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Effect of medicinal plants against common pistachio psyllid under laboratory conditions

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Abstract

Agonoscena pistaciae Burckhardt and Lauterer (Hem.,: Aphalaridae) is the most important key pest of pistachio trees in Iran. Chemical control is the most effective and accessible method to control this pest. But, the environmental problems caused by the overuse of the synthetic insecticide have been a source of concern in recent years. Botanical insecticides are recognized as proper alternatives to conventional insecticides. Therefore, this study was conducted to determine the toxicity of extracts from *Achillea millefolium* L. (Asteraceae) and *Carum copticum* L. (Apiaceae) against 5th instar nymphs of common pistachio psyllid, *Agonoscena pistaciae* Burckhardt and Lauterer (Hemiptera: Aphalaridae) under laboratory conditions.. Bioassay was carried out using a dipping technique. Probit analysis of concentration-mortality data was conducted to estimate the LC_{50} value. The results showed that *A. millefolium* and *C. copticum* extracts were effective on *A. pistaciae. C. copticum* with an LC_{50} value of 749.95 mL/L was more toxic than *A. millefolium* (914.33 mL/L) on 5th instar nymphs of *A. pistaciae*. But, according to the LC_{50} values, no significant differences were observed between *A. millefolium* and *C. copticum* 24 h post-treatment. Our results suggest that the extracts of *A. millefolium* and *C. copticum* are suitable compounds to control of *A. pistaciae*.

Key Words: *Agonoscena pistaciae*, *Achillea millefolium, Carum copticum*, toxicity.

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Introduction

The common pistachio psyllid, *Agonoscena pistaciae* Burckhardt and Lauterer (Hem.,: Aphalaridae), is the most damaging pest of pistachio in Iran (Mehrnejad, 2003; Alizadeh *et al*., 2011). Both adults and nymphs suck leaf sap and produce large amounts of honeydew. Direct feeding of the pest causes defoliation, stunting, the fall of fruit buds, and poor yield (Mehrnejad, 2002). This key pest is generally controlled by insecticides belonging to various classes (Alizadeh *et al*., 2011). The repeated and intense use of synthetic insecticides for several decades has raised long-term human health and environmental concerns, mainly due to their slow degradation in the environment and toxic residues in the products, and the evolution of resistance to pesticides in pest populations (Isman, 2006).Therefore, it is necessary to develop techniques that will provide more efficient *A. pistaciae* control without serious effects on the environment. Biorational insecticides include botanical extracts and their synthetics, bacteria, viruses, fungi, and protozoa, as well as the chemical analogs of naturally occurring biochemicals, such as pheromones and insect growth regulators that are used to control numerous insect pest species (Haseeb *et al.*, 2004). Among current alternative methods aiming at curbing the use of synthetic insecticides, botanicals have been suggested as an alternative source for insect control (Liang *et al*., 2003; Charleston *et al*., 2005), because most botanical insecticides are selective to insect pests and have no or little harmful effects on non-target organisms and the environment. Furthermore, they are easily available and less expensive than synthetic insecticides (Regnault-Roger, 1997; Liang *et al*., 2003; Charleston *et al*., 2005). In addition, in organic agriculture, compounds extracted from plants can be used as biopesticides to kill or repel pest insects (Schmutterer, 1990; Liu & Stansly, 1995; Liang & Liu, 2002) and also, as oviposition deterrents, antifeeding additives, growth regulators, and chemosterilants (Jilani *et al*., 1990; Isman, 2006).

Some medicinal plants have been reported to show insecticidal properties, and they have widely been used as crop protectants (Omotoso, 2004, 2008; Padin *et al*., 2013). Plants of the Asteraceae family contain effective secondary metabolites that could affect insect's behavior and biology (Abd El-Aziz, 2011). It has been shown that some Asteraceae plant extracts (*Rhaponticum acaule* DC and *Mantisalca duriaei* Briq. *et* Cavill.) have antifeedant characteristics towards *Tribolium confusum* Jacquelin du Val so that they affect significantly larval growth and show high toxic effect on the larvae and adult (Boussaada *et al*., 2008). *Achillea* contains various species of perennial plants found worldwide. *Achillea* genus contained terpenoids, lignans, flavonoids and other compounds in its foliage and flowers with different biological activities against insects and microorganisms (Vitalini *et al*., 2011; Zhiani & Moradi, 2014). *Achillea millefolium* L. (Asteraceae), known as "yarrow", is a perennial herb that has been widely used in folk medicine in many countries (Benedek *et al.*, 2008). *A. millefolium* crude extracts have toxic effects against *Phthorimaea operculella* Zeller. (Allahverdizadeh & Mohammadi, 2016) and coffee leaf miner, *Leucoptera coffeella* (Guerin-Meneville and Perrottet, 1842) (Alves *et al*., 2013). Also, it has been reported that essential oils of *A. millefolium* control *Plodia interpunctella* (Hubner) adult (Ebadollahi & Ashouri, 2011). Insecticidal activities of other plant species of Asteraceae, for example, *Ambrosia tenuifolia* Spreng., *Baccharis trimera* (Less.), *Matricaria chamomilla* L., *Tagetes minuta* L. have been demonstrated against pests (Alok-Krishna *et al*., 2005; Juan Hikawczuk *et al.*, 2008; Padin, *et al*., 2013).

