

Oviposition deterrence of shoots and essential oils of *Minthostachys* spp. (Lamiaceae) against the potato tuber moth

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Abstract: The potato tuber moth is a noxious pest of potato in stores, where the use of repellent plants is an environmentally sound alternative to the application of chemical pesticides. We evaluated the protective effect of native *Minthostachys* species (Lamiaceae) against tuber infestation by the potato tuber moth in a rustic store in Cusco, Perú. We covered potato tubers with dried shoots of *Minthostachys spicata* and *Minthostachys glabrescens* and compared tuber damage with a control treatment of maize straw. We also conducted a no-choice oviposition bioassay in the laboratory, testing the oviposition deterrence of essential oils of *M. spicata*, *M. glabrescens* and *Minthostachys mollis* at natural concentrations. We recorded the number of eggs laid by mated moths on filter paper treated with essential oils of each of the three species and on two control treatments: hexane and untreated blank. Finally, we tested for differences in oviposition deterrence among five full-sib families of potato tuber moth raised under identical conditions. We found that dried, chopped leaves and flowers of *Minthostachys* species reduced the percentage of tuber damage in stores in comparison with the control (5% vs. 12%), but no difference in protection was found between species. Essential oils at natural concentrations deterred moth oviposition, reducing the number of eggs laid by about 80% compared with the control treatments; again, there were no significant differences between *Minthostachys* species. Finally, whereas we detected among-family variation in oviposition on filter papers treated with essential oils, no difference was found in the number of eggs laid on control substrates. Therefore, there was genetic variation for oviposition deterrence in the potato tuber moth and resistance to repellent plants might evolve thereafter.

Key words: *Phthorimaea operculella*, Gelechiidae, genetic variation, muña, potato pests, repellent plants, storage

1 Introduction

The potato tuber moth *Phthorimaea operculella* (Lep., Gelechiidae) is a noxious pest of potato under both field and storage conditions (Haines 1977; Raman and Palacios 1982). The larvae feed on leaves and stems but they are most harmful to potato tubers (Varela and Bernays 1988). The heaviest infestations take place when potatoes are kept in stores, where the adult moth lays its eggs on the tubers (Raman et al. 1987). *Phthorimaea operculella* is a nocturnal moth. Therefore, orientation to plant volatiles seems the most likely mechanism of tuber location in stores (Fenemore 1988).

The use of chemical pesticides to control potato tuber moth has resulted in harmful side effects such as health hazards from residues (Dikshit et al. 1985), reduction in populations of natural enemies (Shelton et al. 1981) and the development of insect resistance to pesticides (Haines 1977; Llanderal-Cazares et al. 1996). An alternative method of management of this insect pest in stores is the use of repellent plant species (Das 1995). A cover of chopped and dried leaves of repellent plants may provide tuber protection comparable to

that of chemical pesticides and biopesticides (Bt) (Lal 1987). The mechanistic bases of such oviposition deterrence are, however, poorly understood (Das 1995; Regnault-Roger 1997). One of the relative advantages of the use of repellent plants in pest management (Foster and Harris 1997) is that insects should not develop resistance towards this control method as rapidly as it occurs with chemical pesticides (Lockwood et al. 1984) and biopesticides (Tabashnik et al. 1997). However, similar to any other species, insect pest populations can undergo adaptive changes to cope with new environments (Boivin et al. 2003). Consequently, if there is genetic variation in the response towards oviposition deterrents within the pest population then resistance to repellents might evolve. To our knowledge, this issue has not been addressed for any insect pest-repellent plant system.

The mint family (Lamiaceae) is one of the aromatic plant families that have been proved effective for insect pest management, mainly due to their essential oils (Regnault-Roger 1997). In Perú, the native shrub 'muña', *Minthostachys* spp. (Lamiaceae) has been reported to protect stored tubers against the potato

tuber moth when they are covered with dried and crushed leaves (Ormachea 1979; Raman et al. 1987). Laboratory bioassays have shown insecticidal activity of dichloromethane extracts and essential oils of *Minthostachys* species against insect vectors of human diseases (Fournet et al. 1996; Ciccía et al. 2000).

