

## Larvicidal activity of *Piper tuberculatum* on *Spodoptera frugiperda* (Lepidoptera: Noctuidae) under laboratory conditions

Actividad larvica de *Piper tuberculatum* sobre *Spodoptera frugiperda* (Lepidoptera: Noctuidae) bajo condiciones de laboratorio

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**Abstract:** The larvicidal activity of the neotropical “matico” *Piper tuberculatum* was evaluated. The secondary compounds were extracted of leaves, stems and mature spikes with fruits and seeds from wild plants and *in vitro* plants of *Piper tuberculatum*. The acute toxicities to the fall armyworm, *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae), of extracts of spikes with fruits and seeds and *in vitro* plants of *P. tuberculatum* were evaluated by means of contact bioassays. Only CH<sub>2</sub>Cl<sub>2</sub>:MeOH (2:1) and EtOH extracts of mature spikes and CH<sub>2</sub>Cl<sub>2</sub>:MeOH (2:1) extract from *in vitro* plants showed significant levels of larval mortality. The CH<sub>2</sub>Cl<sub>2</sub>:MeOH (2:1) and EtOH extracts of mature spikes caused 90% mortality when doses of 0.1850 mg/μL were applied to the *S. frugiperda* in 24 and 48 h of exposure, respectively. The CH<sub>2</sub>Cl<sub>2</sub>:MeOH (2:1) extract from *in vitro* plants caused 95% mortality when doses of 0.1850 mg/μL were too applied in 48 h of exposure. The mature spikes test best results were: LD<sub>50</sub> 0.001 mg/μL with EtOH and 0.007 mg/μL with CH<sub>2</sub>Cl<sub>2</sub>:MeOH (2:1) and LD<sub>90</sub> 0.027 mg/μL with EtOH and 0.103 mg/μL with CH<sub>2</sub>Cl<sub>2</sub>:MeOH (2:1); and, in the case of *in vitro* plants, only CH<sub>2</sub>Cl<sub>2</sub>:MeOH (2:1) extract was: LD<sub>50</sub> 0.003 mg/μL and LD<sub>90</sub> 0.060 mg/μL. The potential value of extracts derived from *P. tuberculatum* as efficient insecticides against *S. frugiperda* is discussed.

**Key words:** CH<sub>2</sub>Cl<sub>2</sub>:MeOH (2:1) extract. EtOH extract. *In vitro* propagation. Larval susceptibility. Lethal Dosis.

**Resumen:** Se evaluó la actividad larvica del “matico” neotropical *Piper tuberculatum*. Los compuestos secundarios fueron extraídos de hojas, tallos y espigas maduras (con frutos y semillas) de plantas silvestres y de plantas *in vitro* de *P. tuberculatum*. Fue evaluada la toxicidad aguda de extractos de espigas maduras con frutos y semillas y de plantas *in vitro* de *P. tuberculatum* sobre el “cogollero” *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) mediante bioensayos de contacto. Solamente extractos Diclorometano:metanol (CH<sub>2</sub>Cl<sub>2</sub>:MeOH) (2:1) y etanólicos (EtOH) de espigas maduras y el extracto CH<sub>2</sub>Cl<sub>2</sub>:MeOH (2:1) de plantas *in vitro* mostraron niveles significativos de mortalidad. Los extractos CH<sub>2</sub>Cl<sub>2</sub>:MeOH (2:1) y EtOH de espigas maduras causaron 90% de mortalidad cuando dosis de 0,1850 mg/μL se aplicaron sobre *S. frugiperda* en 24 y 48 h de exposición, respectivamente. El extracto CH<sub>2</sub>Cl<sub>2</sub>:MeOH (2:1) de plantas *in vitro* causó 95% de mortalidad cuando dosis de 0,1850 mg/μL también se aplicaron en 48 h de exposición. Los mejores resultados para las espigas maduras fueron: CL<sub>50</sub> 0,001 mg/μL con EtOH y 0,007 mg/μL con CH<sub>2</sub>Cl<sub>2</sub>:MeOH (2:1) y CL<sub>90</sub> 0,027 mg/μL con EtOH y 0,103 mg/μL con CH<sub>2</sub>Cl<sub>2</sub>:MeOH (2:1); y en el caso de las plantas *in vitro*, solamente el extracto CH<sub>2</sub>Cl<sub>2</sub>:MeOH (2:1) fue: CL<sub>50</sub> 0,003 mg/μL y CL<sub>90</sub> 0,060 mg/μL. Se discute el valor potencial de los extractos de *P. tuberculatum* como un eficiente insecticida sobre *S. frugiperda*.