Ajowan (*Carum copticum* L.) is an annual medicinal plant with white flowers and small brownish fruits. It grows in Iran, Pakistan, Egypt, and India (Sahaf & Moharramipour, 2008) and is cultivated in various areas such as Europe, Egypt, Pakistan, Afghanistan, and Iran (Gersbach & Reddy, 2002). Ajowan fruits with a smell like thymol (Singh et al., 2004) are an important commercial product for the food/flavoring industry (Minija & Thoppil, 2002). Antifungal and antibacterial properties of *C. copticum* and *Cuminum cyminum* L. have, however, been proved (Behtoei *et al*., 2012). Antimicrobial activity of ajowan essential oil against multi-drug resistant *Salmonella typhi* (Schroeter) has been reported (Rani & Khullar, 2004).

Insecticidal activity of *C. copticum* essential oil has been demonstrated by many researchers against different insect species, such as *Callosobruchus chinensis* L., *Callosobruchus maculates* (F.), *Sitophilus oryzae* L., *Rhyzopertha dominica* F., *T. confusum*, *Tribolium castaneum* (Herbst), *Oryzaphilus surinamensis* L. and *P. interpunctella* (Upadhyay *et al*., 2007; Sahaf *et al*., 2007; Sahaf and Moharramipour, 2008; Shojaaddini *et al*., 2008; Habashi *et al*., 2011). However, there are no data on the insecticidal toxicity of the extracts of these plants against *A. pistaciae*. Therefore, the present study was conducted to investigate the insecticidal potential of *A. millefolium* and *C. copticum* extracts against *A. pistaciae* under laboratory conditions.

Material and Methods

Insect rearing

Adults of *A. pistaciae* were collected from an untreated pistachio orchard in Rafsanjan, Iran. Mated females of *A. pistaciae* were placed in pistachio leaf disk and were removed after 24 h. Generally, the eggs and nymphs of *A. pistaciae* were reared in pistachio leaf disk cages under controlled conditions in laboratory. The hatched nymphs were transferred into new pistachio leaf disk. The pistachio leaf disks were replaced in 3 d intervals until the nymphs reached 5th instar. The 5th instar nymphs were used for bioassay experiments. The rearing of insects and bioassay experiments were performed under controlled laboratory conditions (25 ± 1°C, 55 ± 5% RH and a photoperiod of 16:8 [L: D] h).

Plant Extract

The aerial parts of *A. millefolium* and seeds of *C. copticum* were collected from Bibihayat, Khanaman, Rafsanjan, Iran from June to August 2016. Plant materials were air dried and ground in a laboratory mill to a moderately fine powder. The extraction was carried out by the procedure described by Rehman et al. (2009). Powdered material (1 kg) was extracted with 95% ethanol (2.5 L). The solution was held at 25 ± 1 °C for 8 d with the glass jar being shaken twice a day to help dissolve the powdered plant material. The solution was filtered through Whatman No.1 filter paper. The extracts were evaporated under reduced pressure in a rotary evaporator, bulked, and stored in a freezer at -20°C.

