Insecticidal properties of pyripyropene A, a microbial secondary metabolite, against agricultural pests

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We previously reported the strong insecticidal activity of a microbial secondary metabolite, pyripyropene A (PP-A), against aphids. Pyripyropenes (PPs) have been known to show weak feeding inhibition against lepidopteran pests, but their strong aphicidal activities were first reported in our former study. Here we investigated the details of the insecticidal property of PP-A. Our biological evaluation of PP-A found that it shows high insecticidal activities against some sucking pests, such as whiteflies, as well as aphids, and preferable biological profiles as agricultural insecticides. Furthermore, PP-A controlled aphids well under field conditions. © Pesticide Science Society of Japan

Keywords: pyripyropene, aphicide, natural product, hemiptera.

Introduction

Natural products, especially microbial secondary metabolites, are effective resources for new drug discoveries. Many medicines, pesticides, and veterinary drugs have been discovered from microbial metabolites. For example, spinosad and milbemycin had been developed as natural-product pesticides, targeting the control of agricultural pests such as lepidoptera and acari. Even now, many chemicals from microbial extracts with a unique chemical structure or mode of action have been reported to show effective activities to control agricultural pests.1–3 In the former study, we reported that the PP-A shown in Fig. 1, 3,6-bis(acetyloxy)-4-[(acetyloxy)methyl]-1,3,4R,4aR,5,6S,6aS,12R,12aS,12bS-decahydro-12-hydroxy-4,6a,12b-trimethyl-9-(3-pyridinyl)-2H,11H-naphtho[2,1-b]pyrano[3,4-e]pyran-11-one (molecular formula: C31H37NO10; molecular weight: 583.63; water solubility: 132 ppm; Log P: 3.0), with a unique meroterpenoid structure, showed high insecticidal activity against aphids which are important pests causing great damage to crop production.4 PPs, including PP-A, have been demonstrated for the first time to inhibit acetyl-CoA:cholesterol acyltransferase (ACAT),5–7 a target of an antilipotropic drug; however, the insecticidal profiles had not been investigated sufficiently, even though PP-A had been reported as an insecticidal compound against lepidoptera pests.8 Currently, the market of organic and biological pesticides, including natural products, microorganisms such as bacteria, viruses, fungi, natural enemies of insect pests, and insect pheromones, is expanding gradually9 because these pesticides are thought to be degraded naturally in their surroundings and to have relatively low impacts against nontarget organisms.10 On the other hand, the chemical products with high toxicity against nontarget organisms, such as pollinators, aquatic life, and mammals, have been strictly regulated worldwide, and pesticides with high safety for these nontarget organ-

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Fig. 1. Structure of pyripyropene A (PP-A).
isms are strongly desired. Microbial secondary metabolites are thought to be decomposed relatively easily by plants, microbes, and ultraviolet radiation under natural conditions. Our earlier study revealed that PP-A shows insecticidal activity specifically against aphids and exhibits good activity at a low dose in lab tests.

From the viewpoint of negative impacts to the environment, we think the natural compounds with highly effective specifically against targeted pests have high potential for the next generation of pesticides. Therefore, we evaluated whether PP-A itself is an effective compound for controlling agricultural pests. We report the results and discuss the possibility of PP-A as an insecticide.

**Materials and Methods**

1. **Chemicals**

   We used PP-A from Meiji natural compounds library and a typical formulation type in crop protection, wettable powder (WP), in our evaluation. The formulation, including 5% PP-A, was prepared following the method described in the reference. Standards of imidacloprid (IMI), pymetrozine, and flocnicamid for pesticide residue analysis were purchased from Wako Chemicals (Tokyo) and that of fenitrothion from Sigma-Aldrich (Tokyo). An IMI wettable dispersible granule (WDG) and fenitrothion emulsifiable concentrate (EC) formulation available as pesticides were used in the field trials.

