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**Mini-Review**

# Pesticidal Constituents Derived from Piperaceae Fruits

**Hoi-Seon Lee***

*Institute of Bio-Safety Research and Institute of Agricultural Science & Technology, College of Agricultural and Life Science, Chonbuk National University, Chonju 561-756, Korea*

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**Fungicidal, insecticidal, and mosquito larvicidal activities of piperidine alkaloids, pipernonaline and piperoctadecalidine, and isobutylamide alkaloids, pellitorine, guineensine, pipercide, and retrofractaminde A, derived from Piperaceae fruits were studied. Pipernonaline and piperoctadecalidine showed potent fungicidal activities against *Puccinia recondita* with 91 and 80% control values at 500 ppm. Against *Phytophthora infestans*, pipernonaline showed strong fungicidal activity with 91 and 80% control values at 1,000 and 500 ppm. $LD_{50}$ values of pipernonaline and piperoctadecalidine against *Plutella xylostella* were 125 and 95.5 ppm, respectively, and that of piperoctadecalidine against *Tetranychus urticae* was 246 ppm. Against larvae of *Aedes aegypti* and *Culex pipiens pallens*, $LD_{50}$ values of pipernonaline were 0.35 and 0.21 ppm, respectively. Highest larvicidal activities of pipercide and retrofractamide A were found against *A. aegypti*, *A. togoi*, and *C. pipiens pallens*. $LD_{50}$ values of pipercide and retrofractamide A were 0.10 and 0.039 ppm against *A. aegypti*, 0.26 and 0.01 ppm against *A. togoi*, and 0.004 and 0.028 ppm against *C. pipiens pallens*, respectively. Based upon these results and earlier findings, bioactive components derived from Piperaceae fruits may be valuable for development of useful lead product of possibly safer fungicidal, insecticidal, and mosquito larvicidal agents.**

**Key words:** *fungicide, insecticide, larvicide, pipernonaline, piperoctadecalidine, pellitorine, guineensine, pipercide, retrofractaminde A, Piperaceae fruits*

One of the most important and challenging aspects in the pesticidal research is the development of new and effective approaches for controlling various insect pests, plant diseases, and mosquito larvaes.[9,15] Synthetic pesticides have been effectively used to control insects and plant diseases; however, increasing concern over their environmental effects has highlighted the need for the development of alternative types of selective control or methods for crop protection with/without reduced use of conventional pesticides.[15] Researches on plant-derived pesticides in agriculture are currently intensified as it becomes evident that plant-derived pesticides still have enormous potential to inspire and influence modern agrochemical research.[7] Although defining the ecological effects of most synthetic pesticides is difficult, there is a good reason to suppose that the secondary metabolism of plants has evolved to protect them from insects and microbial pathogens.[7,14]

Plant extracts and phytochemicals may be an alternative to currently used pesticides for controlling insect pests, phytopathogenic fungi, and mosquito larvaes, because they constitute a rich source of bioactive chemicals.[44, 48] They are often active against a limited number of specific target species, biodegradable into nontoxic products, and potentially suitable for use in integrated management programs, thus leading to the development of new classes of safer mosquito larvae, plant disease, and insect control agents. Therefore, efforts have been focused on secondary plant metabolites for potentially useful products as commercial pesticides or as lead compounds.[4,7,16] Plant secondary metabolites are synthesized through three synthetic pathways. Terpenoids, phenolics, and nitrogen-based secondary metabolites are synthesized from mevalonate, shikimic acid, and amino acid as precursors, respectively. These metabolites are highly diverse; more than 30,000 ‘natural products’ or ‘secondary metabolites’ have been reported from plants so far.[33,49] However, because only 5-10% of all higher plants, which consist of 300,000 species, have been analyzed phytochemically in some detail, the actual number of secondary products is certain to exceed 100,000 compounds.[33]

Bioactive natural products play an important role as lead compounds in the development of new pesticides, and in understanding the natural phenomenon throughout their biosynthesis, metabolism, and mode of action. However, the researches of bioactive natural products in developed countries are limited to few institutes and universities. Ahn *et al*. (1998) reported the isolation of insecticidal and anti-gnawing compounds from *Thujopsis dolbrata* var. hondae against insect pests and mouse.[3] Establishment of bioassay system significantly contributes to findings of new bioactive compounds. In developed countries, new bioassay systems to examine the natural products have been accumulating for a long time. In our study, the Piperaceae fruit-derived components were examined for fungicidal, insecticidal, and mosquito larvicidal effects.

*Corresponding author
Phone: +82-63-270-2544; Fax: +82-63-270-2550
E-mail: hoiseon@chonbuk.ac.kr

## Biological Activity of Piperaceae Plants

The genus *Piper*, belonging to the Piperaceae, has been receiving considerable attention in recent years due to its biological properties. Various *Piper* species, widely distributed in the tropical and subtropical regions of the world, have been used as spices as well as folk medicines. The fruits of Piperaceae plants have been studied for their biologically active principles including unsaturated amides, which have potent insecticidal activities.[31)] Furthermore, some Piperaceae plant fruits have been used as food-flavoring agents and are also known to possess insecticidal activities[25,43,46)].

