Efficacy of *Piper* (Piperaceae) Extracts for Control of Common Home and Garden Insect Pests

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ABSTRACT   Extracts from three species of the plant family Piperaceae, *Piper nigrum* [L.], *Piper guineense* [Schum & Thonn], and *Piper tuberculatum* [Jacq.], were tested for efficacy against insects from five orders. All three species contain isobutyl amides, plant secondary compounds that act as neurotoxins in insects. These materials are considered safe to mammals because *Piper* spp. were used for centuries for spice and medicinal purposes. When 24-h *P. nigrum* LC_{50} values were compared between common insect pests from eastern Canada and the northeastern United States, the most sensitive species in order of increasing lethal concentration were eastern tent caterpillar, *Malacosoma americanum* (F.) < European pine sawfly larvae, *Neodiprion sertifer* (Geoffroy) < spindle ermine moth larvae, *Yponomeuta cagnagella* [Hübner] < viburnum leaf beetle larvae, *Pyrrhalta viburni* [Paykull] < stripped cucumber beetle adults, *Acalymma vittatum* (F.) < Colorado potato beetle adults, *Leptinotarsa decemlineata* (Say) < Japanese beetle adults, *Popillia japonica* [Newman] < hairy chinch bug, *Blissus leucopterus hirtis* [Montandon]. The life stage tested was the point at which each species causes the greatest amount of damage to the host plant and the point at which most gardeners would likely choose to treat with a conventional synthetic insecticide. Greenhouse trials revealed that the pepper formulations also had a repellent activity, thus protecting plant leaves from 1) herbivory (lily leaf beetle, *Lilioceris lilii* [Scopoli], adults and larvae and stripped cucumber beetle adults) and 2) oviposition [European corn borer, *Ostrinia nubilalis* (Hübner)]. Combinations with other botanical extracts were additive at best in toxicity and repellent trials. Nontarget toxicity to beneficial invertebrates is a possibility because the *P. nigrum* LC_{50} for beneficial ladybird beetles was 0.2%. *P. nigrum* extracts can provide a reasonable level of control against lepidopteran and European pine sawfly larvae and also will work as a short-term repellent and feeding deterrent. It is recommended that the use of *Piper* extracts be restricted to small-scale spot treatments in residential areas where insect pest outbreaks have occurred.

KEY WORDS   Piperaceae, *Piper nigrum*, piperamides, efficacy, repellent effect

BIOPESTICIDES OF PLANT ORIGIN were reviewed recently (Regnault-Roger et al. 2002), and it was concluded that botanicals have considerable market potential as reduced risk control agents. In addition, the National Research Council (2000) in the United States recommended a number of areas where botanicals meet current and future requirements for alternative pest control. Lydon and Duke (1989), Isman (1994), and MacKinnon et al. (1997) surveyed several plant families that show promise as sources of new botanical insecticides. Members of the pepper family Piperaceae produce phytochemicals with insecticidal activity. The most widely recognized species are black pepper, *Piper nigrum* L., and African Guinea pepper, *Piper guineense* Schum & Thonn, but many other species in the family are also insecticidal (Bernard et al. 1995).

Early investigations with *P. nigrum* extracts indicated that isobutyl amides were responsible for the toxicity of the extracts to the adzuki bean weevil, *Callosobruchus chinensis* L. (Miyakado et al. 1979, 1980). Three of the isobutyl amides isolated from *P. nigrum*, pipercide, pellitorine, and piperine, were toxic at 0.15, 2, and 20 µg/male *C. chinensis*, respectively (Dev and Koul 1997). Guineensine, isolated from *P. guineense*, had similar activity to pipercide when tested topically on the cowpea weevil, *Callosobruchus maculatus* F. (0.25 versus 0.84 µg/male 48-h LD_{50} values, respectively). Pepper extracts containing mixtures of isobutyl amides are also highly effective (Scott et al. 2002).

Essential oils of *P. nigrum* were found to effectively protect stored wheat from the stored grain pests *Sitophilus oryzae* L. and *Rhyzopertha dominica* F. at con-

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centrations >100 mg/liter for up to 30 d (Sighamony et al. 1986). Stored beans were protected from the bruchid *Acanthoscelides obtectus* Say by ground black pepper for up to 18 wk (Baier and Webster 1992). *P. guineense*-treated kaolin powder at 150 μl/g reduced the average adult emergence of *C. maculatus* by 100% after 30-d treatment (Kéïta et al. 2000). Dust and ether-extract formulations of *P. guineense* also were effective at controlling *C. maculatus*, at concentrations between 0.5 and 0.75 g/20 g cow pea seed within 36 h after treatment (Mbata et al. 1995). Emergence of adults from treated eggs was prevented successfully with dust and oil treatments at 0.25 g/seed.

The efficacy of *Piper* extracts against a few other insect pests also has been demonstrated. The termite *Macrotermes nigeriensis* Sjostedti was controlled with a 5% aqueous solution of *P. guineense* applied topically (Ivbijaro et al. 1993). Javier and Morallo-Rejesus (1986) determined that semipurified organic solvent extracts of *P. nigrum* were more toxic than crude extracts against the house fly, *Musca domestica* L.; cutworm *Spodoptera litura* F.; black armyworm, *Spodoptera exempta* (Walker); and diamondback moth, *Plutella xylostella* (L.). Ewete et al. (1996) showed that *P. guineense* incorporated into the diet of the European corn borer, *Ostrinia nubilalis* (Hübner), at 300 mg/liter reduced larval growth by 27% and increased the time to adult emergence and reduced egg production at concentrations >10 mg/liter. In terms of nontarget effects, aqueous mixtures of oven-dried and powdered *P. guineense* at 10 mg/liter were found to be effective at controlling fourth instars of mosquito *Aedes aegypti* L., but they were not toxic to other aquatic organisms (Okorie and Ogunro 1992).

Other species of *Piper* that also contain piperamides include long pepper, *Piper longum* L. from South Asia and *Piper tuberculatum* Jacq. from South and Central America. The latter is of particular interest because of high concentrations of piperamides in the leaves (Scott et al. 2002). Along with *P. nigrum* and *P. guineense*, *P. tuberculatum* may offer the most promising and active pepper extract available for the development of a commercial insect control product due to its long traditional use, low associated health and environmental risk, and the relative abundance of material.

Most previous evaluations have focused on major crop and nuisance pests, and little research has been undertaken on insects of the home and garden where a botanical product might gain ready acceptance. We developed a practical formulation that was evaluated against target insect species that are of concern to gardeners and horticulturalists in eastern Canada and the northeastern United States and were selected for use in efficacy trials based on their abundance and availability. These include insects from the orders 1) Coleoptera [Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Chrysomelidae); Japanese beetle, *Popillia japonica* Newman (Scarabaeidae); lily leaf beetle, *Lilioceris lilii* Scopoli (Chrysomelidae); striped cucumber beetle, *Acalymma vittatum* (F.) (Chrysomelidae); and viburnum leaf beetle, *Pyrrhalta*

viburni Paykull (Chrysomelidae)]; 2) Dermaptera [earwig *Forficula auricularia* L. (Forficulidae)]; 3) Hemiptera [hairy chinch bug, *Blissus leucopterus hirtus* Montandon (Lygaeidae)]; 4) Hymenoptera [European pine sawfly, *Neodiprion sertifer* (Geoffroy) (Diprionidae)]; and 5) Lepidoptera [European corn borer (Pyralidae); spindle ermine moth, *Yponomeuta cagnagella* Hübner (Yponomeutidae); and eastern tent caterpillar, *Malacosoma americanum* Fabricius (Lasiocampidae)].

Nontarget toxicity also was evaluated using an insect species that would likely encounter the effects of the botanical treatment. Applications in the garden could affect the ladybird beetle *Hippodamia convergens* (Guérin-Méneville) (Coccinellidae) as beneficials or predators of garden insect pests.

