

Research Article

Effect of pederin on activity of digestive and detoxifying enzymes of *Ephestia kuehniella* (Lep: Pyralidae) and *Tribolium confusum* (Col: Tenebrionidae)

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Abstract: During the last decade, plant and microbial-derived metabolites have received growing attention as potential tools for pest management in agriculture. Pederin $(C_{25}H_{45}NO_9)$ is a vesicant toxin produced by Pseudomonas-like bacterial symbionts of rove beetles within the genus Paederus (Col: Staphylinidae). In this study, the toxicity of pederin to two stored product pests, Ephestia kuehniella Zeller (Lep: Pyralidae) and Tribolium confusum Jacquelin du Val (Col: Tenebrionidae) was evaluated using laboratory bioassays. Probit analysis estimated the median lethal concentrations of pederin as 1311.96 and 596.36ppm for E. kuehniella fourth larval instar and T. confusum adults, respectively. We also measured the activity of two major digestive enzymes (amylases and proteases) as well as three major detoxifying enzymes (P_{450s} monooxygenases, glutathione Stransferases, and carboxyl esterases) in insects treated orally with pederin. Feeding on pederin resulted in significant decrease in the activity of amylolytic, proteolytic, and carboxyl esterase enzymes, but significant increase in the activity of P450s and glutathione S-transferases. Results of this study may highlight pederin as a novel source of pesticides with unique mode of action for use in pest management programs.

Keywords: Digestive Enzyme, Detoxifying Enzyme, Natural Products, *Paederus fuscipes*, pederin, toxin

Introduction

The postharvest losses of stored products caused by insects has been estimated to range from 9% in developed countries to 20% or more in developing countries (Subramanyam, 1995, Phillips and Throne, 2010). For decades, the use of synthetic fumigants has been the major commercial mean of stored product pest control (Fields and White, 2002, Cox *et al.*, 1984, Bell

and Savvidou, 1999). For example, sulfuryl fluoride, methyl bromide and phosphine have long been used for management of stored product pests throughout the world because of their rapid action and activity against a broad spectrum of pests (Fields and White, 2002). effects of these However, the harmful compounds on human health, non-target organisms and the environment as well as low efficiency against the target pest due to cryptic activity and rapid development of resistance have persuaded efforts for development of new classes of pesticides with more specificity against the target pest and less toxic effects to non-target organisms (Gill and Garg, 2014,

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Desneux et al., 2007, Aktar et al., 2009). Natural products, derived from plants, animals, bacteria, and fungi, are a source of novel compounds with insecticidal, fungicidal. herbicidal, and therapeutic properties besides being environmentally safer molecules than many of the currently used pesticides (Duke et al., 2010, Cantrell et al., 2012). Almost 70% of all newly registered active pesticides have their origins in natural products (Dayan and Duke, 2014), indicating growing interest and exigency for development of pesticides with novel modes of action. Successful examples include the insecticide spinosad, the herbicide glufosinate, and the fungicide strobilurin (Duke et al., 2010).

Polyketides are a class of secondary metabolites produced by certain organisms and serve survival functions for the organisms producing them (Demain and Fang, 2000, Chan et al., 2009). The pederin family consists of numerous members of polyketides with potent cytotoxicity and antitumor and antiviral activity, which are mainly based on inhibition of eukaryotic protein biosynthesis (Piel, 2002, Kellner, 2002). Despite the fact that all other members of this family have been known only from marine sponges, pederin (C₂₅H₄₅NO₉) itself occurs exclusively in several species of rove beetles (Col: Staphylinidae) within the two genera Paederus and Paederidus (Piel et al., 2004, Piel, 2002). In Paederus beetles, pederin is biosynthesized by a symbiotic bacterium in the γ subdivision of the Proteobacteria, which is closely related to the ubiquitous species, Pseudomonas aeruginosa (Piel et al., 2004, Piel, 2002). The bacteria, and therefore the ability of pederin biosynthesis, are largely confined to adult females. However, larvae and adult males can also store pederin acquired maternally or by ingestion (Kellner, 1998). Although, the toxic properties of pederin to humans have been recognized for nearly one century (Pirajá Da Silva, 1912), its insecticidal has been very poorly activity studied (Tabadkani and Nozari, 2014, Kellner and Dettner, 1996). Kellner and Dettner (1996) showed that Paederus larvae are deterrent to wolf spiders (Kellner and Dettner, 1996), but not to a range of predatory insects. In another study, Tabadkani and Nozari (2014) showed that the body fluid of *Paederus fuscipes* Curtis is highly toxic to the predatory ground beetle *Chlaenius pallipes* Gebler (Col: Carabidae).