*P. longum* is a slender aromatic climber found in different parts of India. The fruits of the plant, frequently used to treat bronchial trouble, are known to be carminative and analgesic, and widely used as anodynes and for the treatment of stomach disease in China. Furthermore, *P. longum* has inhibitory activity against aflatoxin $B_1$ biosynthesis, coronary-vasodilating activity, antimetastatic activity, and antimutagenic property.[8)] Dry black pepper, *P. nigrum* is a perennial vine with a trailing or climbing stem, round, smooth, flexuous, dichotomously branched, swelling at the joints, and often throwing-out radicles, which adhere to bodies such as the roots of ivy, or become roots themselves, striking into the ground. This plant is a native of the East Indian continent, notably the Malabar Coast, as well as of many islands in the Indian Ocean and West India, where it is extensively cultivated. *P. nigrum* has been reported to be toxic to houseflies (*Musca domestica* L.), rice weevils (*Sitophilus oryzae* L.), and cowpea weevils (*Callosobruchus maculatus* F.).[41-43)] Furthermore, the fruits of *P. nigrum* are not only important as spices and flavoring, but also for cholera, dyspepsia, flatulence, diarrhea, various gastric ailments, and paralytic and arthritic disorders.[34)] Some researches have reported that *P. nigrum* also has anti-inflammatory, tyrosinase-inhibitory, and antimicrobial activities. Additionally, phenolic amides from *P. nigrum* have been known as antioxidative compounds.[27)] Piperine can be easily isolated from the fruit of black pepper plants, although it is apparently inactive as a contact toxicant to houseflies.[43)] Several insecticidal amides, such as pipericide, (*E*,*E*)-*N*-(2-methylpropyl)-2,4,12-tridecadienamide, and (*E*,*E*,*E*)-11-(1,3-benzodioxol-5-yl)-*N*-(2-methylpropyl)-2,4,10-undecatrien-amide, have been isolated from *P. nigrum.*[25,43)] Furthermore, *P. betle* leaves have been traditionally used in India and China for the prevention of oral malodor. The general practice in India is to chew the leaves alone or in combination with areca nut and other spices such as cardamom, clove, and cinnamon, which act as "breath fresheners" and help prevent halitosis.[29)]

## Fungicidal Effects of *P. longum* Fruits

Pre-harvest losses due to fungal diseases in world crop production in developing countries may amount to 12% or even higher.[23)] Furthermore, due to the development of resistance in known fungal pathogens and the emergence of fungal pathogens intrinsically resistant to the currently available antibiotics, identification and development of novel antifungal agents are of utmost importance. Previous studies have confirmed that the antimicrobials can be identified through consultations with traditional healers.[13)] Researches into plant-derived fungicides are now being intensified as it becomes evident that plant-derived fungicides still have enormous potential to inspire and influence modern agrochemical researches.[23)] Members of the Zingiberaceae, in

**Table 1. Test conditions of phytopathogenic fungi in greenhouse**

| Disease | Plant/ Stage | Plant No./pot | Pathogen | Inoculation; Inoculum Dosage | Keeping Period in Humidity Chamber | Chamber Temp.[a] /Period[b] |
|---|---|---|---|---|---|---|
| Rice blast (RCB) | Rice/2-leaf | 3 | Pyricularia grise | Leaf spray; $1 \times 10^6$ spore/ml | 1 day | 25°C/5 |
| Rice sheath Blight (RSB) | Rice/3-leaf | 3 | Rhizoctonia solani | Pouring inoculum[c] on the soil; 10 ml/pot | 7 days | 28°C/7 |
| Cucumber gray Mold (CGM) | Cucumber/1-leaf | 1 | Botrytis cinere | Leaf spray; $1 \times 10^6$ spore/ml | 3 days | 20°C/3 |
| Tomato late Blight (TLB) | Tomato/2-leaf | 2 | Phytophthora infestans | Leaf spray; $1 \times 10^5$ zoosporangia/ml | 4 days | 18°C/4 |
| Wheat leaf Rust (WLR) | Wheat/1-leaf | 4 | Puccinia recondita | Leaf spray; 1.5 mg of uredospores/pot | 1 day | 20°C/10 |
| Barley powdery mildew (BPM) | Barley/1-leaf | 4 | Erysiphe graminis | Dusting[d] the conidia on barley plant | Not need | 20°C/10 |

[a]Chamber temperature kept for treated and control plants. [b]Period (days) from inoculation of pathogen to evaluation of disease severity on host plants, including the days required to keep plants applied with test solution in a humidity chamber. [c]Inoculum of *Rhizoctonia solani* was made by inoculating mycelial plugs in wheat bran medium at 25°C for 7 days, and macerated at the ratio of 500 g medium-incubated *R. solani* per 1 L distilled water in a mixer. [d]Preparation of *E. graminis* conidia, an obligate parasite, was made by dusting inoculation of conidia onto 10 barley plants cultivated in the pot (f 7.5 cm). Treated plants were dusted with *E. graminis* conidia formed on barley leaves at eight tested pots/ maintained pot.