The present study had three aims. First, we evaluated in a rustic store in Cusco, Perú, the protective effect of *Minthostachys* species against tuber infestation by the potato tuber moth *P. operculella*. We covered potato tubers with dried and chopped shoots of *Minthostachys spicata* and *Minthostachys glabrescens* and compared tuber damage with a control treatment of maize straw. Secondly, we conducted a no-choice oviposition bioassay in the laboratory, testing the oviposition deterrence of essential oils of *M. spicata*, *M. glabrescens* and *Minthostachys mollis* at natural concentrations. We recorded the number of eggs laid by mated *P. operculella* moths on filter paper treated with essential oils of each of the three species and on two control treatments: filter paper treated with solvent (hexane) and untreated oviposition substrate. Thirdly, we tested for differences in oviposition deterrence among five full-sib families of potato tuber moth raised under identical conditions. Thus, we estimated whether the response towards repellent essential oils from *Minthostachys* species exhibits genetic differences within an experimental population of the insect pest.

2 Materials and Methods

2.1 Study system and general procedures

In Andean highlands, leaves and flowers of evergreen shrubs from the genus *Minthostachys* (Lamiaceae) and related genera are used in traditional medicine and to protect stored produce (Ormachea 1979; Fournet et al. 1996). In mountain slopes at 3640 m near Qenqo village (3 km north of Cusco City, Perú – 13°32'S, 71°57'W), we collected branches in the upper half of the shoot from several mature individuals (0.90–1.20 m height) of *M. spicata*, *M. glabrescens* and *M. mollis*. Collected samples included stems, leaves and flowers. The plant material was air-dried in darkness, and flowers and leaves were detached and chopped. The experimental population of *P. operculella* was initiated from several adult moths obtained from the Andenes Experimental Station (Instituto Nacional de Investigación Agraria - Cusco, Perú) in February 2004. Moths were kept in 11 plastic containers covered with a net where filter paper discs were placed for oviposition. Laid eggs were collected every 24 h and placed in Petri dishes at room temperature (16–18°C) until the first larval stage emerged. Larvae were then put in 5 l containers with a thin layer of sand and 2 kg of potatoes of the susceptible variety 'Peruanita' where they completed their life cycle. Adult moths were fed a solution of diluted honey.

2.2 Rustic store trial

The trial was conducted in April 2004 in a rustic farm store located at Kayra Experimental Station in San Jerónimo District, Cusco, Perú (3150 m; 13°54'S, 71°89'W). The mean maximum temperature during the rustic store trial was 20°C. Twenty-four wooden crates (45 × 45 × 30 cm), each containing 5 kg of healthy potato tubers (about 30 tubers; 'Revo-

lución' variety) with a straw layer at the bottom, were used. Three treatments ($n = 8$) were applied in a randomized block design. Potato tubers were covered with either a thick layer (≈ 25 mm) of dried leaves and flowers of *M. spicata*, *M. glabrescens*, or a thick layer of maize straw, which constituted a physical barrier. The trial started when 150 *P. operculella* moths were released at the centre of the array of wooden crates. The rustic store was then closed and evaluations were conducted 1 month later. We evaluated the percentage of tubers showing visible damage caused by *P. operculella*. Damage (%) in the different treatments was compared using a one-way ANOVA followed by a Tukey test. Data were arcsin-transformed before analysis to meet ANOVA assumptions of normality and variance homogeneity.

2.3 Essential oils

Essential oils were obtained by hydrodistillation for 5 h of the dry plant material (240 g of each *Minthostachys* species). The volatile fraction was collected in hexane. To eliminate the excess of water we dehydrated the sample with sodium hydroxide. We obtained 1.43, 1.0 and 1.60 ml of essential oils from 240 g of *M. spicata*, *M. glabrescens* and *M. mollis* respectively. Hexane solutions of essential oils at the natural concentrations found in each one of the plant species were prepared and stored at 2°C until the start of bioassays. Thus, we had stock hexane solutions (50 ml) of essential oils of *M. glabrescens*, *M. spicata* and *M. mollis* at 0.40%, 0.29% and 0.44% respectively.