Given the widespread use of natural products in crop protection and medicine, pederin may provide a source of new classes of insecticides with specific mode of action for pest management. In this study, the toxicity of pederin to two stored product pests, the Mediterranean flour moth, Ephestia kuehniella (Zeller) (Lep: Pyralidae) and the confused flour beetle, Tribolium confusum Jacquelin du Val Tenebrionidae) was studied under (Col: laboratory condition. Both species are common important pests of stored cereals and worldwide. In addition to direct damage caused through external feeding of the larvae, the quality of the stored product is severely affected by the presence of living and dead insects, frass, feces, silk, larval exuviae, and chemical excretions and odors (Stejskal and Hubert, 2006, Panizzi and Parra, 2012). We also investigated the activity of common digestive and detoxifying enzymes in response to infection with pederin.

Materials and Methods

Insects

Adult *P. fuscipes* were collected (between 11-15 o'clock) form rice fields at Rasht (37.2 N 49.60E) (Guilan province, north of Iran) using hand, sweep net, and light traps. After species identification, adult females were used for pederin extraction. Adult females are readily identified by absence of a distinct cleft at posterior edge of their last abdominal ventrite, as opposed to males.

E. kuehniella eggs were obtained from a commercial insectariums. A stock colony was established in the laboratory by distributing 0.5g eggs in plastic containers $(10 \times 7 \times 5 \text{ cm})$ containing 1000g wheat flour plus 100g yeast. The colony was maintained at controlled condition $(25 \pm 1 \text{ °C}, 65 \pm 5\% \text{ RH}, \text{ and } 16\text{L}: \text{8D}$

photoperiod). The fourth instar larvae were used for all experiments.

Adult *T. confusum* were hand collected from an infected wheat warehouse and transferred to laboratory for establishment of stock colony. After species identification, by existing keys the beetles were released inside plastic containers $(5.5 \times 15.5 \times 25 \text{ cm})$ containing 500g wheat flour plus 15g yeast. The containers were maintained at growth chambers $(28 \pm 1 \text{ °C}, 70 \pm 5\% \text{ RH}, \text{ and absolute darkness})$. Newly emerged Adult beetles (< 24-h old) were used for all experiments.

Pederin extraction

Pederin extraction was performed following the method of Mohseni et al. (2014) (Mohseni et al., 2014). Briefly, P. fuscipes adult females were finely crushed in Eppendorf micro-tubes containing absolute ethanol (Merck). The mixture was then centrifuged (Heraeus Fresco 21 centrifuge, Fisher Scientific, Germany) at 3,000 ×g for 10 min to remove tissue particles and the resulting supernatant was maintained at 37 °C for ethanol evaporation. After removal of lipids using hexane-methanol (1:1 v/v), the methanol phase was separated using decanter and evaporated by maintaining at 37 °C. The raw extract was dissolved in ethyl acetate (98%, Merck) and stored at 4 °C for further analysis. 10µl of the ethyl acetate extract and authentic pederin were applied to highperformance thin-layer chromatography (TLC) plates (0.2mm silica gel 60, 10 \times 10cm, Merck), which were developed in ethyl acetate. After evaporation of the solvent, they were stained in a dipping solution of anisaldehyde: sulfuric acid: acetic acid (1: 2: 100v/v/v) (99%, Merck) and heated at 90 °C for 2min. Using this procedure, lilac spots of pederin (> 0.05µg) are provided at $R_f = 0.22$ (Kellner and Dettner, 1995).

Concentration-mortality response

The concentration-mortality response of the fourth larval instar of *E. kuehniella* and adult stage of *T. confusum* to the pederin extract was

studied to estimate the median lethal concentration (LC50) for each species. Based concentration-setting pre-tests, on the appropriate concentrations of pederin extract, causing the mortality range of 10-90%, were determined as 300, 448, 670, 1003, and 1500ppm for T. confusum and as 700, 962, 1322, 1818, and 2500 ppm for E. kuehniella. One mL of each concentration was mixed with 5g wheat flour and the resulting toxic diet was distributed at the floor of a Petri-dish (9cm diameter). Twenty E. kuehniella fourth larval instar or T. confusum adults, which had been starved for 24 hours, were released inside each Petri-dish. The Petri-dishes were maintained at controlled condition and mortality was recorded 24 hours post treatment. Both bioassays were conducted in a completely randomized design (CRD) with three replications. The percentage of mortality was corrected using Abbott's formula as follow:

Corrected mortality =
$$\left(\frac{T-C}{100-C}\right) \times 100$$

Where T and C are the numbers of dead insects in treatment and control, respectively (Abbott, 1925).