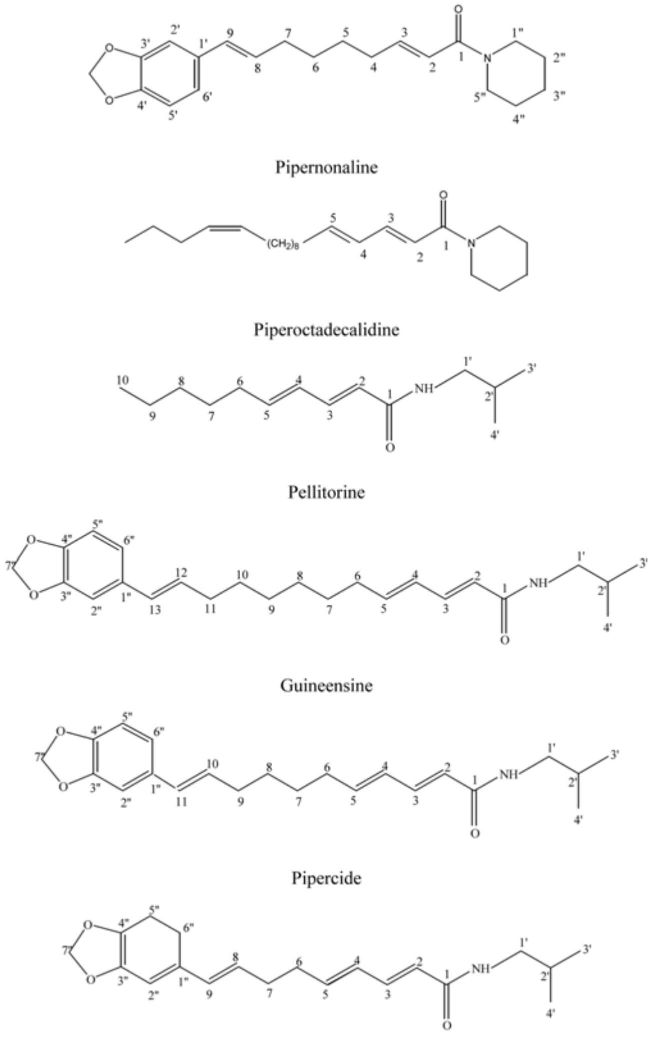


**Fig. 1. Chemical structures of bioactive constituents derived from Piperaceae fruit.**

particular *Ziniber officinale*, have a long history of use in traditional medicines. In a recent assessment of ethnobotanical uses of plants, *Z. officinale* was found to significantly inhibit a wide range of fungi pathogenic to humans.[13] Because the members of Zingiberaceae are generally regarded as safe for human consumption, they are good candidates for development as antifungal therapeutics.

Fungicidal effects were examined through a whole plant method in greenhouse. Test conditions of phytopathogenic fungi in greenhouse is shown in Table 1. Against six phytopathogenic fungi, methanol extract of *P. longum* fruits possessed fungicidal activity against *P. recondite* with 100% control value at 1,000 ppm, whereas showed no activity against *P. grisea*, *R. solani*, *B. cinerea*, *P. infestans,* and *E. graminis*. Further solvent fractionation showed strong fungicidal activity in the hexane fraction against *P. recondita* with 100% control value at 1,000 ppm, and moderate against *P. grisea* and *P. infestans* at the same concentration. However, low fungicidal activities were observed in other organic solvent fractions, and no activities were observed in the butanol and water fractions. Two active isolates from the hexane fraction showed potent fungicidal activities against *P. grisea*, *R. solani*, *P. infestans*, and *P. recondita*, and were characterized by the spectral analyses as pipernonaline and piperoctadecalidine (Fig. 1). Piperoctadecalidine isolated from *P. longum* fruits was identified through comparison with the published mass-spectroscopic properties (Table 6).[5,45] The isolations and the spectral analyses of pipernonaline and piperoctadecalidine from *P. longum* have already been reported for anodyne and in the treatment of stomach disease.[5,45] These data are identical to those of Tabuneng *et al*.[45] The fungicidal activities of pipernonaline and piperoctadecalidine isolated from *P. longum* fruits against six phytopathogenic fungi when treated at 1,000, 500, and 250 ppm were determined *in vivo* (Table 2). Pipernonaline showed strong fungicidal activities against *P. grisea*, *P. infestans*, and *P. recondita*, except for cucumber gray mold caused by *B. cinerea* and barley powdery mildew caused by *E. graminis*. Furthermore, piperoctadecalidine showed a potent fungicidal activity against *P. recondita* with 100 and 80% control values at 1,000 and 500 ppm, respectively.

To study for the fungicidal activities of other components derived from *P. longum*, four commercially available compounds derived from this plant species[35] were examined against six phytopathogenic fungi (Table 2). Piperettine showed 100% control values against *E. graminis* when treated at 2,000 ppm (not shown). However, little activity was observed for eugenol, piperine, piperlongumine, and piperettine when treated at 1,000, 500, and 250 ppm. Due to the fungicidal activity of pipernonaline against *P. infestans*, and *P. recondita*, this compound was compared with synthetic fungicides such as chlorothalonil, dichlofluanid, and mancozeb (Table 2). Potent fungicidal was activities were observed with chlorothalonil against *P. infestans* and dichlofluanid against *B. cinerea* at 50 ppm, and mancozeb against *P. recondite* at 10 ppm. On the other hand, no activity was produced with chlorothalonil against *B. cineria, P. grisea, R. solani*, and *P. recondita*, dichlofluanid against *P. grisea, P. infestans, P. recondita*, and *R. solani*, and mancozeb against *B. cineria, P. grisea*, and *P. infestans*. Therefore, although their fungicidal activities are over 50 times less potent than those of the synthetic fungicides, pipernonaline and piperoctadecalidine may be useful as lead products for developing new types of fungicides to control plant pathogens on crops and may be excellent candidates for development as antifungal agents.

