the reduction of ATP production leading to depletion of the cellular energy source.

The principal insecticidal compounds in Pr fruit extract belong to piperamide compounds, including piperine, piperlonguminine, guineensine, pipericides, and retrofractamide A (Kikuzaki *et al.*, 1993; Parmar *et al.*, 1997). Piperamide compounds which have a methylenedioxyphenyl (MDP) moiety in their chemical structure, including those five compounds, exhibit dual action as neurotoxicants and metabolic poisons (Miyakado *et al.*, 1989; Scott *et al.*, 2008). Like dillapiole, piperamides possessing an MDP moiety can inhibit the detoxification function of cytochrome P450 (Scott *et al.*, 2008).

The main chemical substances in *S. rarak* fruits are saponins (Morikawa *et al.*, 2009). Methanolic and aqueous *S. rarak* fruit extracts were reported to have moderate insecticidal activity against CHC (Syahroni & Prijono, 2013). Saponins could disrupt cell membrane integrity and damage the mucous lining of insect digestive system cells (Francis *et al.*, 2002; Qasim *et al.*, 2020).

The synergistic action of the test extract mixtures might be contributed by MDP-possessing compounds, such as dillapiole in *P. aduncum* and piperamides in *P. retrofractum* extract, which inhibited the activity of cytochrome P450 detoxification enzymes (Bernard *et al.*, 1995; Scott *et al.*, 2008). Inhibition of cytochrome

Table 5. Joint effect of *P. retrofractum* (Pr), *S. rarak* (Sr) and *T. vogelii* (Tv) extract mixtures on *C. pavonana* larvae at 96 h after treatment

Concentration ratio of Pr, Sr and Tv extract	Expected $LC_{50}^a$ (%)	Co-toxicity ratio at $LC_{50}^a$	Expected $LC_{95}^a$ (%)	Co-toxicity ratio at $LC_{95}^a$
1:5:1	0.424	1.94	1.127	1.13
1:5:2	0.244	1.43	0.854	1.88
2:5:1	0.417	1.92	0.867	1.44

<sup>a</sup>Expected  $LC_x$  and co-toxicity ratio at  $LC_x$  were calculated according to Robertson & Smith (1984) as described in Materials and Methods.

P450 by those compounds might retain the action of active compounds in other extracts so as to impart synergistic action of the test extract mixtures. Furthermore, disruption of cell membrane integrity by saponins in *S. rarak* extract might facilitate the entry of active compounds of other extracts in the mixtures through the insect midgut wall and this process might also contribute to the synergistic action of the test extract mixtures.

A probable explanation for the antagonistic action of Pa, Sr and Tv extract mixtures at the 1:5:1 (LC<sub>95</sub> level) and 1:5:2 ratio (LC<sub>50</sub> and LC<sub>95</sub> level) against CHC was that the proportion of Pa extract relative to Tv extract in those mixtures was not sufficient to provide appropriate amounts of compounds that were needed to proportionately inhibit toxin detoxifying enzymes in the insect body. The proportion of Pa extract relative to Tv extract in the Pa, Sr and Tv extract mixtures at the 1:5:1 and 1:5:2 ratios was lower than that in the synergistic 2:5:1 mixture. Nailufar & Prijono (2017) reported that in Pa and Tv extract mixtures, the higher the proportion of Pa extract relative to Tv extract, the stronger their synergistic activity.

Most plant extract mixtures studied so far containing extracts from two different plant species or binary mixtures. In this study, extracts from three different plant species (ternary mixtures) were combined. Active constituents of each extract in the ternary extract mixtures tested have different modes of action (Hollingworth, 2001; Scott *et al.* 2008; Qasim *et al.*, 2020). Thus, ternary extract mixtures containing compounds with more than two different modes of action are expected to exert more multiple attacks on their target sites than binary extract mixtures. Moreover, these actions could be potentiated by different synergism mechanisms as described above.

Four plant species used in this study could grow well in many parts of Indonesia (Sunarno, 1997; Jansen, 1999; Utami & Jansen, 1999; Widowati, 2003). For mass-production of botanical insecticides, the source plants could be cultivated in a selected suitable location around the production site. The use of synergistic plant extract mixtures constitutes one of the attempts to enhance the performance of botanical insecticides. A further enhancement of the botanical insecticide performance might be achieved through the application of recent development in insecticide formulation technologies including nanotechnology (Gahukar & Das, 2020). Nuryanti *et al.* (2018a) reported that a nanoemulsion formulation of *P. retrofractum* and

*Tagetes erecta* extract mixtures showed a strong insecticidal activity against *N. lugens* (LC<sub>95</sub> 0.15%).

The use of extract mixtures offers some advantages over single extracts (Nailufar & Prijono, 2017). It might increase the spectrum of activity of extract mixtures against target pests. A lower concentration is needed to achieve a certain level of control if the extract mixture was synergistic. Further, lower extract application rates might minimize the risk of poisoning non-target organisms and the environment in general. Application of synergistic botanical insecticides at lower rates may reduce application costs. The use of synergistic extract mixtures could delay the development of insecticide resistance in target pests, if any. Moreover, the use of mixed extracts would harness better the existing rich botanical diversity and as such would reduce the dependence on a single plant species as sources of botanical insecticides (Isman, 2006).

## CONCLUSION

Mixtures of *P. aduncum* fruit, *S. rarak* fruit, and *T. vogelii* leaf extract at appropriate proportions were synergistic against *P. xylostella* and *C. pavonana* larvae. *P. retrofractum* fruit, *S. rarak* fruit, and *T. vogelii* leaf extract mixtures at proper proportions were also synergistic against *C. pavonana* larvae. Those synergistic extract mixtures are potential alternatives for the control of *P. xylostella* and *C. pavonana*.

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