Laboratory effectiveness of some insecticides and *Piper aduncum* fruit extract against diamondback moth (*Plutella xylostella* [L.]) from Cisarua-Bogor and Pangalengan-Bandung, West Java, Indonesia

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Abstract. This work was done to evaluate the laboratory effectiveness of 5 commercial insecticides, i.e. abamectin, chlorantraniliprole, chlorfenapyr, methomyl, and spinetoram, against diamondback moth (DBM), *Plutella xylostella*, from Cisarua District, Bogor Regency and Pangalengan District, Bandung Regency, West Java, Indonesia. Emamectin benzoate and *Piper aduncum* (Piperaceae) fruit extract were also tested against *P. xylostella* from Cisarua, Bogor. The results of feeding of DBM larvae with treated broccoli leaves showed that chlorfenapyr, emamectin benzoate, and spinetoram were still effective against DBM from Cisarua (their LC95 were lower than their respective recommended field rates). Spinetoram was also still effective but chlorfenapyr was rather ineffective against DBM from Pangalengan. Abamectin was rather effective (LC95 was 1.8- and 1.5 times as high as its field rate) but methomyl was not effective against the 2 populations (LC95 was more than 4-fold higher than its field rate). Meanwhile, chlorantraniliprole was rather ineffective against DBM from Pangalengan and was not effective against the Cisarua population (LC95 was about 3.6-fold and 8.3-fold higher than its field rate, respectively). Furthermore, LC95 of *P. aduncum* extract at 96 h after treatment was 0.47% (w/v) (< 0.5%) suggesting that this botanical insecticide is potential to be used as an alternative means for controlling DBM.

1. Introduction
The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is considered as the most important pest of cruciferous crops worldwide [1, 2]. This pest attacks all growth stages of cruciferous crops from early planting to harvest. Severe infestation of *Brassica* crops by DBM can cause crop harvest failure [3, 4].

Like in other countries, integrated pest management (IPM) has been adopted as the basis for managing DBM population in Indonesia, with emphasis on encouragement of the function of *Diadegma semiclauzus* parasitoid as a major natural enemy of DBM [5]. However, until now many farmers still use synthetic insecticides to control DBM [6, 7]. Continuous use of synthetic insecticides...
may interfere with the function of DBM natural enemies [8] and cause the development of resistant DBM population [9].

Two older insecticides, i.e. deltamethrin (pyrethroid) and profenofos (organophosphate), were reported to be ineffective against DBM from Pangalengan-Bandung and Batu [10], Kejajar Dieng-Wonosobo [11], and Cipanas-Cianjur [12]. On the other hand, 2 newer insecticides, i.e. emamectin benzoate and spinetoram (semisynthetic analogues of soil bacterial metabolites), were reported to be still effective against DBM from some cabbage growing areas in West Java and Central Java [11, 12, 13, 14].

Effectiveness of insecticides commonly-used by farmers should be monitored regularly to ensure their feasibility to be used at farmer level. In this study, 6 insecticides registered for controlling DBM in Indonesia, i.e. abamectin, chlorantraniliprole, chlorfenapyr, emamectin benzoate, methomyl, and spinetoram [15], were tested. Methomyl is a carbamate insecticide, while the other 5 insecticides belong to newer insecticide groups [16].

In addition to the above 6 insecticides, spiked-pepper (Piper aduncum, Piperaceae) fruit extract was also tested in this study. Ethyl acetate spiked-pepper fruit extract was reported to have a strong insecticidal activity against DBM with LC₅₀ of 0.23% [11]. Spiked-pepper fruit extract contained dillapiole (a lignan) as its main insecticidally active substance [17]. Dillapiole can inhibit polysubstrate monoxygenases (PSMO) that commonly detoxify foreign compounds or toxic metabolic wastes in insect cells. Inhibition of PSMO activity can result in accumulation of toxic compounds in insect cells which eventually can lead to insect death [18].

This study was done to evaluate the laboratory effectiveness of 5 commercial insecticides, i.e. abamectin, chlorantraniliprole, chlorfenapyr, methomyl, and spinetoram, against DBM from Cisarua District, Bogor Regency and Pangalengan District, Bandung Regency, West Java, Indonesia. Emamectin benzoate and spiked-pepper fruit extract were also tested against DBM from Cisarua, Bogor.