Bioassay Technique

Responses of 5th instar nymphs of *A. pistaciae* to various concentrations of plant extracts were determined using a dipping bioassay technique that had been reported by previous studies (Amirzade et al., 2014). The technical material was not soluble in water, so firstly, the amount required to make the basal solution was dissolved in 1ml of acetone and then, the Twin 80 (concentration = 2%) was used to solve it. The preliminary tests to determine the concentration which causes 5-95% of population death were done for the final test. The final concentrations (472, 910, 1754, 3381 and 6516 ml/L for *A. millefolium* and 113, 330, 967, 2832 and 8291 mL/L for *C. copticum*) were calculated using logarithmic distance (Robertson & Preisler, 1991) following preliminary trials. One-day-old 5th instar nymphs of common pistachio psyllid were dipped in each concentration of each plant extract for 3 s. The treated nymphs were air-dried and then, they were placed on the pistachio leaf disk and assayed. Agar beds were layered in the base of each Petri-dish for keeping the humidity of the pistachio leaves. Freshly cut leaf discs of pistachio were re-sized to fit in the base of each Petri-dish and then, placed on the agar beds. The bioassays were done at three replications. Each replication consisted of 20 5th instar nymphs per Petri-dish. Distilled water was used as the control. Mortality of nymph was recorded after 24 h. Leaf disks were maintained in a growth chamber at 25 ± 1°C.

Statistical Analysis

The LC_{50}, 95% Confidence Interval (CI), the slope and intercept of the probit mortality regression and the relevant statistical tests were estimated by probit analysis using POLO – PC Software. Differences in LC_{50} values were considered significant between pesticides and insect species if there was no overlap of 95% fiducial limits.

Failure of 95% to overlap was used as the criterion for the significant difference at LC_{50}. Efficacy data were analyzed by analysis of variance (ANOVA) for a completely randomized design with mean separation by Tukey (P = 0.05) (SPSS, 16).

Results

The toxicity of botanical extracts from *A. millefolium* and *C. copticum* against 5[th] instar nymphs of *A. pistaciae* were evaluated using dipping bioassay methods. Bioassay of the *A. millefolium* and *C. copticum* extracts on the 5[th] instar nymphs of *A. pistaciae* indicated that there were significant differences among different concentrations of *A. millefolium* ($F = 46.20$; df = 5, 12; $P < 0.0001$) and *C. copticum* ($F = 113.26$; df = 5, 12; $P < 0.0001$). Morality percentages were significant among different treatments and were classified into different groups. The percent mortality caused by the extracts ranged from 35.85 to 86.79% for *A. millefolium*, and from 24.52 to 100% for *C. copticum* (Table 2).

The results indicated that the extracts of *A. millefolium* and *C. copticum* were effective on 5[th] instar nymphs of *A. pistaciae*. The LC_{50} of *A. millefolium* and *C. copticum* extracts was obtained at 914.33 and 749.95 mL/L, respectively. According to the LC_{50} values, no significant differences were observed between *A. millefolium* and *C. copticum* 24 h after treatment based on the overlap of 95% CL (Table 1).

Table 1. The toxicity of the extracts of *A. millefolium* and *C. copticum* on 5[th] instar nymph of *A. pistaciae* using a dipping bioassay method 24 h after treatment.

	n	Slope ± SE	LC_{50} mL/L	95% Confidence Interval	χ^2
A. millefolium	360	1.24 ± 0.22	914.33	511.66-1333.80	0.48(3)
C. copticum	360	1.38 ± 0.24	749.95	370.75-1172.32	9.65(3)

During the experiment, a difference was observed between the levels of mortality percentage induced by different concentrations of these extracts. Naturally, the extracts became more effective as the concentrations were increased. The mortality percentage of *A. pistaciae* nymph was enhanced with insecticide concentration (Table 2).

Table 2. The mortality percentage of 5[th] instar nymphs of *A. pistaciae* treated with *A. millefolium* and *C. copticum* after 24 h

A. millefolium		***C. copticum***	
Concentration (mL/L)	**Mean ± Std. Error**	**Concentration (mL/L)**	**Mean ± Std. Error**
472	35.85 ± 1.67[a]	113	24.52 ± 4.99[a]
910	52.83 ± 3.33[ab]	330	30.19 ± 4.99[a]
1754	60.37 ± 2.89[b]	967	54.71 ± 3.27[b]
3381	75.47 ± 4.41[bc]	2832	69.81 ± 1.89[c]
6516	86.79 ± 4.41[c]	8291	100.00 ± 0.00[d]

The dose-response curves for two extracts were similar. This suggests that the two extracts either contain similar active ingredients or possess a similar mode of action (Fig. 1).

