Insecticidal activity of the extracts of Piper retrofractum fruit and Tephrosia vogelii leaf and their mixtures against Crocidolomia pavonana

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ABSTRACT

This laboratory work was carried out to evaluate the insecticidal activity of the extracts of Piper retrofractum (Piperaceae) fruit and Tephrosia vogelii (Fabaceae) leaf and their mixtures against the cabbage head caterpillar, Crocidolomia pavonana. Ground plant materials of the two plant species were extracted separately with n-hexane and methanol. The results of leaf-residue feeding bioassays showed that P. retrofractum (Pr) and T. vogelii (Tv) hexane extracts had strong insecticidal activity against C. pavonana larvae (LC95 < 0.5%) and were more active than their respective methanol extracts. Pr and Tv hexane extract had a moderate and a rather weak contact effect on C. pavonana larvae, respectively. In feeding tests, Pr + Tv (1:1) hexane and methanol extract mixtures indicated synergistic joint effect both at LC95 and LC50 level, whereas in the contact test, the mixture of Pr + Tv (1:1) hexane extract was synergistic at the LC50 level but antagonistic at the LC95 level.

In choice tests, antifeedant effects of Pr and Tv hexane extracts at LC50 to LC95 levels on C. pavonana larvae followed a concentration-dependent fashion. Thus, separate or mixed P. retrofractum and T. vogelii extracts are potential alternatives for the control of C. pavonana.

Keywords: Antifeedant, Botanical insecticides, Cabbage pest, Extract mixtures, Joint action

ABSTRAK

Aktivitas insektisida ekstrak buah Piper retrofractum dan daun Tephrosia vogelii serta campuran kedua ekstrak terhadap Crocidolomia pavonana

Penelitian ini dilakukan untuk menguji aktivitas insektisida ekstrak buah Piper retrofractum (Piperaceae) dan daun Tephrosia vogelii (Fabaceae) serta campuran kedua ekstrak tersebut terhadap ulat krop kubis, Crocidolomia pavonana. Serbuk bahan tumbuhan uji diekstrak secara terpisah dengan pelarut n-heksana dan metanol. Hasil pengujian dengan metode residu feeding bioassays yang kuat terhadap larva C. pavonana (LC95 < 0.5%) serta lebih aktif daripada ekstrak methanol masing-masing. Ekstrak heksana Pr dan Tv masing-masing memiliki efek kontak sedang dan agak lemah terhadap larva C. pavonana. Pada pengujian dengan metode residu pada daun, campuran ekstrak Pr + Tv (1:1), baik yang disiapkan dengan pelarut heksana maupun metanol, bersifat sinergistik pada taraf LC50 dan LC95, sedangkan secara kontak, campuran ekstrak heksana Pr + Tv (1:1) bersifat sinergistik pada taraf LC50 tetapi antagonistik pada taraf LC95. Pada pengujian aktivitas makan dengan metode pilihan, efek penghambatan makan ekstrak heksana Pr dan Tv pada konsentrasi yang setara LC25 sampai LC50 makin meningkat dengan makin tinginya konsentrasi yang diuji. Dengan demikian, ekstrak tunggal atau campuran ekstrak P. retrofractum dan T. vogelii potensial untuk digunakan sebagai bahan insektisida alternatif dalam pengendalian hama C. pavonana.

Kata Kunci: Campuran ekstrak, Hama kubis, Insektsida nabati, Penghambatan makan, Sinergisme

INTRODUCTION

Javanese long pepper (JLP) Piper retrofractum Vahl (Piperaceae), a source of spices and herbal medicines, is a potential source of botanical insecticides that has long been cultivated in Java, Indonesia (Heyne, 1987; Utami & Jansen, 1999). JLP fruit extracts have been reported to possess strong insecticidal activity against various insect pests including European earwigs Forficula auricularia (Assabgui et al., 1997), cabbage head caterpillar Crocidolomia pavonana (Prijono et al., 2006; Nurfajrina & Prijono, 2015), green stinkbug Nezara viridula (Hasnash & Rusdy, 2015), tropical armyworm Spodoptera litura (Yooboon et al., 2019), papaya mealybug Paracoccus marginatus (Asnan et al., 2015), tea mosquito bug Helopeltis antonii (Indriati et al., 2015), and rice brown planthopper Nilaparvata lugens (Nuryanti et al., 2018).