2. Methods

Six commercial insecticide formulations, i.e. Agrimec 18 EC (abamectin 18 g a.i./L), Endure 120 SC (spinetoram 120 g a.i./L), Metindo 25 WP (methomyl 25% a.i.), Prevathon 50 SC (chlorantraniliprole 50 g a.i./L), Proclaim 5 SG (emamectin benzoate 50 g a.i./kg), and Rampage 100 EC (chlorfenapyr 100 g a.i./L), were purchased from pesticide kiosks in Bogor. Spiked-pepper fruits were collected from bushes in Darmaga campus of IPB University, Bogor.

Broccoli (Brassica oleracea L. var. italica) leaves used for DBM rearing and as treatment substrates in insecticide toxicity tests were obtained from Bina Sarana Bhakti organic farm, Cisarua, Bogor. Chinese cabbage (Brassica juncea [L.] Czern.) seedlings, 10-14 d after sowing, were used as oviposition sites for DBM females [11].

DBM larvae used in this study were offsprings of DBM colony collected from farmer’s fields at Cisarua District, Bogor Regency (S 6°42’22”, E 106°57’27”) and Pangalengan District, Bandung Regency (S 7°13’31”, E 107°34’3’”), West Java. The test insects were maintained in the laboratory following the procedures of Prijono et al [14]. Briefly, the larvae were fed broccoli leaves and the adults were fed 10% honey solution impregnated in a piece of cotton wool. Third-instar DBM larvae from the second laboratory generation were used in all tests.

2.1. Extraction of spiked-pepper fruits

Spiked-pepper fruits were cut into small pieces, then the cut pieces were air-dried in the laboratory for one week. Air-dried spiked-pepper fruit pieces were ground into powder with a blender. Ground spiked-pepper fruits were then extracted with n-hexane (1:8, w/v) by immersion method following the procedures of Nailufar and Prijono [19]. The extract obtained was kept in refrigerator (ca. 4 °C) until being used for bioassay.
2.2. Insecticide testing method
Toxicity bioassays of six insecticides against DBM larvae were done by a leaf-dip feeding method following the procedures of Prijono et al [14]. Each test insecticides were tested at 5 concentration levels which were expected to give between 15% and 95% insect mortality. The test concentration ranges were determined in preliminary tests. Each insecticide formulation was diluted to the desired volume with distilled water containing an insecticide sticker alkylarylpolyglycol ether 80 µg/L (Agristick 400 L 0.2 ml/L). Distilled water containing Agristik 400 L 0.2 ml/L served as a control solution.

 Portions of broccoli leaves (4×4 cm) were dipped separately in particular insecticide dilutions to complete wetness and then the treated leaves were air-dried. Two portions of treated or control leaves were placed in an upside-down petri dish lined with a napkin, then ten third-instar DBM larvae were put into the dish. Five replicates were used for each treatment. The test larvae were allowed to feed on treated or control leaves for 48 h, then treated leaf remains were replaced with untreated leaves for the following 48 h. Larval mortality was recorded at 48 and 96 h after treatment (HAT) and then the data was analysed with the probit method using PoloPlus [20]. LC95 of each insecticide was compared with its recommended field rate to determine its relative effectiveness against DBM.

 Spiked-pepper fruit extract was also tested against DBM larvae by a leaf-dip feeding method as described above. The extract was tested at 5 concentration levels as determined in a preliminary test. Spiked-pepper extract was mixed with methanol and Tween 80 (5:1, v/v; final concentration 1.2%) and then diluted with distilled water to the desired volume [11]. Distilled water containing 1% methanol and 0.2% Tween 80 served as a control solution. Methods of treatment, mortality assessment, and data analysis were the same as those in the aforementioned toxicity testing of commercial insecticides.

3. Results and discussion
LC50 and LC95 of all test insecticides on DBM from Cisarua-Bogor and Pangalengan-Bandung at 96 HAT were lower than those at 48 HAT (Table 1 and Table 2). These data indicate that mortality level of the test larvae still increased between 48 and 96 HAT. Treated leaves were given to the test larvae for the first 48 h and then the larvae were fed untreated leaves for the next 48 h. Mortality of the test larvae that occurred after treated leaf remains were replaced with untreated leaves was probably due to insecticide residues that were still present in the body of the test larvae.

 Based on toxicity of methomyl at the level of LC50 - 96 HAT as the basis for comparison, emamectin benzoate, spinetoram, chlorfenapyr, abamectin, and chlorantraniliprole were about 6200-, 1550-, 43-, 35-, and 5-fold, respectively, more toxic than methomyl against DBM from Cisarua-Bogor (Table 1). Furthermore, spinetoram, abamectin, chlorantraniliprole, and chlorfenapyr were about 620-, 85-, 10-, and 9-fold, respectively, more toxic than methomyl against DBM from Pangalengan-Bandung (Table 2). Methomyl is the oldest compound among the 6 commercial insecticides tested in this study. Pesticide companies generally develop newer insecticides that are more toxic than the older ones [16].