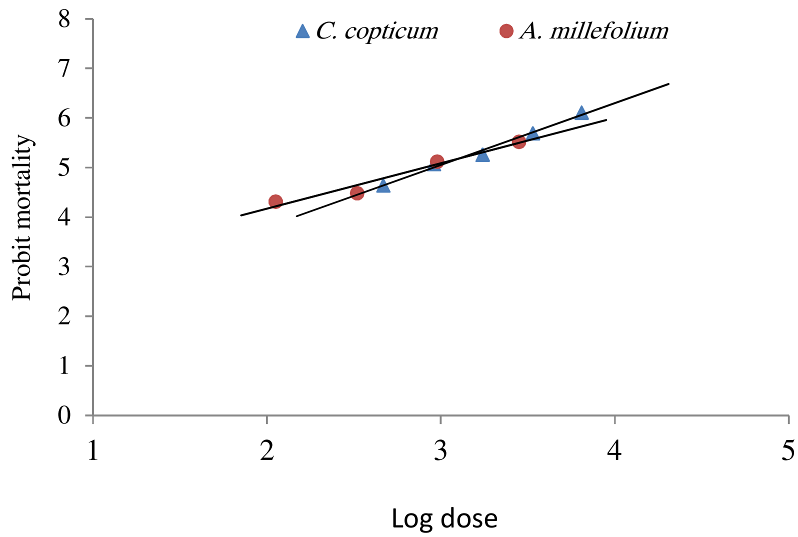

Fig. 1. Dose-response relationship of the log dose and mortrality percentage probit of *A. pistaciae* exposed to extracts of *A. millefolium* and *C. copticum*.

Discussion

Effective control of the target insect pests was achieved as the detrimental impacts were minimized to non-target beneficial arthropods, the environment, and humans. This approach has drawn attention to the use of biorational control of the insect pests in the agricultural ecosystems. Consequently, botanicals have become more desirable than conventional synthesized broad-spectrum chemical pesticides due to their low toxicity to the environment and beneficial arthropods (Yang *et al*., 2010). In this bioassay, the extracts of *A. millefolium* shoot and *C. copticum* seeds were effective on *A. pistaciae* nymphs after 24 h under laboratory conditions. Probit analysis revealed that *C. copticum* (LC_{50} = 749.95 mL/L) was more toxic to *A. pistaciae* than *A. millefolium* (LC_{50} = 914.33 mL/L) (Table 1). Therefore, the extracts of *A. millefolium* and *C. copticum* showed good potential as toxicant agents to 5th nymph *A. pistaciae.* This confirms the findings of several studies which have demonstrated the highly lethal effect of some of these species on different pests. The effect of *A. millefolium* could be attributed to antifeedant effect so that antifeedant effects of various plants extracts have been reported with promising results against different pests in previous studies (Kutas & Nadasy, 2005; Szczepanik *et al*., 2005). Also, this is rendered quite possible due to the existence of compounds such as flavonoids, terpenoids and lignans with different biological activities against insects (Vitalini *et al*., 2011; Zhiani & Moradi, 2014) and 1, 8-cineol, camphor and borneol (Haziri *et al*., 2010). Ateyyat and Abu-Darwish (2009) revealed that *A. millefolium* contains compounds, such as flavonoids, which are soluble in polar solvents such as acetone and ethanol. Flavonoids have a catecholic B-ring that seems to be responsible for the toxicant activity against insects (Onyilagha *et al*., 2004). Nadim *et al*. (2011) reported that the predominant constituents of *A. millefolium* were sabinene, cineole, borneol, bornyl acetate, pinene, terpinine and chamazulene. It has been shown that toxicity of essential oil of *A. millefolium* was related to 1, 8-cineol, camphor and borneol (Haziri *et al*., 2010). Moreover, 1,8-cineole has been shown to inhibit acetylcholinesterase (AChE) from *S. oryzae* adults and *T. castaneum* larvae (Abdelgaleil *et al*., 2009). Contact toxicity and repellent activity of 1,8-cineole have been reported in studies with *Sitophilus granarius*, *Sitophilus zeamais*, *T. confusum* and *Prostephanus truncatus* (Horn) (Obeng-Ofori *et al*., 1997). Dehghan & Elmi (2014) reported that chemical compounds of essential oils of *Achillea* species were highly variable, which may be due to the differences in their chemical polymorphic structure and environmental conditions. Effects of *A. millefolium* extract on different developmental stages of insects were investigated and in some cases, the compounds showed an acceptable control on pest insects (Conti *et al*., 2010; Zoubiri & Baaliouamer, 2014). Allahverdizadeh & Mohammadi (2016) showed *A. millefolium* crude extracts had toxic effects on *P. operculella*. Also, the extracts from *A. millefolium* have shown high mortality at the coffee leaf miner, *L. coffeella* (Alves *et al*., 2013). The essential oils of *A. millefolium* exhibited an acceptable fumigant toxicity against adult *P. interpunctella* with a 24-h LC_{50} value of 34.80 μL/L (Ebadollahi & Ashouri, 2011).