**Palabras clave:** Extracto CH<sub>2</sub>Cl<sub>2</sub>:MeOH (2:1). Extracto EtOH. Propagación *in vitro*. Susceptibilidad larval. Concentración letal.

### Introduction

*Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae), the fall armyworm, is a polyphagous insect of enormous agricultural importance, not only because of the damage it provokes, but also because of control difficulties (Santos *et al.* 2003). The species is a migratory pest endemic to the Western Hemisphere that occurs from Southern Canada to Argentina and causes considerable economic losses in several important crops such as maize, sorghum, rice, cotton, alfalfa, forage grasses, and occasionally other crops in most of the countries of its range (Clark *et al.* 2007). In its distribution area, two genetically distinct strains are found that differed in their plant host distribution (Pashley 1986); one strain feeds primarily on maize and sorghum (corn strain), and the other strain feeds on rice and bermuda grass (rice strain) (Pashley *et al.* 1985; Pashley 1986).

The indiscriminate use of synthetic insecticides has caused environmental contaminations and toxicity to living organisms (Nakata *et al.* 2005), indicating the need for the development of products that not hazardous to the environment, target-specific and biodegradable. Thus, the development of new insecticides from plant extracts sources can be an alternative for the control of *Spodoptera* bugs.

Species of different plant families and their derived products have received increased attention from scientists and more than 2000 plant species are already known to have insecticide properties (Sukamar *et al.* 1991). Among these families the Piperaceae family has been investigated, especially the species of the genus *Piper* (Marquis 1991; Bernard *et al.* 1995). More than 15 species of *Piper* have been reported in the literature to have insecticidal activity (Bernard *et al.* 1995; Parmar *et al.* 1997). For example, the Amazonian species, *Piper rotundistipulum* (Trel. and Yunck., 1950), is

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locally used as insecticide and fish poison (Schultes and Raufauf 1990). *Piper guineense* (Schumacher, 1827) and *Piper nigrum* (Linnaeus, 1753) are used as insecticide and molluscicide in several parts of Africa (Ivbijaro and Bolaji 1990). The Indian species *Piper longum* (Linnaeus, 1753), *Piper betle* (Linnaeus, 1753), *Piper peepuloides* (Roxb., 1820) and *Piper cubeba* (Linnaeus, 1753) have demonstrated insecticidal activity against mosquitos and flies (Miyakado *et al.* 1989).

The benzene extract of the leaves of *Piper futokatsura* (Sieb., 1900) from Taiwan and Japan are known as a feeding deterrent to *Spodoptera litura* (Fabricius, 1775) larvae (Matsui and Munskata 1975), and the leaves of *Piper umbellatum* (Linnaeus, 1753), *Piper hispidum* (Sw., 1788), *Piper auritum* (Kunth, 1816), and others *Piper* spp., which are native to Central America and the Northwest Amazonian basin, are used by indigenous peoples to prevent malaria and removing head lice (Schultes 1980). Likewise, the leaf and stem petrol and dichloromethane extracts of *Piper falconeri* (C.DC., 1925) have shown insecticidal activity against *Musca domestica* (Linnaeus, 1758) (flies) and *Aedes aegypti* (Linnaeus, 1762) (mosquitoes); the *Piper acutisleginum* (DC., 1869) dichloromethane extract has also been reported to show insecticidal activity against *M. domestica* and *A. aegypti* (Parmar *et al.* 1997) and the dichloromethane and ethanolic extracts of spikes and *in vitro* plants of *Piper tuberculatum* (Jacq., 1795) have shown insecticidal activity against *Diatraea saccharalis* (Fabricius, 1794) (Soberón *et al.* 2006).