2.4 Oviposition bioassay

We conducted a no-choice oviposition test under laboratory conditions (16–22°C; 12 : 12 L : D; 40% RH). One female and one male moth from the experimental population were placed into a 500-ml plastic cylinder with two opposite holes (5 cm Ø) covered with mesh. The top of the cylinder was covered with a Petri dish that had a filter paper disc (10 cm Ø) attached to its lower side. We applied five treatments ($n = 20$ full replicates per treatment). In the first three treatments paper discs were soaked in hexane solutions of essential oils of each of the three *Minthostachys* species (*M. glabrescens*, *M. spicata* and *M. mollis*). The fourth and fifth treatments served as controls: paper discs were either soaked in hexane or left untreated. The number of eggs laid on each treatment was evaluated 24 h after moths were confined into the plastic containers. This was done because within this time period essential oils at natural plant concentrations evaporated from the paper disc surface. There is evidence that the peak of oviposition of *P. operculella* under similar environmental conditions occurs within the first 5 days after confinement (Gamboa and Notz 1990). Data were analysed with a one-way ANOVA and differences among treatments were determined with a Tukey test. Data were log-transformed before analysis to meet ANOVA assumptions.

2.5 Genetic variation bioassay

We first took several couples of moths from the experimental population and isolated them into plastic containers. We randomly chose five pairs and collected all the eggs laid within 5 days, and then raised them separately until adulthood, thus obtaining five full-sib families. We replicated the no-choice test described above but only using essential oils from *M. spicata*. This was done because results of the oviposition bioassay showed no difference in deterrence among *Minthostachys* species. Likewise, we used only the

hexane treatment as control because results showed no effect of this solvent on moth oviposition (see below). Consequently, there were two treatments: paper discs treated with essential oils of *M. spicata* at natural concentrations and paper discs soaked in hexane. Twelve replicates (couples) from each family were assigned to each of the two treatments. The number of eggs laid on each treatment was evaluated 24 h after moths were confined into the plastic containers. Data from each treatment were analysed separately using one-way ANOVA (main factor: moth family). Whereas the control treatment analysis tested whether families had intrinsic differences in fecundity, the essential oil treatment analysis tested for genetic variation in the response of *P. operculella* to *M. spicata*, a natural oviposition deterrent. Data were log-transformed before analysis to meet ANOVA assumptions.

3 Results

3.1 Rustic store trial

A cover of dried leaves and flowers of *Minthostachys* species reduced the percentage of tuber damage caused by potato tuber moth in stores compared with the control, a layer of maize straw ($F_{2,21} = 5.53$; $P < 0.012$; fig. 1). No significant difference in percentage of damaged tubers was found between *M. glabrescens* and *M. spicata* ($P > 0.05$, Tukey test).

3.2 Oviposition bioassay

Treatment of paper discs with essential oils of *Minthostachys* species at natural concentrations significantly deterred oviposition by *P. operculella* ($F_{4,95} = 15.91$; $P < 0.001$; fig. 2), reducing the number of eggs laid by about 80% compared with the controls. There were no significant differences in oviposition neither between *M. glabrescens*, *M. spicata*

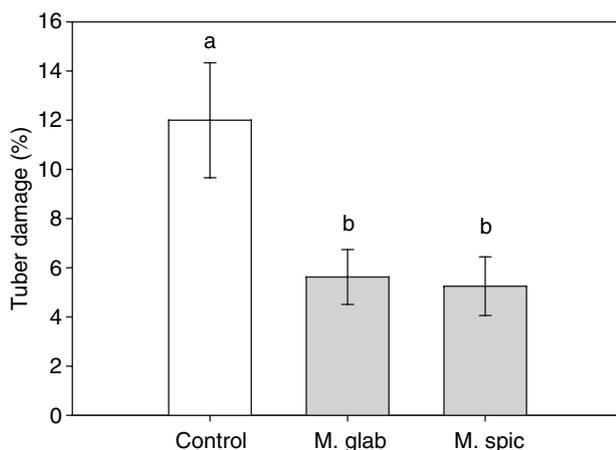


Fig. 1. Percentage of damaged tubers by the potato tuber moth, *Phthorimaea operculella*, in stored potato covered by a layer of maize straw (control), dried foliage of *Minthostachys glabrescens* (*M. glab*), or dried foliage of *M. spicata* (*M. spic*). Bars indicate mean \pm SE. Means sharing a letter are not significantly different ($P > 0.05$, Tukey test after one-way ANOVA)