Enzyme assays

The effect of feeding on P. fuscipes body fluid on activity of digestive enzymes (amylases and and detoxifying proteases) enzymes (glutathione S-transferase (GST), cytochrome P₄₅₀, and carboxyl esterase) was studied in last larval instar of E. kuehniella and adult T. confusum. One mL of the LC_{30} of pederin extract was mixed with 5g wheat flour and the resulting toxic diet was distributed at the base of a Petri-dish (9cm diameter). The last larval instar of E. kuehniella and adult T. confusum were then released inside Petri-dishes and allowed to feed on the toxic diet for 24 hours. The same volume of diet, without pederin contamination, was provided for these species as control. Treated insects were used for enzyme assays after 24 hours of feeding on toxic diet and control insects on ethyl acetatic diet were investigated too.

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Protein quantification

Total protein concentration of samples was determined according to Bradford method (Bradford, 1976) at 630nm, with bovine serum albumin as standard.

Digestive enzyme assays Sample preparation

After feeding on toxic diet for 24 hours, 10 fourth larval instars of *E. kuehniella* were dissected under a stereomicroscope in saline solution (NaCl, 10mM) to extract guts. In case of adult *T. confusum*, the whole body (n = 20) was used for enzyme sample preparation. The samples were homogenized in pre-cooled homogenizer in distilled water (w/v). The homogenates were then centrifuged at 16,000 × g for 10min at 4 °C and the resultant supernatants were stored at -20 °C for subsequent analysis of digestive enzyme activities.

Amylase activity

The total amylase activity of last larval instar of E. kuehniella and adult T. confusum was measured using a colorimetric method with 3,5dinitrosalicylic acid (DNS) reagent and 1% (w/v) soluble starch as substrate according to the method of Bernfeld (1955) with slight modifications. The reaction mixture, containing 70µl of Tris-HCl buffer (20 mM, pH 7), 10µl of the enzyme solution, and 20µl of 1% starch, was incubated for 10 min at 35 °C. The reaction was stopped by the addition of 50µl DNS reagent and heating in boiling water for 10 min. The absorbance of the resultant complex was read at 540nm using a microplate reader (ELx808). One unit of a-amylase activity was defined as the amount of enzyme required to produce 1 mg maltose in 30 min at 37 °C. The negative control contained all reaction mixtures except enzyme source to prove the enzyme presence in the samples. This assay was carried out in triplicate.

Protease activity

The total proteolytic activity was quantified using azocasein as the substrate according to Elpidina *et al.*, (2001) with slight modifications. J. Crop Prot.

The reaction mixture, consisting of 40μ l of Tris-HCl buffer (20mM, pH 7), 5μ l of 2% (w/v) azocasein (pH 5.0), and 10μ l of the enzyme solution, was incubated for 60 min at 37 °C. The reaction was stopped by adding 100µl 30% TCA (trichloroacetic acid). The samples were incubated at 4 °C for 30min to precipitate non-hydrolyzed substrate, then centrifuged at 15,000 × g for 15 min at 4 °C. One hundred µl of the resultant supernatant was mixed with equal volume of KOH 2M and the absorbance was read at 405nm. One unit of enzyme activity (U) was defined as a change in absorbance at 405nm of one mg protein after one minute. This assay was carried out in triplicate.

Detoxifying enzyme assays Sample preparation

Bodies of 10 last larval instar of *E. kuehniella* and 20 adult *T. confusum* were homogenized in 400µl phosphate buffer (0.04 M, pH 7.0) containing Triton X-100 (0.1% v/v) and centrifuged at 10,000 × g for 20 min at 4 °C. The supernatant was transferred to new tubes and preserved at -20 °C for analysis of the activity of carboxyl esterase, glutathione transferase and P450 and all steps were performed for controls too.