The target insect pests and nontarget invertebrates were treated with *Piper* extract formulations to achieve the following objectives: 1) establish concentration levels for the target insects and toxicity values to protect the nontarget invertebrate; 2) evaluate knockdown, repellent action, and feeding deterrent effect under controlled treatment conditions; and 3) determine residue levels for *Piper* active compounds on contact surfaces. The overall objective was to provide an in-depth evaluation of the efficacy, toxicity, and environmental fate of pepper extracts.

Materials and Methods

Piper Extracts and Commercial Botanical Formulations

Seed material for both *P. nigrum* and *P. guineense* were purchased from commercial suppliers in Canada, the United States, and Togo, West Africa. Leaves of *P. tuberculatum* were collected in Costa Rica by Pablo Sanchez and Luis Poveda near San Carlos. Voucher specimens have been placed in the University of Ottawa and the Universidad Nacional (Heredia, Costa Rica) herbaria. *P. nigrum* and *P. guineense* peppercorns and *P. tuberculatum* leaf material were ground and the active constituents extracted following the methods described in Scott et al. (2002). Natural solvents and emulsifiers were incorporated into the formulation to reduce the risk of toxicity to the applicator and the environment. The *Piper* extracts were formulated by R. Bradbury (EcoSafe Natural Products, Saanichton, British Columbia, Canada) as follows: 20% extract, 70% tetrahydrofurfuryl alcohol (THFA; Penn Specialty Chemicals, Memphis, TN), and 10% emulsifier (Alkamuls EL-719 ethoxylated castor oil, a gift of Rhodia, Cranbury, NJ). The piperamide concentration in the extracts and formulations was analyzed based on the methods of Scott et al. (2002)). High-performance liquid chromatography (HPLC) analysis was conducted using a Prostar model pump, model 330 UV/Vis, photodiode array detector and model 410 autosampler (Varian Chromatography Systems, Walnut Creek, CA).

Other botanical extracts were obtained to act as synergists or to compare with the insecticidal and

repellent activity of the *Piper* extracts. These included Central American *Cedrela odorata* L. (Meliaceae) extract; dillapiol, 95% dill oil from *Anethum sawa* Roxb. (Umbelliferae); neem oil *Azadiracta indica* Juss (Meliaceae) (Ahimsa Alternative Inc., Oklahoma City, OK); Garlic Barrier concentrated garlic *Allium sativum* L. (Alliaceae) (a gift of Garlic Barrier Labs, Glendale, CA), and Ropel, lemon grass oil *Cymbopogon citratus* [DC] Stapf. (Poaceae) (Burlington Scientific, Farmingdale, NY). All commercial products were applied at rates recommended by the manufacturer.

Effect of Sunlight and UV Radiation Exposure to *Piper* Extracts

The effect of sunlight and UV radiation on exposed piperamides either alone or in *Piper* extracts was assessed following the methods described in Scott et al. 2003. Degradation of piperamides exposed to full-sunlight conditions was determined by placing 50-μl aliquots of 20% extract on glass microscope slides and allowing them to dry overnight on the bench top. The slides were then exposed to full sunlight for 6 h during peak daylight hours. Readings of solar radiation were taken at 3-h intervals. The glass slides were then rinsed with 5 ml of 99% ethanol to wash off the extract residue. Treated control slides not exposed to full sunlight were rinsed using the same method. The ethanol solutions (1 ml) were then filtered through a 0.2-μm polypropylene filter and placed in a 1.5-ml HPLC vial in preparation for analysis. Analysis was conducted according to Scott et al. (2002); however, a Varian Prostar model pump, model 330 UV/Vis, photodiode array detector and model 410 autosampler, and a Varian reverse-phase C18 column (Varian Chromatography Systems), were used. The compounds were eluted with a binary gradient of acetonitrile and water, where acetonitrile was increased from 30 to 90% in 12 min, as described by Scott et al. (2002). Solar radiation readings were taken at each time period with a Li-Cor Quantum model LI-192SB sensor (Li-Cor, Omaha, NE). The UV light was an Industrial F20T12/BLB blacklight blue lamp that emits in the near UV, 315–400 nm. The lamp has a deep blue filter to absorb visible light and transmit near UV at a 3.7-W output.

Insect Species Collected or Cultured

Coleoptera. *P. japonica* adults were collected at one site in Ottawa, Ontario, Canada, in August 2001 and 2002. Adults were kept at 10°C and a photoperiod 16:8 (L:D) h and fed crab apple, *Malus sylvestris* L. Miller (Rosaceae), or mountain ash, *Sorbus americana* Marsh. (Rosaceae), leaves until bioassays were initiated. *L. lilii* eggs and adults were collected from several gardens in May and June 2001 and 2002 and at the Central Experimental Farm in Ottawa during June and July 2001 and May 2002. Adults were kept at 10°C and a photoperiod of 16:8 (L:D) h until bioassays were initiated or kept on Asiatic lily plants (Liliaceae) in the greenhouse to produce eggs. Larvae were reared on

plants until bioassays were initiated. *P. viburni* larvae were collected from the Kemptville Agricultural College Campus, Kemptville, Ontario, and The Log Farm, Ottawa, Ontario, in May 2002. Larvae were kept in containers with fresh European highbush cranberry, *Viburnum opulus* L. (Caprifoliaceae), leaves at 10°C and a photoperiod of 16:8 (L:D) h until bioassays were initiated. *A. vittatum* adults were collected from the Gloucester allotment gardens in August 2002, in Ottawa and kept at 10°C and a photoperiod of 16:8 (L:D) h and fed cucumber slices until bioassays were initiated. Wild-type adult *L. decemlineata* were collected from organic potato fields, Hatley, Québec, and urban allotment gardens having pesticide use restrictions, Ottawa, Ontario, in July 2002.

Dermaptera. *F. auricularia* were collected from a garden site in Ottawa, Ontario, in July 2001. The earwigs were fed carrot and apple slices and were at held at 10°C and a photoperiod of 16:8 (L:D) h until the test was initiated.

Hemiptera. *B. leucopterus hirtus* nymphs and adults were collected in residential turf in Gatineau, Québec, in August 2002. The chinch bugs were kept in a mixture of soil and grass mulch at 10°C until tests were initiated.

Hymenoptera. *N. sertifer* larvae were collected from Mugo pine, *Pinus mugo* variety Mugo (Pinaceae), trees near Almonte and Pakenham, Ontario, in May 2002. The larvae were fed freshly cut Mugo pine boughs and were held at 10°C and a photoperiod of 16:8 (L:D) h until bioassays were initiated.

Lepidoptera. *M. americanum* larvae were collected near Almonte and Pakenham, Ontario, in May 2002. Larvae were kept at 10°C and fed *M. sylvestris* leaves until trials began. Larvae were separated into instars, and bioassays were conducted with the different age classes where numbers permitted. *Y. cagnagella* were collected from the Kemptville Agricultural College Campus, Kemptville, Ontario, once in June 2001 and twice in May 2002. The individuals collected in June 2001 at the prepupal, nonfeeding larval stage, whereas the earlier larval stages were fed burning bush, *Euonymus alatus* Thunb. Siebold variety Compactus (Celastraceae). The larvae were kept at 10°C and a photoperiod of 16:8 (L:D) h until trials began. *O. nubilalis* larvae and adults were obtained from the University of Ottawa culture. Second instars were selected 6 d after eggs hatched and the adults within 24 h of emerging from the pupal stage.

Voucher specimens for all insects collected and used in efficacy bioassays were placed in the Insect Biosystematics collection, Eastern Cereal and Oilseed Research Centre (ECORC), Agriculture and Agri-Food Canada, Ottawa, Ontario.