In addition, natural insecticides such as pyrethrum, rotenone and nicotine, among others, have been extensively used until recently; these substances can affect the feeding behavior and growth regulators, disrupting the insect hormonal balances (Balandrin *et al.* 1985). An example is azadirachtin, a biopesticide obtained from the neem tree *Azadirachta indica* (A. Juss., 1830), which could be readily biodegradable, selective, non-mutagenic, with low toxicity to mammals, and causes minimal effects on the environment (Gupta 2004); however, this substance is very expensive, it cannot be synthesized chemically and has to be purified, using costly and sophisticated methods, from large quantities of a seasonally produced seed (Allan *et al.* 1999). An alternative are the plant tissue culture methods. In effect, plant cell cultures have been actively studied as a potential source of high-value biological compounds (Eda Hiro and Seki 2006). The major advantages of a cell culture system over the conventional cultivation of whole plants are: useful compounds can be produced under controlled conditions independent of climatic changes or soil conditions; cultured cells would be free of microbes and insects; the cells of any plants could easily be multiplied to yield their specific metabolites; automated control of cell growth and rational regulation of metabolite processes would reduce of labor costs and improve productivity (Vanisree *et al.* 2004).

Chemical studies carried out on Brazilian Piperaceae species have revealed the occurrence of pyrones, lignoids and chromenes besides various amides bearing isobutyl, pyrrolidine, dihydropyridone and piperidine moieties (Parmar *et al.* 1997; Baldoqui *et al.* 1999; Navickiene *et al.* 2000; da Silva *et al.* 2002). These amides have generated interest as a result of their potent insecticidal and antifungal properties (Miyakado *et al.* 1989; Bernard *et al.* 1995; Navickiene *et al.* 2000; da Silva *et al.* 2002). *P. tuberculatum* known as "matico" or "cordoncillo", a species quite abundant in the West Indies and it is widely distributed from Brazil to Mexico, has

been described seven isolated amide structures from seeds with CH<sub>2</sub>Cl<sub>2</sub>:MeOH (2:1) (Navickiene *et al.* 2000). These amides, are active against the fungus *Cladosporium cladosporioides* (Fres., de Vries, 1952) ranged from 5.0 to 10.0 µg (da Silva *et al.* 2002) and *Cladosporium sphaerospermum* (Penz., 1882) ranged from 0.1 to 5.0 µg (Navickiene *et al.* 2000).

The objective of this research was to investigate the insecticidal activity of extracts of leaves, stems and mature spikes, with fruits and seeds, of wild plants and *in vitro* plants of *P. tuberculatum* on third instar larval of *S. frugiperda*.

## Materials and Methods

**Plant material.** Spikes with mature seeds, leaves and stems of *P. tuberculatum* were collected in November 2003 from 'Cumbil' river (Lambayeque, Peru). Botanical identification was performed by Doctor Guillermo E. Delgado from Universidad Nacional Pedro Ruiz Gallo (UNPRG) based on taxonomic description realized by Yuncker (1973). The botanic specimen vouchers were deposited at same herbarium of the institution (HPR).

***In vitro* micropropagated plants.** The culture was initiated from axenic seedlings explants. A total of 50 seeds per flask were surface-sterilized by 70% ethanol (v/v) 1 min followed by 2-2.5% sodium hypochlorite (w/v) for 20 min and then washed three times with sterile water. Floating seeds were discarded; about 3-10 of them were transferred to glass test tubes containing 20 mL of MS medium (Murashige and Skoog 1962) and 2% sucrose. Shoot-tip and nodal segments, 1 cm length containing a lateral bud, taken from three-month-olds *in vitro* seedlings, were used as explant source. MS medium, supplemented with 0.02 mg/L indoleacetic acid (IAA), 0.02 mg/L gibberellic acid (GA<sub>3</sub>) and 3% sucrose was used to initiate cultures, and were maintained by subculturing every six months on a fresh medium containing the same formulation. In all cultures, the same MS medium was supplemented with 100 mg/L m-inositol and 1 mg/L thiamine.HCl, adjusted to pH 5.7 ± 0.1, solidified with "Phytigel" 0.3% prior to autoclaving, dispensed into tubes (150 x 25 mm) containing 20 mL MS medium and covered with polypropylene caps. All cultures were incubated at 24-28°C in a 16-h light, 8 h dark photoperiod provided by cool white fluorescent tubes, with 5 µmol m<sup>-2</sup> s<sup>-1</sup>, for seed germination and 30 µmol m<sup>-2</sup> s<sup>-1</sup> for clonal propagation.