2. **Insecticidal spectrum by foliar application**

   The insecticidal assays of PP-A against the green peach aphid (*Myzus persicae*), cotton aphid (*Aphis gossypii*), comstock mealybug (*Pseudococcus comstocki*), rice leaf bug (*Trigonotylus caelestialium*), brown plant hopper (*Nilaparvata lugens*), green rice leafhopper (*Nephotettix cincticeps*), diamondback moth (*Plutella xylostella*), common cutworm (*Spodoptera litura*) and western flower thrips (*Frankliniella occidentalis*) were conducted, following the methods reported previously. The activities were calculated as 50% lethal concentration (LC50) values.*

3. **Insecticidal activity against the greenhouse whitefly**

   (Trialeurodes vaporariorum) and sweetpotato whitefly
   (Bemisia tabaci biotype B and Q)

   Each leaf disk, having a diameter of 1.4 cm, was cut from leaves of the kidney bean for the greenhouse whitefly and biotype Q or of the cabbage for biotype B and placed in a 5.0 cm plastic cup containing 2.5 mL of agar gel. Then 0.5 mL of the test compound in a 50% aqueous acetone solution containing 0.05% Tween 20 was applied to the dish with an airbrush and dried at room temperature. Ten adults were released onto the leaf disk. A lid was placed on the cup, and the cup was placed in a temperature-controlled room (light period, 16 hr; dark period, 8 hr; 25°C). The survival of adults was observed after 2 days, and the adult mortality rate was calculated using the following equation:

   \[
   \text{mortality} = \frac{\text{number of dead adults}}{\text{number of surviving adults+number of dead adults}}
   \]

   Each compound was tested in duplicate. The LC50 was calculated via probit analysis.

4. **Insecticidal activity against the two-spotted spider mite**

   (*Tetranychus urticae*)

   One leaf disk having a 2.8 cm diameter was cut from a kidney bean plant grown in a plastic pot; the leaf was placed in a plastic cup containing 40 mL of agar gel. Then 10 adult mites were released onto the leaf disk. After their release, 0.5 mL of the test compound in a 50% aqueous acetone solution containing 0.05% Tween 20 was applied to the disk with an airbrush and dried at room temperature. The cup was placed in a temperature-controlled room (light period, 16 hr; dark period; 8 hr; 25°C). The survival of adults was observed after 2 days, and the adult mortality rate was calculated using the same equation as in the test against the whitefly. Each compound was tested in duplicate.

5. **Insecticidal activity against 2nd, and 3rd instar larvae and the adult of green peach aphid**

   (M. persicae)

   The assays were conducted using the same method as that used for the green peach aphid reported previously with 2nd and 3rd instar larvae and adults. In the adult assay, the number of aphids released was 4 per leaf disk. The LC50 was calculated via probit analysis.

6. **Efficacy of PP-A to the next generations of green peach aphid**

   (M. persicae) by topical application

   A topical treatment of PP-A was applied to adults: a 25 µL Hamilton syringe was used to administer 0.5 µL of acetone, including a determined amount of PP-A, onto the backs of adult aphids. After treatment, 5 adults were released onto each cabbage leaf disk. The condition of breeding was the same as in the method above. Three days after treatment, the surviving adults and larvae and the larvae laid onto the leaf disks were counted. Each compound was tested in five replications. The adult and larva mortality rates were calculated using the same equation as in the test against the whitefly.

7. **Translaminar efficacy of PP-A against the green peach aphid**

   (M. persicae)

   Cabbage, which was grown for 5 weeks after seeding and naturally infested with aphids in a greenhouse, was used in this efficacy test. The 3 mL test compound in a 10% aqueous acetone solution was sprayed only onto the upper side of the leaves on the cabbage plants in a plastic pot using an airbrush. After treatment, 20 aphid larvae were released, and the pot was placed in a temperature-controlled room under the same conditions as above. The aphids per pot were counted before application and 1, 3, 6, and 7 days after application. The test was conducted in duplicate.
8. Systemic efficacy of PP-A against the wheat aphid (Rhopalosiphum padi) by root dipping
Two days after seeding, wheat seedlings grown in 24-well Greiner plates were dipped into the test compound, which was dissolved in 200 µL of a 10% acetone/aqueous solution to a concentration of 100 mg/L. After treatment, the seedlings were infested with 5 aphid larvae, covered with a plastic tube well, by well and placed in a temperature-controlled room under the same conditions as above. The aphids per pot were counted 6 days after infestation. Each compound was tested in duplicate. The larva mortality rate was calculated using the same equation as in the test against whiteflies.