 LC95 of chlorfenapyr, emamectin benzoate, and spinetoram against DBM from Cisarua-Bogor at 96 HAT was about 2, 19, and 21 times lower than their respective recommended field rates. Meanwhile, LC95 of abamectin, chlorantraniliprole, and methomyl at 96 HAT was about 2-, 8-, and 5-fold higher than their respective field rates (Table 1). Moreover, LC95 of spinetoram against DBM from Pangalengan-Bandung at 96 HAT was about 23 times lower than its field rate whereas that of abamectin, chlorantraniliprole, chlorfenapyr, and methomyl at 96 HAT was about 2-, 4-, 2-, and 5-fold higher than their respective field rates (Table 2). Thus, chlorfenapyr, emamectin benzoate, and spinetoram were still effective against DBM from Cisarua-Bogor. Spinetoram was also still effective but chlorfenapyr was rather ineffective against DBM from Pangalengan-Bandung. Abamectin was rather effective but methomyl was not effective against the 2 populations. Chlorantraniliprole was rather ineffective against DBM from Pangalengan-Bandung and was not effective against the Cisarua-Bogor population.
LC₉₅ of the spiked-pepper extract against DBM from Cisarua-Bogor at 96 HAT was 4662.5 mg/L or about 0.47% (w/v). This data indicates that spiked-pepper extract is potential to be used for the control of DBM. Plant extracts prepared with organic solvent were considered feasible to be used in the field if they can yield at least 80% insect mortality at extract concentrations of less than 0.5% (w/v) [21]. However, the spiked-pepper extract used in this study (LC₉₅ 0.47%) was about 2-fold less toxic than the spiked-pepper extract used by Chenta and Prijono (LC₉₅ 0.23%) [11]. This difference might be due to the difference in the content of active compounds in the source plant materials and the difference in the susceptibility of DBM larvae used in the 2 studies to the test extracts.

Table 1. Toxicity of 6 commercial insecticides and P. aduncum extract on P. xylostella larvae from Cisarua, Bogor.

| Insecticide | Field rate (mg a.i./L) | Date of larval collection | Date of testing | Time of assessment (HAT) | b ± SE | LC₉₀ (95% CI) (mg a.i./L) | LC₉₅ (95% CI) (mg a.i./L) |
|-------------|------------------------|--------------------------|----------------|-------------------------|--------|--------------------------|--------------------------|
| Abamectin   | 18                     | 6/20/2017                | 8/2/2017       | 48                      | 1.816 ± 0.310 | (14.68) | (95% CI): (11.53–21.21) | (60.0–446.7)             |
|             |                        |                          |                | 96                      | 2.564 ± 0.357 | 7.11   | (5.94–8.43)             | (22.22–54.85)            |
| Emamectin  benzoate | 10                   | 6/29/2017                | 8/12/2017      | 48                      | 0.768 ± 0.123 | 0.16   | (0.09–0.32)             | (5.50–312.93)            |
|             |                        |                          |                | 96                      | 1.458 ± 0.195 | 0.04   | (0.03–0.05)             | (0.29–1.32)              |
| Chlorantraniliprole | 50                   | 5/21/2017                | 7/31/2017      | 48                      | 2.025 ± 0.297 | 91.90 | (95% CI): (51.67–164.0) | (278.3–5190.4)           |
|             |                        |                          |                | 96                      | 1.778 ± 0.249 | 49.42  | (25.74–87.05)           | (187.1–3580.2)           |
| Chlorfenapyr | 125                  | 6/29/2017                | 8/11/2017      | 48                      | 1.523 ± 0.310 | 20.99  | (95% CI): (15.45–32.81) | (107.3–1784.4)           |
|             |                        |                          |                | 96                      | 1.676 ± 0.217 | 5.83   | (1.84–11.94)            | (22.1–1896.7)            |
| Methomyl    | 1000                   | 5/8/2017                 | 7/2/2017       | 48                      | 0.863 ± 0.142 | 2281.5 | (95% CI): (1277.9–5442.7) | (42857–2.9 x 10⁵)         |
|             |                        |                          |                | 96                      | 1.274 ± 0.167 | 248.0  | (167.1–361.2)           | (2479.8–14078)           |
| Spinetoram  | 60                     | 5/8/2017                 | 7/4/2017       | 48                      | 1.756 ± 0.295 | 0.26   | (95% CI): (0.13–0.50)   | (0.94–41.26)             |
|             |                        |                          |                | 96                      | 1.329 ± 0.190 | 0.16   | (95% CI): (0.10–0.24)   | (1.43–8.62)              |

| P. aduncum extract | 29/6/2017 | 1/8/2017 | 48 | 3.389 ± 0.484 | 2115.7 | (95% CI): (1378–3489) | (3781.3–50320) |
|                   |                        |            |    |               | 6467.2 | (95% CI): (795–1937)   | (2709.7–25307) |

