

SYNERGISTIC ACTIVITY OF *PIPER ADUNCUM* FRUIT AND *TEPHROSIA VOGELII* LEAF EXTRACTS AGAINST THE CABBAGE HEAD CATERPILLAR, *CROCIDOLOMIA PAVONANA*

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ABSTRACT

One of the strategies for increasing the biological activity of botanical insecticides is through the synergistic mixtures of plant extracts. In order to assess the insecticidal joint action of both *Piper aduncum* (Pa) fruit and *Tephrosia vogelii* (Tv), leaf extract mixtures were assayed on the cabbage head caterpillar, *Crociodolomia pavonana*. This study was conducted at Bogor Agricultural University, Indonesia, from October 2010 to August 2011. Pa fruit and Tv leaf powder were extracted using ethyl acetate (1:8 w/v) through maceration done four times for Pa fruit, and three times on Tv leaf. Pa and Tv extracts, and the mixture of both extracts were assayed through leaf-feeding of the second-instar larvae of *C. pavonana* with a 48 h feeding treatment. The mixtures were tested at three concentration ratios, i.e. 1:5 (Pa:Tv), 1:1, and 5:1 (w/w). Based on a comparison of LC₅₀ at 72 h after treatment, mixtures of both *P. aduncum* and *T. vogelii* extracts at ratios of 1:5, 1:1, and 5:1 were 3.0, 3.1, and 4.3 times more toxic than *P. aduncum* extract, respectively. Meanwhile, the same mixtures of both plant extracts at the same ratios of 1:5, 1:1, and 5:1, was 2.4, 2.5, and 3.4 times more toxic than *T. vogelii* extract, respectively. Based on the independent joint action model, *P. aduncum* and *T. vogelii* extract mixtures at the three concentration ratios showed a strong synergistic effect on *C. pavonana* larvae, at both LC₅₀ and LC₉₅ levels, in which the 5:1 mixture was the most synergistic. Thus, the use of synergistic Pa and Tv extract mixtures was more effective than individual Pa or Tv extracts in controlling *C. pavonana*.

Key words: botanical insecticides, cabbage pest, extract mixtures, synergism, tropical plants

INTRODUCTION

Endowed with rich botanical resources, Indonesia is a source of plants purportedly possessing insect control properties. Insecticidal preparations from plants, or botanical insecticides, are biodegradable and mostly safe to non-target organisms (Prakash and Rao, 1997). Because of such, they can be compatibly incorporated into an integrated pest management program. Moreover, the increase in organic farming practices, which precludes use of synthetic insecticides, has brought back the demand for botanical insecticides (Isman, 2006). Society's heightening awareness on food safety has also increased the need for safe and biodegradable insecticides, including botanicals, which are expected to leave only negligible amounts of insecticide residues, if any, in food (Koivunen, 2013).

A potential source for botanical insecticides is the fish-poison bean, *Tephrosia vogelii* (Leguminosae) (Sunarno 1997). Easily grown in Indonesia, their leaves contain the insecticidal rotenone and other rotenoids, including deguelin and tephrosin (Delfel *et al.*, 1970; Marston *et al.*,

1984). Rotenone is an important botanical insecticide commonly used in crop pest management (Cavosky *et al.*, 2011).

Another abundant and potential source for botanical insecticides in Indonesia is the spiked pepper, *Piper aduncum* (Piperaceae) (Jansen, 1999). Bernard *et al.* (1995) reported an active ethanol extract from *P. aduncum* leaves against both the European corn borer, *Ostrinia nubilalis*, and rock hole breeding mosquito, *Aedes atropalpus*. Syahroni and Prijono (2013) reported an ethyl acetate extract from *P. aduncum* fruits exhibiting a strong insecticidal activity against the cabbage head caterpillar, *Crociodolomia pavonana*. A phenylpropanoid compound, dillapiole, was isolated and identified by Bernard *et al.* (1995) as the main insecticidal constituent from a *P. aduncum* leaf extract. A treatment containing dillapiole at a concentration of 0.1 ppm caused a 92% mortality in *A. atropalpus* larvae. Hasyim (2011) also reported that dillapiole was the main insecticidal component from *P. aduncum* fruit extract.