## Insecticidal Effects of *P. longum* Fruits

Over past several decades, various attempts have been made to control insect diseases using effective eradicate and synthetic pesticides,[1,3,6] including laboratory strains of five agricultural insect pests, brown planthopper (*Nilaparvata lugens* Stål), diamondback moth (*Plutella xylostella* L.), green

**Table 2. Antifungal activities of constituents derived from *P. longum* fruits on plant**

| Material | Control Values (%)[a] | | | | | | |
|---|---|---|---|---|---|---|---|
| | Conc.[b] | RCB[c] | RSB | CGM | TLB | WLR | BPM |
| Eugenol | 1000 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 500 | 0 | 0 | 0 | 0 | 0 | 0 |
| Pipernonaline | 1000 | 71 | 35 | 0 | 91 | 100 | 0 |
| | 500 | 51 | 0 | 0 | 80 | 91 | 0 |
| | 250 | 26 | 0 | 0 | 55 | 80 | 0 |
| Piperine | 1000 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 500 | 0 | 0 | 0 | 0 | 0 | 0 |
| Piperoctadecalidine | 1000 | 33 | 0 | 0 | 0 | 100 | 0 |
| | 500 | 0 | 0 | 0 | 0 | 80 | 0 |
| | 250 | 0 | 0 | 0 | 0 | 62 | 0 |
| Piperlongumine | 1000 | 10 | 0 | 0 | 0 | 0 | 0 |
| | 500 | 0 | 0 | 0 | 0 | 0 | 0 |
| Piperettine | 1000 | 0 | 0 | 0 | 0 | 0 | 60 |
| | 500 | 0 | 0 | 0 | 0 | 0 | 40 |
| Chlorothalonil[d] | 50 | - | - | - | 95 | - | - |
| Dichlofluanid | 50 | - | - | 92 | - | - | - |
| Mancozeb | 10 | - | - | - | - | 95 | - |
| LSD (0.05)[e] | | 10.1 | 9.2 | 2.6 | 7.9 | 4.1 | 4.0 |

[a]Activity is preventive value (%), 100% complete killing, 0% zero killing; [b]ppm. [c]RCB, rice blast caused by *Pyricularia grisea* on rice; RSB, rice sheath blight caused by *Rhizoctonia solani* on rice; CGM, cucumber gray mold caused by *Botrytis cinerea* on cucumber; TLB, tomato late blight caused by *Phytophthora infestans* on tomato; WLR, wheat leaf rust caused by *Puccinia recondita* on wheat; BPM, barley powdery mildew caused by *Erysiphe graminis* on barley; [d]commercial name; [e]LSD, least significant difference.

peach aphid (*Myzus persicae* Sulzer), tobacco cutworm (*Spodoptera litura* Fab.), and two-spotted spider mite (*Tetranychus urticae* Koch). Nearly 20 million hectares of rice are estimated to be infested by *N. lugens*, with an annual loss of some half a million tones of grain.[17,50] *P. xylostella* L. is a cosmopolitan species that probably originated in the Mediterranean region. Host plants include both cultivated and wild plants of the family Cruciferae, as well as several ornamentals, such as wallflower, candytuft, stocks, and alyssum. *M. persicae* Sulzer is distributed worldwide and is common on seedlings, young plants, and lower leaves of older plants. Aphids vector many plant viruses. This is potentially the greatest consequence of aphid infestations. The green peach aphid vectors virus diseases in more than 30 plant families, including beans, sugar beet, sugarcane, brassica sp., citrus, and tobacco. *S. litura* is an economically important pest of vegetable and tobacco crops in Southeast Asia, India, China, and Japan.[19]

Adult moths of the common cutworm *S. litura* migrate overseas; males have been caught on weather-forecasting ships on oceans,[26] and sudden and coincidental occurrences of these moths have often been recorded in two locations, 180 km apart at the time of typhoon approaches to these sites.[26] The two-spotted spider mite, *T. urticae* Koch, is an important pest of ivy geranium and other ornamental plants, and is a generalist that can feed on several hundreds of host plant species.[30,47]

Against these five agricultural insect pests, the methanol extract of *P. longum* fruits possessed insecticidal activity against *M. persicae*, *S. litura*, and *T. urticae* with 100% control value at 1,000 ppm. Further solvent fractionation showed strong fungicidal activities in the hexane fraction against *M. persicae*, *S. litura*, and *T. urticae* with 100% control value at 1,000 ppm. However, little activity was found in the other organic solvent fractions except chloroform fraction, in which 50% control value against *M. persicae*, *S. litura*, and *T. urticae* was observed. Two active isolates from the hexane fraction showed potent insecticidal activities, and was characterized by spectral analyses as pipernonaline and piperoctadecalidine (Table 6).