9. Field efficacy of PP-A foliar application against the green peach aphid (Myzus persicae) on the eggplant
The field trial was conducted in an eggplant (Solanum melongena cv. Senryou 2) field in Odawara, Kanagawa Prefecture, Japan (10 plants per plot, 2 plots per treatment). The determined concentration of diluted solution of PP-A WP with tap water was applied to eggplants by portable MARUYAMA sprayer (spray volume; 200 L/ha). The dominant insect occurring in the field was the green peach aphid. Before application and 5, 8, and 15 days after application, the aphids in each plot were counted. The corrected density index was calculated using Formula 1, below. Then the percentage of control was calculated using Formula 2.

Formula 1: \( \text{corrected density index} = \frac{(\text{number of aphids in the treated plot at each observation date})}{(\text{number of aphids in the untreated plot at each observation date})} \times 100 \)

Formula 2: \( \% \text{control} = 100 - \text{corrected density index} \)

10. Effect against the housefly (Musca domestica) by oral application
The determined amount of PP-A dissolved in 10% sucrose/deionized water was absorbed into cotton wool in the plastic Falcondon shale. This shale was placed into the bigger plastic cup and five female adults (Yumenoshima population) were released into the cup. One day after treatment, symptoms in the adults were observed. The test was conducted in duplicate. The adult mortality rate and abnormality rate were calculated similarly as in the whitefly test.

Results
In the insecticidal spectrum evaluation results, PP-A showed good aphicidal activity against M. persicae and A. gossypii, which commonly occur on a variety of crops such as vegetables and fruits worldwide. Against other hemiptera, PP-A showed good efficacy against the mealybug (P. comstocki) and whitefly (T. vaporariorum and B. tabaci). On the other hand, PP-A did not show such as any activity against T. caelestialium, N. lugens, or N. cincticeps even though they are the same hemiptera. Neither did it show any activity against lepidoptera (P. xylostella or S. litura),

| Insect name         | Source of insects | Growth stage | LC₅₀ (mg/L) |
|---------------------|-------------------|--------------|-------------|
| Hemiptera           |                   |              |             |
| Green peach aphid   | Myzus persicae    | Odawara (2002) | 1st | 0.98 |
| Cotton aphid        | Aphis gossypii    | Purchased    | 1st | 0.30 |
| Comstock mealybug   | Pseudococcus comstocki | Kanagawa (before 2001) | 1st | 6.4  |
| Greenhouse whitefly | Trialeurodes vaporariorum | Odawara (2001) | Adult | 2.5 |
| Sweetpotato whitefly| Bemisia tabaci (biotype B) | Purchased | Adult | 1.8  |
| Rice leaf bug       | Trigonotylus caelestialium | Odawara (1995) | 2nd | >100 |
| Brown planthopper   | Nilaparvata lugens | Purchased    | 2nd | >100 |
| Green rice leafhopper| Nephotettix cincticeps | Purchased | 2nd | >100 |
| Lepidoptera         |                   |              |             |
| Diamondback moth    | Plutella xylostella | Yokohama (1991) | 2nd | >100 |
| Common cutworm      | Spodoptera litura | Purchased    | 3rd | >100 |
| Thysanoptera        |                   |              |             |
| Western flower thrips| Frankliniella occidentalis | Purchased | 1st | >100 |
| Acari               |                   |              |             |
| Two-spotted spider mite | Tetranychus urticae | Purchased | Adult | >500 |

| Sample            | Dose (ng/adult) | Effect against adult | Effect against larvae |
|-------------------|-----------------|----------------------|-----------------------|
| PP-A              | 500             | 100                  | 94                    |
| Hionicamid        | 500             | 92                   | 92                    |
| Control (Acetone) | 12              | 15                   | 359                   |