Botanical insecticides are generally applied at relatively high application rates; however, the synergistic mixtures of plant extracts can lower the application rate. Scott *et al.* (2002) reported tertiary and quaternary mixtures of piperamides from *Piper tuberculatum* exhibiting a greater-than-additive activity compared with both single compounds and binary mixtures. A mixture of both *Piper nigrum* extract and pyrethrum showed a synergistic effect on *Drosophila melanogaster*, with a synergist ratio of 11.6 (Jensen *et al.* 2006). Abizar and Prijono (2010) reported that a mixture of ethyl acetate extract containing both *T. vogelii* leaf and *Piper cubeba* fruit (5:9, w/w) was more toxic against *C. pavonana* larvae than separate extracts of both samples, furthermore, the mixture indicated a synergistic action. Also, a mixture of an ethyl acetate extract from *P. aduncum* and a methanol extract containing *Sapindus rarak* fruit (1:10, w/w) exhibited synergistic activity on *C. pavonana* larvae (Syahroni and Prijono 2013). In another study, Lina *et al.* (2013) reported a synergistic action from a tertiary mixture of *Brucea javanica*, *P. aduncum* and *T. vogelii* extract against *C. pavonana* larvae. The joint action of binary mixtures from *P. aduncum* and *T. vogelii* extracts, however, has never been reported.

Dillapiole found in *P. aduncum* inhibits the activity of a xenobiotic detoxifying enzyme called polysubstrate monooxygenase (PSMO) located in the midgut of *O. nubilalis* (Bernard *et al.*, 1990). This compound contains a methylenedioxyphenyl moiety, which is common among numerous synergistic insecticidal compounds. It also inhibits the oxidative metabolism of specific insecticides through PSMO, and as such, synergist compounds can retain their insecticidal toxic activity (Bernard and Philogène, 1993). *P. aduncum* extract is thus expected to show a synergistic activity when mixed with extracts from other botanical insecticides, such as *T. vogelii*. This study sought to assess the joint insecticidal action of *P. aduncum* fruit and *T. vogelii* leaf extract mixtures on *C. pavonana* larvae.

MATERIALS AND METHODS

Collection of insecticidal plant materials

T. vogelii leaves were collected from the Agropolitan Area (6°43'23" S, 107°0'26" E, 1283 m asl) of Cipanas District, West Java, Indonesia, while *P. aduncum* fruits were gathered from the campus area of Bogor Agricultural University, Bogor, Indonesia. *T. vogelii* leaves were immediately cut to small pieces and air-dried for one week, and were protected from direct exposure to sunlight. Whole *P. aduncum* fruits were also air-dried in the laboratory for one week.

Rearing of test insects

A *C. pavonana* colony was maintained at the Laboratory of Insect Physiology and Toxicology of the Department of Plant Protection, Bogor Agricultural University, following procedures as described by Prijono and Hassan (1992). *C. pavonana* larvae were briefly fed with pesticide-free

broccoli leaves. The adults were provided with a 10% honey solution dipped in a cotton swab. The second-instar larvae were used for bioassays.

Extraction of plant material

After air-drying, both the *T. vogelii* leaves and *P. aduncum* fruit were ground separately using a blender and then sieved through a 0.5 mm mesh. After grinding, 25 g of *T. vogelii* leaves and 25 g of *P. aduncum* fruit were macerated in 200 ml ethyl acetate for 3 h, with the mixture stirred every 30 minutes. The use of ethyl acetate as an extraction solvent was based from our previous studies (Abizar and Prijono, 2010; Syahroni and Prijono, 2013). The extract was filtered with Whatman filter paper no. 41 (diameter 185 mm) and the marc was macerated again using 200 ml of ethyl acetate. The solvent was evaporated using a rotary evaporator set at 50 °C and reduced pressure. The aforementioned maceration was repeated for two to six times and the whole extraction experiment was replicated three times.

Each extract was weighed, then the percentage yield of plant extract relative to dry weight of extracted plant material was calculated. Extract yield data was transformed to $\arcsin\sqrt{\text{proportion}}$, then the transformed data was analyzed using analysis of variance based on a completely randomized design. The Duncan's multiple range test was used to compare means.

