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A review of *Piper* spp. (Piperaceae) phytochemistry, insecticidal activity and mode of action

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Abstract The tropical plant family Piperaceae has provided many past and present civilizations with a source of diverse medicines and food grade spice. The secondary plant compounds that produce these desired qualities function also as chemical defenses for many species in the genus *Piper*. The compounds with the greatest insecticidal activity are the piperamides. Many studies have shown the effectiveness of *Piper* spp. extracts for the control of stored products pests and recently studies from our laboratory group have tested the extracts of *Piper. nigrum*, *P. guineense* and *P. tuberculatum* against insect pests of the home and garden. These results and those from investigations that examined the biochemical and molecular modes of action of the piperamides singly or in combination will be the focus of this review. The conclusions of our current work with Piperaceae are that *Piper* extracts offer a unique and useful source of biopesticide material for controlling small-scale insect out-breaks and reducing the likelihood of resistance development when applied as a synergist with other botanical insecticides such as pyrethrum.

Keywords Enzyme inhibition · Gene expression · Insecticidal activity · Piperaceae · Piperamides

Abbreviations

Kdr	Knock-down resistance
MDP	Methylenedioxyphenyl
PBO	Piperonyl butoxide
PSMO	Polysubstrate monooxygenase

Introduction

In many parts of the developed world there has been a definite shift in public opinion regarding the use of pesticides in municipal areas. There is a desire to do away with chemical control strategies for weeds, herbivorous insect pests and plant pathogens, and to find new solutions to control pests and disease in agriculture and urban areas. In Canada, concerns for human and environmental health have led to the banning or severely restricting of synthetic insecticide use in municipal areas. Such legislation has been upheld by a federal judicial decision (Supreme Court of Canada 2001) and the federal agency that regulates pesticides is developing a low risk, non-conventional pesticide

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initiative (P.M.R.A. 2006). In the United States, regulatory changes have led to streamlining of pesticide registration processes to favour low-risk products (E.P.A. 2006), which include products that are Generally Regarded As Safe (G.R.A.S.) and allow biopesticides and botanicals as a category different from conventional pesticides. Furthermore, these products are recognized for use in agricultural produce grown under certified organic practices where synthetic pesticides are not allowed.

In assessing current and future requirements for alternatives for pest control (National Research Council 2000), botanical products are prominent. Over the past two decades surveys of plant families (Lydon and Duke 1989; Isman 1994; MacKinnon et al. 1997; Regnault-Roger et al. 2002) have promoted sources for new botanical insecticides that could possibly meet some of this demand. Piperaceae is one family that has many promising phytochemicals with insecticidal activity (Arnason et al. 2002), the most recognized insecticidal compounds being from *Piper nigrum* L., *P. guineense* Schum and Thonn and *P. tuberculatum* Jacq.. The unique *Piper* secondary plant compounds have several modes of action including contact toxicity (Scott et al. 2004, 2005a), synergism (Scott et al. 2002, 2003; Jensen et al. 2006b), repellent and antifeedant (Scott et al. 2004) properties.

This review will detail the recent findings that signify the potential for Piperaceae in the development of a botanical formulation as a reduced risk biopesticide.

The Piperaceae

The Piperaceae family is considered to be among the most archaic of pan-tropical flowering plants (Burger 1971). The genus *Piper* contains approximately 1,000 species of herbs, shrubs, small trees and hanging vines. Several *Piper* spp. from India, southeast Asia and Africa are of economic importance since they are used as spices and traditional medicines (Simpson and Ogorzaly 1995). Many plant families have a global distribution, but few have the rich ethnobotanical and ethnopharmaceutical history of Piperaceae. Since the latter has been used for centuries both medicinally and as a food additive, the associated health risk to humans is generally regarded as low (Tripathi et al. 1996; Parmar et al. 1997). The U.S. Food and Drug Administration (F.D.A.) considers black pepper as a food grade spice categorized as G.R.A.S. (CITE:21CFR182.10).