**Insects.** Eggs of *S. frugiperda* were collected from a corn crop during its vegetative to early reproductive stage, in the Fundo La Peña, UNPRG - Lambayeque, Peru, and were reared in the Laboratorio de Entomología of the Facultad de Agronomía (UNPRG), under laboratory conditions. Insects were maintained in Petri dishes lined with damp filter paper (one fall armyworm per dish to avoid cannibalism) under a controlled environment (26 ± 2°C, 80 ± 5% relative humidity, 16:8 h light:dark photoperiod). Third instar larvae of *S. frugiperda* were fed to repletion with fresh leaves of maize.

**Extraction of the constituents.** Spikes, leaves and stems (45 g, respectively) of wild plants of *P. tuberculatum* were oven dried at 40°C, milled and submerged three times in CH<sub>2</sub>Cl<sub>2</sub>:MeOH (2:1) and EtOH 96%, respectively, at room temperature, yielding between 1.6 to 11.8% (0.72 to 5.31 g)

of extract; likewise, *in vitro* micropropagated plants (9 g) yielding 6.3% (0.57 g) of extract with CH<sub>2</sub>Cl<sub>2</sub>:MeOH (2:1). In the case of extraction with boiling water, 10 g of dried spikes, leaves and stems were supplemented with 100 mL of distilled water and submitted to boiling (up to 100°C) by 10 min; the extracts obtained were evaporated at reduced pressure (45°C).

**Topical test.** Bioassays were carried out at in the Laboratorio de Entomología of UNPRG. The stock solutions of extracts were prepared by dissolving 100 mg of dry extract in 1 mL of MeOH-water to obtain a concentration of 100 mg/mL. After 24 h, and using and Eppendorf® 0-10 µL pipette, 6.5 µL of the solution, containing an aliquot of each one of the treatments, was applied directly on the larval mesothorax of *S. frugiperda*. The plant extract was tested at doses of 0.0, 0.0007, 0.0014, 0.0029, 0.0057, 0.0115, 0.0230, 0.0460, 0.920 and 0.1850 mg/µL. Twenty larvae were tested per treatment and the experiment was carried out twice. The control insects received a topic application with MeOH-water alone. Larval mortality was recorded at 24, 48 and 72 hour post-treatments, under the same conditions of temperature and humidity described above. The larvae were considered dead if they displayed no observable response to a mechanical stimulus, i.e. short-term pressure applied with a spatula.

A dose - response correlation was obtained using a linear regression model to fit the probit data to the log of the dose of each extract applied. LD<sub>50</sub> and LD<sub>90</sub> values were determined used the software US. EPA Probit Program Version 1.5 (2003).

**Results**

CH<sub>2</sub>Cl<sub>2</sub>:MeOH (2:1) and EtOH extracts of leaves and stems, and boiling water extracts of leaves, stems and spikes from wild plants of *P. tuberculatum* did not show larvicidal activity against the third instar larval of *S. frugiperda* tested at dose ranging from 0.0007 to 0.1850 mg/µL (data not shown).

The response of fall armyworm to the topical applications of CH<sub>2</sub>Cl<sub>2</sub>:MeOH (2:1) and EtOH extracts from mature spikes of wild plants and CH<sub>2</sub>Cl<sub>2</sub>:MeOH (2:1) extract from *in vitro* plants of *P. tuberculatum* showed a positive relationship between dose and mortality. The responses varied with the time of exposure.

The resultant regression lines for all the extracts appeared to be very similar, showing a relatively fast intoxication pro-

cess on the insects exposed to *P. tuberculatum* extracts. In a general way, the LD<sub>50</sub> and LD<sub>90</sub> values decreased when the time between application and evaluation increased (Table 1). The data presented confirm that mature spikes and *in vitro* plants extracts from *P. tuberculatum* presented potential insecticide activities.