Toxicity tests

Both *P. aduncum* and *T. vogelii* extracts were tested separately and in combination with each other for their toxicity against *C. pavonana* larvae. During preliminary tests, *P. aduncum* fruit extracts were tested at a concentration of 0.1% (w/v) while *T. vogelii* leaf extracts were tested at 0.14% (w/v), with six replications each. All bioassays were done using a leaf-dip feeding method. Each test extract was mixed with methanol, Solvesso R-100 (a light aromatic petroleum solvent, primarily C9-10 dialkyl and trialkylbenzenes), and an emulsifier Tween 80 2-[2-[3,4-bis(2-hydroxyethoxy)oxolan-2-yl]-2-(2-hydroxyethoxy)ethoxy] ethyloctadec-9-enoate (9:1:5; final concentration 0.96% v/v), then diluted with distilled water to the desired volume. A solution containing distilled water, methanol, Solvesso R-100, and Tween 80 was used as a control.

Fresh broccoli leaf portions (4 cm x 4 cm) were dipped separately into an extract preparation until complete wetness. Control leaves were dipped into the control solution. Treated and control leaves were placed separately into glass petri dishes (diameter 9 cm) placed upside-down and lined with tissue paper, extending up to the space separating the top and bottom of each petri dish. Fifteen freshly-molted second-instar larvae of *C. pavonana* were placed into each petri dish containing either a treated or control leaf portion. Test larvae were allowed to feed on the leaves for 48 h, then were fed with non-treated leaves for the next 48 h. Dead larvae was counted daily up to 96 h after treatment (HAT). Analyses conducted on insect mortality data were the same as for extract yield data.

The number of macerations of both *P. aduncum* and *T. vogelii* extracts which provided the best yield and lethal effect was used for further testing. During bioassays with separate extracts, *P. aduncum* and *T. vogelii* extracts after a specific number of macerations were tested at six concentration levels, which were expected to result in an insect mortality range of 15% to 95%. Extract treatment procedures were the same as the preliminary tests. The number of dead larvae was counted daily until 96 HAT and insect mortality data at 96 HAT were analyzed using the probit method (Robertson *et al.*, 2002-2003).

The *P. aduncum* and *T. vogelii* extract mixtures were tested at three concentration ratios (1:5, 1:1, and 5:1 w/w). Each extract mixture was tested at six different concentration levels, which were expected to result in an insect mortality range of 15% to 95%. Procedures for treatment, insect mortality count, and mortality data analysis were the same as those conducted for separate extracts.

The joint action between *P. aduncum* and *T. vogelii* extracts was determined based on the independent joint action model. This was through calculation of the combination index (CI) at both LC₅₀ and LC₉₅ levels following Chou and Talalay (1984). The type of joint action for extract mixtures is classified as follows: (1) if CI < 1.00, then the joint action is synergistic; (2) if CI = 1.00, then the joint action is additive; and (3) if CI > 1.00, then the joint action is antagonistic (Chou and Talalay, 1984).

RESULTS AND DISCUSSION

Extract yield and preliminary test results

In general, yields from both *P. aduncum* fruit and *T. vogelii* leaf extracts increased with increasing number of macerations in ethyl acetate solvent. The relationship between yield and number of macerations followed a quadratic regression line with a high determination coefficient (*R*), i.e. 0.899 for *P. aduncum* extract and 0.994 for *T. vogelii* extract (Fig. 1).

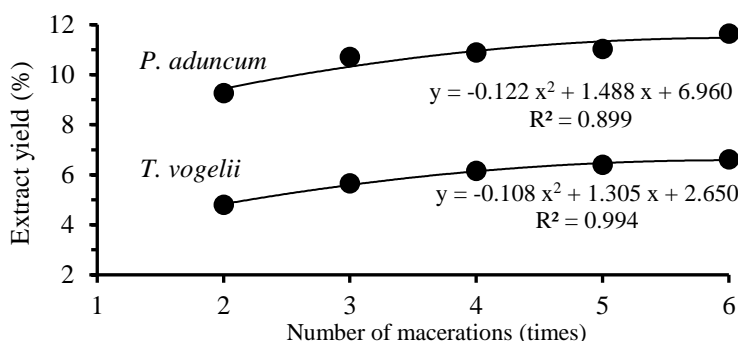


Fig. 1. Relationship between number of macerations and yield from both *P. aduncum* fruit and *T. vogelii* leaf extracts.