 ŁLC₉₀ of the spiked-pepper extract against DBM from Cisarua-Bogor at 96 HAT was 4662.5 mg/L or about 0.47% (w/v). This data indicates that spiked-pepper extract is potential to be used for the control of DBM. Plant extracts prepared with organic solvent were considered feasible to be used in the field if they can yield at least 80% insect mortality at extract concentrations of less than 0.5% (w/v) [21]. However, the spiked-pepper extract used in this study (LC₉₀ 0.47%) was about 2-fold less toxic than the spiked-pepper extract used by Chenta and Prijono (LC₉₀ 0.23%) [11]. This difference might be due to the difference in the content of active compounds in the source plant materials and the difference in the susceptibility of DBM larvae used in the 2 studies to the test extracts.

Table 1. Toxicity of 6 commercial insecticides and P. aduncum extract on P. xylostella larvae from Cisarua, Bogor.

Previously, emamectin benzoate and spinetoram were also reported to be still effective against DBM from some cabbage-growing areas in West Java and Central Java [11, 12, 13, 14]. Meanwhile, abamectin and chlorfenapyr were reported to be ineffective against DBM from Pacet-Cianjur and Cisarua-Bogor, West Java [14]. Four years ago, a relatively new insecticide chlorantraniliprole was reported to be ineffective against DBM from Cipanas-Cianjur, West Java [12].
Table 2. Toxicity of 5 commercial insecticides on P. xylostella larvae from Pangalengan, Bandung.

| Insecticide       | Field rate (mg a.i/L) | Date of larval collection | Date of testing | Time of assessment (HAT) | LC50 (95% CI) (mg a.i/L) | LC95 (95% CI) (mg a.i/L) |
|-------------------|-----------------------|----------------------------|-----------------|-------------------------|--------------------------|--------------------------|
| Abamectin         | 18                    | 2/18/2017                  | 4/23/2017       | 48                      | 1.989 ± 0.251            | 9.5                      | 63.5                     |
|                   |                       |                            |                 |                         | (5.9 - 17.2)             | (28.4-803.3)             |
|                   |                       |                            |                 |                         | 2.828 ± 0.319            | 7.2                      | 27.4                     |
|                   |                       |                            |                 |                         | (6.2-8.4)                | (20.6-41.9)              |
| Chlorantraniliprole| 50                    | 4/22/2017                  | 5/24/2017       | 48                      | 3.566 ± 0.415            | 71.1                     | 205.4                    |
|                   |                       |                            |                 |                         | (62.2-81.9)              | (160.6-298.5)            |
|                   |                       |                            |                 |                         | 3.682 ± 0.434            | 63.8                     | 178.4                    |
|                   |                       |                            |                 |                         | (55.9-73.2)              | (141.2-254.7)            |
| Chlorfenapyr      | 125                   | 2/18/2017                  | 4/5/2017        | 48                      | 3.109 ± 0.394            | 88.2                     | 298.1                    |
|                   |                       |                            |                 |                         | (69.3-123.3)             | (185.3-929.2)            |
|                   |                       |                            |                 |                         | 2.882 ± 0.373            | 68.4                     | 254.5                    |
|                   |                       |                            |                 |                         | (59.3-79.6)              | (187.4-417.9)            |
| Spinetoram        | 60                    | 5/27/2017                  | 7/3/2017        | 48                      | 3.140 ± 0.384            | 1.1                      | 3.66                     |
|                   |                       |                            |                 |                         | (0.97-1.26)              | (2.791-5.6)              |
|                   |                       |                            |                 |                         | 3.946 ± 0.457            | 0.99                     | 2.58                     |
|                   |                       |                            |                 |                         | (0.78-1.29)              | (1.774-6.673)            |
| Methomyl          | 1000                  | 10/16/2017 11/28/2017      |                 | 48                      | 2.105 ± 0.280            | 922.25                   | 5573.7                   |
|                   |                       |                            |                 |                         | (724.5-1196.5)           | (3542.5-11616.5)         |
|                   |                       |                            |                 |                         | 1.871 ± 0.216            | 614.25                   | 4651.5                   |
|                   |                       |                            |                 |                         | (368.5-1164.5)           | (2007.25-45956)          |

*HAT: hours after treatment.
*b: slope of the probit regression line, SE: standard error, CI: confidence interval.