Another plant species that has long been used as a source of botanical insecticides is fish-poison bean (FPB), Tephrosia vogelii J.D. Hooker (Fabaceae) (Gaskins et al., 1972; Sunarno, 1997; Stevenson et al., 2017). FPB leaf preparations have been reported to have good insecticidal activity...
against some field crop and stored-product pests (Prakash & Rao, 1997; Dadang & Prijono, 2011; Kamanula et al., 2011; Stevenson & Belmain, 2017). Rotenoids such as deguelin, tephrasin, and rotenone are the primary constituents responsible for the insecticidal activity of FPB leaves (Delfel et al., 1970; Stevenson et al., 2012).

JLP fruits have been reported to contain more than 15 piperamides (Kikuzaki et al., 1993; Parmar et al., 1997) including guineensine, pellitorine, pipericine, piperonine, and retrofractamide A. These compounds have also been isolated from other Piperaceae plants and were shown to have insecticidal activity (Miyakado et al., 1989; Scott et al., 2008). Moreover, a mixture of pipericine, dihydro-piperonine, and guineensine showed high synergistic activity against the pulse beetle Callosobruchus chinensis (Miyakado et al., 1989). Three piperamides have methylenedioxyphenyl (MDP) moieties in their molecules which is a characteristic of insecticide synergists that can inhibit cytochrome P450 enzymes (Bernard & Philogène 1993). These enzymes commonly involve in the detoxification of toxic compounds in the body (Chen, 2020). Thus, in addition to possessing insecticidal activity itself, JLP extract which contains substances possessing MDP moieties is also expected to yield synergistic activity when mixed with other plant extracts.

Previously, Dadang and Prijono (2011) reported that mixtures of methanol extracts of JLP and FPB at concentration ratios of 2:1, 1:1, and 1:2 were more toxic than separate extracts to C. pavonana. They, however, did not analyze joint action of those extract mixtures quantitatively. This work was conducted to evaluate the insecticidal activity of hexane and methanol extracts of JLP fruits and FPB leaves as well as to analyze joint action of JLP and FPB extract mixtures against C. pavonana larvae. Extracts of the two species were also tested separately for their antifeedant effect.

MATERIALS AND METHODS

Collection of Insecticidal Plant Materials

Dry JLP fruits were purchased from a medicinal herb kiosk at a local market in Bogor. FPB leaves were collected from Caringin District, Bogor Regency, West Java (6° 44' 44.7" S and 106° 49' 57.5" E longitude; 636 m asl). They were immediately cut to small pieces and air-dried for one week in a laboratory space protected from direct exposure to sunlight.

Rearing of Test Insects

C. pavonana colony was maintained at the Laboratory of Insect Physiology and Toxicology, Department of Plant Protection, Bogor Agricultural University following the procedures as described by Prijono and Hassan (1992). Briefly, C. pavonana larvae were fed pesticide-free broccoli leaves and the adults were provided with 10% honey solution absorbed to a cotton swab.

Extraction of Plant Materials

Air-dried JLP fruit cuts and FPB leaf cuts were pulverized separately with a blender and then sieved with a 0.5 mm mesh sieve. Ground JLP fruits and FPB leaves, 300 g each, were separately extracted with 2 l of n-hexane or methanol by infusion method (Houghton & Raman, 1998). Each extract was filtered with Whatman No. 41 filter paper and the extraction was repeated until the filtrate was clear. The filtrates were pooled and the solvent was evaporated to dryness at 50 °C under reduced pressure in a rotary evaporator. After evaporation of the solvent, the JLP hexane extract consisted of two parts, i. e. liquid and solid parts, which were then separated physically. All extracts were kept in refrigerator (4 ± 0.5 °C) until used for bioassays.

Toxicity Tests

The JLP hexane extract used in all bioassays was only the liquid fraction of the initial JLP hexane extract since its solid fraction was not active. Hereafter, the term JLP hexane extract refers to the liquid fraction of the initial JLP hexane extract.

Leaf-dipping Tests

Hexane and methanol extracts of JLP and FPB were tested separately against second-instar larvae C. pavonana at five concentration levels which were expected to give insect mortality in the range of about 15% to 95% as determined in preliminary tests (Robertson et al., 2017). Hexane extracts were mixed with acetone, methanol and an emulsifier Tween 80 (5:5:2, final concentration 1.2% v/v), whereas methanol extracts were mixed with methanol and Tween 80 (5:1, 1.2%), then were diluted with distilled water to the desired volume. Distilled water containing acetone, methanol and Tween 80 (5:5:2, 1.2%) or methanol and Tween 80 (5:1, 1.2%) was used as control solution.