Glutathione S-transferase

Glutathione S-transferase (GST) activity was 2,4-Dinitrochlorobenzene measured using (CDNB) according to the method of Habig et al. (1974). The total reaction volume per well of a 96-well microplate was 200µl, consisting of 10µl of enzyme solution, 2µl of 200mM CDNB (containing 0.1% (v/v) ethanol)), and 188µl of 10mM GSH in Tris-HCl buffer (0.05 M; pH 8). GST activity was determined by the change in absorbance as measured every 30 s for 5 min at 340nm. Control wells consisted of 2µl of CDNB, 188µl of GSH, and 10µl of Tris-HCl buffer (0.05 M; pH 8) (Habig et al., 1974) and all steps were performed for controls too.

Cytochrome P₄₅₀

Cytochrome P_{450} activity was measured in terms of general oxidase level using heme

peroxidation method with 3,3'5,5'-tetramethylbenzidine (TMBZ) (Sigma Aldrich) as the substrate (Brogdon et al., 1997) (William and Janet, 1997). The total reaction volume per well of a 96-well microplate was 325µl, including 20µl of enzyme solution, 80µl of 0.625 M potassium phosphate buffer (pH 7.2), 200µl of TMBZ solution, and 25µl of hydrogen peroxide (H₂O₂) (3%). Plates were incubated at room temperature for 2h, after which the absorbance was read at 450nm. Control wells consisted of 20µl of distilled water, 80µl of 0.625M potassium phosphate buffer, 200µl of TMBZ solution, and 25µl of H_2O_2 (3%). A standard curve for heme peroxidase activity was prepared using different concentrations of cytochrome C (0.15, 0.3, 0.6, 0.8, 1, and 1.2) from horse heart (Sigma Aldrich). The activity of cytochrome P₄₅₀, obtained from plate reading, was expressed as equivalent units (EU) of cytochrome P₄₅₀ per milligram of protein using the standard curve of cytochrome C (William and Janet, 1997) and all steps were performed for controls too.

Carboxyl esterase

The general esterase activity of E. kuehniella and T. confusum were quantified using α naphthyl-acetate and *β*-naphthyl acetate as substrates according to the method of Van Asperen with slight modifications (Van Asperen, 1962). The total reaction volume per well of a 96-well microplate was 270µl, consisting of 20µl of enzyme solution, 90µl of substrate, 70µl of phosphate buffer (0.2 M, pH 7.0), and 90µl of fast blue RR salt. Plates were incubated at 37 °C for 30min, after which, the absorbance was read at 450nm for α -naphthylacetate and at 540nm for β -naphthyl acetate every 2min for 10min (Van Asperen, 1962) 37.2 N 49.60 E and all steps were performed for controls too.

Data analysis

All experiments were carried out in completely randomized design (CRD) with

two treatments (control and treated insects) and three replications. The corrected mortality data of *E. kuehniella* larvae and *T. confusum* adults were used to estimate the median lethal concentration (LC₅₀) using Probit analysis in POLO-PC 2002 software (California, USA). The average enzyme activity was compared between control and treated larvae using independent *t*-tests of SPSS software (version 19).

Results and Discussion

The TLC yielded lilac spots of pederin at $R_f = 0.22$, indicating that pederin was successfully extracted using our procedure (Fig. 1). Our results revealed high toxicity of *P. fuscipes* body extract for both *E. kuehniella* and *T. confusum* via ingestion. The median lethal concentration (LC₅₀) of pederin for last larval instar of *E. kuehniella* and adult *T. confusum* was estimated as 1311.96 and 596.36ppm, respectively (Table 1).



Figure 1 Part of TLC plate showing successful extraction of pederin from *Paederus fuscipes*. Extracts of adult females were applied together with known amounts of authentic pederin to the starting line (bottom), developed in ethyl acetate and stained with anisaldehyde/sulfuric acid/acetic acid.

Table 1 Results of probit analysis (mean \pm 95% CI) for estimation of median lethal concentration (LC₅₀) of pederin against last larval instar of *Ephestia kuehniella* and adult *Tribolium confusum*.

Insect	n	Slope \pm SE	LC ₅₀ (95% lower-upper) (ppm)	χ^2	df	Heterogeneity
E. kuehniella	300	4.01 ± 0.46	1311.96 (1187.17-1444.43)	8.11	13	0.624
T. confusum	300	3.17 ± 0.38	596.36 (514.21-679.79)	6.69	13	0.515

In agreement with these results, Tabadkani and Nozari (2014) showed that *P. fuscipes* body fluid is highly toxic to the predatory ground beetle, *Ch. pallipes*. When the beetles fed a diet containing *P. fuscipes* body fluid, they became torpid within 24-36 hours and started to die after 48-60 hours of oral treatment (Tabadkani and Nozari, 2014). Pederin is expected to act against a wide range of eukaryotic organisms with respect to its unique mode of action. Generally, pederin inhibits mitosis in eukaryotic cells by blocking the synthesis of particular proteins in ribosomes (Pavan, 1963). Therefore, any type of living tissue is expected to be affected by this compound.