Both pipernonaline and piperoctadecalidine showed potential insecticidal activities against *M. persicae* and *P. xylostella*, but little against *N. lugens* and *S. litura* (Table 3). Both compounds resulted in 100% mortality at 1,000 ppm against *M. persicae* and *P. xylostella*. The second larvae of *P. xylostella* were most susceptible to both compounds at 100 ppm. However, both compounds show little insecticidal activity against *S. litura*, a lepidopteron species. This indicates that the insecticidal activities of both piperidine alkaloid compounds from *P. longum* fruits varied with the species tested. In addition, piperoctadecalidine showed a potent acaricidal activity against *T. urticae*. This is a very interesting phenomenon, because both piperidine alkaloids showed similar insecticidal activities against the same insect species. However, the significant difference from the mortality of the piperidine alkaloids against *T. urticae* may lead us to

**Table 3. Insecticidal and acaricidal activities of constituents derived from *P. longum* fruits**

| Compound | Motality (%)[a] | | | | | |
|---|---|---|---|---|---|---|
| | Conc.[b] | BPH[c] | GPA | DBM | TCW | TSSM |
| Pipernonaline | 1000 | 0 | 80 | 100 | 0 | 0 |
| | 500 | 0 | 50 | 100 | 0 | 0 |
| | 250 | 0 | 20 | 90 | 0 | 0 |
| Piperine | 1000 | 0 | 10 | 20 | 0 | 10 |
| | 500 | 0 | 0 | 0 | 0 | 0 |
| | 250 | 0 | 0 | 0 | 0 | 0 |
| Piperoctadecalidine | 1000 | 0 | 90 | 100 | 0 | 80 |
| | 500 | 0 | 60 | 100 | 0 | 50 |
| | 250 | 0 | 30 | 80 | 0 | 30 |
| Piperlongumine | 1000 | 0 | 15 | 10 | 0 | 18 |
| | 500 | 0 | 0 | 0 | 0 | 0 |
| | 250 | 0 | 0 | 0 | 0 | 0 |
| Piperettine | 1000 | 0 | 10 | 10 | 0 | 0 |
| | 500 | 0 | 0 | 0 | 0 | 0 |
| | 250 | 0 | 0 | 0 | 0 | 0 |

[a]Activity is mortality (%), 100% complete killing, 0% zero killing; [b]ppm. [c]BPH, *Nilaparvata lugens*; GPA, *Myzus persicae*; DBM, *Plutella xylostella*; TCW, *Spodoptera litura* and TSSM, *Tetranychus urticae*.

quantitative structure-activity relationships against *T. urticae.* Using the same bioassay method, Ahn *et al.*[2] found that AC 303630, flucycloxuron, and other acaricides, when used alone, were highly effective against egg, immature, and adult stages of *T. urticae* at 50 ppm. However, their mixture (flucycloxuron + bifenthrin) was toxic to immature stages only, showing little effect on adults at 80 ppm. The other acaricides, Bromopropylate and Azocyclotin, showed 100% mortality at 450 and 116 ppm, respectively, suggesting that more study is necessary on their structure-activity relationships against *T. urticae*.

Various essential oils have been documented to exhibit acute toxic effects against insects. Lee *et al.*[22,23] and Park *et al.*[32] demonstrated the toxicity of a number of essential oil constituents against the western corn rootworm, *Diabrotica virgifera*, the two-spotted spider mite, *T. urticae*, and the common house fly, *Musca domestica*. Contact toxicity to the American cockroach, *Periplaneta americana*, has been demonstrated in eugenol, and carvacrol is acutely toxic to the German cockroach, *Blattella* germanica[28] as well as to many other insects. The toxicity of anethole has been demonstrated against a number of species, including various beetles, weevils, mosquitoes, and moths.[18,21,38,39] These studies strongly support the possibility of piperoctadecalidine as a new acaricide. In this regard, further studies are underway to determine the insecticidal mode of action of both alkaloids and acaricidal mode of action of piperoctadecalidine

## Lavicidal Activity of *P. longum* and *P. nigrum* Fruits

Mosquitoes are insects belonging to the order Diptera. Over 3,000 different species of mosquitoes exist worldwide, with 51 species belonging to nine genera found in Korea.[10] Mosquitoes are hosts to a variety of pathogens and parasites, including virus, bacteria, fungi, protoctistans, and nematodes.[11] Some of these organisms alternate a parasitic phase with a free-living phase; others are entirely parasitic, many of which alternate between their mosquito hosts and other invertebrate or vertebrate host. The blood-sucking habit renders adult mosquitoes prone to acquiring pathogens and parasites from one vertebrate host to pass them on to other hosts. In Korea, outbreak of encephalitis occurs in many patients, mainly children, annually. Moreover, annual number of *Plasmodium vivax* malaria is reemerging, just two cases in 1993 have tripled each year since then, with more than 1,600 cases reported in 1997.[12,37,40] The vector-borne disease by mosquitoes is one of the serious problems of human sanitation. Worldwide, millions of people are threatened with the mosquito-transmitted disease annually. Indirect damages by mosquito are also significant. Itchiness caused by biting and sleeping interference restrict human activities, inflict economic loss to the tourist and leisure industries, and also inflict agricultural loss in terms of weight loss of domestic animals and interference with many physiological processes of the animals.