The larval mortality at 90% was reached after 24 h when using 0.1850 mg/µL of CH<sub>2</sub>Cl<sub>2</sub>:MeOH (2:1) extract from mature spikes; and a mortality of 100% was reached with 0.1850 mg/µL EtOH extract from mature spikes in 72 h. In reference to the *in vitro* plants, the extract obtained with CH<sub>2</sub>Cl<sub>2</sub>:MeOH (2:1) alone generated a 95% larval mortality with 0.1850 mg/µL in 48 h. The mortality of the control group was 0%.

The small variations in LD<sub>50</sub> and LD<sub>90</sub> values of both extracts with respect to time of exposure suggest a rapid toxic action. Similar to what happens with larvae of *Anticarsia gemmatalis* (Hubner, 1818) (Navickiene *et al.* 2007), almost immediately following the application of doses of each treatment, larval movement decreased and feeding practically ceased. Also, typical intoxication symptoms, as described by Marchini *et al.* (1992), such as spasmodic movements, regurgitation and faecal elimination, were observed, thus confirming the acute toxicity of these extracts to fall armyworm.

**Discussion**

Preliminary tests have demonstrated that CH<sub>2</sub>Cl<sub>2</sub>:MeOH (2:1) and EtOH extracts of leaves and stems, and boiling water extracts of leaves, stems and spikes from wild plants of *P. tuberculatum* did not show larvicidal activity against the third instar larval of *S. frugiperda* tested at dose ranging from 0.0007 to 0.1850 mg/µL. Results agree with dose reported in the control of third instar larval of *D. saccharalis* (Soberón *et al.* 2006) and second and third instar larval and adult stage of *Aedes aegypti* and *Anopheles pseudopunctipennis* Theobald, 1901 (Bazán-Calderón *et al.* 2011); however, disagree with the results reported for extracts of leaves and stems of *P. tuberculatum* used in control of *Aedes atropalpus* (Coquille, 1902) (Bernard *et al.* 1995) and *A. gemmatalis* (Navickiene *et al.* 2007). According to Scott *et al.* (2002; 2003) interplant differences related to the efficacy of extracts may be due to the large variability observed with the individual piperamide concentrations, especially 4,5-dihydropiperlonguminine, in leaves. It is also important to decide where and when plants should be collected to obtain material with the highest biological activity; in this case, the geographical region may not

**Table 1.** Components of the probit analyses and LD<sub>50</sub> and LD<sub>90</sub> values for the fall armyworm *Spodoptera frugiperda* exposed to three extracts from *Piper tuberculatum*.

Extract	Time after treatment (h)	Slope (± SE)	(µg insect <sup>-1</sup> )				Significance		
			LD <sub>50</sub>	LD <sub>50</sub> 95% FL		LD <sub>90</sub>	LD <sub>90</sub> 95% FL	t ratio <sup>a</sup>	X <sup>2</sup> (g.I.) <sup>a</sup>
				Lower-upper	Lower-upper				
CH <sub>2</sub> Cl <sub>2</sub> :MeOH (wild plants)	24	1.10 (± 0.15)	0.012	0.001-0.015		0.230	0.230-0.741	0	5.964 ns
	48	1.17 (± 0.15)	0.009	0.005-0.014		0.123	0.068-0.403	0	4.265 ns
	72	1.19 (± 0.17)	0.007	0.003-0.011		0.103	0.052-0.316	0.04	3.536 ns
EtOH (wild plants)	24	0.90 (± 0.14)	0.015	0.009-0.026		0.393	0.155-2.038	0	10.270 ns
	48	1.10 (± 0.15)	0.005	0.003-0.008		0.079	0.042-0.217	0	6.977 ns
	72	1.51 (± 0.23)	0.001	0.000-0.002		0.027	0.016-0.056	0.05	3.513 ns
CH <sub>2</sub> Cl <sub>2</sub> :MeOH (in vitro plants)	24	0.78 (± 0.13)	0.006	0.003-0.011		0.253	0.096-1.557	0	4.924 ns
	48	0.90 (± 0.14)	0.003	0.002-0.005		0.082	0.039-0.296	0	3.082 ns
	72	0.99 (± 0.15)	0.003	0.002-0.005		0.060	0.031-0.181	0	4.505 ns

<sup>a</sup>Significance level: ns = not significant (P > 0.05).

matter, but site-specific properties could affect piperamide levels: soils nutrients, microclimate and levels of herbivory. Thus, as is the case with *Piper* species, there can be a selective advantage in producing different compounds and, in addition, compounds that interfere with detoxification but are not metabolized, even if they themselves are not toxic (Navickiene *et al.* 2007).