Table 2. Efficacy of PP-A against each growth stage of M. persicae (LC₅₀ mg/L)

| Insect stage | Sample |
|--------------|--------|
| 1st instar larva | PP-A 0.38, Hionicamid 1.2 |
| 2nd instar larva | PP-A 0.14, Hionicamid 0.39 |
| 3rd instar larva | PP-A 2.7, Hionicamid 2.1 |
| Adult         | PP-A 5.0, Hionicamid >5.0 |

Table 3. Effect of PP-A against the next generation of M. persicae

| Sample            | Dose (ng/adult) | Effect against adult | Effect against larvae |
|-------------------|-----------------|----------------------|-----------------------|
| PP-A              | 500             | 100                  | 94                    |
| Hionicamid        | 500             | 92                   | 92                    |
| Control (Acetone) | 12              | 15                   | 359                   |
thysanoptera (*F. occidentalis*), or acari (*T. urticae*) (Table 1).

In order to confirm the efficacy of PP-A against each growth stage of aphid, we evaluated the activities against 1st, 2nd, and 3rd instar larvae and adults of *M. persicae*. PP-A showed better efficacy than flonicamid against all stages except 3rd instar larvae and the efficacy equivalent to that of flonicamid against 3rd instar larvae (Table 2). From the viewpoint of efficacy to adults, although PP-A exhibited relatively slow action, which led to the occurrence of many 1st instar larvae, almost all of the larvae laid by the treated adults failed to infest the leaves and died (Table 3). This symptom of decreasing the number of laid larvae was similarly observed in the aphids treated with other PP analogues, but PP-A had the strongest effect (data not shown). Moreover, we evaluated this PP-A activity against a less susceptible population of aphids in comparison with the activity of an existing organophosphate (OP) insecticide. PP-A showed high efficacy against the resistant population of aphids, with an R/S ratio of less than 1.0, in contrast to the efficacy of fenitrothion (Table 4).

Among sucking pests, nowadays whiteflies are destructive pests that affect the production of cotton, soybeans and a variety of vegetables worldwide, and they often develop resistance to major insecticides. Therefore, we confirmed the efficacy of PP-A against some populations of whiteflies. In the assay, PP-A showed good efficacy against biotype Q of *B. tabaci*, which is less susceptible to existing insecticides, including neonicotinoids, as well as against biotype B, which is a species susceptible to those insecticides (Table 5). The insecticide commonly used for sucking-pest control, IMI, showed lower efficacy than PP-A against biotype Q of *B. tabaci*.

Aphids and whiteflies, which are target pests for PP-A, tend to infest on the underside of leaves or on the upper, young leaves of crops. Therefore, to control them well, it is important for treatment to permeate from the upper side to the underside of leaves (translaminar efficacy) and/or to show insecticidal activities against these pests on stems and leaves, absorbing from the roots (systemic efficacy). In our evaluation of the translaminar efficacy, PP-A showed slower but better efficacy against *M. persicae* on cabbages than did a commercial standard, pymetrozine (Fig. 2). As to the systemic efficacy, PP-A showed good efficacy against *R. padi* (collected in Yokohama in 2006) when the wheat roots were dipped in a solution concentration of 100 mg/L, decreasing the number of aphids to one-fifth (20%) of the density in the untreated plots.

Through these insecticidal tests, we could confirm narrow but preferable systemic properties and efficacy against resistant pests, and we further evaluated the field efficacy of PP-A by foliar application to confirm its utility as a pesticide. In the field trial against *M. persicae* on eggplant, regardless of the fact that less-OP-susceptible populations of aphids appeared, PP-A showed good control against aphids. However, it had lower efficacy, and the dose was much higher than that of one of best

### Table 4. Efficacy of PP-A against less-OP-susceptible population of *M. persicae* (LC$_{50}$ mg/L)

| Sample   | OP-less susceptible | Susceptible | R/S |
|----------|---------------------|-------------|-----|
| PP-A     | 0.65                | 0.98        | 0.66 |
| Fenitrothion | 232              | 5.3         | 44  |