Fresh broccoli leaf portions (4 cm × 4 cm) were dipped separately in a particular extract preparation to complete wetness. Control leaves were dipped in the relevant control solution. Treated and control leaf portions were placed separately in upside-down glass petri dishes (9 cm diameter) lined with tissue paper which extended to the space between top and bottom parts of each petri dish. Fifteen freshly-moulshed second-instar larvae C. pavonana were placed in each petri dish containing a treated or control leaf portion. Each treatment was done with five replicates. Test larvae were allowed to feed on treated or control leaves for 48 h and then were fed untreated leaves for the next 24 h. The number of dead larvae was counted daily until 72 h after treatment (HAT) and insect mortality data at 72 HAT were analyzed with probit method using PoloPlus (Robertson et al., 2003).
Mixtures of JLP and FPB hexane extract as well as JLP and FPB methanol extract, at a 1:1 concentration ratio each, were also tested. Each extract mixture was tested at six concentration levels which were expected to give about 15% to 95% insect mortality. Each concentration treatment was done with five replicates. Procedures for treatment, insect mortality counts, and analysis of mortality data were the same as those in the tests with separate extracts.

The type of joint action of each extract mixture was determined based on the independent joint action model by calculating co-toxicity ratio (CTR) of each mixture following Robertson and Smith (1984). The type of joint action of extract mixtures was categorized as follows: (1) if CTR > 1.0, then synergistic joint action is indicated; (2) if CTR = 1.0, then additive joint action is indicated; and (3) if CTR < 1.0, then antagonistic joint action is indicated.

Contact Tests
Hexane extracts of JLP and FPB were tested separately and as a 1:1 mixture at 5-6 concentration levels which were expected to give 15% to 95% insect mortality. Each test extract was diluted with acetone to the desired concentration. A particular extract solution at a volume of 0.5 ml was pipetted into a glass vial (2.2 cm diameter and 5.8 cm high). The vial was then closed and swirled slantwise to coat the inner surface of the vial with the test extract solution. Excess of liquid was discarded and the vial was air-dried in a fume hood. After the extract deposit in the vial dried up, 15 second-instar larvae \textit{C. pavonana} (2–4 h after moultling) was put into the vial. The vial was then closed with muslin cloth and placed upside down in a tray. Control insects were placed in the vial treated with acetone only. After being exposed to the extract thin film for 2 h, the test larvae were transferred to an upside-down petri dish sealed with tissue paper and fed with a fresh broccoli leaf portion (4 cm × 4 cm). Insect mortality was recorded at 24 HAT and the mortality data was analyzed with probit method using PoloPlus (Robertson et al., 2003). The type of joint action of the extract mixture was determined by calculating co-toxicity ratio of the mixture as in the leaf-dipping tests.

Antifeeding Tests
Hexane extracts of JLP and FPB were used in the choice feeding tests against second-instar larvae \textit{C. pavonana}. Each extract was tested at four concentrations equivalent to its LC_{95} to LC_{70} based on the results of the aforementioned leaf-dipping tests. Procedures for extract dilution were the same as in the leaf-dipping tests and five replicates were used for each extract concentration treatment.

Four broccoli leaf portions (2.5 cm × 2.5 cm) were cut out from broccoli leaves on both sides of the leaf midrib. Leaf portions from one side of the midrib was used for the treatment and those from the other side for the corresponding control. Procedures for leaf treatment were the same as in the leaf-dipping tests. After air drying, two treated and two control leaf portions were placed alternately in an upside-down petri dish sealed with tissue paper. Ten second-instar larvae \textit{C. pavonana} were released in the center of the petri dish. After 24 h, areas of treated and control leaves eaten were measured using 1-mm square graph paper. Differences in areas of treated and control leaves eaten were analyzed by using paired t-test (Ott & Longnecker, 2016).

The antifeedant effect (AF) of test extracts at each concentration level was calculated as follows (Dadang & Prijono, 2008): \[ AF (\%) = \frac{(A_{\text{CL}} - A_{\text{TL}})}{(A_{\text{CL}} + A_{\text{TL}})} \times 100\% \] where \( A_{\text{CL}} \) and \( A_{\text{TL}} \) were the area of control and treated leaves eaten (mm²), respectively. The antifeedant levels among different concentration treatments with each extract were analyzed by analysis of variance followed by Tukey’s test (Ott & Longnecker, 2016) using SAS statistical software package (SAS Institute 2018).