The insecticidal constituents of many plant extracts and essential oils are monoterpenoids (Fang *et al*., 2010; Wang *et al*., 2014). Thymol, as a monoterpenoid, is the major component in *C. copticum* essential oil. One of the chemical compounds with high repellent activity is thymol (Nerio *et al*., 2010). There are numerous reports on the insecticidal activity of thymol. Regnault-Roger & Hamraoui (1995) tested 22 essential oils for their fumigant toxicity to the bean weevil, *Acanthoscelides obtectus* (Say), and found thymol, carvacrol, and terpineol to be effective in inhibiting beetle reproduction. Erler (2005) reported the fumigant activity of thymol against adults and eggs of *T. confusum* and larvae and eggs of *Ephestia kuehniella* Zeller. *β*-cymene (21.67%), γ-terpinene (15.85%), α-pinene (4.86%), β-pinene (3.62%), and 4- terpineol (1.65%) are the other components of *C. copticum* oil that have insecticidal activity (Erler, 2007). For instance, it was reported that γ-terpinene and thymol were the most active constituents among the major monoterpenoids of essential oil against adults of the rice weevil, *S. oryzae* (Erler, 2007). So, the toxic effects of *C. copticum* on *A. pistaciae* could be attributed to thymol and other components. The rapid action against *A. pistaciae* may be indicative of a neurotoxic mode of action. There is evidence for the octopaminergic system as a target for some monoterpenoids (Enan, 2001). However, it is necessary to elucidate whether the toxic action of thymol and γ-terpinene is mediated by octopamine. The octopamine is a multifunctional, naturally occurring biogenic amine that plays a key role as a neurotransmitter, neurohormone, and neuromodulator in invertebrate systems, such as in insects (Evans, 1980; Orchard, 1982). Fumigant toxicity of the essential oil of *C. copticum* has been studied on five stored-product beetles including, *C. maculatus*, *S. oryzae*, *T. castaneum* (Sahaf *et al*., 2007), *R. dominica*, and *O. surinamensis* (Habashi *et al*., 2011). Sahaf *et al*. (2007) studied fumigant toxicity of essential oil from *C. copticum* against *S. oryzae* and *T. castaneum.* They observed that *S. oryzae* (LC_{50} = 0.91 μL/L) were significantly susceptible than *T. castaneum* (LC_{50} = 33.14 μL/L). Also, the essential oil of *C. copticum* had activity against growth stages of *P. interpunctella* (Shojaaddini *et al*., 2008). Ziaee & Moharramipour (2013) reported that 3500 mg/kg of *C. copticum* caused 80% mortality in *S. granarius* 7 days after treatment; while for *T. confusum*, 29% mortality was achieved and it was increased to 100% after 14 d.

The acceptable effects of other extracts have been proved to control *A. pistaciae.* Razavi & Mahdian (2015) showed that *Viola odorata* L. extract is effective on *A. pistaciae* mortality percentage. Sheibani & Hassani (2014) investigated the effect of Sirinol (garlic extract), Tondexir (pepper extract), and Palizin (eucalyptus extract) on *A. pistaciae.* The results showed the highest mortality in Palizin treatment after 2 and 7 d. But, the sampling of 14, 21 and 28 d post-treatment showed the highest and lowest mortality in Sirinol and Tondexir treatments, respectively. These researchers reported no significant differences between Sirinol and Palizin 28 d post-treatment, but these compounds showed significant differences with Tondexir.