### Table 5. Efficacy of PP-A against each biotype of susceptible of *B. tabaci*<sup>a</sup>  

| Sample | Concentration (ppm) | *B. tabaci* (biotype B) | *B. tabaci* (biotype Q)<sup>b</sup> |
|--------|---------------------|------------------------|----------------------------------|
| PP-A   | 5.0                 | 100.0                  | 100.0                            |
|        | 1.3                 | 67.3                   | 41.8                             |
| IMI    | 50                  | ND                     | 100.0                            |
|        | 20                  | ND                     | 62.9                             |
|        | 5.0                 | 100.0                  | ND                               |
|        | 1.3                 | 100.0                  | ND                               |

<sup>a</sup> % Mortality at 6 days after application. ND: no data.  
<sup>b</sup> The population was collected in Ibaraki (2007).
commercial products, IMI (Fig. 3).

Regarding the mode of action, PP derivatives have been reported not to show any respiratory inhibition but to act on the proteins involved in the nervous system of the insect.\(^{11}\) In our study, the housefly orally treated with PP-A showed a unique symptom that was apparently not fatal but that affected its flying ability. All treated adult flies exhibited the disability to fly 1 day after application, although death was not observed.

With regard to the safety of PP-A to mammals, PP-A by oral application showed no acute toxicity against rats at a dose of 2000 mg/kg. Moreover, in an Ames test against Salmonella typhimurium TA98 and TA100 with or without an S9mix addition, no mutagenicity was observed at 5000 µg/plate.

Regarding ecotoxicological aspects, the acute toxicities against aquatic organisms Oryzias latipes, Daphnia magna, and Pseudokirchneriella subcapitata were all low (tests were conducted according to guidelines from the Japanese Ministry of Agriculture, Forestry, and Fisheries). The LC₅₀ value of PP-A for O. latipes was over 20 mg/L, and the 50% effective concentration (EC₅₀) values for D. magna and P. subcapitata were also over 20 mg/L. In evaluating the effect against the beneficial insect Aphidius colemani, which is commonly used as a biological pesticide for aphid control, PP-A showed low acute toxicity in foliar application to crops even at 1000 mg/L (data not shown).

Discussion

We evaluated the insecticidal properties of PP-A discovered from microbial metabolites. During evaluation of the insecticidal spectrum, we found that PP-A showed high aphicidal activity, especially against M. persicae and A. gossypii. PP-A showed that good efficacy against whiteflies as well. On the other hand, it did not show high activity against other hemiptera such as the brown planthopper, green rice leafflyer and rice leaf bug, lepidoptera, thysanoptera and acari. Although the insecticidal spectrum is narrow, aphids and whiteflies commonly occur on a variety of crops worldwide and damage crop production. We thought PP-A would be an effective tool for controlling these pests and conducted an evaluation to see if it is suitable as an aphicide. Further evaluation revealed that PP-A showed preferable systemic properties, such as transaminar efficacy on the treated leaves and systemic efficacy from roots. While the activity against adults was low in the tests against each growth stage of aphids and the treated adults still laid larvae, interestingly, almost all of the larvae showed some abnormality in their behavior and died within a few days.

The mode of action seems to be different from that of the existing insecticides acting on the central nervous system, such as synthetic pyrethroids and neonicotinoids. In our lab and field tests, a unique symptom was observed: the aphids treated with PP-A could not stay on the leaves, and many of them drop to the ground within three hours after treatment. The aphids on the ground displayed excessive wandering for a while but finally died. Such an observation also supports that the mode of action for PP-A is unique. In relation to this uniqueness, PP-A showed good activity against a less-OP-susceptible population of aphids and a less-IMI-susceptible one of whiteflies, which are major problems in crop cultivation.\(^{15,16}\) These results accelerated our investigation of PP-A as a tool to control these sucking pests. In the field trial against aphids, PP-A showed good control against OP-resistant aphids, but the efficacy was lower than that of the best commercial standard, IMI, and a higher dose for aphid control was needed in the field trials than that expected from the lab tests. The moderate field efficacy is likely to result from low stability in field conditions. Our test of PP-A stability when exposed to sunlight indicated that it degraded relatively easily within 3 days (data not shown). While the environmental impact seems low due to the narrow spectrum and relatively low persistence in the field, improved field efficacy is desired for better control of sucking pests and the dosage administered in the field should be reduced.