We measured the activity of two common digestive enzymes (amylases and proteases) in *E. kuehniella* last larval instar and *T. confusum* adults in response to oral exposure to *P. fuscipes* body extract (Fig. 2). Feeding on pederin resulted in significant decrease in the activity of amylases in both species in comparison with control (Fig. 2a). Similarly, the activity of proteases significantly decreased in both *E. kuehniella* and *T. confusum* as a result of feeding on pederin extract (Fig. 2b). These results imply that pederin strongly interferes with normal function of digestive enzymes in the gut.

Despite the abundance of studies on insecticidal activity and enzyme inhibitory effects of a wide variety of plant-derived toxins/inhibitors (Carlini and Grossi-De-Sá, 2002), the effect of bacterial-derived toxins on digestive enzyme of insects have been rarely studied. However, in several studies, the oral insecticidal effects of bacterial toxins with different modes of actions have been demonstrated (Khandelwal et al., 2004, Hinchliffe et al., 2010, Bowen and Ensign, 1998, Andrejko et al., 2013). For example, a pilin subunit of the nematode symbiotic bacterium, Xenorhabdus nematophilus, has been reported to have strong oral toxicity to the larvae of Helicoverpa armigera (Hübner) (Lep: Noctuidae), causing extensive damage to the midgut epithelial membrane (Khandelwal *et al.*, 2004). Transformation of a 537 bp *mrxA* gene, encoding the pilin subunit, into the genome of tobacco and tomato, was reported to cause 100% mortality in larvae of *H. armigera* and reduce damage on genetically manipulated plants (Kumari *et al.*, 2015). Lv *et al.* (2014) reported significant decrease in the amylase activity (Lv *et al.*, 2014), beside increase in protease activity of the 5th larval instar of the oriental armyworm, *Mythimna separata* (Walker) (Lep: Noctuidae) when reared on fraxinellone, a toxin extracted from *Dictamnus dasycarpus*.

Insects have developed a variety of metabolic responses in order to deal with pesticides and other xenobiotic compounds. In most species, these responses include two distinct phases I and II. In phase I, the foreign compound(s) is oxidized to create a reactive site (electrophilic site) by the action of detoxification enzymes such as P_{450s} and esterases, while phase II involves the conjugation of phase I products with endogenous substances such as glutathione. This process transforms the lipophilic xenobiotic compounds into more water soluble derivatives and thus, facilitates excretion of the xenobiotic compound (Mamidala et al., 2011; Reddy et al., 2012). We measured the activity of three major enzymes (monooxygenases, GSTs, and esterases) in E. kuehniella larvae and T. confusum adults in response to oral exposure to pederin extract (Fig. 3). As expected, feeding on pederin caused significant increase in the activity of P_{450s} monooxygenase of both E. kuehniella larvae and T. confusum adults when compared with control (ttest: df = 4, t = 22.99 and 15.58 for T. confusum and *E. kuehniella*, respectively, P < 0.01) (Fig. 3a). Similarly, the activity of glutathione S-transferases significantly increased following oral was treatment of T. confusum with pederin extract (ttest: t = 7.83, df = 4, P < 0.01) and tended to increase in *E. kuehniella* larvae (*t*-test: t = 2.44, df

= 4, P = 0.071) (Fig. 3b). By contrast, a significant decrease in the activity of carboxyl esterase was detected in both species, when either α -naphthyl-acetate or β -naphthyl acetate was used as substrate for the enzyme (Fig. 3c and d). These results may imply that oxygenases and glutathione S-transferases are the major groups of detoxifying

enzymes involved in metabolism of pederin in treated insects. Feeding on fraxinellone has been shown to result in increased activity of carboxylesterase and glutathione S-transferase, but decreased activity of other detoxifying enzymes such as NADPH-P450 reductase and O-demethylase (Lv *et al.*, 2014).



Figure 2 (a): Relative activity of amylolytic and (b): proteolytic enzymes of *Ephestia kuehniella* last larval instar and *Tribolium confusum* adults in response to oral treatment with pederin, ** show significant difference treatment and control at P < 0.01 (t-student test).