As a spice, black pepper has been traded world-wide for many centuries and represents a highly important cash crop for many tropical countries including India, Indonesia, Vietnam, Malaysia and Brazil (Simpson and Ogorzaly 1995). The unripe fruit is the source of black pepper, while the ripened fruit is the source of white pepper (Parmar et al. 1997). The most familiar medicinal Piperaceae in Africa is the Ashanti or Guinea pepper, *P. guineense* (Iwu 1993). The leaves and fruits are used as a cough remedy and the seeds for treating stomach-aches. The Piperaceae from tropical America have the same aromaticity as those of Indian origin and have also found their way into folk-medicine. Many *Piper* spp. are represented, as well as the genera *Lepianthes* (Rafinesque) and *Peperomia* (Ruiz et Pavón), among the plants that have documented medicinal use (Shultes and Raffauf 1990). Various classifications of use include abortifactants, antibiotic, arrow or fish poisons, diuretic, toothache remedy, tobacco snuff substitute and insect repellant. A recent ethnobotanical study by our group shows a large number of species used for treatment of anxiety and epilepsy (Bourbonnais-Spear et al. 2005).

The anti-bacterial and fever-reducing activities of *Piper* extracts are well known from ancient Asian medicinal practices in South Asia as well as in other parts of the world. The compounds providing the medicinal properties are also important chemical defenses for the plants in order to repel insects, nematodes and fungal pathogens (Evans et al. 1984; Parmar et al. 1997; Lee et al. 2001).

Phytochemistry

The phytochemistry of most of the *Piper* spice plants is well documented (Parmar et al. 1998; Tripathi et al. 1996; Dyer and Palmer 2004). The