The yellow fever mosquitoes, *Aedes aegypti* (L.), *Aedes togoi* (Theobald), and *Culex pipiens pallens* (Coquillett), are widespread and serious primary medical insect pests. Control of these mosquito larvaes can be achieved by repeated applications of organophosphates such as temephos and fenthion, and insect growth regulators such as diflubenzuron and methoprene. Although effective, their repeated use has disrupted natural biological control systems, leading to outbreaks of insect species, sometimes resulted in the widespread development of resistance, had undesirable effects on nontarget organisms, and fostered environmental and

**Table 4. Regression parameters for mortality responses of *A. aegypti* and *C. pipiens pallens* larvae exposed to constituents derived from *P. longum* fruits**

| Sample | Tested Mosquito | $LD_{50}$ | $LD_{95}$ |
|---|---|---|---|
| Pipernonaline | *Aedes aegypti* | 0.35 | 0.67 |
| | *Culex pipiens pallens* | 0.21 | 0.52 |
| Piperine | *Aedes aegypti* | 5.10 | 9.84 |
| | *Culex pipiens pallens* | 3.21 | 6.31 |
| Piperoctadecalidine | *Aedes aegypti* | 0 | 0 |
| | *Culex pipiens pallens* | 0 | 0 |
| Piperlongumine | *Aedes aegypti* | 0 | 0 |
| | *Culex pipiens pallens* | 0 | 0 |
| Piperettine | *Aedes aegypti* | 0 | 0 |
| | *Culex pipiens pallens* | 0 | 0 |

Doses expressed in ppm: $LD_{50}$, median lethal dose; $LD_{95}$, 95% lethal dose.

**Table 5. Regression parameters for mortality response of *A. aegypti*, *A. togoi*, and *C. pipiens pallens* larvae exposed to constituents derived from *P. nigrum* fruits**

| Sample | Tested Mosquito | $LD_{50}$ | $LD_{95}$ |
|---|---|---|---|
| | *Aedes aegypti* | 0.92 | 1.76 |
| Pellitorine | *Aedes togoi* | 0.71 | 1.30 |
| | *Culex pipiens pallens* | 0.86 | 1.52 |
| | *Aedes aegypti* | 0.89 | 1.20 |
| Guineensine | *Aedes togoi* | 0.75 | 1.13 |
| | *Culex pipiens pallens* | 0.17 | 0.29 |
| | *Aedes aegypti* | 0.10 | 0.17 |
| Pipercide | *Aedes togoi* | 0.26 | 0.41 |
| | *Culex pipiens pallens* | 0.004 | 0.007 |
| | *Aedes aegypti* | 0.039 | 0.069 |
| Retrofractamide A | *Aedes togoi* | 0.01 | 0.016 |
| | *Culex pipiens pallens* | 0.028 | 0.043 |
| | *Aedes aegypti* | 5.10 | 9.84 |
| Piperine | *Aedes togoi* | 4.60 | 8.77 |
| | *Culex pipiens pallens* | 3.21 | 6.31 |

Doses expressed in ppm: $LD_{50}$, median lethal dose; $LD_{95}$, 95% lethal dose.

human health concerns. These problems have highlighted the need for the development of new strategies for selective mosquito larval control.[24,34,51)]

Against the larvae of *A. aegypti* and *C. pipiens pallens*, methanolic extract of *P. longum* fruits possessed a strong mosquito larvicidal activity at 40 ppm. Further solvent fractionation showed strong larvicidal activity in the hexane fraction, which showed 100% mortality at 40, 20, and 10 ppm. Chloroform fraction showed 31 and 39%, and water fraction exhibited 15 and 22% mortality against *A. aegypti* and *C. pipiens pallens* at 40 ppm, respectively. Little activity

**Table 6 . $^{1}H$-NMR and $^{13}C$-NMR of pipernonaline and peroctadecalidine**

| No | Pipernonaline | | Piperoctadecalidine | |
|---|---|---|---|---|
| | $^{13}C$ | $^{1}H$ | $^{13}C$ | $^{1}H$ |
| 1 | 165.2 | - | 165.3 | - |
| 2 | 120.5 | 5.90 1H *d* (*J* = 15.0 Hz) | 118.3 | 6.27 *d* (*J* = 14.8 Hz) |
| 3 | 145.2 | 6.80 1H *dt* (*J* = 15.0, 4.0 Hz) | 142.5 | 7.24 1H *dd* (*J* = 14.8, 10.6 Hz) |
| 4 | 32.2 | 2.21 2H *m* | 128.6 | 6.16 1H *dd* (*J* = 15.1, 10.6 Hz) |
| 5 | 27.9 | 1.47 4H *m* | 142.1 | 6.04 1H *m* |
| 6 | 28.9 | | 32.7 | 2.14 1H *m* |
| 7 | 32.6 | 2.17 2H *m* | 28.9-29.5 | 1.42-1.28 |
| 8 | 128.6 | 6.07 1H *dt* (*J* = 6.6, 15.4 Hz) | 28.9-29.5 | 1.42-1.28 |
| 9 | 129.5 | 6.32 1H *d* (*J* = 15.4 Hz) | 28.9-29.5 | 1.42-1.28 |
| 10-12 | | 1.47 4H *m* | 28.9-29.5 | 1.42-1.28 |
| 13 | | | 26.9 | 2.00 1H *m* |
| 14 | | | 129.7 | 5.35 1H *m* |
| 15 | | | 129.3 | 5.35 1H *m* |
| 16 | | | 28.6 | 2.00 1H *m* |
| 17 | | | 22.6 | 1.28 1H *m* |
| 18 | | | 15.5 | 0.90 3H *t* (*J* = 7.3 Hz) |
| 1' | 132.2 | - | 46.5 | 3.61 2H *br s* |
| 2' | 105.2 | 6.90 1H *d* (*J* = 1.6 Hz) | 26.4 | - |
| 3' | 146.5 | - | 24.4 | 1.65-1.56 6H *m* |
| 4' | 147.9 | - | 25.4 | - |
| 5' | 108.0 | 6.71 1H *d* (*J* = 8.0 Hz) | 42.9 | 3.49 2H *br s* |
| 6' | 120.1 | 6.76 1H *d* (*J* = 1.5, 8.0 Hz) | | |
| -$OCH_2O$- | 100.8 | 5.92 2H *s* | | |
| 1" | 42.9 | 3.48 2H *br s* | | |
| 2" | 26.5 | 1.63 2H *m* | | |
| 3" | 24.6 | 1.56 4H *m* | | |
| 4" | 25.6 | | | |
| 5" | 46.6 | 3.48 2H *br s* | | |