The results confirm that CH<sub>2</sub>Cl<sub>2</sub>:MeOH (2:1) and EtOH extracts of mature spikes from wild plants and CH<sub>2</sub>Cl<sub>2</sub>:MeOH (2:1) extracts from *in vitro* plants of *P. tuberculatum* showed a potent insecticidal activity on third instar larval of this Lepidoptera species. The CH<sub>2</sub>Cl<sub>2</sub>:MeOH (2:1) and EtOH extracts of mature spikes showed higher toxicity than CH<sub>2</sub>Cl<sub>2</sub>:MeOH (2:1) extract from *in vitro* plants and also EtOH extract was more effective than CH<sub>2</sub>Cl<sub>2</sub>:MeOH (2:1) extract.

The results suggest two possibilities for the direct use: insecticidal activity of mature spike EtOH extracts from *P. tuberculatum* that allows rural people to freely use the extract obtained with traditional alcoholic drinks as “aguardiente, yonque or cañazo”. The second possibility, even though the *in vitro* plant extracts showed lower toxicity than mature spike extracts, may be an active compound biosynthesis at large scale using the establishment of cellular suspensions (Danelutte *et al.* 2005).

Several studies have shown insecticidal activity of plants extracts against *S. frugiperda*. For instance, the antifeedant activity of *Citrus*-derived limonoids limonin, nomlin, and obacunone and their semisynthetic derivatives, obtained from seeds of *Citrus limonoides* = *C. limon* (L., Burm. F., 1768) (Ruberto *et al.* 2002); the insecticidal activity of crude ethanolic seed extracts of *Annona muricata* (Linnaeus, 1753), *A. squamosa* (Linnaeus, 1753) (Annonaceae), *Lansium domesticum* (Corrêa, 1807) and *Sandoricum koetjape* (Merr., 1912) (Meliaceae) (Leatemia and Isman 2004a); and the potential use of Asteraceae extracts to control *S. frugiperda* and selectivity to their parasitoids *Trichogramma pretiosum* (Riley, 1879) and *Telenomus remus* (Nixon, 1937) (de Souza Tavares *et al.* 2009). On the other hand, when added to the diet, *Melia azedarach* (Linnaeus, 1753) (Meliaceae) SLE (senescent leaves extracts) showed lower toxicity than *Jatropha gossypifolia* (Linnaeus, 1753) (Euphorbiaceae) SLE; however, after two weeks on the diet, the *M. azedarach* SLE proved lethal to 100 percent of the larval population; likewise, acute toxicity after topical application in a dipping assay was relatively low for both *J. gossypifolia* or *M. azedarach* SLEs (LC<sub>50</sub> of 2.6 and 1.4 g L<sup>-1</sup>, respectively, after 24 h) (Bullangpoti *et al.* 2012). These results were lightly similar with the results obtained in our work. The insecticidal and insectistatic activities of the seed extract and the three main constituents, oleic, palmitic and stearic acids of *Carica papaya* (Linnaeus, 1753) (Caricaceae), were tested; larval viability values were 0%, 29.2%, and 50% when the seed extract was applied at 24,000, 16,000, and 9,600 ppm, respectively, and the larval viability of the main compounds was 33.3%, 48.5%, and 62.5% when exposed to 1,600 ppm of palmitic acid, oleic acid, or stearic acid, respectively (Pérez-Gutiérrez *et al.* 2011). In our study were obtained similar results with same doses. To determine the insecticidal and insectistatic activities of methano, hexane and ethyl acetate extracts of the seeds and leaves of *Ricinus communis* (Linnaeus, 1753) (Euphorbiaceae), castor oil and ricinine were tested at different concentrations against *S. frugiperda*; the half maximum larvae viability concentration (LVC<sub>50</sub>) were 0.38 x 10<sup>3</sup> ppm for the ricinine, 0.75 x 10<sup>3</sup>