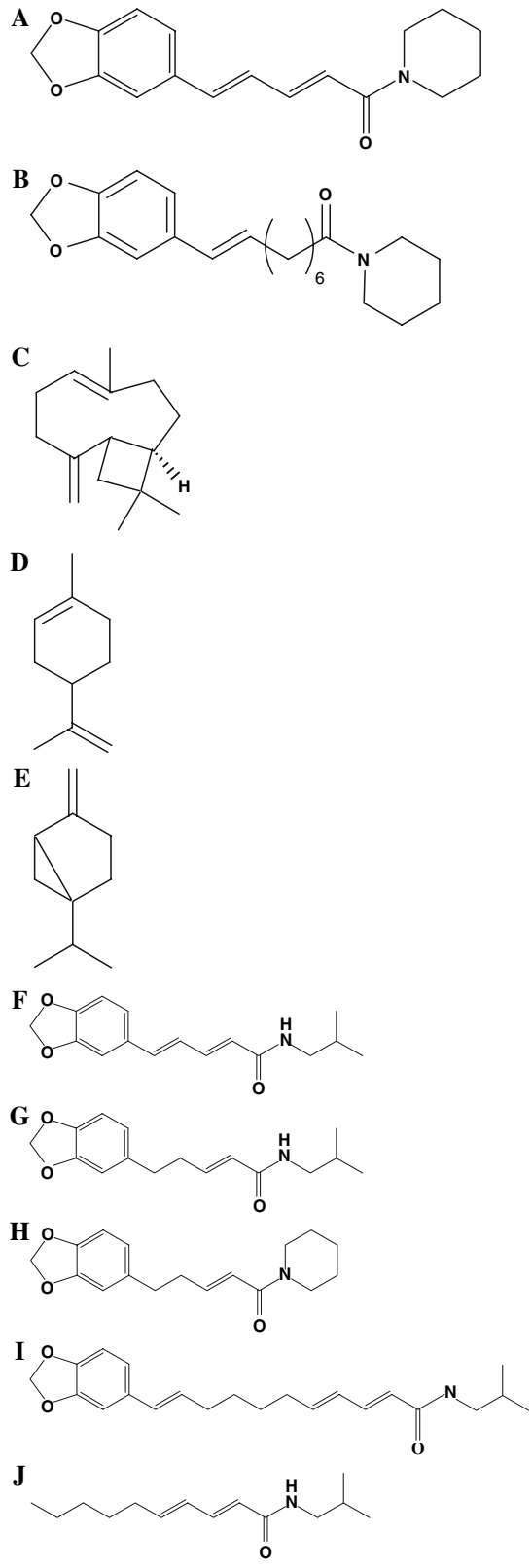

Fig. 1 Structure of *Piper* compounds: piperine (**A**); piperolein B (**B**); *β*-caryophyllene (**C**); limonene (**D**); sabinene (**E**); piperlonguminine (**F**); dihydropiperlonguminine (**G**); dihydropiperine (**H**); pipercide (**I**); pellitorine (**J**); guineensine (**K**); piperiline (**L**); *N*-substituted amides (**M**); *N*, *N*-disubstituted amides (**N**); methylenedioxyphenyl group (**O**); piperovatine (**P**); piperonyl butoxide (**Q**); safrole (**R**); dillapiol (**S**); piperettine (**T**)

most recognized compound from *P. nigrum* seed is the amide, piperine (Fig. 1**A**). It is present in the highest concentration of all secondary compounds in the seed of the plant, but 2 to 10-fold differences can occur between samples (Semler and Gross 1988). An analysis of *P. nigrum* germplasm from different North American distributors showed that the levels of piperine in dried seed material fluctuated along with the total piperamide content (Scott et al. 2005b). *P. nigrum* ground seed, peppercorn or dried berry contains 10.8 wt% oleoresin, 5.7 wt% piperine, 1.5 (v/w)% essential oil and 9 wt% water (Perakis et al. 2005). The most abundant compounds in the *P. nigrum* essential oil acetone extract were piperine (33.5%) and piperolein B (13.7%) (Fig. 1**B**) as well as unidentified alkaloid constituents (5.5 and 6.3%) and other identified piperamides (<3.4%) (Singh et al. 2004). In the essential oil 49 compounds have been identified, representing 99.4% of the total oil, while 18 compounds were identified in the acetone extract accounting for 75.6% of the total. In the essential oil the major components were *β*-caryophyllene (24.2%) (Fig. 1**C**), a sesquiterpene, limonene (16.9%) and sabinene (13%) (Fig. 1**D**, **E** respectively), both monoterpenes. Similar results were observed using a supercritical fluid extraction (SFE) of the essential oil (Perakis et al. 2005), however the SFE extraction increased the level of sesquiterpene hydrocarbons compared to hydrodistillation.

The efficacy of *Piper e*xtracts as botanical insecticides has been correlated with the concentration of piperamides in the extract (Scott et al. 2005b). Five piperamides, including piperine, commonly present in *P. nigrum* seed and *P. tuberculatum* leaf extract were used as standards to measure the efficiency of solvent extraction: piperlonguminine; dihydropiperlonguminine; dihydropiperine and pipercide (Fig. 1**F**–**I** respectively). Ethyl