Bioassays for Assessing Insecticidal and Repellent Effects

Twenty-four-hour LC$_{50}$ Determination. With the exception of *B. leucopterus hirtus* adults, *F. auricularia* adults, *N. sertifer* larvae, and *Y. cagnagella* larvae, all toxicity trials were conducted by spraying to runoff on

individual host plant leaves allowed to dry at room temperature for 30 min. Test insect larvae were then treated by spray to drip, patted dry, and placed on the leaves with the same treatment inside a glass petri plate or 500-ml Mason jar. Each species was treated with six *P. nigrum* concentration levels, including a formulation blank, based upon the results of a range-finding test. Ten larvae were used per two to three leaves, and each treatment was replicated at least two times where numbers permitted. Test containers were kept at room temperature. The LC_{50} value refers to mortality after 24 h, determined by touching the larvae with a probe to elicit a response.

Dermaptera. *F. auricularia* were sprayed to runoff with solutions of *P. nigrum* and *P. guineense* extracts at concentrations of 0.1, 0.5, and 1% and then placed individually in plastic cups, 10 replicates per treatment. Controls consisted of water only and a formulation blank consisted of a 5% formulation mixture in water. Mortality was observed after 24 h.

Hemiptera. *B. leucopterus hirtus* adults were treated with the spray to drip method but were then placed in petri plates with Whatman filter paper. Ten chinch bugs were used per treatment with two and three replicates for the first and second trials, respectively. Six *P. nigrum* concentration levels were tested, including a formulation blank or emulsifiable concentrate (EC), based upon the results of a range-finding test. After 24 h, the number of dead adults on the filter paper was determined by touching the chinch bug with a probe to elicit a response.

Hymenoptera. *N. sertifer* larvae were separated into instars, and bioassays were conducted with the fourth or fifth instars where numbers permitted. *N. sertifer* larvae were placed onto the tips of needles of *P. mugo* boughs freshly cut and then were sprayed to runoff with the same range of *P. nigrum* concentrations. The boughs were then placed inside a 500-ml Mason jar to allow them to remain upright and covered with a mesh lid. After 24 h, the number of dead or moribund larvae at the bottom of the jars was determined.

Lepidoptera. Prepupal *Y. cagnagella* larvae collected in 2001 were treated with control (water only), 1% formulation blank, and *P. nigrum* at 0.01, 0.05, and 0.1%. Larvae were dipped into the solutions and then placed in a covered petri plate. Ten larvae per replicate were used, with three replicates per treatment. Survival of larvae was noted 48 h after treatment, and then the number of adults that emerged successfully within the following 2-wk period.

Repellent, Antifeedant, and Oviposition Deterrent Effects

All Piper spp. repellent trials were conducted in the greenhouse at Carleton University.

Coleoptera. *P. japonica* adults were caged on Explorer rose plants (Rosaceae) variety Louis Jolliet to determine the repellent effect of *P. nigrum* by comparing damage to leaves caused by feeding. Treated plants were sprayed either with 100 ml of water only, or water combined with 2.5% formulation blank or

0.5% *P. nigrum.* Each treatment was replicated three times with 20 *P. japonica* per plant. Each plant was individually caged with a wire frame and mesh covering. The number of leaves on each plant was standardized by removing all but 10 undamaged leaves per plant. A leaf was considered damaged if *L. decemlineata* feeding caused partial or complete surface area loss compared from the start of the trial. The plants were checked visually for damage to leaves after 96 h.

Prepupal *L. lilii* larvae and adults were tested using potted Asiatic lily plants treated with *P. nigrum* extracts. Asiatic *Lilium* (Liliaceae) were purchased in the spring as bulbs, Orange Pixie, Butter Pixie, and Latoya varieties, or as plants, Cancun, Orange Pixie, Butter Pixie, and Lennox varieties, and were treated with formulated *P. nigrum* extract in the range of 0.125, 0.25, 0.5, and 1%. At each concentration level three replicate plants were treated along with three replicate controls: a formulation blank of equal EC concentration. Ten prepupal *L. lilii* larvae were placed on each plant, which was checked after 24 h to assess larval mortality, movement from the treated leaves, and feeding damage to leaves. Adults also were placed in three large cages where three plants per cage were treated either with 1% *P. nigrum*, neem oil, or Ropel. The choice test was checked after 96 h to assess damage to leaves from feeding as described previously.

A choice test using cucumber plants, *Cucumis* spp. (Cucurbitaceae), variety Bush Pickle, was conducted where plants were sprayed to runoff with either 0.1 or 0.5% *P. nigrum* extract and placed in a cage with both a water and formulation blank control. Sixty *A. vittatum* adults were released in each of three replicate cages. Each plant was surrounded with a plastic collar, which was capped at the end of 96 h to remove each plant but not lose the insects feeding on it. The plants were then cooled to 10°C so that the number of adult beetles on each plant could be collected and the number of damaged leaves counted.

Lepidoptera. Green pepper plants, *Capsicum annum* L. (Solanaceae), were grown to the early fruiting stage and then chosen on the basis of damage-free fruits. Ten plants per treatment were sprayed with either *C. odorata* extract plus dillapiol, 95% dill oil from *A. sawa* (1:1 for the highest concentration and 0.6:1 for the lowest, due to availability of extract) at 0.1 and 0.03% extract or *P. tuberculatum* extract at 0.1 and 0.05%. The control plants were sprayed with 4:1 95% ethanol:distilled water. The following day, the plants were hand infested with three second instars of *O. nubilalis* larvae plus one egg mass. On the third day, the plants were resprayed. After 11 d, the fruits and plants were tallied for surviving larvae.

O. nubilalis adults were collected within 24 h of emerging from the pupal stage. Five female and five male adults were aspirated into a 500-ml flask. Green pepper plants at the mature fruiting stage were treated with either water, formulation blank, 0.5% *P. guineense* extract, Ropel or Garlic Barrier, both at the recommended application rates. Each plant was sprayed until runoff (≈100 ml), with four replicates for the control-, EC blank-, and *P. guineense*-treated plants

Table 1.   Range of piperamide concentrations (milligrams per gram) in *P. nigrum*, *P. guineense*, and *P. tuberculatum* extracts used in efficacy trials 2001 and 2002

Extract	Year	Dihydropiper longuminine	Piperlon guminine	Dihydropiperine	Piperine	Total
P. nigrum	2002	0.4	1.9–2.9	17.7–22	370–427	391–451
	2001	0.5–1.8	0.9–5.1	10.4–23.7	288–514	299–538
P. guineense	2002	31.2	28.1	84.4	53.5	197
	2001	3.6–8	2.7–7.7	9.8–29.9	33.4–68.8	49–114
P. tuber	2002	59–65.5	2.7–7.5	6–15.5	4.7–13.5	72–102

and three for the remaining treatments. Plants were left to dry and then caged by enclosing the plants with a metal frame and mesh net. The adult *O. nubilalis* were added to the cages and left in the greenhouse for 96 h. The cages were reopened and the surviving adults, the number of leaves with egg masses, and the total number of egg masses were counted.

Nontarget Invertebrates

Ladybird Beetles. Adult *H. convergens* were purchased from Biobest, Leamington, Ontario, through Plant Products, Brampton, Ontario. The adults were kept at 4°C and were not fed before or during the test. All *H. convergens* tests were initiated within 2 wk of obtaining the adults. The toxicity test procedure followed that previously described for the 24-h LC_{50} determination. Adults were dipped into *P. nigrum* solutions ranging from 0.01 to 1%, patted dry on filter paper, and then placed into a plastic petri plate with Whatman #1 filter paper. Ten *H. convergens* were treated per plate with three replicate plates per concentration.