Yield from *P. aduncum* extracts was not significantly different among the different number of times macerated. On the other hand, yield from *T. vogelii* extract after macerating six times was significantly higher than after macerating two or three times. Extract yield after macerating four times was significantly higher than after macerating for two times, but there was not significant difference with other maceration treatments, including after macerating six times (Table 1).

Table 1. The effect of the number of macerations on the yield from *P. aduncum* fruit (0.10%) and *T. vogelii* extract (0.14%) and on larval mortality (*C. pavonana*).

Number of macerations	Extract yield (%) ^{a,c}		Larval mortality (%) ^{b,c}	
	<i>P. aduncum</i>	<i>T. vogelii</i>	<i>P. aduncum</i>	<i>T. vogelii</i>
2x	9.26 ± 0.99a	4.80 ± 0.26a	33.7 ± 44.7a	45.2 ± 30.3a
3x	10.71 ± 1.94a	5.65 ± 0.57ab	38.5 ± 30.6a	53.0 ± 33.2a
4x	10.89 ± 1.87a	6.15 ± 0.57bc	23.3 ± 33.9a	53.0 ± 36.0a
5x	11.03 ± 1.88a	6.39 ± 0.56bc	24.1 ± 7.2a	38.1 ± 31.6a
6x	11.64 ± 1.89a	6.62 ± 0.31c	17.8 ± 17.2a	45.2 ± 24.1a

^aBased on air-dried weight of plant material, moisture content of *P. aduncum* fruit and *T. vogelii* leaf powder was 6.89% ± 1.42% and 7.37% ± 2.04% (n = 3), respectively.

^bCumulative mortality 96 h after treatment. Extract yield and larval mortality are expressed as mean ± standard deviation.

^cMeans in the same column followed by the same letter are not significantly different according to Duncan's multiple range test ($\alpha = 0.05$). Data was transformed to $\arcsin \sqrt{\text{proportion}}$ before analysis of variance.

Unlike yield from *P. aduncum* and *T. vogelii* extracts which exhibited an increase with number of macerations, *C. pavonana* larvae mortality was not significantly different with increasing number of macerations (Table 1). Low insect mortality was observed for both *P. aduncum* extracts obtained after more than three macerations and in *T. vogelii* extracts obtained after more than four macerations.

A probable explanation for the increase in extract yield and insect mortality with increasing number of macerations is the large proportion of active substances present upon three macerations, while further increasing the number of macerations can increase the yield of inactive substances. It seems that the proportion of active substances from the extracts was lower, and thus, the test insect mortality was also lower, as the number of macerations was done for more than three times for *P. aduncum* extract. Meanwhile, insect mortality was lower after four macerations for the *T. vogelii* extract. Based on yield and test insect mortality data, *P. aduncum* extracts after three macerations and *T. vogelii* extracts after four macerations were used for further testing.

Extract toxicity and synergism

Both *P. aduncum* and *T. vogelii* extracts both represented slow acting toxicants against *C. pavonana* larvae, with *P. aduncum* extract acting slightly quicker than *T. vogelii* extract (Fig. 2). At 24 HAT, larval mortality was generally still low with the *P. aduncum* extract treatment, and negligible in the *T. vogelii* extract treatment. Larval mortality increased sharply from 24 to 48 HAT for both extracts. After the treated leaves were replaced with untreated ones at 48 HAT, larval mortality increased only very slightly from 48 to 72 HAT for the *P. aduncum* extract, but still increased sharply in the *T. vogelii* extract. There was no increase in larval mortality from 72 to 96 HAT in *P. aduncum* extract treatment, while mortality only slightly increased for the *T. vogelii* extract. Thus, larval mortality from both test extracts has reached a constant level at 96 HAT, and during this time, larval mortality increased with extract concentration. Thus, probit analysis was performed against larval mortality data at 96 HAT to determine the quantitative relationship between extract concentration and insect mortality.