Generally, plant extracts are effective against sucking pests and other pests.Yang *et al.* (2010) reported that MOI-201 (a Chinese medicine plant extract) and Requiem (a plant extract of *Chenopodium ambrosioides* L.) had significant repellency to *Bactericera cockerelli* (Sulc) adults and deterred oviposition. It has been proved that MOI-201 caused > 97% mortality of young nymphs (1^{st} to 3^{rd} instar) of the potato psyllid in the laboratory bioassays (Yang *et al*., 2010). Jazzar & Hammad (2003) showed that extracts of leaves and fruits of *Melia azedarach* L. (Chinaberry) with Tween-20 caused a mortality range of 34.6 to 74.1% at 3^{rd}- 4^{th} instar nymphs of *Bemisia tabaci* (Gennadius). Kestenholz et al. (2007) showed that extracts of *Cassia sophera* L. was more effective than the powdered leaves on alleviating the infestations of *C. maculatus* and *S. oryzae*. In another experiment using extract of *Foeniculum vulgare* Gaetner

(Apiaceae) fruit, over 90% mortality was achieved in adults of *S. oryzae* and *C. chinensis* at 3 or 4 days after treatment (Kim et al., 2003). Ho et al. (1995) reported that non-polar extracts of *Illicium verum* Hook f. completely suppressed F1 adult emergence in *T. castaneum* and *S. zeamais* while polar extracts only caused a significant reduction in F1 adult emergence. Lu et al. (2012) reported that *Alpinia officinarum* Hance rhizome extract exhibited strong fumigant and repelled the pests. Padin et al. (2013) reported that extracts of *Viola arvensis* Murray, *Matricaria chamomilla* L., *Brassica campestris* L., and *Jacaranda mimosifolia* D. Don caused 68, 57, 56 and 49% mortality on *T. castaneum* after 7 days, respectively. Also, ethanol extract of *Peganum harmala* L. (seed) has caused a mortality of 66 and 100% in the third instar larvae that had been fed for two days on the cabbage leaves treated with the ethanol extract at concentrations of 30 and 40 mg/ml, respectively (Abbasipour et al., 2010).

In summary, results of the present study clearly showed that the extracts of *A. millefolium* and *C. copticum* had significant effect on the control of *A. pistaciae. C. copticum* had greater potential to control *A. pistaciae.* We believe that biorational insecticides, including the extracts of *A. millefolium* and *C. copticum*, have great potential for the management of *A. pistaciae* although more insecticides should be screened and evaluated under both laboratory and field conditions for additional control of this pest. Furthermore, an integrated *A. pistaciae* management strategy should take into consideration the use of insect-resistant varieties, conserving and augmenting biological control agents and avoiding applications of broad-spectrum insecticides.

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تاثیر گیاهان دارویی روی پسیل معمولی پسته در شرایط آزمایشگاهی

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چکیده

پسیل معمولی پسته *Agonoscena pistaciae* Burckhardt and Lauterer (Hem.,: Aphalaridae) آفت کلیدی درختان پسته در ایران است. کنترل شیمیایی موثرترین و در دسترس‌ترین روش برای کنترل این آفت است. اما به‌دلیل مضرات متعدد حشره‌کش‌های شیمیایی، ترکیبات مشتق شده از گیاهان می‌توانند جایگزین مناسبی برای آن‌ها باشند. لذا در این تحقیق اثر سمیت عصاره دو گیاه بومادران (Asteraceae) *Achillea* millefolium L. و زنیان *Carum copticum* L. (Apiaceae) روی پوره سن پنجم پسیل معمولی پسته در شرایط آزمایشگاهی مورد بررسی قرار گرفت. پاسخ پوره سن پنجم پسیل معمولی پسته در برابر غلظت‌های متفاوت عصاره گیاهان بومادران و زنیان با استفاده از روش غوطه-ورسازی پوره سن پنجم در محلول تهیه شده با غلظت‌های مشخص بررسی شد. تجزیه پروبیت دز – پاسخ، برای تخمین LC_{50} هر یک از عصاره ها استفاده شد. تجزیه پروبیت نشان داد که زنیان با LC_{50} برابر با ۷٤۹/۹۵ میلی‌لیتر بر لیتر برای پوره های سن پنجم پسیل معمولی پسته نسبت به بومادران با LC_{50} برابر با ۹۱٤/۳۳ میلی‌لیتر بر لیتر سمیت بیشتری برای پسیل معمولی پسته دارد. البته طبق مقادیر LC_{50} به دست آمده، تفاوت معنی‌داری بین عصاره گیاه بومادران و زنیان پس از گذشت ۲٤ ساعت مشاهده نگردید. بنابراین طبق نتایج به‌دست آمده هر دو عصاره گیاه بومادران و زنیان در کنترل موثر پوره‌های پسیل معمولی پسته نقش دارند.

واژه‌های کلیدی: پسیل معمولی پسته، بومادران، زنیان، سمیت

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