RESULTS AND DISCUSSION

Extract Toxicity

In leaf-dipping tests, hexane extracts of both JLP fruits and FPB leaves were about twice as active as their respective methanol extracts (Table 1). LC_{95} of both JLP and FPB hexane extracts were less than 0.5% suggesting that both extracts had strong insecticidal activity against \textit{C. pavonana} larvae. Prijono (1999) indicated that a plant extract is considered to have strong insecticidal activity if the extract at concentrations of no more than 0.5% can give at least 80% kill in the test insect. Referring to this criteria, JLP and FPB methanolic extracts were considered to have sufficiently strong (LC_{95} 0.6%) and moderately strong (LC_{95} 1.3%), respectively, against \textit{C. pavonana} larvae (Table 1). Thus, at the LC_{95} level JLP methanolic extract was more than twice as active as FPB methanolic extract. Previously, Dadang & Prijono (2011) reported that in the feeding tests JLP methanolic extract was more active than FPB extract on \textit{C. pavonana} larvae.

JLP and FPB hexane extracts which were more active than their respective methanol extracts might indicate that the former extracts contained proportionately higher amounts of more nonpolar active compounds. Kikuzaki et al. (1993) reported that JLP fruits contained a higher amount of guineensine which is more nonpolar and more active than the other known insecticidal constituents of JLP. In another study, Stevenson et al. (2012) reported that FPB chemotype 1 leaves contained more deguelin which is more nonpolar than the other insecticidal rotenoids such as tephrosin and rotenone.
Table 1. Results of probit analysis of *C. pavonana* larval mortality treated with *P. retrofractum* fruit and *T. vogelii* leaf extracts and their mixtures in leaf-dipping tests, 72 h after treatment

| Extract | b ± SEa | LC50 (95% CI)b (%) | LC95 (95% CI)b (%) |
|---------|---------|---------------------|---------------------|
| *P. retrofractum* (JLP) | | | |
| Hexane | 3.97 ± 0.44 | 0.12 (0.09–0.15) | 0.31 (0.22–0.73) |
| Methanolic | 4.32 ± 0.39 | 0.25 (0.20–0.30) | 0.60 (0.47–1.00) |
| *T. vogelii* (FPB) | | | |
| Hexane | 4.23 ± 0.45 | 0.14 (0.10–0.21) | 0.34 (0.22–0.57) |
| Methanolic | 2.56 ± 0.31 | 0.30 (0.26–0.34) | 1.30 (0.94–2.18) |
| JLP + FPB (1:1) | | | |
| Hexane | 7.26 ± 0.84 | 0.15 (0.14–0.16) | 0.26 (0.23–0.31) |
| Methanolic | 4.37 ± 0.40 | 0.16 (0.10–0.21) | 0.38 (0.27–0.97) |

a: slope of the probit line, SE: standard error.
b: CI: confidence interval.

In leaf-dipping tests, FPB hexane extract had about the same level of activity as JLP hexane extract as shown by their similar LC50 and LC95 (Table 1). In contact tests (contact to extract thin film on a glass surface), however, JLP hexane extract was more active than FPB extract as indicated by the lower LC50 and LC95 of the former extract than the latter (Table 2). This difference was probably due to the better penetration of active compounds in JLP extract than those in FPB extract through the insect cuticle. This, in turn, could be due to the difference in polarity of the applied compounds (Matsumura, 1985). Dadang & Prijono (2011) reported that JLP methanolic extract had a good contact effect (by topical application) on *C. pavonana* larvae but they did not test FPB extract.

In leaf-dipping tests, mixtures of JLP and FPB hexane and methanolic extracts had strong insecticidal activity (LC95 < 0.5%) against *C. pavonana* larvae. Both extract mixtures indicated synergistic joint action at both LC50 and LC95 levels (Table 3). Dadang and Prijono (2011) reported that JLP and FPB methanolic extract mixtures at 1:2, 1:1, and 2:1 concentration ratios were more toxic than FPB extract alone and had comparable activity with JLP extract alone but they did not perform quantitative analysis of joint action of those extract mixtures.

