Appl Biol Chem (2018) 61(3):337–343
https://doi.org/10.1007/s13765-018-0363-7

Online ISSN 2468-0842
Print ISSN 2468-0834

ARTICLE

# Effects of *Opuntia ficus-indica* lectin on feeding, survival, and gut enzymes of maize weevil, *Sitophilus zeamais*

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Received: 23 January 2018 / Accepted: 19 March 2018 / Published online: 27 March 2018


**Abstract** In this study, the effects of *Opuntia ficus-indica* lectin (OfiL) on the survival and nutritional parameters of *Sitophilus zeamais* (maize weevil) adults were evaluated. OfiL was incorporated into the artificial diets at concentrations of 15, 60, and 95 mg/g (mg of lectin per g of wheat flour). Mortality was evaluated after 7 and 15 days, and the amount of food ingested and the weight of the insects were determined on the 7th day. In addition, the in vitro effects of OfiL on the gut enzymes of the insect were investigated. The ingestion of OfiL did not show any significant difference in the mortality rates compared to control. The relative consumption rate was also similar to that of the control, and no deterrent effect was detected. However, the values of the relative biomass variation and the efficiency of ingested food conversion were negative in the treatments at 60 and 95 mg/g, showing that lectin ingestion resulted in weight loss. OfiL exhibited a stimulatory effect on the protease activity from *S. zeamais* gut extract, which may cause uncontrolled hydrolysis of proteins in the digestive tract. This lectin did not promote significant alteration in the amylase activity. In conclusion, OfiL was able to exert anti-nutritional effects without causing a deterrent effect.

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**Keywords** Indian-fig · Lectin · Insecticidal activity · Greater rice weevil · Agricultural pest

## Introduction

The weevils belonging to *Sitophilus* genus (Family: Curculionidae) are cosmopolitan insects found in the tropical regions and are able to infest grains of major importance such as rice, wheat, and maize [1]. The grains are the nutrient source for these insects and serve as the shelter for the immature forms, which develop inside them [2].

*Sitophilus zeamais* is one of the main pests of maize; however, it also attacks other crops such as rice, wheat, and sorghum, in addition to fruits and processed foods [3, 4]. The potential to promote cross-infestation and the high capacity for penetration and destruction of the grains account for the high economic impact of this insect [1, 5]. In addition, a remarkable plasticity at the individual and population levels renders the control of this pest very difficult [6]. The presence of *S. zeamais* also facilitates the dissemination of pathogens and contamination by fungal toxins at storage [7, 8].

The control of stored grain pests has been mainly performed by cleaning of the grains, aeration, temperature and moisture regulation, and application of insecticides [1]. The use of methyl bromide and phosphine is commonly used as the main strategy for the control of storage pests. However, these compounds were reported to be highly toxic to the environment and humans, which has led to the search for alternative non-chemical methods of control (e.g., heat treatments and entomopathogenic fungi) and for more environmentally friendly insecticides (e.g., plant essential oils and entomotoxic proteins) [9–12]. In

addition, there are reports on the insecticidal-resistant populations of *S. zeamais* exposed to selective pressures under laboratory conditions or found directly in the field [13–16].

Lectins are carbohydrate-binding proteins broadly found in plants. One of the roles of these proteins is the defense against pathogens, predators, and herbivores [17, 18]. They have been described as insecticidal agents against species of the orders Coleoptera, Diptera, Hemiptera, Homoptera, Hymenoptera, Isoptera, Lepidoptera, and Neuroptera, acting on both immature and adult forms [19]. Many of the insecticidal lectins have a chitin-binding ability, which allows them to interact with important structures of the insect body that are composed of this polysaccharide [20]. In addition, lectins may interfere with insect physiology and behavior. A lectin from *Myracrodruon urundeuva* leaf was reported to be a strong feeding deterrent to *S. zeamais* adults and was able to impair the digestive process of this insect by enzyme modulatory effects [10].

The cladodes of *Opuntia ficus-indica* (L.) Mill. (Cactaceae) contain a chitin-binding lectin (deemed OfiL) that has been previously isolated and characterized [21]. OfiL showed antifungal activity against phytopathogens and insecticidal activity against *Nasutitermes corniger* termite [21, 22]. In this work, the effects of OfiL on the survival and nutritional parameters of *S. zeamais* adults were evaluated.