ppm for a methanol extract of seeds, 1.97 x 10<sup>3</sup> ppm for an ethyl acetate seed extract and 2.69 x 10<sup>3</sup> ppm for the castor oil (Ramos-López *et al.* 2010), doses too very similar with the used in our study. In other work, of the 20 species tested, 7 showed mortality for caterpillars *S. frugiperda*: *Petiveria alliacea* (Linnaeus, 1753) (98%), *Malva sylvestris* (Linnaeus, 1753) (90%), *Artemisia verlotorum* (Lamotte, 1876) (90%), *Baccharis genistelloides* (Lam., Pers., 1807) (80%), *Zingiber officinale* (Rosc., 1807) (70%), *Cymbopogon citratus* (DC., Stapf, 1906) (60%) and *Ruta graveolens* (Linnaeus, 1753) (58%) (Tagliari *et al.* 2010); however, not were indicated the doses applied. Likewise, using fruits of *Moringa oleifera* (Lam., 1785) (Moringaceae), the highest total correct mortality percentage was recorded with the highest concentration of moringa oil (100%) and unsaponifiable matter (80.7%); it was concluded that moringa oils at 10% concentration could be applied as botanical insecticide to prevent the plants from *S. frugiperda* attack (Kamel 2010). These results not could be comparable with our work.

Only some species of *Piper*, of the flora of Peru, as *Piper aduncum* (Linnaeus, 1753), *Piper aequale* (Vahl., 1797), *P. hispidum*, *Piper reticulatum* (Linnaeus, 1753) and *P. tuberculatum* showed significative activity against the mosquito *A. atropalpus* (Bernard *et al.* 1995). In this paper was reported that 100 mg/L (0.1 mg/mL or 0.0001 mg/μL) hexanic crude extract of *P. tuberculatum* leaves presented most intense activity with 54% mortality in second instar larval of *A. atropalpus*, after 24 h of exposure; this mortality was attributed to isobutylamide 4,5-dihydropiperlonguminine (pure substance isolated from the active fraction) because caused 47% mortality of mosquito larvae at 0.01 mg/L in the same time of exposure. Comparing these results, in our work was showed that intermediate doses of extract, from mature spikes as 0.0115 mg/μL and 0.0230 mg/μL extract, from *in vitro* plants, produced a mortality exceeds 50% in *S. frugiperda* at 24 h of exposure.

In previous studies with seed extracts of *P. tuberculatum* were isolated several amides, mainly bearing isobutyl, pyrrolidine, dihydropyridone and piperidine moieties (Navickiene *et al.* 2000; da Silva *et al.* 2002). The antifungal activity of each amide was determined by direct bioautography against *C. sphaerospermum* and *C. cladosporioides* with 1-5 μg and 5-10 μg, respectively, as the minimum quantity of compounds, specially piperine and 5,6-dihydropiperlonguminine, necessary to inhibit growth of the fungus (Navickiene *et al.* 2000; da Silva *et al.* 2002). Likewise, extracts of *P. tuberculatum* were used in control of dermatophyte fungi *Microsporum canis* (Bodin, 1902), *Microsporum gypseum* (Bodin, Guiart & Grigorakis, 1907) and *Trichopyton rubrum* (Malmsten, 1845) (Palacios *et al.* 2009) therefore, these amides have shown to have a potent fungicidal activity as well as an insecticidal activity as reported in the control of the third instar larval of *D. saccharalis* (Soberón *et al.* 2006) and control of II and III instar larval and adults of *A. aegypti* and *A. pseudopunctipennis* (Bazán-Calderón *et al.* 2010).