Table 2. Results of probit analysis of *C. pavonana* larval mortality treated with hexane extract of *P. retrofractum* fruits and *T. vogelii* leaves and their mixture in contact tests, 24 h after treatment

| Extract | b ± SEa | LC50 (95% CI)b (%) | LC95 (95% CI)b (%) |
|---------|---------|---------------------|---------------------|
| *P. retrofractum* (JLP) | | | |
| 4.32 ± 0.43 | 0.80 (0.70–0.95) | 1.92 (1.44–3.31) |
| *T. vogelii* (FPB) | | | |
| 2.68 ± 0.37 | 1.27 (0.96–2.47) | 5.20 (2.60–44.38) |
| JLP + FPB (1:1) | | | |
| 3.76 ± 0.54 | 1.16 (1.04–1.37) | 3.18 (2.36–5.37) |

a: slope of the probit line, SE: standard error.
b: CI: confidence interval.

Table 3. Joint action of *P. retrofractum* (Pr) dan *T. vogelii* (Tv) extract mixtures (1:1) on *C. pavonana* larvae in leaf-dipping and contact tests

| Extract mixturesa | Assessment time (HAT)b | Level of toxicity | Expected LC50 (%)c | Co-toxicity ratio at LC50 (%)c | Indication of joint action |
|-------------------|------------------------|------------------|-------------------|-----------------------------|---------------------------|
| Feeding test      |                        |                  |                   |                             |                           |
| Pr + Tv hex       | 72                     | LC50             | 0.19              | 1.27                        | Synergistic                |
|                   |                        | LC95             | 0.39              | 1.50                        | Synergistic                |
| Pr + Tv met       | 72                     | LC50             | 0.37              | 2.31                        | Synergistic                |
|                   |                        | LC95             | 0.87              | 2.29                        | Synergistic                |
| Contact test      |                        |                  |                   |                             |                           |
| Pr + Tv hex       | 24                     | LC50             | 1.31              | 1.13                        | Synergistic                |
|                   |                        | LC95             | 2.99              | 0.94                        | Antagonistic               |

a: hex: hexane extract, met: methanol extract, all mixtures were in 1:1 concentration ratios.
b: HAT: hours after treatment.
c: Expected LC50 and co-toxicity ratio at LC50 were calculated according to Robertson & Smith (1984) as described in Materials and Methods.
In contact tests, LC₉₅ and LC₉₅ of JLP and FPB hexane extract mixture were more or less in between those of single FPB and JLP extracts (Table 2). This mixture indicated synergistic action on C. pavonana larvae at the LCₙ₀ level but antagonistic at the LCₚ₅ level. At higher concentrations, variation in the difference of penetration rates among JLP and FPB active compounds through the insect cuticle, as assumed above, was probably wider than that at lower concentrations. As such, the proportion of some active compounds that penetrated the insect cuticle more slowly was less than that in the original extract mixture. This might result in antagonistic effect.

FPB leaves containing rotenoids, primarily deguelin, tephrosin, and rotenone, have long been known for their insecticidal activity both as stomach and contact poison (Delfel et al., 1970; Stevenson et al., 2012; Zhang et al., 2020). The lethal effect of rotenone is due to its action as a cellular respiratory poison through the inhibition of electron transfer between NADH dehydrogenase and coenzyme Q at complex I in mitochondria (Hollingworth 2001). This causes a significant reduction in ATP production which eventually leads to insect death.

Previously, JLP extract has been reported to act as a stomach poison (being active through feeding treatment) against European earwigs Forficula auricularia (Assabgui et al., 1997), cabbage head caterpillar, Crocidolomia pavonana, and diamondback moth Plutella xylostella (Zarkani et al., 2009; Dadang & Prijono, 2011). This extract has also been reported to have contact action against some sucking pests including green stinkbug Nezara viridula (Hasnah & Rusdy, 2015), papaya mealybug Paracoccus marginatus (Asnan et al., 2015), tea mosquito bug Helopeltis antonii (Indriati et al., 2015), and rice brown planthopper Nilaparvata lugens (Nuryanti et al., 2018).

JLP fruits have been reported to contain more than 10 piperamides, including guineensine, piperamide, and retrofractamide A, which possess methylenedioxyphenyl group(s) (Kikuzaki et al. 1993; Parmar et al. 1997). It seems that these compounds contributed to the synergistic activity of FPB and JLP extracts reported in this study, especially in feeding tests. The presence of a methylenedioxyphenyl group is a characteristic of insecticide synergists which can inhibit cytochrome P450 that metabolizes toxic compounds in the body to lesser toxic substances (Bernard & Philogène 1993). The inhibition of this enzyme allows toxic compounds in the mixture to retain their activity.