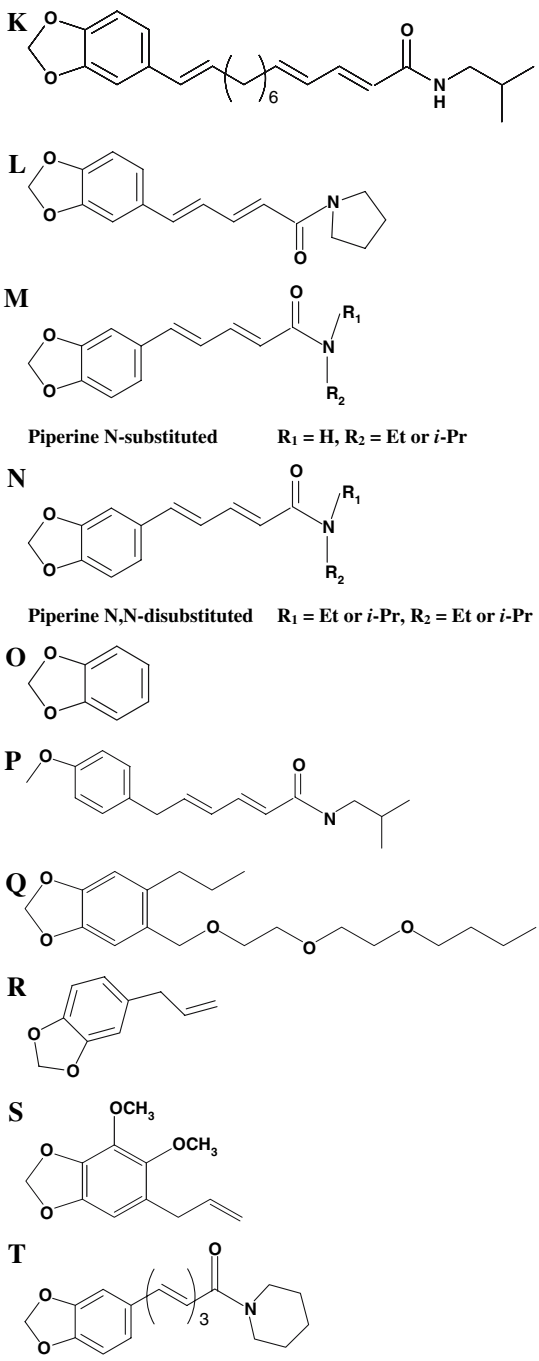

Fig. 1 continued

acetate extraction (125 ml) of *P. nigrum* seed (50 g) was shown to have a high efficiency (>80%) of piperamide extraction especially when refluxed, using a water-cooled condenser, at a boil for 20 min (Scott et al. 2005b). After filtering the peppercorn-ethyl acetate slurry and rinsing the filter cake 4 × with 30 ml ethyl acetate, the ethyl acetate filtrate was transferred to a separatory funnel and washed twice with 75 ml water. The ethyl acetate fraction was separated and dried with anhydrous magnesium sulfate and re-filtered. The filtrate was evaporated to dryness with a rotary evaporator and the dried extract was analyzed. To reduce solvent use for this process, SFE for piperamide extraction is currently being investigated. High performance liquid chromatography (HPLC)–mass spectrometry (MS) was applied as it is a sensitive analytical method with high resolution that can separate the co-eluting amides in *P. nigrum*, *P. guineense* and *P. tuberculatum* (Scott et al. 2005b).

Insecticidal activity

The wide variety of secondary plant compounds found in *Piper* were suggested as potential leads for novel insecticides (Miyakado et al. 1989), while many varieties are used in traditional control of insects that are vectors of disease (Okorie and Ogunro 1992) and damage stored crops (Sighamony et al. 1986; Baier and Webster 1992; Mbata et al. 1995; Kéïta et al. 2000). Early investigations with *P. nigrum* seed extracts indicated that piperamides were responsible for the toxicity of the extracts to the adzuki bean weevil *Callosobruchus chinensis* L. (Miyakado et al. 1979, 1980). *P. nigrum* seed oil formulations were found to effectively protect stored wheat from both stored grain pests, *Sitophilus oryzae* (L.) and *Rhyzopertha dominica* (F.), at concentrations above 100 mg/l for up to 30 days (Sighamony et al. 1986). Stored beans were protected from the bruchid *Acanthoscelides obtectus* Say by ground black pepper for up to 18 weeks (Baier and Webster 1992). Three of the piperamides isolated from *P. nigrum*, pipercide, pellitorine (Fig. 1**J**) and piperine ranged in toxicity from 0.15, 2 and 20 μg/ male *C. chinensis* respectively (Dev and Koul 1997). Guineensine (Fig. 1**K**), isolated from *P. guineense* seed, had relatively the same activity as pipercide when tested topically on the cow pea weevil *Callosobruchus maculatus* (F.), 0.25 vs. 0.84 μg/male (48 h LD_{50}). Essential oils from *Piper guineense* seed mixed with kaolin powder