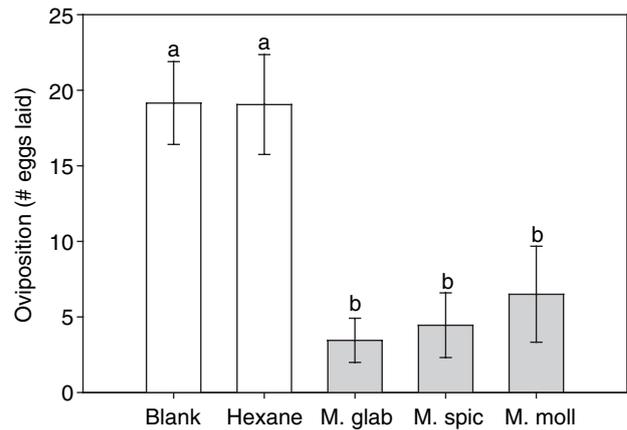


Fig. 2. Oviposition by the potato tuber moth, *Phthorimaea operculella*, on filter paper discs untreated (blank), treated with hexane (Hexane), or treated with hexane solutions of essential oils from *Minthostachys glabrescens* (*M. glab*), *M. spicata* (*M. spic*), or *M. mollis* (*M. moll*). Bars indicate mean \pm SE. Means sharing a letter are not significantly different ($P > 0.05$, Tukey test after one-way ANOVA)

and *M. mollis* treatments ($P > 0.05$, Tukey test) nor between the hexane and the untreated controls ($P > 0.05$, Tukey test).

3.3 Genetic variation bioassay

There was genetic variation for the oviposition response of *P. operculella* to essential oils of *M. spicata* (fig. 3). We detected significant among-family variation in oviposition on paper discs treated with essential oils of *M. spicata* at natural concentrations ($F_{4,55} = 4.08$; $P < 0.006$). In contrast, no difference

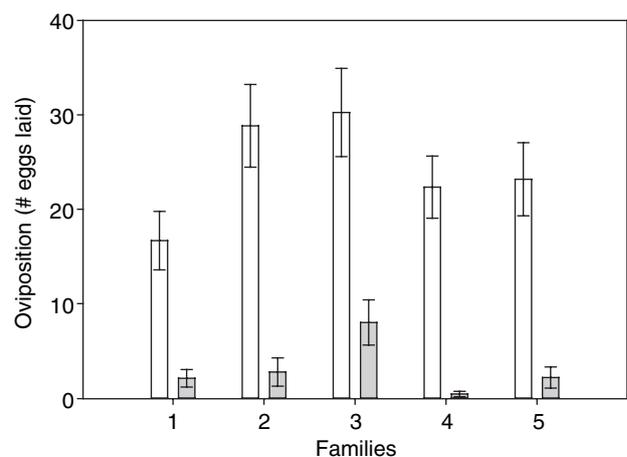


Fig. 3. Oviposition by five *Phthorimaea operculella* full-sib families on filter paper discs treated with hexane (white bars) or treated with hexane solutions of essential oils from *Minthostachys spicata* (grey bars). Bars indicate mean \pm SE (see text for statistical analysis)

among full-sib families was found in the number of eggs laid on control paper discs ($F_{4,55} = 1.95$; $P > 0.11$). Thus, families did not differ in their intrinsic fecundity.