### Antifeedant Activity

The treatment with both FPB and JLP hexane extract at LC₂₅ to LC₇₀ reduced feeding by C. pavonana larvae. The amount of treated leaves eaten by C. pavonana larvae was much less than that of their respective control (Table 4 and Table 5). The feeding reduction increased with the increase in extract concentration but differences in feeding reduction among extract concentration treatments of both extracts were not statistically significant due to large variations in feeding activity (Table 4 and Table 5).

#### Table 4. Effect of FPB hexane extract on feeding activity of C. pavonana larvae in a choice-test

| Concentration (%) | Leaf area eaten (mm²) (Mean ± SD) | Antifeedant effect (%) (Mean ± SD) |
|-------------------|-----------------------------------|-----------------------------------|
|                   | Control                           | Treatment                         |
| LC₁₀ (0.10)       | 62.47 ± 31.89                     | 20.65 ± 8.91                      | 45.9a ± 26.5                      |
| LC₁₂ (0.12)       | 49.82 ± 41.37                     | 6.49 ± 2.77                       | 52.9a ± 45.2                      |
| LC₁₅ (0.15)       | 38.96 ± 23.61                     | 8.65 ± 4.98                       | 58.4а ± 20.2                      |
| LC₂₀ (0.18)       | 44.58 ± 22.45                     | 5.70 ± 4.23                       | 79.6a ± 17.3                      |

$^a$In each concentration, leaf area eaten in the treatment is significantly different from its control if $P < 0.05$ (paired t-test).

$^b$Means followed by the same letter are not significantly different (Tukey test, $\alpha = 0.05$).

#### Table 5. Effect of JLP hexane extract on feeding activity of C. pavonana larvae in a choice-test

| Concentration (%) | Leaf area eaten (mm²) (Mean ± SD) | Antifeedant effect (%) (Mean ± SD) |
|-------------------|-----------------------------------|-----------------------------------|
|                   | Control                           | Treatment                         |
| LC₀₇₅ (0.075)     | 51.5 ± 5.9                        | 18.8 ± 26.3                       | 42.1a ± 19.2                      |
| LC₁₀₀ (0.100)     | 68.8 ± 7.2                        | 16.0 ± 23.4                       | 60.3a ± 22.1                      |
| LC₁₂₅ (0.125)     | 71.0 ± 7.4                        | 11.1 ± 30.2                       | 66.2a ± 31.1                      |
| LC₁₆₀ (0.160)     | 82.1 ± 6.6                        | 9.7 ± 51.3                        | 72.4a ± 27.9                      |

$^a$In each concentration, leaf area eaten in the treatment is significantly different from its control if $P < 0.05$ (paired t-test).

$^b$Means followed by the same letter are not significantly different (Tukey test, $\alpha = 0.05$).
The decreased feeding activity of *C. pavonana* larvae on treated broccoli leaves can be the result of stimulation of deterrent sense cells which may be complemented by suppression of phagostimulant sense cells in insect taste organs (Koul 2008). Intoxication of *C. pavonana* larvae by FPB and JLP extracts, as initially indicated by the insect decreased mobility, could further reduced feeding activity. Moreover, weak insects due to poisoning and starvation would be more vulnerable to attacks by their natural enemies. Thus, the treatment with toxic plants extracts which also possess antifeedant activity is expected to reduce pest population and crop damage substantially.

**CONCLUSION**

In the feeding treatment, *P. retrofractum* (Pr) and *T. vogelii* (Tv) hexane extracts had strong insecticidal activity against *C. pavonana* larvae (*LC*<sub>95</sub> < 0.5%) and were more active their respective methanol extracts. Pr and Tv hexane extract had a moderate and a rather weak contact effect, respectively, on *C. pavonana* larvae. In feeding tests, Pr + Tv (1:1) hexane and methanol extract mixtures indicated synergistic joint effect both at LC<sub>50</sub> and LC<sub>95</sub> level, whereas in the contact test, the mixture of Pr + Tv (1:1) hexane extract was synergistic at the LC<sub>50</sub> level but antagonistic at the LC<sub>95</sub> level. In choice tests, antifeedant effects of Pr and Tv hexane extracts at LC<sub>25</sub> to LC<sub>70</sub> levels on *C. pavonana* larvae followed a concentration-dependent manner.

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