at 150 μl/g reduced the average adult emergence of *C. maculatus* by 100% after 30 days treatment (Kéïta et al. 2000). Dust and ether-extract formulations of *P. guineense* dried fruit were also 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 extracted oil treatments at 0.25 g/seed. Formulated ethyl acetate extracts of *P. nigrum* seed were most effective against lepidopteran and hymeopteran herbivorous insects: eastern tent caterpillar *Malacosoma americanum* F., forest tent caterpillar *Malacosoma disstria* Hubner, introduced pine sawfly *Diprion similis* Hartig and European pine sawfly *Neodiprion sertifer* Geoffroy (Scott et al. 2004, 2007) The 24 h LC_{50}s for late instar larvae of *M. americana*, *N. sertifer*, *D. similis* and *M. disstria* were between 0.012 and 0.053% *P. nigrum* as shown in Table 1. Typically the concentration required for other insects tested was between 0.1 and 0.5% *P. nigrum* for the LC_{50} dose: Viburnum leaf beetle *Pyrrhalta viburni* Paykull larvae and striped cucumber beetle *Acalymma vittatum* F. adults and Colorado potato beetle *Leptinotarsa decemlineata* Say larvae and adults (Table 1). Although these are 10–50-fold greater than the more susceptible species, the concentrations would still be acceptable for a botanical product.

The behaviour modification (antifeedant and repellent effects) effects of *Piper* spp. extracts were also examined in greenhouse trials: pepper seed extracts deterred Lily leaf beetles *Lilioceris lilii* Scopoli and *A. vittatum* from damaging leaves of lily and cucumber plants respectively at concentrations in the 0.1–0.5% range (Scott et al. 2004). The repellent activity was observed to benefit the plant for up to 4 days post-spraying. However the residual repellent effect of *P. nigrum* was much less under full sunlight, and herbivore damage resumed shortly after application (Scott et al. 2003).

Environmental persistence

The observation that piperamides are degraded quickly under full sunlight (Scott et al. 2003, 2004) indicates that the use of these extracts to protect stored grains or around the home and garden are more promising than crop protection applications. Under ultraviolet (UV) lamp exposure, pure piperine degraded quickly with a half-life of approximately 40 min (Scott et al. 2004). This indicated that photolysis was likely responsible for the degradation rather than a photosensitized reaction from pigment in the peppercorn extract. A similar half-life ($t_{0.5}$ = 49 min) was determined for piperine in *P. guineense* seed extract after exposure to full sunlight. The residual activity of *Piper* components is lengthened in grain bins as reported by Baier and Webster (1992), Kéïta et al. (2000) and Sighamony et al. (1986). It was also observed that under conditions where *P. nigrum* extracts were applied to the soil, the residual activity was longer: the half-life for piperamides was one to several days depending on the time of year (Scott et al. 2005a). The benefit of short residual activity is that *Piper* seed extracts are more acceptable for organic certification.

Table 1 *Piper nigrum* L. LC_{50} values for selected insect larvae and adults

Insect	Order: Family	*P. nigrum* LC_{50} (%)	Reference
D. similis larva	Hymenoptera: Diprionidae	0.012	Scott et al. (2007)
M. americanum larva	Lepidoptera: Lasiocampidae	0.018	Scott et al. (2004)
N. sertifer larva	Hymenoptera: Diprionidae	0.046	Scott et al. (2004)
M. disstria larva	Lepidoptera: Lasiocampidae	0.053	Scott et al. (2007)
P. viburni larva	Coleoptera: Chrysomelidae	0.103	Scott et al. (2004)
vittatum adult	Coleoptera: Chrysomelidae	0.103	Scott et al. (2004)
L. decemlineata adult	Coleoptera: Chrysomelidae	0.498	Scott et al. (2003)
M. domestica adult	Diptera: Muscidae	0.82	Jensen et al. (2006a)

Mode of action

Piperamides are known to act as neurotoxins in the insect. Lipid amides were initially observed to modify axonal excitability by an effect upon sodium currents, at first described as ''pyrethroid-like'' (Lees and Burt 1988). However, later it was determined to be a mechanism distinct from that of the pyrethroids when a CNS preparation from American cockroaches, *Periplaneta americana*, resistant to pyrethroids were affected by the same doses of pipercide that affected susceptible cockroaches (Miyakado et al. 1989) and when isobutyl amides were shown to be more potent than pyrethroids against a resistant (*super-kdr*) strain of house fly (Elliott et al. 1986).