In terms of toxicity and ecotoxicology, PP-A showed preferable toxic profiles, that is, low toxicity to mammals and nontarget organisms. Furthermore, PP-A did not show any phytotoxicity to dicotyledons, such as the eggplant, cabbage, kidney bean, and cucumber, or to monocotyledons, such as the rice and wheat, even at concentrations over 100 mg/L with foliar application in the lab or field tests. Recently, safety to nontarget organisms has become a key factor. Therefore, from this standpoint, our study supports PP-A as a useful insecticide candidate.

In summary, we evaluated the possibility of the practical use of PP-A. Although the efficacy of PP-A itself was not sufficient and would need to be improved, we confirmed that PP-A would be a useful lead compound with eco-friendly, preferable properties for the control of sucking pests and unique insecticidal symptoms.

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References

1) C. L. Cantrell, F. E. Dayan and S. O. Duke: J. Nat. Prod. 75, 1231–1242 (2012).
2) R. Horikoshi, M. Nomura, T. Nakamura, M. Hayashimoto, M. Tsuchida, K. Oyama and M. Mitomi: PCT Int. Appl. WO2010/071218 (2010)
3) Y. Xu, S. Furutani, M. Ihara, Y. Ling, K. Kai, H. Hayashi and K. Matsuda: PLoS One 10, e0122629 (2015).
4) R. Horikoshi, K. Goto, M. Mitomi, K. Oyama, T. Sunazuka and S. Ōmura: J. Antibiot. (Tokyo) 70, 272–276 (2017).
5) S. Ōmura, H. Tomoda, Y. K. Kim and H. Nishida: J. Antibiot. (Tokyo) 46, 1168–1169 (1993).
6) H. Tomoda, Y. K. Kim, H. Nishida, R. Masuma and S. Ōmura: J. Anti- biot. (Tokyo) 47, 148–153 (1994).
7) Y. K. Kim, H. Tomoda, H. Nishida, T. Sunazuka, R. Obata and S. Ōmura: J. Antibiot. (Tokyo) 47, 154–162 (1994).
8) Y. K. Kim, H. S. Lee, M. C. Rho, K. H. Kim, H. Y. Song and S. U. Kim: (Korea Research Institute of Bioscience and Biotechnology): PCT Int. Appl. WO2004/060065 (2004)
9) PhillipsMcDougall: *AgriFutura* No. 170 (2013)

10) J. Leahy, M. Mendelsohn, J. Kough, R. Jones and N. Berckes: *Environmental Protection Agency publication*, doi: 10.1021/bk-2014-1172. ch001 (Published 23 Oct., 2014)

11) K. Goto, R. Horikoshi, M. Tsuchida, K. Oyama, S. Ōmura, H. Tomoda, T. Sunazuka, et al.: (Meiji Seika Kaisha Ltd. and the Kitasato Institute): *Jpn Kokai Tokkyo Koho* JP 4015182 (2006) (in Japanese)

12) S. Kagabu, M. Mitomi, S. Kitsuda, R. Horikoshi, M. Nomura and Y. Onozaki: (Meiji Seika Pharma Co., Ltd.): *US Patent Appl.* US9073866 (2013)

13) D. Fuog, S. J. Fergusson and C. Flückiger: “Insecticides with Novel Modes of Action Mechanisms and Application” ed. by I. Ishaaya and D. Degheele, Springer, Berlin, pp. 40–49, 1998.

14) C. A. Leichter, N. Thompson, B. R. Johnson and J. G. Scott: *Pestic. Biochem. Physiol.* 107, 169–176 (2013).

15) C. Bass, A. M. Puinean, C. T. Zimmer, I. Denholm, L. M. Field, S. P. Foster, O. Gutbrod, R. Nauen, R. Slater and M. S. Williamson: *Insect Biochem. Mol. Biol.* 51, 41–51 (2014).

16) E. Fernández, E. C. Grávalos, P. J. Haro, D. Cifuentes and P. Bielza: *Pest Manag. Sci.* 65, 885–891 (2009).