Both *P. aduncum* and *T. vogelii* extracts had strong insecticidal activity against *C. pavonana* larva, with LC₉₅ values at 0.317% and 0.290%, respectively (Table 2). Based on a comparison between LC₅₀ at 96 HAT, *T. vogelii* extract was 1.27 times more toxic on *C. pavonana* larvae than the *P. aduncum* extract. The difference in toxicity between the two extracts was seemingly due to the difference in toxicity and/or amount of active compounds present. The main insecticidal compounds in *T. vogelii* leaves include rotenone, deguelin, and tephrosin, which belong to the rotenoid class (Delfel *et al.*, 1970; Marston *et al.*, 1984). Rotenone has good insecticidal activity against various insect pests which could act as stomach and contact poison (Yu, 2008). At the cellular level, it acts by inhibiting electron transfer between NADH dehydrogenase and coenzyme Q in the Complex I of the mitochondrial electron transport chain (Hollingworth, 2001), leading to the depletion of ATP as the cellular energy carrier. This eventually immobilizes the affected insects leading to death (Yu, 2008).

Hasyim (2011) reported dillapiole as the main constituent of the most active fraction from a hexane extract of *P. aduncum* fruits, with a GC peak area of 68.8%. That active fraction had strong insecticidal activity against *C. pavonana* larvae, with LC₉₅ of about 0.077%. More than 40 years ago, dillapiole was reported to possess a strong insecticidal and synergistic activity against fruit flies, *Drosophila melanogaster* (Lichtenstein *et al.*, 1974). This compound has also been reported as the main active component from *P. aduncum* leaves (Bernard *et al.*, 1995). In addition to dillapiole, myristicin was also present in the most active fraction from the hexane extract of *P. aduncum* fruits, with a GC peak area of 4.9% (Hasyim, 2011). This compound has also long been known for its insecticidal and synergistic activity (Lichtenstein and Casida, 1963).

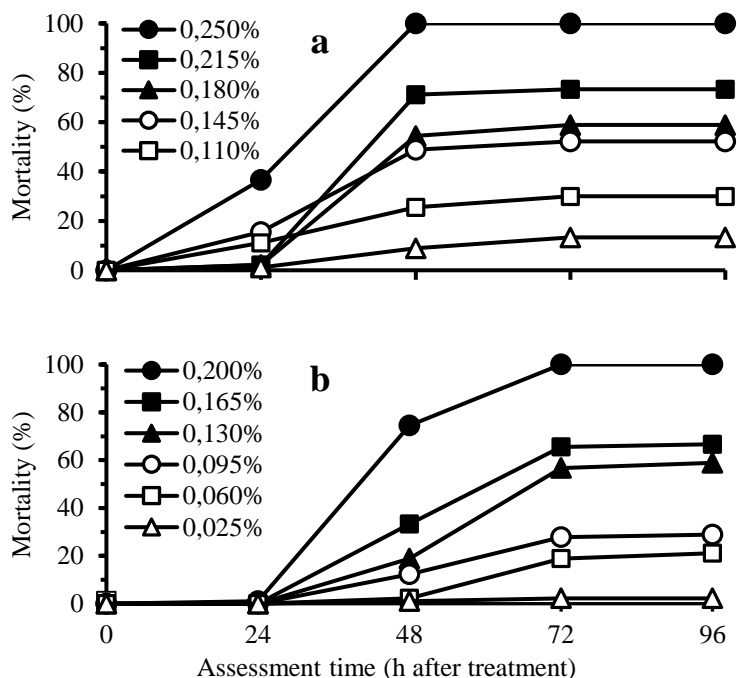


Fig. 2. Time-course mortality of *C. pavonana* larvae in *P. aduncum* (a) and *T. vogelii* (b) extracts. No mortality was observed in control larvae up to 96 h post-treatment.

Table 2. Toxicity of *P. aduncum* fruit, *T. vogelii* leaf extracts, and their mixtures against *C. pavonana* larvae 96 h after treatment.