Isobutyl amides from black pepper and their synthetic derivatives (de Paula et al. 2000) and from guinea pepper (Gbewonyo et al. 1993) were characterized by structure-activity relationships. Synthetic piperamides, derived from natural piperine and piperiline (Fig. 1**L**) by substitutions at the *N*–position (Fig. 1**M**), showed no clear correlation with structure and activity, although *N*, *N*-disubstituted amides (Fig. 1**N**) were most active against the lepidopteran *Ascia monuste orseis* (Pieridae) (de Paula et al. 2000; Table 2). The results of this study were in contrast to the previous work of Miyakado et al. (1985, in de Paulo et al. 2000) which showed that *N*-isobutyl substitution had the highest activity. Gbewonyo et al. (1993) found that compounds with the methylenedioxyphenyl (MDP) (Fig. 1**O**) group, pipercide and guineensine, were more toxic but did not have the knockdown toxicity of the piperamides without the MDP group, pellitorine (Table 2).

Further work with piperovatine (Fig. 1**P**), an isobutyl amide from *P. piscatorum*, found that concentrations greater than 10 μM increased neuronal intracellular calcium concentrations in cultured *P. americana* neuronal cells (McFerren et al. 2002). It was also observed that the increased Ca^{2+} concentration was not affected by a muscarine acetylcholine receptor (mAchR) agonist, atropine, indicating that isobutyl amides do not interact with that neurotransmitter system. In contrast, the piperovatine Ca^{2+} effect was eliminated by the application of a voltage-gated sodium channel blocker, tetrodotoxin (TTX), indicating the importance of the voltage-gated Na^{+} channel directly or indirectly controlling the release of intracellular Ca^{2+} stores (McFerren et al. 2002).

Insecticide manufacturers routinely combine nontoxic MDP containing compounds such as piperonyl butoxide (PBO) (Fig. 1**Q**), a derivative of safrole (Fig. 1**R**), with formulations to enhance the toxicity (Hodgson and Levi 1998). Along with piperamides, Piperaceae also produce a variety of lignans and neolignans which possess the MDP functionality (Arnason et al. 2002). Dillapiol (Fig. 1**S**), from *P. aduncum*, has been shown to have an optimal structure for polysubstrate monooxygenase (PSMO) inhibition (Bernard et al. 1989; Budzinski et al. 2000). *P. nigrum* extract also functions as a highly effective synergist for pyrethrum: a synergist ratio of 11.6 was obtained in trials with adult *Drosophila melanogaster* (Jensen et al. 2006b), which is substantially better than PBO. The activity of *P. nigrum* was synergistic rather than merely additive, since a sublethal concentration was combined with pyrethrum. At 0.2 mg/ml *P. nigrum*, double the concentration used in the synergism trial, only three genes were differentially expressed, compared to the many up-regulated by 0.9 mg/ml (Table 3). One of the genes up-regulated with known function is related to nicotinic acetylcholine receptor activity of cationic channel activity. This gene expression may be a compensation for the effect piperamides have on the voltage-gated sodium channel inactivation (Jensen et al. 2006b). The combination of *P. nigrum* and pyrethrum resulted in the up-regulation of five genes and the down-regulation of two genes. This compares strikingly with the 70 genes up-regulated and 25 genes down-regulated by pyrethrum alone at the same concentration. Confirmation of the differential gene expression was performed by RT-qPCR. It confirmed that the pyrethrum treatment indeed produced an up-regulation of the lethal (2) essential for life (l(2)efl) gene (CG4533) and oxidoreductase activity (CG13091) and the down-regulation of the *Tom* gene (CG5185) (Fig. 2). RT-qPCR also confirmed that the combination of pyrethrum and *P. nigrum* was responsible only for down-regulation of the *Tom* gene and not up-regulation