4 Discussion

Muña, *Minthostachys* species demonstrated repellent properties against attack by the potato tuber moth under traditional store conditions in Peruvian Andes. Differences among treatments were detected after only 1 month of storage. The use of a neutral control treatment allowed us to estimate the repellent properties of the test plants besides the unavoidable protective effect of a physical barrier (Das 1995). A similar store trial has shown that rather inert substrates, such as sand, ash and rice husk, confer protection against the potato tuber moth (2–4% tuber infestation vs. 100% tuber infestation in untreated control; Das and Rahman 1997). The protective effect of neutral substrates explains why we found a rather low absolute level of tuber infestation (12%) in our control treatment. The $\approx 50\%$ decrease in tuber infestation herein found is very similar to that earlier reported for dried foliage of *Minthostachys* species in stored potatoes (Raman et al. 1987). Our results confirm the validity of ancestral practices of Andean people regarding the use of muña in crop protection and strongly suggest the inclusion of these species in pest management strategies.

Bioassays showed that essential oils from *Minthostachys* species effectively deterred oviposition by the potato tuber moth. Earlier studies have demonstrated the biological activity of extracts and essential oils from these species against noxious insects. Exposure to essential oils of *Minthostachys andina* impregnated in filter papers caused 30–50% mortality of two triatomine bugs, vectors of Chagas disease (Fournet et al. 1996). Dichloromethane extracts of *Minthostachys setosa* showed high insecticidal activity against larvae of the mosquito *Aedes aegypti*, which is a vector of yellow fever and dengue and has shown increased resistance to synthetic insecticides (Ciccía et al. 2000). Essential oils of the same species showed insecticidal activity against the common house fly *Musca domestica* (Solís-Ramos 1979). Finally, essential oils from *M. mollis* had repellent effects against the mite *Varroa destructor*, which causes a severe disease in honey bees (Ruffinengo et al. 2005).

As was the case in the store trial, where there were no differences in protective effects between the *Minthostachys* species evaluated, we found similar levels of repellence in the essential oils of the three *Minthostachys* species tested. This suggests that these species share one or several semiochemicals occurring in their essential oils. Earlier studies have reported pulegone and menthone, two oxygenated monoterpenes, as major components of the essential oils of *M. glabrescens*, *M. spicata* and *M. mollis* (Senatore 1995, 1998; Malagón et al. 2003). Pulegone and menthone have shown to be toxic against several insect taxa (Ellis and Baxendale 1997; Franzios et al. 1997; Pavlidou et al.

2004). Interestingly, both compounds are induced by mechanical and natural damage in *M. mollis* (Banchio et al. 2005a,b). Using thin layer chromatography, we have detected pulegone as one of the main components in these *Minthostachys* species and related Lamiaceae species (P.C. Guerra et al., unpublished data).

One of the most widely cited advantages of natural pesticides over synthetic insecticides is the lack of selection of insecticide resistance in the target pest (Lockwood et al. 1984; Foster and Harris 1997). However, insect pests can readily develop resistance against various plant defences (Berenbaum and Zangerl 1992), including Bt-toxins (Whalon et al. 1993; Tabashnik et al. 1997). The mechanisms underlying this phenomenon are not different from 'common' evolution of insecticide resistance (McKenzie and Batterham 1994): provided that there is heritable variation in a trait that confers relative fitness advantage within a population against a selective factor, resistance to this factor may evolve. Despite the limited sample of moth families and the probable reduction in genetic variability in laboratory-reared populations compared with field populations (Foley 1986), we detected genetic variation in the response to oviposition-deterrent essential oils in the potato tuber moth; resistance to *Minthostachys*-repellent plants might evolve thereafter. There is evidence of heritable variation in pheromone response in the related species *Pectinophora gossypiella* (Lep., Gelechiidae) (Collins and Cardé 1989), and a recent study has demonstrated experience-induced behavioural responses to oviposition repellents in the moth species *Plutella xylostella* (Liu et al. 2005). However, to our knowledge, this is the first report of genetic variation in the oviposition response to a repellent plant compound within an insect population.