Extract	$a \pm SE^a$	$b \pm SE^a$	LC ₅₀ (95% CI) ^b (%)	LC ₉₅ (95% CI) ^b (%)
<i>P. aduncum</i> (Pa)	3.982 ± 0.322	4.684 ± 0.384	0.141 (0.110–0.173)	0.317 (0.235–0.742)
<i>T. vogelii</i> (Tv)	3.769 ± 0.317	3.949 ± 0.330	0.111 (0.072–0.163)	0.290 (0.186–2.014)
Pa + Tv 1:5	6.896 ± 0.544	5.192 ± 0.417	0.047 (0.037–0.057)	0.097 (0.075–0.190)
Pa + Tv 1:1	6.041 ± 0.418	4.474 ± 0.324	0.045 (0.029–0.059)	0.104 (0.075–0.232)
Pa + Tv 5:1	6.647 ± 0.518	4.494 ± 0.374	0.033 (0.021–0.043)	0.077 (0.057–0.162)

^a a = intercept of probit regression b = slope of probit regression, SE = standard error, ^bCI = confidence interval.

Table 3. Type of joint action of mixtures of *P. aduncum* fruit and *T. vogelii* leaf extracts against *C. pavonana* larvae 96 h after treatment.

Extract mixture ^a	Combination index at		Type of joint action at	
	LC ₅₀	LC ₉₅	LC ₅₀	LC ₉₅
Pa + Tv 1:5	0.428	0.344	Synergistic	Synergistic
Pa + Tv 1:1	0.394	0.373	Synergistic	Synergistic
Pa + Tv 5:1	0.254	0.256	Synergistic	Synergistic

Based on the LC₅₀ value at 96 HAT (Table 2), mixtures of *P. aduncum* and *T. vogelii* extracts at concentration ratios of 1:5, 1:1, and 5:1 were more toxic than *P. aduncum* extract alone at 3.0, 3.1, and 4.3 times, respectively. Meanwhile, mixtures of both extracts at the same concentration ratios of

1:5, 1:1, and 5:1, were more toxic than *T. vogelii* extract alone at 2.4, 2.5, and 3.4 times, respectively. Based on the LC₉₅ values, the respective figures were 3.3, 3.0, and 4.1 times more toxic than *P. aduncum* extract alone, and 3.0, 2.8, and 3.8 times more toxic than *T. vogelii* extract alone. Furthermore, based on the combination indices (CI) at both LC₅₀ and LC₉₅ levels, *P. aduncum* and *T. vogelii* extract mixtures at the three concentration ratios were synergistic (CI < 1.00) on *C. pavonana* larvae, with the mixture at a ratio of 5:1 (Pa:Tv) being the most synergistic (Table 3).

P. aduncum and *T. vogelii* extract mixtures at the three concentration ratios tested, i.e. 1:5, 1:1 and 5:1, were synergistic against *C. panonana* larvae. It seems that the synergistic property of the extract mixtures was contributed by dillapiole and, to a much lesser extent, by myristicin, which were present in the *P. aduncum* extract (Hasyim, 2011). Dillapiole and myristicin have a methylenedioxyphenyl (MDP) moiety in their respective molecular structures, which is typically present in various insecticide synergists (Bernard and Philogène, 1993). Compounds possessing an MDP moiety can inhibit the activity of polysubstrate monooxygenase (PSMO) enzymes that commonly break down toxic compounds or metabolites in the body (Scott *et al.*, 2008), so that the compounds or other simultaneously administered toxins can still exert their action on their targets. In addition, if PSMO is inhibited, various indigenous toxic metabolic wastes will accumulate in the body leading to organismal death (Bernard *et al.*, 1995).

Bernard *et al.* (1990) reported that dillapiole inhibited the activity of PSMO in the microsomal midgut cells from *Ostrinia nubilalis*, in such a way that other insecticidal compounds present in combination with dillapiole were not degraded by PSMO. Likewise, inhibition of PSMO by dillapiole present in *P. aduncum* extracts seemed to retain the toxic activity of active compounds present in the *T. vogelii* extract, so as to impart the synergistic action of both extract mixtures. The greatest synergism between *P. aduncum* and *T. vogelii* extracts (5:1 ratio) could be due to the greatest inhibition of PSMO by dillapiole present in the *P. aduncum* extract, so that active compounds from the *T. vogelii* extract can still interact with the target site.