Moekasan et al. [10] and Udiarto et al. [22] also reported that abamectin was no longer effective against DBM from some cabbage growing regions in Java. DBM resistance to abamectin has been well reported from some countries including Brazil, China, Malaysia, Pakistan and Taiwan [1]. In China, 29 out of 31 DBM populations collected from 18 different geographical regions from 2012 to 2013 showed more than 100-fold resistance to abamectin with the highest resistance of nearly 1500-fold [23].

Chlorantraniliprole has also been extensively used by cabbage farmers in Cisarua-Bogor and Pangalengan-Bandung, but the effectiveness of this insecticide dropped quickly over a relatively short period of its commercialisation. In some other countries, intensive use of chlorantraniliprole for DBM control has resulted in rapid development of DBM resistance to this insecticide [24]. Populations of DBM showing high resistance to chlorantraniliprole have been reported from Thailand (RF = 775) [25], Philippines (RF > 4100) [26], Southern China (RF = 2000) [27], and Brazil (RF = 4000) [28].

Resistance of DBM to chlorfenapyr has never been reported in Indonesia. Some DBM populations from Southern China have been reported to have evolved 136- to 334- fold resistance to chlorfenapyr [29]. A further study with a highly resistant field population indicated that resistance to chlorfenapyr was autosomally inherited and incompletely dominant.

Methomyl, an old carbamate insecticide, was not effective against DBM from both Cisarua-Bogor (Table 1) and Pangalengan-Bandung (Table 2). This insecticide has long been used by cabbage farmers in the 2 locations to control cabbage pests including DBM so that the effectiveness of methomyl against DBM from the 2 locations gradually decreased. An insecticide belonging to another group, i.e. profenofos (an organophosphate), has long been reported to be ineffective against DBM from some cabbage growing regions in Pangalengan, Lembang-Bandung, Garut (West Java), Batu (East Java), and Buleleng (Bali) [10, 22]. Recently, profenofos was also reported to be ineffective against DBM from Kejajar Dieng-Wonosobo, Central Java [11] and Cipanas-Cianjur, West Java [12]. Methomyl and profenofos have the same mode of action, i.e. they inhibit acetylcholinesterase enzyme.
in the nerve synapse [30]. The ineffectiveness of methomyl to DBM can be due to regular use of that insecticide for DBM control and/or cross-resistance to profenofos. There have been no reports yet that reveal DBM resistance to emamectin benzoate and spinetoram. Kayani dan Ahmad [31] in Pakistan reported that laboratory selection of a DBM population for 5 generations with emamectin benzoate did not increase the tolerance level significantly. Zhang et al [32] reported that some field populations of DBM collected from Central China from 2013 to 2014 were susceptible to spinetoram (RF = 0.88–2.35). In Brazil, DBM populations from the Agreste region of Pernambuco, Brazil, were reported to show a large variation in their susceptibility to spinetoram and there was cross-resistance between spinosad (the parent compound of spinetoram) and spinetoram [33].

Insecticides that are not effective against DBM as shown in this study should be withdrawn from use to remove further resistance selection pressures. In addition, insecticides that have shown decreased effectiveness should be used more prudently to lessen resistance development. For example, rotational use of insecticides with different mode of actions can be implemented [34]. On the other hand, 2 newer insecticides that are still effective against DBM, i.e. emamectin benzoate and spinetoram, should be used wisely in the context of appropriate pesticide management strategies so that their usability can be maintained over an extended period of time. In the context of IPM, use of these effective insecticides should consider the control threshold of DBM and the population of parasitoids of the pest [5].

4. Conclusion

The order of effectiveness of the test insecticides against DBM from Cisarua-Bogor and Pangalengan Bandung followed the sequence of their introduction or the history of their uses. The oldest insecticides tested in this study, i.e. a carbamate insecticide methomyl, was not effective against DBM from both Cisarua-Bogor and Pangalengan-Bandung. There were mixed results with 3 newer insecticides, i.e. abamectin, chlorantraniliprole and chlorfenapyr, regarding their effectiveness against DBM. The 2 newest insecticides, i.e. emamectin benzoate and spinetoram, were still effective against the test insect. Moreover, P. aduncum extract was effective against DBM so that this botanical insecticide is potential to be used as an alternative means for controlling DBM.

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