Figure 3 Activity of (a): P_{450s} monooxygenase, (b): glutathione S-transferase, and (c): carboxyl esterase and for (d): alpha and beta naphtyl acetate as substrate enzymes of *Ephestia kuehniella* last larval instar and *Tribolium confusum* adults in response to oral treatment with pederin, ** show significant difference between treatment and control at P < 0.01 (t-student test).

Altogether, results of the current study indicate high oral toxicity of pederin for two distantly related insects (a beetle and a moth). However, the mechanism(s) and the precise mode of action through which pedrin acts against insect digestive tract remains to be discovered in future studies. A possible explanation may be disruption of midgut peritrophic membrane as a barrier that protects the epithelium from mechanical damage by food, exposure to ingested toxins as well as microbial invasion besides concentrating food and digestive enzymes (Shao et al., 2001). Plant and microbial toxins have recently attracted increasing attention as tools for control of insect pests. Current methods of administration are either expression of the toxin in genetically modified plants or dispersing the toxin over plant surfaces (Hinchliffe et al., 2010). Pederin, as a novel compound with unique mode of action, may provide new insights for control of insect pests in agriculture and homes. This persuades researches for development of appropriate formulations in order to deliver sufficient amounts of the toxin to the target pest, and at the same time, avoid the harmful effects of this compound on non-target organisms including natural enemies as well as the end users by whom the crop is intended to be consumed (Hinchliffe et al., 2010). Given the high oral toxicity of pederin to insects and available routes for synthesis (Wan et al., 2011, Mosey and Floreancig, 2012), forthcoming works may evaluate the efficiency of pederin as baited traps for control of specific pests, such as cockroaches, termites, ants, in homes.

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تأثیر پدرین بر فعالیت آنزیمهای گوارشی و سمزدا در لاروهای *Ephestia kuhniella* (Coleoptera: Tenebrionidae) و (Lepidoptera: Pyralidae)

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چکیده: در دهه گذشته متابولیتهای مشتق شده از میکروب و گیاه توجه روبه رشدی را بهعنوان یک ابزار قدرتمند برای کنترل آفت در کشاورزی دریافت کردهاست، پدرین (Col: Staphylinidae) یک ماده تاول زاست که از باکتری شبه سودوموناس همزیست با سوسک دونده جنس (Ephestia kuehniella (Lep: Pyralidae) و Tribolium در این پژوهش سمّیت پدرین بر دو آفت انباری (Asympilica) (Col: Staphylinidae) و Tribolium (Col: Tenebrionidae) و Tribolium (Col: Tenebrionidae) و Tribolium (Col: Tenebrionidae) و Tribolium (Col: Tenebrionidae) و Repestia kuehniella (Lep: Pyralidae) و Tribolium (Col: Tenebrionidae) (تمایش میّیت پدرین بر دو آفت انباری (Asympilica) آزمایشگاهی ارزیابی شدهاست. آنالیز پروبیت میانگین غلظتهای کشنده پدرین ۱۹۲۱/۹۶ و ۵۹۶/۳۶ و ۵۹۶/۳۶ (قسمت در میلیون) بهترتیب برای لارو سن پروبیت میانگین غلظتهای کشنده پدرین ۲۰ ما ۲۰ مهمچنین فعالیت دو آنزیم گوارشی اصلی (آمیلاز و پروتئاز) و سه آنزیم سمزدای اصلی (مونواکسیژنازهای ۲۹۵۵ یکوناتیوناس ترانسفرازها و کربوکسیل استرازها) را در حشراتی که بهصورت خوراکی با پدرین تیمار شدند اندازه گیری کردیم. تغذیه با پدرین منجر به کاهش معنی دار در فعالیت آنزیمهای آمیلولتیک، پروتئولتیک و کربوکسیل استراز شد، اما افزایش معنی دار در فعالیت معنی دار در فعالیت آنزیمهای آمیلولتیک، پروتئولتیک و کربوکسیل استراز شد، اما افزایش معنی دار در فعالیت معنی دار در فعالیت آنزیمهای آمیلولتیک، پروتئولتیک و کربوکسیل استراز شد، اما افزایش معنی دار در فعالیت

واژگان کلیدی: آنزیم گوارشی، آنزیم سمزدا، محصولات طبیعی، Paederus fuscipes، پدرین، سم