Statistics

Probit analysis (Hubert and Carter 1990a) was used to determine the LC_{50} values for *P. nigrum.* Comparison of the LC_{50} values between trials for each species tested was conducted using a χ^2 or Z-test (Hubert and Carter 1990b). Results of all other bioassays were tested for normality, and the data were transformed if necessary. A one-way analysis of variance was performed with a post hoc Tukey's multiple comparison of means test (SYSTAT 1999).

Results

Pepper Formulation Development

Black pepper extracts contained large amounts of piperine and smaller amounts of piperlonguminine, dihydro-piperine, and dihydro-piperlonguminine (Table 1). The four piperamides from each batch were totalled to provide an estimate of the total amide content, although a few minor compounds were not quantified. The total ranged from 299 to 588 mg/g in *P. nigrum*, from 49 to 197 mg/g in *P. guineense*, and from 72.4 to 102 in *P. tuberculatum.* Piperamide levels from *P. nigrum* batches prepared for the efficacy trials in both 2001 and 2002 were found to have an overlapping range of concentrations, whereas the *P. guineense* ex-

tracts used in 2002 had higher piperamide levels than in 2001, probably due to a difference in the source of the peppercorns.

Tests with different formulation components indicated that 1) the three pepper extracts were soluble in ethanol and THFA, and 2) the two emulsifiers selected, Jeneil Co. biosurfactant JBR-325 and -425, and Rhone-Poulenc castor oil Alkamus EL-719, rated consistently high in terms of emulsion bloom in water, emulsion suspensibility, and spreading area. THFA was chosen over ethanol based on the lower flashpoint and potential for registration, and Alkamus EL-719, a synthetic ethoxylated castor oil, was preferred over JBR-425, a natural rhamnolipid, because it was less toxic in house fly toxicity trials (unpublished data). The final composition of the pepper formulation for testing was pepper extract 20%, THFA 70%, and emulsifier 10%. The formulation was diluted in water at the time of spraying to the appropriate concentration.

Effect of Sunlight and UV Radiation on *Piper* Extracts

The piperamide content of extracts was found to be stable over several months in the laboratory at room temperature, but the formulations are susceptible to photodegradation. Piperine in the extracts degraded quickly after exposure to sunlight (Fig. 1). Pure piperine also degraded quickly under UV lamp exposure with a half-life of $\approx$39 min, suggesting that the degradation was a direct photolysis, not a photosensitized reaction from some pepper pigment. After 6-h exposure to sunlight, all amides in the *P. guineense* extract, including piperine ($t_{0.5}$ = 49 min), were below detection (data not shown). Light peak light levels were measured between 1,230 and 1,410 μEinsteins m^{-2} s^{-1}.

Insecticidal Activity

Accurate LC_{50} values for the *P. nigrum* formulation were obtained with seven selected urban insect pest species and one beneficial insect (Tables 2 and 3). In general, the extracts were more toxic to larval insects where LC_{50} values ranged from 0.018 to 0.103% (Table 2) than adult insects, where LC_{50} values ranged from 0.103 to 0.746% (Table 3). Lepidoptera and one hymenopteran species were more susceptible (0.018–0.075%) than Coleoptera or the one Hemiptera (0.103–0.746%) to the *P. nigrum* extracts. No direct comparisons were made between life stages for the
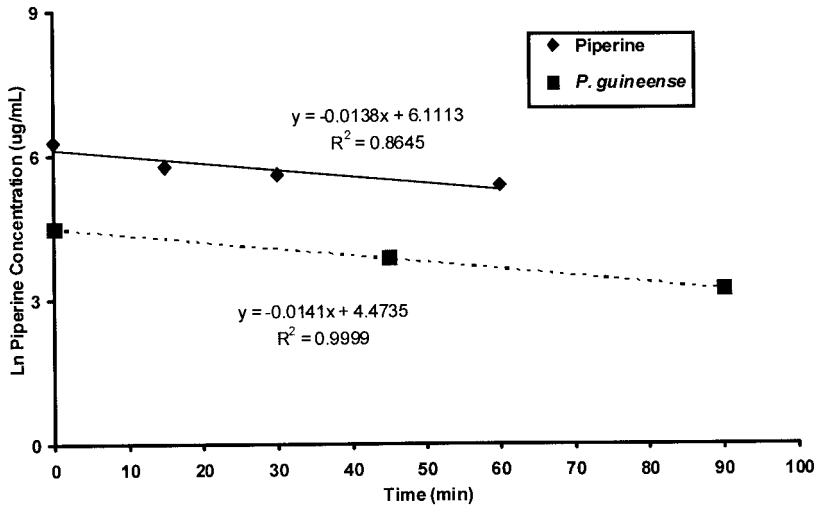
Fig. 1. Natural log of piperine concentration after exposure of *P. guineense* extract (dashed line) to full sunlight and piperine to UV radiation (solid line) over 1.5 h.

same insect species in this study; however, it was observed previously that sensitivity to *P. nigrum* extracts was less for Colorado potato beetle late instars and adults compared with early instars (Scott et al. 2003).

Although LC_{50} data were not available, toxicity assessments at several concentrations were made for other insects. Survival of adult earwigs *F. auricularia* decreased significantly at 0.5% and 1% *P. nigrum* ($F = 14.7$; df $= 7, 16$; $P < 0.008$) and 1% *P. guineense* (Tukey's multiple range test, $P = 0.001$) after 24 h (Fig. 2). These latter results also show comparable insecticidal activity for both the African and black pepper. *P. nigrum* extracts up to 0.1% did not reduce late instar, prepupal *Y. cagnagella* survival significantly after 48 h ($F = 13.712$; df $= 3, 8$; $P = 0.855$), but effects were manifested later in the life cycle where 0.01 and 0.1% *P. nigrum* reduced survival to adult emergence significantly (Tukey's multiple range test, $P = 0.058$ and 0.004, respectively) (Fig. 3).

Repellent, Antifeedant, and Oviposition Deterrent Effects

Coleoptera. Behavior modification effects (repellent and antifeedant effects) of pepper formulations were clearly evident with some situations, but not others. In a no-choice experimental design with *L. lilii* larvae, fewer larvae remained on treated lily plants (expressed as mobile insects) and fewer damaged leaves were observed ($F = 11.189$; df $= 15, 32$; $P < 0.001$) compared with controls as the *P. nigrum* dose was increased from 0.125 to 0.5% (Fig. 4). These two concentration-dependent relations were significant ($P = 0.05$) with $R^2 = 0.86$ for leaves damaged and $R^2 = 0.94$ for insect mobility. Pepper treatment also reduced the survival of all exposed larval insects ($F = 13.223$; df $= 3, 8$; $P = 0.002$) but not the number of dead or moribund larvae after 24 h ($F = 18.073$; df $= 3, 8$; $P = 0.131$).