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References

- Banchio E, Zygadlo J, Valladares GR, 2005a. Effects of mechanical wounding on essential oil composition and emission of volatiles from *Minthostachys mollis*. *J. Chem. Ecol.* **31**, 719–727.
- Banchio E, Zygadlo J, Valladares GR, 2005b. Quantitative variations in the essential oil of *Minthostachys mollis* (Kunth.) Griseb. in response to insects with different feeding habits. *J. Agric. Food Chem.* **53**, 6903–6906.
- Berenbaum MR, Zangerl AR, 1992. Genetics of secondary metabolism and herbivore resistance in plants. In: *Herbivores: their interactions with secondary plant metabolites*. Ed. by Rosenthal GA, Berenbaum MR, Academic Press, San Diego, CA, 415–438.
- Boivin T, Bouvier JC, Chadœuf J, Beslay D, Sauphanor B, 2003. Constraints on adaptive mutations in the codling

- moth *Cydia pomonella* (L.); measuring fitness trade-offs and natural selection. *Heredity* **90**, 107–113.
- Ciccia G, Coussio J, Mongelli E, 2000. Insecticidal activity against *Aedes aegypti* larvae of some medicinal South American plants. *J. Ethnopharmacol.* **72**, 185–189.
- Collins RD, Cardé RT, 1989. Heritable variation in pheromone response of the pink bollworm, *Pectinophora gossypiella* (Lepidoptera: Gelechiidae). *J. Chem. Ecol.* **15**, 2647–2659.
- Das GP, 1995. Plants used in controlling the potato tuber moth, *Phthorimaea operculella* (Zeller). *Crop Prot.* **14**, 631–636.
- Das GP, Rahman MM, 1997. Effect of some inert materials and insecticides against the potato tuber moth, *Phthorimaea operculella* (Zeller), in storage. *Int. J. Pest Manage.* **43**, 247–248.
- Dikshit AK, Misra SS, Lal L, 1985. Persistence of chlorpyrifos residues in potatoes and the effect of processing. *Potato Res.* **28**, 461–468.
- Ellis MD, Baxendale FP, 1997. Toxicity of seven monoterpeneoids to tracheal mites (Acari: Tarsenomidae) and their honey bee (Hymenoptera: Apidae) hosts when applied as fumigants. *J. Econ. Entomol.* **90**, 1087–1091.
- Fenimore PG, 1988. Host-plant location and selection by adult potato moth, *Phthorimaea operculella* (Lepidoptera: Gelechiidae): a review. *J. Insect Physiol.* **3**, 175–177.
- Foley DH, 1986. Allozyme variation in laboratory-reared and field-collected Potato Tuberworm Moth (Lepidoptera: Gelechiidae). *Ann. Entomol. Soc. Am.* **79**, 80–83.
- Foster SP, Harris MO, 1997. Behavioural manipulation methods for insect pest management. *Annu. Rev. Entomol.* **42**, 123–146.
- Fournet A, Rojas de Arias A, Charles B, Bruneton J, 1996. Chemical constituents of essential oils of Muña, Bolivian plants traditionally used as pesticides, and their insecticidal properties against Chagas' disease vectors. *J. Ethnopharmacol.* **52**, 145–149.
- Franzios G, Mirotsoy M, Hatziaepostolou E, Kral J, Scouras ZG, Mavragani-Tsipidou P, 1997. Insecticidal and genotoxic activities of mint essential oils. *J. Agric. Food Chem.* **45**, 2690–2694.
- Gamboa M, Notz A, 1990. Biología de *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) en papa (*Solanum tuberosum*). *Rev. Fac. Agron. (Maracay)* **16**, 245–257.
- Haines CP, 1977. The potato tuber moth, *Phthorimaea operculella* Zell.: a bibliography of recent literature and a review of its biology and control on potatoes in the field and in store. Tropical Products Institute, London.
- Lal L, 1987. Studies on natural repellents against potato tuber moth (*Phthorimaea operculella* Zeller) in country stores. *Potato Res.* **30**, 329–334.