The synergistic *P. aduncum* and *T. vogelii* extract mixture will need a lower extract concentration or dosage to achieve a certain level of control than the two extracts applied separately. Moreover, the use of extract mixtures may increase their spectrum of activity against target pests. On the other hand, lower extract application rates may reduce the risk of poisoning non-target organisms and the environment in general (Prakash and Rao, 1997). Application of synergistic insecticides at lower rates may also reduce application cost, making it more economical (Stone *et al.*, 1988). The use of synergistic extract mixtures can also be incorporated into an insecticide resistance mitigation program (Scott *et al.* 2003). Furthermore, the use of extract mixtures will decrease the dependence on a single plant species as a source for botanical insecticides (Isman, 2006). Thus, use of a synergistic *P. aduncum* and *T. vogelii* extract mixture is more efficient than either single *P. aduncum* or *T. vogelii* extracts, and the further development of a more synergistic extract mixture is still worth pursuing.

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REFERENCES

- Abizar, M. and D. Prijono. 2010. Insecticidal activity of leaf and seed extracts of *Tephrosia vogelii* J. D. Hooker (Leguminosae) and fruit extract of *Piper cubeba* L. (Piperaceae) on the cabbage head caterpillar, *Crociodolomia pavonana* (L.) (Lepidoptera: Crambidae) [in Indonesian]. JHPT Trop. 10:1-12.

- Bernard, C.B. and B.J.R. Philogène. 1993. Insecticide synergists: role, importance, and perspectives. *J. Toxicol. Environ. Health.* 38:199-223.
- Bernard, C.B., H.G. Krishnamurty, D. Chauret, T. Durst, B.J.R. Philogène, P. Sanchez-Vindas, C. Hasbun, L. Poveda, L. San Roman and J.T. Arnason. 1995. Insecticidal defenses of Piperaceae from the Neotropics. *J. Chem. Ecol.* 21:801-814.
- Bernard, C.B., J.T. Arnason, B.J.R. Philogène, J. Lam and T. Waddell. 1990. In vivo effect of mixtures of allelochemicals on the life cycle of the European corn borer, *Ostrinia nubilalis*. *Entomol. Exp. Appl.* 57:17-22.
- Cavoski, I., P. Caboni and T. Miano. 2011. Natural pesticides and future perspectives, pp 169-190. In M. Stoytcheva (eds.). *Pesticides in the Modern World – Pesticides Use and Management*. InTech, Rijeka, Croatia.
- Chou, T.C. and P. Talalay. 1984. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. *Adv. Enzyme Regl.* 22:27-55.
- Delfel, N.E., W.H. Tallent, D.G. Carlson and I.A. Wolff. 1970. Distribution of rotenone and deguelin in *Tephrosia vogelii* and separation of rotenoid-rich fractions. *J. Agric. Food Chem.* 18:385–390.
- Hasyim, D.M. 2011. Potential of spiked pepper (*Piper aduncum*) as a botanical insecticide against *Crociodolomia pavonana* larva [in Indonesian]. MS. Thesis, Bogor Agricultural University, Bogor, Indonesia. 56 p.
- Hollingworth, R.M. 2001. Inhibitors and uncouplers of mitochondrial oxidative phosphorylation, pp 1169-1261. In R.I. Krieger (ed.). *Handbook of Pesticide Toxicology*. Vol 2: Agents. Academic Press, San Diego, California.
- Isman, M.B. 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annu. Rev. Entomol.* 51:45–66.
- Jansen, P.C.M. 1999. *Piper aduncum* L., p 260. In C.C. de Guzman and J.S. Siemonsma (eds.). *Plant Resources of South-East Asia No. 13: Spices*. Backhuys Publisher, Leiden.
- Jensen, H.R., I.M. Scott, S. Sims, V.L. Trudeau and J.T. Arnason. 2006. The effect of a synergistic concentration of a *P. nigrum* extract used in conjunction with pyrethrum upon gene expression in *Drosophila melanogaster*. *Insect Molec. Biol.* 15:329–339.
- Klocke, J.A. 1987. Natural plant compounds useful in insect control, pp 396–415. In G.R. Waller (ed.). *Allelochemicals: Role in Agriculture and Forestry*. American Chemical Society, Washington, D.C.
- Koivunen M., S.O. Duke, J.C. Coats and J.J. Beck. 2013. Pest management with natural products, pp 1-4. In J.J. Beck, J.R. Coats, S.O. Duke and M.E. Koivunen (eds.). *Pest Management with Natural Products*. ACS Symp. Ser. 1141. American Chemical Society, Washington, DC.
- Lichtenstein, E.P. and J. Casida. 1963. Myristicin, an insecticide and synergist occurring naturally in the edible parts of parsnips. *J. Agric. Food Chem.* 11:410-415. doi: 10.1021/jf60129a017.