Table 2. *P. nigrum* LC_{50} values, 95% confidence intervals, and slope of probit lines for selected insect larvae

Insect	Order: Family	n	*P. nigrum* LC_{50} (%)	95% C.I. (%)	Slope
Eastern tent caterpillar	Lepidoptera: Lasiocampidae	358	0.018	0.015, 0.022	2.84
European pine sawfly	Hymenoptera: Diprionidae	354	0.046	0.04, 0.054	2.72
Spindle ermine moth	Lepidoptera: Yponomeutidae	357	0.075	0.054, 0.124	1.15
Viburnum leaf beetle	Coleoptera: Chrysomelidae	160	0.103	0.071, 0.137	2.03

Table 3. *P. nigrum* LC_{50} values, 95% confidence intervals, and slope of probit lines for selected insect adults

| Insect	Order: Family	n	*P. nigrum* LC_{50} (%)	95% C.I. (%)	Slope
Striped cucumber beetle	Coleoptera: Chrysomelidae	240	0.103	0.087, 0.13	3.16
Convergent lady beetle	Coleoptera: Coccinellidae	540	0.213	0.173, 0.276	2.03
Colorado potato beetle	Coleoptera: Chrysomelidae	300	0.498	0.363, 0.652	1.45
Japanese beetle	Coleoptera: Scarabidae	539	0.532	0.446, 0.616	2.11
Hairy chinch bug	Hemiptera: Lygaeidae	214	0.746	0.518, 1.361	1.49
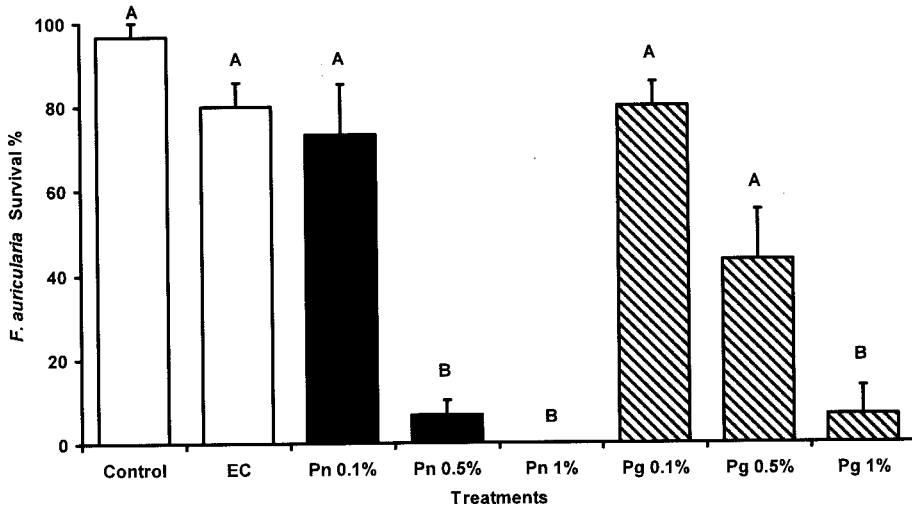
Fig. 2. Adult *F. auricularia* percentage of survival ± SE 24 h after water control; formulation control (EC); and 0.01, 0.1, and 1% *P. nigrum* (Pn) and *P. guineense* (Pg) extract treatment. The treatment means with the same letter are not significantly different (Tukey's test, $P > 0.05$).

In a choice experiment with *L. lilii* adults, *P. nigrum* at 1% did not reduce adult feeding damage after 96 h significantly compared with the formulation blank ($F = 3.542$; df $= 3, 11$; $P = 0.074$) (Fig. 5), and *P. nigrum* at 1% was not significantly more effective than either Ropel or neem oil (Tukey's multiple range test, $P > 0.108$). The 1% pepper spray caused phytotoxicity: burning of lily leaf tips, which was not observed at lower concentrations that effectively repelled larval *L. lilii*.

Rose plants sprayed with 0.5% *P. nigrum* had fewer adult *P. japonica* present after a 96-h choice test compared with water-treated (control) plants ($F = 7.57$; df $= 5, 12$; $P = 0.001$), but there was no significant difference compared with formulation-treated (EC)

plants (Tukey's multiple range test, $P = 0.243$) (Fig. 6). More importantly, there was no difference in the number of leaves damaged by the beetles between treatments (Tukey's multiple range test, $P > 0.953$).

Both 0.5 and 0.1% *P. nigrum* extracts reduced the number of *A. vittatum* adults found on cucumber plants after a 96-h choice test ($F = 8.563$; df $= 7, 16$; $P < 0.007$) (Fig. 7A and B, respectively). The *A. vittatum* adults not found on plants were considered mobile and factored into the analyses but were not shown in Fig. 7A and B. In this situation, no phytotoxicity to cucumber leaves was observed with 0.1% *P. nigrum* formulation.

Lepidoptera. The mixture of *C. odorata* and dillapiol at 0.1% had a significant impact on *O. nubilalis* larval
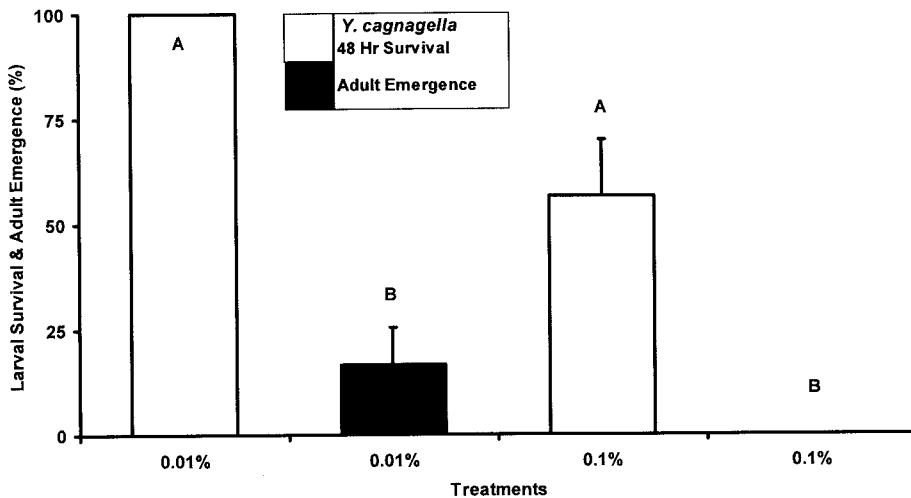

Fig. 3. Prepupal *Y. cagnagella* larvae percentage of survival and adult emergence ± SE after 0.01 and 0.1% *P. nigrum* treatment. The treatment means with the same letter are not significantly different (Tukey's test, $P > 0.05$).
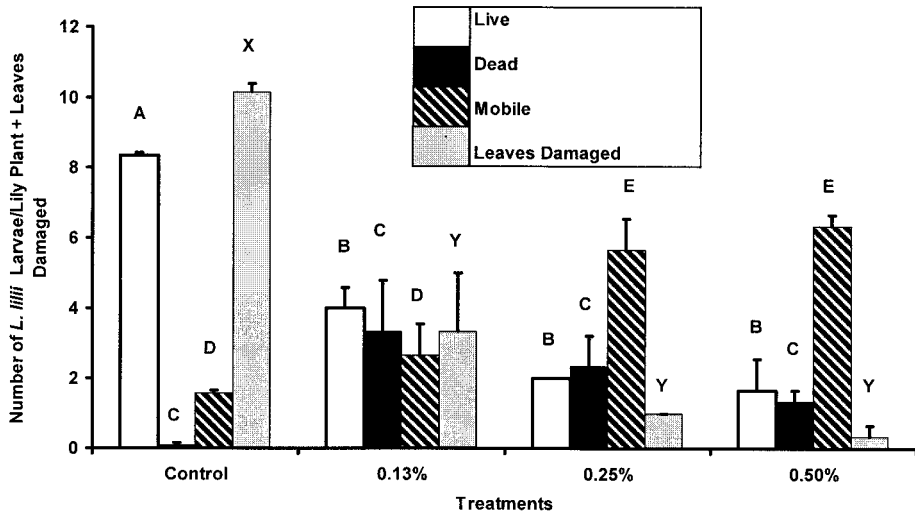
Fig. 4. Mean number of live, dead, and mobile *L. lilii* larvae ± SE on *P. nigrum*-treated Asiatic *Lilium* plants along with the mean number of damaged leaves ± SE per plant. The treatment means for survival (clear bar), mortality (dark bar), mobile *L. lilii* larvae (hatched bar), and number of damaged leaves (shaded bar) with the same letter are not significantly different (Tukey's test, $P > 0.05$).

survival ($P < 0.01$), but *P. tuberculatum* at 0.05 and 0.1% had no significant effect (Fig. 8). When higher concentrations of *P. nigrum* and *P. guineense* alone were tested, the results were similar (unpublished data). However *P. guineense* at 0.5% reduced oviposition compared with control ($F = 3.182$; df = 4, 13; $P = 0.043$) but not the formulation blank, Ropel, or Garlic Barrier ($P > 0.113$) (Fig. 9).