- Liu S-S, Li Y-H, Liu Y-Q, Zalucki MP, 2005. Experience-induced preference for oviposition repellents derived from a non-host plant by a specialist herbivore. *Ecol. Lett.* **8**, 722–729.
- Llenderal-Cazares C, Lagunes-Tejada A, Carrillo-Sánchez JL, Sosa-Moss C, Vera-Graziano J, Bravo-Mojica H, 1996. Susceptibility of *Phthorimaea operculella* (Zeller) to insecticides. *J. Entomol. Sci.* **31**, 420–426.
- Lockwood JA, Sparks TC, Story RN, 1984. Evolution of insect resistance to insecticides: a reevaluation of the roles of physiology and behaviour. *B. Entomol. Soc. Am.* **30**, 41–57.
- Malagón O, Vila R, Iglesias J, Zaragoza T, Cañigueral S, 2003. Composition of the essential oils of four medicinal plants from Ecuador. *Flavour Fragr. J.* **18**, 527–531.
- McKenzie JA, Batterham P, 1994. The genetic, molecular, and phenotypic consequences of selection or insecticide resistance. *Trends Ecol. Evol.* **9**, 166–169.
- Ormachea EC, 1979. Usos tradicionales de la “muña” (*Minthostachys* spp., Labiatae) en aspectos fitosanitarios de Cusco y Puno. *Rev. Per. Entomol.* **22**, 67–70.
- Pavlidou V, Karpouhtsis L, Franzios G, Zambetaki A, Scouras Z, Mavragani-Tsipidou P, 2004. Insecticidal and genotoxic effects of essential oils of Greek sage, *Salvia fruticosa*, and mint, *Mentha pulegium*, on *Drosophila melanogaster* and *Bactrocera oleae* (Diptera: Tephritidae). *J. Agric. Urb. Entomol.* **21**, 39–49.
- Raman KV, Palacios M, 1982. Screening potato for resistance to potato tuberworm. *J. Econ. Entomol.* **75**, 47–49.
- Raman KV, Booth RH, Palacios M, 1987. Control of potato tuber moth *Phthorimaea operculella* (Zeller) in rustic potato stores. *Trop. Sci.* **27**, 568–569.
- Regnault-Roger C, 1997. The potential of botanical essential oils for insect pest control. *Integr. Pest Manage. Rev.* **2**, 25–34.
- Ruffinengo S, Eguaras M, Floris I, Faverin C, Bailac P, Ponzi M, 2005. LD50 and repellent effects of essential oils from Argentinian wild plant species on *Varroa destructor*. *J. Econ. Entomol.* **98**, 651–655.
- Senatore F, 1995. Composition of the essential oil of *Minthostachys spicata* (Benth.) Epl. *Flavour Fragr. J.* **10**, 43–45.
- Senatore F, 1998. Volatile constituents of *Minthostachys setosa* (Briq.) Epl. (Lamiaceae) from Perú. *Flavour Fragr. J.* **13**, 263–265.
- Shelton AM, Wyman JA, Mayor AJ, 1981. Effects of commonly used insecticides on the potato tuberworm and its associated parasites and predators in potatoes. *J. Econ. Entomol.* **74**, 303–308.
- Solís-Ramos N, 1979. Estudio toxicológico del aceite esencial de muña en la *Musca domestica* L. BSc Thesis, Facultad de Ciencias Agrarias, Universidad Nacional San Antonio Abad, Cusco, Perú.
- Tabashnik BE, Liu Y-B, Finson N, Masson L, Heckel DG, 1997. One gene in diamondback moth confers resistance to four *Bacillus thuringiensis* toxins. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 1640–1644.
- Varela LG, Bernays EA, 1988. Behavior of newly hatched potato tuber moth larvae, *Phthorimaea operculella* Zell. (Lepidoptera: Gelechiidae), in relation to their host plants. *J. Insect Behav.* **1**, 261–275.
- Whalon ME, Miller DL, Hollingworth RM, Grafius EJ, Miller JR, 1993. Selection of a Colorado potato beetle (Coleoptera: Chrysomelidae) strain resistant to *Bacillus thuringiensis*. *J. Econ. Entomol.* **86**, 226–233.

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