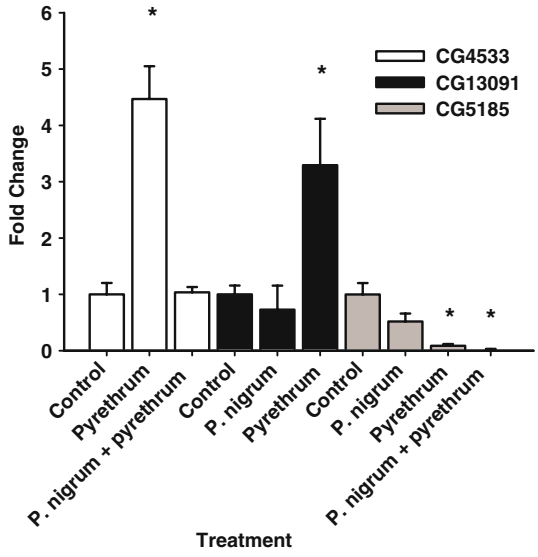

Fig. 2 The mean fold-change in expression (+SEM) of the CG4533 (l(2)efl), CG13091 (oxidoreductase activity) and CG5185 (*Tom*) genes in *D. melanogaster* after exposure to pyerthrum, *P. nigrum* or a combination of both compared with a 99% ethanol control as confirmed by RT-qPCR. Asterick indicates a significant difference from the control (One way ANOVA, Dunnett's one-sided pairwise comparison, $P < 0.05$). Figure reprinted from Jensen et al. 2006b with permission from Insect Molecular Biology

of the other two genes. These results are a further indication that, at the molecular level, *P. nigrum* extract at 0.1 mg/ml acted as a true synergist since there was minimal impact on gene expression yet there was a substantial increase in the toxicity of pyrethrum.

These findings are consistent with a study that demonstrated a synergist ratio of 14 between pyrethrum and the piperamide, piperettine (Fig.1**T**), with the housefly *Musca domestica* (L.) (Gersdorff and Piquett 1957). The PSMO inhibitory activity of piperine, just one individual piperamide selected from *P. nigrum*, was demonstrated to be almost as effective as PBO against multi-insecticide resistant *M. domestica* and *L. decimlineata* (Scott et al. 2003). Piperine inhibited methoxyresorufin *O*-demethylation (MROD) activity in *M. domestica* microsomes in vitro with an IC_{50} of 1.2 μM piperine, comparable to 0.43 μM for PBO. These findings were the first to demonstrate PSMO inhibition by piperine in insects and to explain the synergism with pyrethrum. Piperine had previously been shown to inhibit the activities of other mammalian detoxification enzymes: arylhydrocarbon hydroxylase (AHH) and 7-ethoxycoumarin deethylase (7ECDE) (Reen and Singh 1991); methoxycoumarin demethylase (MOCD) activity (Singh and Reen 1994); and the major drug metabolizing enzyme Cyp3a4 (Bhardwaj et al. 2002). Piperine was also demonstrated to induce phase I (cytochrome b5, cytochrome P450) and phase II (gluathione S-transferase GST, acid-soluble sulfhydryl –SH, malondialdehyde MDA) PSMO enzyme levels (Singh and Rao 1993). Induction of gene transcription for phase I and II enzymes was confirmed recently through an examination of *P. nigrum* on *D. melanogaster* gene expression (Jensen et al. 2006a). An ethanolic extract of pepper upregulated cytochrome P450s *Cyp6a8*, *Cyp9b2*, *Cyp12d1*, *Cyp6d4*, *Cyp6d5* and *Cyp6w1* along with glutathione-S-transferase S1 and glutathione-S-transferase E7 (Table 3). The upregulation of *Cyp* genes is consistent with the biphasic effect of MDP containing compounds: initial inhibition of Cyp enzymes followed by induction

Table 2 Structure-activity relationships between piperamide structure and insecticidal activity