Nontarget Toxicity

The 24-h LC_{50} for *H. convergens* adults was 0.21% (Table 3). Within 1 h, adults treated with concentra-

tions >0.1% were knocked down, but some had recovered by 24 h in the 0.1% treatment.

Discussion

Botanical insecticides have been used for centuries for crop protection. Only with the development of synthetic insecticides in the mid-1900s did their use drop as more effective products took their place. Within a relatively short time, problems arose with the synthetic products: environmental contamination, poisonings of nontarget species, and resistance. This led many to reconsider botanical formulations as nat-
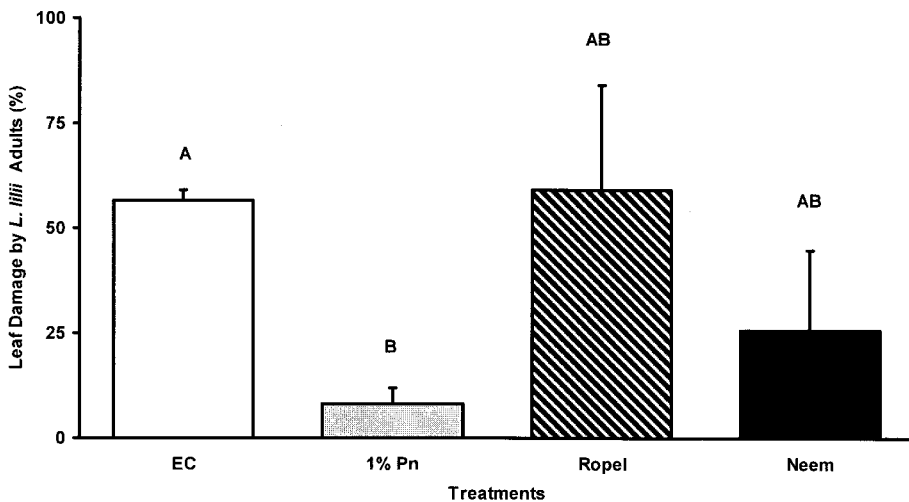
Fig. 5. Percentage of leaf damage ± SE on Asiatic *Lilium* plants by *L. lilii* adults during 96-h choice test: plants treated with either formulation control (EC), 1% *P. nigrum* (1% Pn), *C. citratus* oil (Ropel), or *Azadirachta indica* oil (neem). The treatment means with the same letter are not significantly different (Tukey's test, $P > 0.05$).
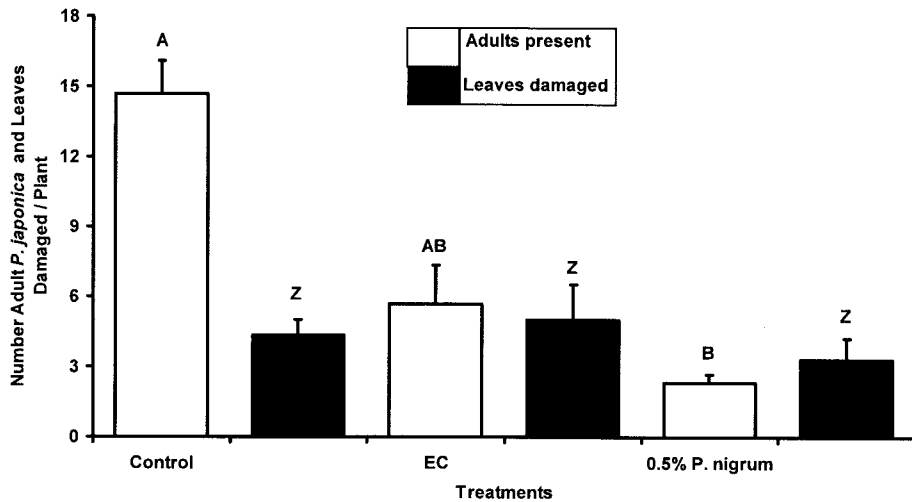
Fig. 6. Mean number of *P. japonica* adults and leaves damaged ± SE on rose plants treated with water (control), formulation control (EC), and 0.5% *P. nigrum*. The treatment means for adults/plant (clear bar) and leaves damaged (dark bar) were compared separately where the same letter indicates no significant difference (Tukey's test, $P > 0.05$).

ural alternatives because they are less toxic. However, these have always had varying degrees of success, and recently even their continued safe use has been questioned. Rotenone and pyrethrum, two of the most commonly applied by home gardeners and organic farmers, are being reevaluated by the U.S. EPA-based on concerns regarding health effects from long-term exposure (Khera et al. 1982, Betarbet et al. 2000).

Plants with an established record for culinary or medicinal use that therefore offer a safer starting material have been evaluated in terms of their potential application as insecticides. For the current study, these plant compounds were considered not as leads for synthetic insecticides, but for extract-based formulations that combine all of the co-occurring secondary plant compounds. The advantage of whole extracts over single active ingredients was demonstrated by Feng and Isman (1995), who showed that resistance development occurred with pure azadirachtin alone but not neem seed extract containing numerous compounds besides azadirachtin. From the regulatory perspective, this complicates the chemistry but simplifies the processing and allows for a unique mixture of actives. The results of the current study agree with many of the previous studies (Miyakado et al. 1979, 1980; Sighamony et al. 1986; Baier and Webster 1992; Ivbijaro et al. 1993; Mbata et al. 1995; Kéïta et al. 2000) in terms of the promising potential efficacy found in *P. nigrum* and *P. guineense* extracts.

P. nigrum extracts were found to provide excellent knockdown of the lepidopteran species and European pine sawfly tested. The larvae were sensitive to *P. nigrum* and *P. guineense* treatments <0.1% if applied as a contact insecticide, although some consumption of treated plant material cannot be discounted. Both *M. americanum* and *N. sertifer* are pests of ornamental trees and shrubs (Johnson and Lyon 1994) and thus could be controlled quickly by the homeowner when

damage by larvae becomes apparent. A spray mixed between 0.05 and 0.1% *P. nigrum* would initially knock the larvae off leaves and branches and the neurotoxic activity would prevent them from returning to the plant. Similarly, gardeners could repel *L. lilii* larvae and *Y. cagnagella* larvae from plants with 0.1% extract formulations. However, larger hard-bodied coleopterans, such as *L. decemlineata* and *P. japonica* adults, require higher doses, probably due to their relatively greater body mass and the difficulty of penetrating the thicker cuticle. A post hoc hypothesis worthy of future examination is larger larval insects are more sensitive to pepper treatments than smaller ones, regardless of insect order. This is based on the observation that the size of the larval insects (Table 4) was inversely correlated with the toxicity ($R^2 = 0.913$, $Y = -0.002X + 0.091$): the smaller *P. viburni* were less sensitive than the much larger *M. americanum* and *N. sertifer*. Although concentrations of 0.5% *P. nigrum* were required to knockdown both *P. japonica* and *L. decemlineata* adults, this is still a practical concentration for botanical insecticides.