Recently, was evaluated the toxicity of extracts and two isobutyl amides (pellitorine and 4,5-dihydropiperlonguminine) from *P. tuberculatum* in velvetbean caterpillar, *A. gemmatilis*; the extracts caused 80% of mortality when doses higher than 800.00 μg insect<sup>-1</sup> of extract of seeds, leaves and stems were administered; pellitorine and 4,5-dihydropiperlonguminine showed 100% mortality at doses of 200 and 700 μg insect<sup>-1</sup> respectively (Navickiene *et al.* 2007). In

our work, the extracts caused 90 – 100% of mortality when doses of 600.00 – 1200.00  $\mu\text{g insect}^{-1}$  (0.6 – 1.2 mg/6.5  $\mu\text{L}$ ) of  $\text{CH}_2\text{Cl}_2$ :MeOH (2:1) and EtOH extracts of mature spikes from wild plants and  $\text{CH}_2\text{Cl}_2$ :MeOH (2:1) extract from *in vitro* plants of *P. tuberculatum* were administered in 72 h of exposure.

This fact has a profound ecological significance since it presupposes an advantage to using plant extracts as a source of complex molecules that exhibit various bioactivities, raising the levels of toxicity in relation to chemically pure individual compounds, in addition to the risk of induce resistance (Bobadilla *et al.* 2005). It is known, chemical studies on Piperaceae species have revealed the occurrence of various compounds as alkaloids, amides, propenylphenols, lignans, neolignans, terpenes, steroids, kawapyrone, piperolides, chalcones, dihydrochalcones, flavones, flavanones and miscellaneous compounds (Parmar *et al.* 1997).

Several of these compounds are known to synergize natural and synthetic insecticides (Bernard *et al.* 1995), for instance, the phenylpropanoid dillapiol, related to piperonyl butoxide, synergizes not only pyrethrins but also several carbamates and organochlorates (Parmar and Tomar 1983). Recently, has been proposed as work strategy the use of heterogeneous extracts of total plant biomass to induce a synergistic effect over some specific organism (Leatemia and Isman 2004b).

In this context Scott *et al.* (2002) demonstrated that the amides presents in *P. tuberculatum* plants has higher toxicity when combined in binary, tertiary and quaternary mixtures compared to single compounds or binary mixtures; one of the four amide compounds, 4,5-dihydropiperlonguminine, was the most toxic in mosquito larvae bioassays. Navickiene *et al.* (2007) reported that seed extracts of *P. tuberculatum* may be more powerful than the pellitorine isolated, therefore, would be advisable the preferential use of crude extracts. There are no simple explanations for the observed differences in the efficacy of the whole extract from different parts of the plant and the isolated piperamides. Variations in the concentration of the insecticide compounds among the plant tissues suggest that varied selective pressures operate in the plants, and a great number of combinations of compositions can arise inside individuals in certain species (Jones and Firn 1991), which can provide a higher protection level to the plant against herbivores (Berenbaum and Zangerl 1996). Our results make it possible to conclude that *Piper* extracts may be good candidates for use in crop protection.

The action mechanism of the pellitorine, 4,5-dihydropiperlonguminine and other related compounds (piperamides) found in *Piper* species on third instar larval of *S. frugiperda* is not well known, however could be attributed the toxicity at the presence of methylenedioxyphenyl ring (MDP) in the molecular structure (Bernard *et al.* 1995; Scott *et al.* 2003), like just as reported to other compounds of similar structure at piperidine, guineensinamide, guineensina, pellitorine and kalecida isolated from *P. guineense* and very actives in the control of adults of *M. domestica* (Gbewonyo *et al.* 1993).

The first three amides were the most active against adults of *M. domestica* and each of these contain a MDP structural moiety. The 5,6-dihydropiperlonguminine, isolated from *P. tuberculatum*, also has an MDP ring and is the main component of the active fraction of the spikes extract (Navickiene *et al.* 2000). Greger (1988) pointed out that these types of amides, including olefinic and alkyl isobutylamides, are com-

mon to a restricted number of related plant families, namely the Piperaceae, Asteraceae, and Rutaceae. In all these families are abundant in the tropics particularly in the humid tropics in the case of the Piperaceae, where herbivory is a selective potent force.

In addition, the piperamides presents dual biological activities, being neurotoxic, affecting the activity of the central nervous system, and also as inhibitors of cytochrome P450 enzymes; these characteristics are too useful to plants of *Piper* genus as a defence strategy against herbivores (Navickiene *et al.* 2007).

In conclusion, *P. tuberculatum* is an abundant species and is semi-domesticated in Peru where is used as a hedge plant. It is for the reasons that the potential use of this species as a source of botanical insect control material looks promising.

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