Piperamide Structure	Examples	Figure	Relative dose[1]	Reference
Amide + MDP, short chain[2]	Piperine,	1**A**	1[a,b]	[a]de Paula et al. (2000)
	Piperiline	1L	0.1–10[a]	
Amide, no MDP, med. Chain[3]	Pellitorine	1**J**	0.1[b,c]	[b]Dev and Koul (1997)
Amide + MDP, long chain[4]	Pipercide,	1**I**	<0.01[b,c]	[c]Gbewonyo et al. (1993)
	Guineensine	1**K**	<0.01[c]	
Amide + MDP, short chain, *N*-substitution	Derived from piperine	1**M**	0.1–10[a]	de Paula et al. (2000)
Amide + MDP, short chain, *N*, *N*-disubstituted	Derived from piperine	1**N**	0.1–1[a]	de Paula et al. (2000)

[1] Dose is based on LD_{50} estimates for each compound relative to piperine; [2]short chain refers to <5 carbons; [3] medium chain refers to 6–10 carbons; [4]long chain refers to 10–12 carbons; [a,b,c] letter refers to reference for the relative dose

Table 3 *Drosophila melanogaster* genes up-regulated or down- regulated by two concentrations of *P. nigrum* L. extract

P. nigrum conc'n. (mg/ml)	Genes up-regulated & function	Genes down-regulated & function	Fold change	CG identifier	Reference
0.2	Oxidoreductase activity		1.73	CG13091	Jensen et al. (2006b)
	SnRNP70K Pre-mRNA splicing factor activity		1.6	CG8749	
	NAcRβ-21C Nicotinic acetylcholine-activated cation-selective channel activity		1.44	CG11822	Jensen et al. (2006b)
		Tom Notch signalling pathway	–1.58	CG5185	Jensen et al. (2006b)
0.9	Cyp9b2, Cyp6a8, Cyp12d1, Cyp6d5, Cyp6w1, Cyp6d4 Monooxygenase activity		3.12, 3.53, 2.51, 1.74, 1.8, 1.44	CG4486, CG10248 CG18240, CG3050 CG8345, CG12800	Jensen et al. (2006a)
	GstS1, GstE7 Glutathione transferase activity		2.22, 1.41	CG8938, CG17531	Jensen et al. (2006a)
		Genes related to starvation and embryonic development	–2.08, –2.79	CG5107, CG11892	Jensen et al. (2006a)

(Dalvi and Dalvi 1991). The observed gene expression profile is indicative both of generalized stress and of a specific response to a toxin. The induction of *Cyp6* genes has previously been observed in the swallowtail butterfly (*Papilio polyxenes*) in response to furanocoumarins from plants belonging to the Apiaceae (Petersen et al. 2001). In certain *D. melanogaster* populations the evolved constitutive overexpression of *Cyp6a8* is associated with DDT resistance (Maitra et al. 1996). Selection for overexpression or increased inducibility of Cyp 6 enzymes in insect populations can confer a broad range of substrate specificity including metabolism of plant secondary metabolites, for example furanocoumarins (Petersen et al. 2001).

Conclusions

These findings suggest ways by which insects could metabolize and detoxify piperamides through further induction of specific enzymes in the Cyp 6 family. In fact, numerous commercial insect pests and wild pepper specialist insects are reported feeding on *P. nigrum* and *P. betle* in India (Ranjith et al. 1991; Devasahayam and Abdullah Koya 1994; Santhosh-Babu 1994; Raut and Bhattacharya 1999). Therefore, despite the evidence that *Piper* spp. contain such seemingly potent natural defense compounds, it would appear that *Piper* herbivores can feed successfully even on the fruit. However, piperamides singly, or more importantly in combination, could still replace contact insecticides, specifically neurotoxic compounds such as carbamates, organophosphates and pyrethroids, for which resistance has developed. A combination of these amides within a botanical formulation would thus provide the advantage of all of the previously mentioned attributes: novel target site, enzyme inhibition, and low mammalian toxicity. The important next step is to develop a commercial product that will be acceptable to a global market.

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