A more suitable explanation of the greater sensitivity of lepidopterans and the European pine sawfly larvae may be greater absorption through the cuticle compared with the coleopteran larvae. Based upon visual observations the coleopteran, *P. viburni*, had a thicker, tougher cuticle. Structural difference in insect cuticle between different species has been documented as the reason for different rates of penetration (Smagghe et al. 1997, Teal et al. 1999). This was observed with cuticle preparations from the adult tobacco budworm, *Heliothis virescens* (F.), and the adult American cockroach, *Periplaneta americana* (L.). The obviously thicker cockroach cuticle had a slower penetration rate compared with the moth (Teal et al. 1999). When the toxicity between the adult coleopterans are compared, cuticle thickness may not play as
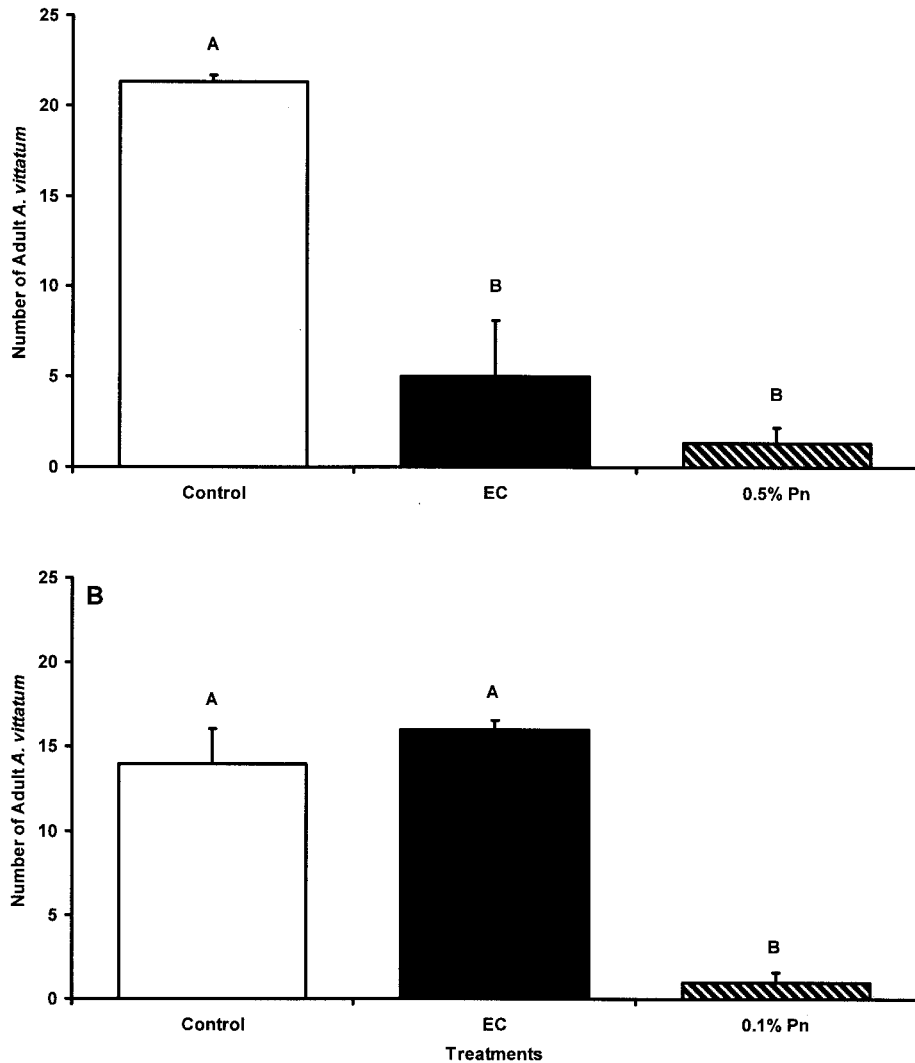
Fig. 7. Mean number of *A. vittatum* adults + SE found on cucumber plants after two 96-h choice test. (A) Plants treated with water (control), formulation control (EC), and 0.5% *P. nigrum*. (B) Plants treated with water (control), formulation blank (EC), and 0.1% *P. nigrum*. The treatment means with the same letter are not significantly different (Tukey's test, $P >$ 0.05).

great a factor as size ($R^2 = 0.967$, $Y = 0.021X + 0.03$) because the larger *P. japonica* and *L. decemlineata* required a 5 times higher dose compared with the proportionally smaller *A. vittatum* (Table 4).

The use of *Piper* extracts for both antifeedant and oviposition deterrent activity has been well documented for stored grain insects (Ivbijaro 1990, Ekesi 2000, Lale and Alaga 2001). In the current study, repellent activity by *Piper* extracts alone or in combination with other botanicals was observed against some common garden insect pests. The most promising results were seen when 0.125% *P. nigrum* treatments protected lily plants. The majority of *L. lilii* larvae moved or dropped off plants and their feeding was reduced (Fig. 4). This treatment level is consid-

ered safe for repeated daily applications because no phytotoxicity was noted at concentrations <0.5%.

In the current study, *A. vittatum* adults were deterred from cucumber plants with a 0.1% *P. nigrum* spray for a 4-d period (Fig. 7A and B). In practical application, this may prevent or reduce the infestation of the Cucurbitaceae roots and fruit by the larvae as well as the spread of the cucumber mosaic virus. Antifeedant activity also was noted for both *P. nigrum* and *P. guineense* as low as 0.01% in a *F. auricularia* feeding trial, but this was attributed as much to the formulation as to the *Piper* constituents (unpublished data). The repellent effect was confirmed when 50 mg of *P. guineense* per 30 cm^2 repelled the red flour beetle,
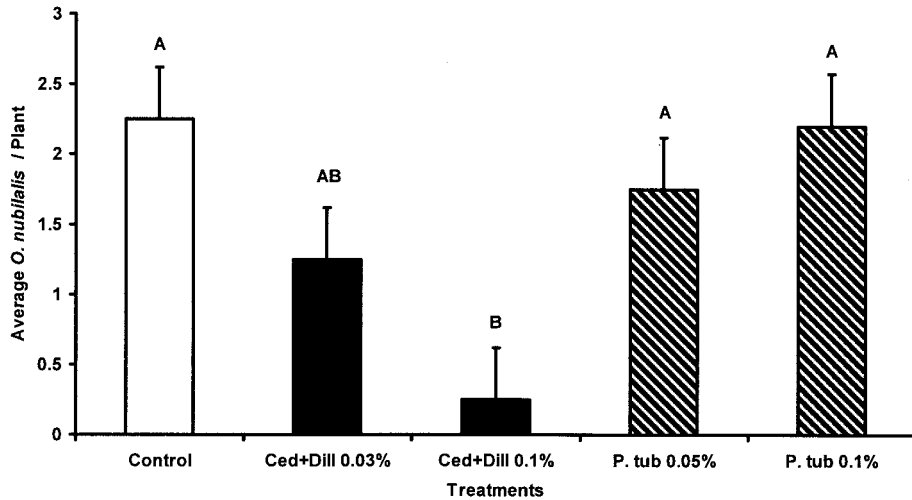
Fig. 8.   Mean number of *O. nubilalis* larvae ± SE found on bell pepper plants 11 d after treatment with water (control), combined *C. odorata* and 0.03 or 0.1% Dillapiol (Ced + Dill), and 0.1 or 0.05% *P. tuberculatum* (P. tub). The treatment means with the same letter are not significantly different (Tukey's test, $P > 0.05$).

Tribolium castaneum (Herbst), from treated paper discs (Lale and Alaga 2001).

In the current study, oviposition by *O. nubilalis* on green pepper plants was reduced compared with controls by a 0.5% *P. guineense* spray for a 4-d period under greenhouse conditions (Fig. 9). Similarly, studies with *P. guineense* and *A. sativum* were equally effective at reducing the egg hatch of the legume pod borer, *Maruca vitrata* F. (Ekesi 2000), and oviposition of *C. maculatus* was reduced by 2 and 3 ml *P. guineense*/kg cowpea seeds compared with control and neem seed oil (Ivbijaro 1990). This suggests that not only would *P. guineense* extracts reduce lepidopteran pest oviposition but also the hatching success of any eggs that were placed on treated leaves.