**Table 7. $^{1}$H-NMR and $^{13}$C-NMR of pellitorine and guineensine**

| No | Pellitorine | | Guineensine | |
|---|---|---|---|---|
| | $^{13}$C | $^{1}$H | $^{13}$C | $^{1}$H |
| 1 | 166.40 | - | 166.35 | - |
| 2 | 121.76 | 5.76 1H *d* ($J$ = 15.0 Hz) | 121.74 | 5.74 1H *d* ($J$ = 15.0 Hz) |
| 3 | 141.27 | 7.19 1H *d* ($J$ = 15.0 Hz) | 141.29 | 7.19 1H *dd* ($J$ = 15, 5 Hz) |
| 4 | 128.20 | 6.09 1H *m* | 129.35 | 6.12 1H *d* ($J$ = 15.0 Hz) |
| 5 | 128.20 | 6.09 1H *m* | 129.35 | 6.07 1H *d* ($J$ = 15.0 Hz) |
| 6 | 32.92 | 2.13 2H *dd* ($J$ = 7.0, 13.8 Hz) | 32.85 | 2.15 2H *m* |
| 7 | 28.49 | 1.37 2H *m* | 29.00 | 1.42 2H *m* |
| 8 | 31.37 | 1.28 2H *m* | 28.72 | 1.32 2H *m* |
| 9 | 22.47 | 1.28 2H *m* | 28.94 | 1.32 2H *m* |
| 10 | 14.01 | 0.88 3H *s* | 29.33 | 1.42 2H *m* |
| 11 | | | 32.90 | 2.15 2H *m* |
| 12 | | | 143.10 | 6.02 1H *d* ($J$ = 15.0 Hz) |
| 13 | | | 129.31 | 6.27 1H *d* ($J$ = 15.0 Hz) |
| 1' | 46.92 | 3.16 2H *t* ($J$ = 6.4, 12.9 Hz) | 46.91 | 3.16 2H *t* ($J$ = 6.4, 12.9 Hz) |
| 2' | 28.63 | 1.76 1H *m* | 28.63 | 1.79 1H *m* |
| 3' | 20.13 | 0.91 3H *s* | 20.13 | 0.91 3H *s* or 0.93 3H *s* |
| 4' | 20.13 | 0.93 3H *s* | 20.13 | 0.93 3H *s* or 0.91 3H *s* |
| NH | - | 5.60 *br s* | - | 5.50 *br s* |
| 1" | | | 132.45 | - |
| 2" | | | 105.37 | 6.89 1H *s* |
| 3" | | | 146.52 | - |
| 4" | | | 149.90 | - |
| 5" | | | 108.22 | 6.74 1H *m* |
| 6" | | | 120.19 | 6.74 1H *m* |
| 7" | | | 100.90 | 5.93 2H *s* |

was found in other organic solvent fractions. A compound isolated from the hexane fraction showed a potent larvicidal activity, and was characterized by the spectral analyses as pipernonaline. This compound had a potent larvicidal activity against larvae of *A. aegypti* and *C. pipiens pallens*, giving $LD_{50}$ values of 0.35 and 0.21 ppm, respectively (Table 4). This $LD_{50}$ value of pipernonaline against *C. pipiens pallens* larvae was 27-, 110-, and 245-fold higher than those of obacunone, nonmilin, and limonin, respectively.[20)]

Methanolic extract of *P. nigrum* fruits showed a strong mosquito larvicidal activity at 100 ppm. Further solvent fractionation showed a strong larvicidal activity in the hexane and chloroform fractions against *A. aegypti*, *A. togoi,* and *C. pipiens pallens*. Bioassay-guided fractionation of the *P. nigrum* fruit extract exhibited four active constituents identified by spectroscopic analyses as pellitorine, guineensine, pipercide, and retrofractaminde A (Fig. 1) (Tables 7 and 8). A known insecticidal amide, pellitorine has served as a standard of comparison in toxicity tests. Against the larvae of *A. aegypti* and *A. togoi*, retrofractamide A was the most effective, with $LD_{50}$ values of 0.039 and 0.01 ppm, and pipercide showed 0.10 and 0.26 ppm, respectively. Larvicidal activity of guineensine was comparable to that of pellitorine. Furthermore, retrofactamide A and pipercide showed 24- and 9-fold higher activity of pellitorine against *A. aegypti*, and 71- and 3-fold against *A. togoi*, respectively. Among the four test compounds, pipercide had a potent larvicidal activity against larvae of *C. pipiens pallens* with $LD_{50}$ value of 0.004 ppm. On the basis of $LD_{50}$ values, the compound most toxic to *C. pipiens pallens* larvae was pipercide followed by retrofractamide A ($LD_{50,}$ 0.028 ppm), guineesine ($LD_{50,}$ 0.17 ppm), and pellitorine ($LD_{50,}$ 0.86 ppm) (Table 5).