- Lichtenstein, E.P., T.T. Liang, K.R. Schulz, H.K. Schnoes and G.T. Carter. 1974. Insecticidal and synergistic components isolated from dill plants. *J. Agr. Food Chem.* 22:658-664.
- Lina, E.C., Dadang, S. Manuwoto, G. Syahbirin and D. Prijono. 2013. Synergistic action of mixed extracts of *Brucea javanica* (Simaroubaceae), *Piper aduncum* (Piperaceae), and *Tephrosia vogelii* (Leguminosae) against cabbage head caterpillar, *Crociodolomia pavonana*. *J. Biopestic.* 6:77-83.
- Marston, A., J.D. Msonthi and K. Hostettmann. 1984. On the reported molluscicidal activity from *Tephrosia vogelii* leaves. *Phytochemistry* 23:1824–1825.
- Prakash, A. and J. Rao J. 1997. *Botanical Pesticides in Agriculture*. CRC Press, Boca Raton, Florida. 480 p.
- Prijono, D. and E. Hassan. 1992. Life cycle and demography of *Crociodolomia binotalis* Zeller (Lepidoptera: Pyralidae) on broccoli in the laboratory. *Indon. J. Trop. Agric.* 4:18–24.
- Robertson, J.L., H. K. Preisler and R.M. Russell. 2002-2003. *PoloPlus: Probit and Logit Analysis. User's Guide*. LeOra Software, Petaluma, California. 36 p.
- Scott, I.M., E. Puniani, T. Durst, D. Phelps, S. Merali, R.A. Assabgui, P. Sánchez-Vindas, L. Poveda, B.J.R. Philogène and J.T. Arnason. 2002. Insecticidal activity of *Piper tuberculatum* Jacq. extracts: synergistic interaction of piperamides. *Agric. For. Entomol.* 4:137-144.
- Scott, I.M., H. Jensen, J.G. Scott, M.B. Isman, J.T. Arnason and B.J.R. Philogène. 2003. Botanical insecticides for controlling agricultural pests: piperamides and the Colorado Potato Beetle *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae). *Arch. Insect Biochem. Physiol.* 54:212–225.
- Scott, I.M., H.R. Jensen, B.J.R. Philogène and J.T. Arnason. 2008. A review of *Piper* spp. (Piperaceae) phytochemistry, insecticidal activity and mode of action. *Phytochem. Rev.* 7:65-75.
- Stone, N.D., M.E. Makela and F.W. Plapp. 1988. Nonlinear optimization analysis of insecticide mixtures for the control of the tobacco budworm (Lepidoptera: Noctuidae). *J. Econ. Entomol.* 81:989-994.
- Sunarno, B. 1997. *Tephrosia vogelii* J.D. Hooker, pp 248-251. In: I.F. Hanum and L.J.G. van der Maesen (eds.). *Plant Resources of South-East Asia No. 11: Auxiliary Plants*. Backhuys Publisher, Leiden.
- Syahroni, Y.Y. and D. Prijono. 2013. Insecticidal activity of *Piper aduncum* L. (Piperaceae) and *Sapindus rarak* DC. (Sapindaceae) fruit extracts and their mixtures on *Crociodolomia pavonana* (F.) (Lepidoptera: Crambidae) [in Indonesian]. *Indon. J. Entomol.* 10:39-50.
- Yu, S.J. 2008. *The Toxicology and Biochemistry of Insecticides*. CRC Press, Boca Raton, Florida. 296 p.