The repellent effect of several nonhost volatiles was tested (Held et al. 2003) to determine whether rose plants could be protected from *P. japonica*. None of the treatments designed to mask the host plant volatiles [red cedar, *Juniperus virginiana* L.; Osage orange, *Maclura pomifera* (Raf.) Schneid; ginko, *Ginkgo biloba* L.; red pepper, *Capsicum frutescens* L.; fennel seeds, *Foeniculm vulgare* Miller; and spearmint, *Mentha spicata* L.] were effective at repelling *P. japonica*. Similarly, the current study determined that *P. japonica* given a choice between *P. nigrum*-treated roses and controls would still feed on the treated plants (Fig. 6). Based upon these results *P. japonica* does not seem to be a pest insect that *Piper* extracts would affect greatly.
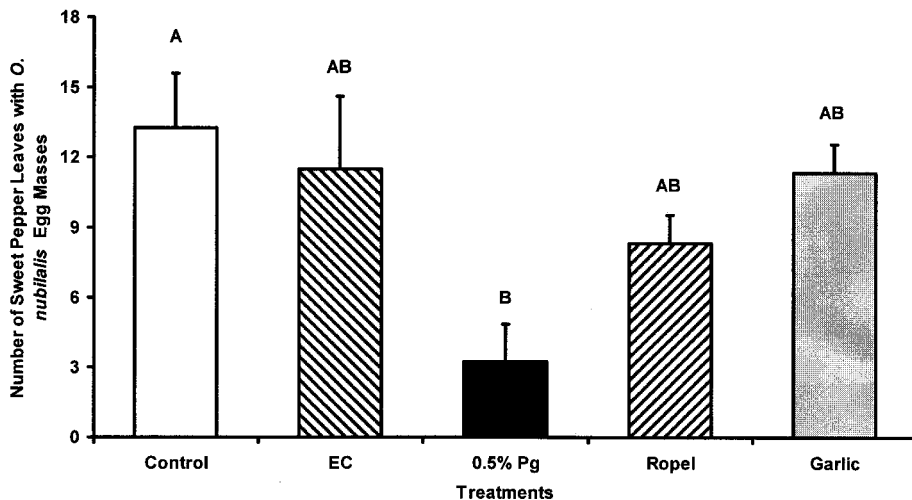

Fig. 9.   Mean number of *O. nubilalis* egg masses found on bell pepper plants 96 h after treatment with either water (control), formulation blank (EC), 0.5% *P. guineense* (0.5% Pg), *C. citratus* (Ropel), or *A. sativum* (Garlic Barrier). The treatment means with the same letter are not significantly different (Tukey's test, $P > 0.05$).

Table 4. Relationship between *P. nigrum* toxicity and insect mean dry weight

Insect	Order	LC$_{50}$ (%)	Mean wt. (mg)
M. americanum larvae	Lepidoptera	0.018	35.9
N. sertifer larvae	Hymenoptera	0.046	17.8
Y. cagnagella larvae	Lepidoptera	0.075	2.3
P. viburni larvae	Coleoptera	0.103	0.4
A. vittatum adults	Coleoptera	0.103	4.1
L. decemlineata adult	Coleoptera	0.498	20.1
P. japonica adults	Coleoptera	0.532	25.7

According to Torto et al. (1992), the most potent amide in *P. nigrum* tested against sorghum stem borer *Chilo partellus* Swinhoe is piperine. It was suggested that the methylenedioxybenzene (MDP) group common to the piperamide molecules is an important factor in antifeedant activity. In both *P. nigrum* and *P. guineense* extracts, piperine is the major amide present (Scott et al. 2002), although several other active amides are present with the MDP group.

Keeping larvae off the plants is an environmental and practical benefit of the *Piper* extracts especially because the larvicidal effects of these compounds on lepidopteran larvae feeding inside the plant are low. As was observed in this study, *P. tuberculatum* at 0.1% did not repel or effect *O. nubilalis* larvae placed on treated green peppers 1 d after spraying (Fig. 9). However, *C. odorata* was more effective as a result of the systemic activity recognized for extracts containing liminoids such as azadirachtin from *A. indica* (Gagnon 1992). When the same trial was repeated with double the concentration of *P. nigrum* and *P. guineense*, no significant effect on *O. nubilalis* survival was noted (unpublished data).

Both a disadvantage and advantage of using a *Piper* extract is the short residual activity, especially under full sunlight. This was further documented under UV radiation as evidenced in Fig. 1. It was shown that under field conditions when *P. nigrum* was applied to deter *L. decemlineata* adults from feeding on potato plants, there was a rapid loss in residual antifeedant activity within 3 h after application, corresponding to an increase in leaf damage (Scott et al. 2003). Thus, *Piper* formulations, unless prepared with a sunscreen or sunblock to extend the life of the active ingredients, will not provide an adequate deterrent unless the plants are in shaded areas. Applications could be timed for later in the day to work as repellents for night-flying or -feeding insects or possibly slugs (Mollusca: Gastropoda). This may explain why *Piper* extracts have been tested mainly against stored-product insects, because sunlight is not a factor in the degradation, and residual activity has been observed for up to 1 mo (Sighamony et al. 1986, Kéïta et al. 2000, Ashamo and Odeyemi 2001). However, the use of *Piper* extracts, primarily for their knockdown and acute toxicity, does not require the actives to remain on the plant surface for longer than the time exposed insects need to absorb them.

Another potential disadvantage is the effect upon nontarget insect species. *H. convergens* were affected by *P. nigrum* concentrations in the same range as those required to knockdown other phytophagous insect species (Tables 2 and 3). Thus, ladybird beetles, considered beneficial and popular biocontrol species, could be affected when pests are being targeted. Certainly, if *H. convergens* are sprayed directly with *P. nigrum* concentration between 0.1 and 0.5%, they will be susceptible. However, the risk to these predators exposed to homopterans is probably lessened considering that the extracts degrade quickly and ladybird beetles do not feed on treated foliage. Therefore, in an integrated pest management context, gardeners could apply the *P. nigrum* extracts to knock down the pest insect population, wait several hours for the actives to degrade under full sunlight, and then release *H. convergens*.

Recommendations for Successful Insect Control by Using *Piper* Extracts

In terms of a beneficial insect control alternative for organic growers and home gardeners, this study found that Lepidoptera and phytophagous Hymenoptera could be controlled with *P. nigrum* extracts at <0.1%. Other important garden vegetable and ornamental pests such as *L. lilii* and *P. viburni* larvae, and *A. vittatum* adults were controlled with a concentration range between 0.1 and 0.2%. *P. nigrum* extracts can knock down adult *L. decemlineata* at 0.5%; however, gardeners would need to scout the field and spot spray plants when the larvae and adults become active.

Because the half-life of piperine and other piperamides is <1 h under full sunlight, residual activity on plant surfaces is very short. Repellent effects were observed, but due to the short half-life of active components (Scott et al. 2003), daily applications would be necessary for plants exposed to full sunlight. The formulations prepared and tested indicate that knock down of insects requires only a short residual effect but care should be taken to apply them during periods of less intense sunlight. Repeated sprays at concentrations below 0.1% would not harm the plant but would not be practical for large scale agriculture. Similarly, concentrations of ≥1% have been found to be phytotoxic under greenhouse conditions; however, turfgrass was not harmed by 4% *P. nigrum* well watered after application (unpublished data). Treatments to protect shaded plants or when used indoors or in greenhouses would likely extend the residual activity and thus the antifeedant and oviposition deterrent effect observed.

Piper extracts, like other insecticides, can be hazardous unless the applicator takes precautions: use of safety glasses, nose and mouth mask, and gloves. *Piper* actives are known irritants (Sigma Aldrich 2002), but the risk to human health is much reduced because the active components have had a safe history as food additives and spices, and the odor of these extracts is familiar to most people. This, therefore, indicates that *P. nigrum* extracts will not only be beneficial as botanical insect control products but also will provide a safe alternative to conventional synthetic insecticides.

Acknowledgments

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