Recent reports showed that extract of *Tagetes minuta* L. had strong biocidal effects on both the larvae and adults of *A. aegypti* and *Anopheles stephensi* L.,[36)] and the insecticidal components isolated from the plant extract were 4 thiophenes, 5-(but-3-ene-1-ynyl)-2,2'-bithiophene, 5-(but-3-ene-1-ynyl)-5'-methyl-2,2-bithiophene, 2,2',5',5"-terthiophene, and 5-methyl-2,2',5',2"-ter-thiophene.[6)] These compounds may be considered as alternatives to the synthetic insecticides. These results open up the possibility of using pellitorine, guineensine, pipercide, and retrofractaminde A as lead compounds for a selective mosquito larval control agent synthesis.

## Conclusion

Based upon these results and earlier findings, the potent fungicidal, insecticidal, and mosquito larvicidal activities of pipernonaline and piperoctadecalidine derived from *P. longum* L. fruit, and mosquito larvicidal activities of four isobutylamides

**Table 8. $^{1}$H-NMR and $^{13}$C-NMR of pipercide and retrofractamide A**

| No | Pipercide | | Retrofractamide A | |
|---|---|---|---|---|
| | $^{13}$C | $^{1}$H | $^{13}$C | $^{1}$H |
| 1 | 166.31 | - | 166.27 | - |
| 2 | 121.87 | 5.75 1H *d* (*J* = 14.8 Hz) | 122.23 | 5.76 1H *d* (*J* = 14.8 Hz) |
| 3 | 141.20 | 7.19 1H *dd* (*J* = 14.8, 15.0 Hz) | 141.00 | 7.19 1H *dd* (*J* = 14.8, 15.0 Hz) |
| 4 | 128.40 | 6.14 1H *d* (*J* = 15.0 Hz) | 128.80 | 6.16 1H *d* (*J* = 15.0 Hz) |
| 5 | 142.77 | 6.07 1H *d* (*J* = 15.0 Hz) | 141.75 | 6.01 1H *m* |
| 6 | 32.78 | 2.17 2H *m* | 32.85 | 2.30 2H *d* (*J* = 3.1 Hz) |
| 7 | 28.31 | 1.46 2H *m* | 32.18 | 2.31 2H *d* (*J* = 3.1 Hz) |
| 8 | 28.94 | 1.46 2H *m* | 127.70 | 5.97 1H *m* |
| 9 | 32.67 | 2.17 2H *m* | 130.18 | 6.30 1H *d* (*J* = 15.0 Hz) |
| 10 | 128.95 | 5.98 1H *d* (*J* = 15.0 Hz) | | |
| 11 | 129.55 | 6.28 1H *d* (*J* = 15.0 Hz) | | |
| 1' | 46.91 | 3.16 2H *t* (*J* = 6.4, 12.9 Hz) | 46.93 | 3.16 2H *t* (*J* = 6.4, 13.0 Hz) |
| 2' | 28.63 | 1.78 1H *m* | 28.63 | 1.79 1H *m* |
| 3' | 20.12 | 0.91 3H *s* or 0.93 3H *s* | 20.13 | 0.91 3H *s* or 0.93 3H *s* |
| 4' | 20.12 | 0.93 3H *s* or 0.91 3H *s* | 20.13 | 0.93 3H *s* or 0.91 3H *s* |
| NH | - | 5.49 *br s* | - | 5.52 *br s* |
| 1" | 132.34 | - | 132.08 | - |
| 2" | 105.38 | 6.89 1H *d* (*J* = 1.0 Hz) | 105.43 | 6.87 1H *s* |
| 3" | 147.92 | - | 147.94 | - |
| 4" | 146.58 | - | 146.74 | - |
| 5" | 108.22 | 6.74 1H *m* | 108.23 | 6.74 1H *m* |
| 6" | 120.23 | 6.74 1H *m* | 120.39 | 6.74 1H *m* |
| 7" | 100.91 | 5.93 2H *s* | 100.95 | 5.93 2H *s* |

identified in *P. nigrum* L. fruit may be valuable to the development of insect control agents. Further studies on the fungicidal, insecticidal, and mosquito larvicidal mode of actions of Piperaceae species fruit-derived compounds, their effects on non-target organisms and the environment, and formulations for improving the potency and stability are needed for their practical use as naturally occurring fungi, insect, and mosquito larval control agents. In addition, the wound-healing responses to the extracts of Piperaceae plants should be evaluated in the event that remedies that incorporate Piperaceae species also act to enhance the host natural defenses.

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