## Materials and methods

### Plant material and insects

Cladodes of *O. ficus-indica* were collected in Limoeiro, Pernambuco, Brazil, with authorization (36301) of the *Instituto Chico Mendes de Conservação da Biodiversidade* (ICMBio). The cladodes were dried for 7 days (27 ± 2 °C; relative humidity of 70 ± 5%), powdered, and stored at 28 °C. The insects are reared in the *Laboratório de Bioquímica de Proteínas*, *Departamento de Bioquímica*, *Universidade Federal de Pernambuco* since 2012. The colonies were maintained at 28 ± 2 °C in glass containers (capacity, 1 L) sealed with unwoven fabric to allow aeration. The diet consisted of maize grains (100 g per container), which was selected based on the integrity, sanitary conditions, size, and absence of contamination with other insects.

### Isolation of OfiL

OfiL was isolated according to Santana et al. [21], initiating with the extraction of proteins from the cladodes using 0.15 M NaCl, followed by chromatography using a chitin column. Lectin concentration was determined according to Lowry et al. [23] using bovine serum albumin (31.25–500 μg/mL) as the standard. Carbohydrate-binding ability was monitored through the hemagglutination assay, which was carried out in microtiter plates according to Procópio et al. [24] using rabbit erythrocytes treated with glutaraldehyde [25]. The collection of erythrocytes was approved by the Ethics Committee on Animal Experimentation of the *Universidade Federal de Pernambuco* (23076.033782/2015-70).

### Insecticidal assay

Insecticidal activity was evaluated using a modified version of the method of Xie et al. [26], as described by Napoleão et al. [10]. First, wheat flour suspensions were prepared, each one consisting of 2.0 g of autoclaved wheat flour (Bunge Alimentos S.A., Benevides, Brazil), homogenized in 5 mL of sterile distilled water (control) or a solution containing OfiL diluted in the sterile water. In each assay, five aliquots (200 μL) were placed on a petri dish (90 × 100 mm) to form flour disks after overnight incubation at 56 °C. Next, each dish containing the disks was weighed. Twelve *S. zeamais* adults with known weight were then transferred to each dish. The assays were maintained at 25 ± 2 °C in the dark for 7 days. After this period, the mortality rate and the weights of dishes (containing the broken flour disks) and insects were recorded again. Mortality was also evaluated on the 15th day. The assays were performed in quadruplicate, and the tested lectin concentrations (mg of lectin/g of wheat flour) were 15, 60, and 95 mg/g.

### Feeding deterrence evaluation

The feeding deterrence index (FDI) was calculated as follows: FDI (%) = 100 × $(X - Y)/(X)$, where $X$ is the mass of the food ingested by the insects in the control assay and $Y$ is the mass of the food ingested by the insects in the lectin assay [27]. According to the FDI value, the lectin was classified as: non-deterrent (FDI < 20%), weak deterrent (50% > FDI ≥ 20%), moderate deterrent (70% > FDI ≥ 50%), or strong deterrent (FDI ≥ 70%).

### Nutritional parameters

The data recorded at the end of the insecticidal assay were used to calculate the following nutritional parameters [26]: (1) the relative consumption rate = $A/(B \times \text{days})$, where $A$ is the mass of the ingested food in mg and $B$ corresponds to the initial insect biomass in mg; (2) the relative biomass variation = $C/(B \times \text{days})$, where $C$ corresponds to the insect biomass variation in mg, after 7 days from the

beginning of the experiment; and (3) the efficiency of conversion of ingested food = $C/(A \times 100)$.

### Gut preparations from *S. zeamais*

Groups of fifty *S. zeamais* adults were collected and immobilized by exposure to − 20 °C for 20 min. The guts were dissected by hand and homogenized in 1 mL of Tris buffer (0.1 M Tris–HCl, pH 8.0, containing 0.02 M $CaCl_2$ and 0.15 M NaCl) or acetate buffer (0.1 M sodium acetate, pH 5.5, containing 0.02 M $CaCl_2$ and 0.15 M NaCl) using a 3-mL tissue grinder. The homogenates were centrifuged at 9000×*g* at 4 °C for 15 min. The supernatants (gut extracts) were collected, and the protein concentration [23] and enzyme activity (as described below) were evaluated.

### Enzyme assays

Protease activity was determined according to Azeez et al. [28]. Briefly, the gut extract in Tris buffer (50 µL; 350 µg of protein) was previously incubated (15 min, 28 °C) with 50 µL of OfiL (1.5–20 µg) or distilled water (100% activity control) and then mixed with 300 µL of 0.1 M sodium phosphate, pH 7.5, containing 50 µL of 0.6% (w/v) azocasein. The mixture was supplemented with 100 µL of 0.1% (v/v) Triton X-100 and incubated at 37 °C for 3 h. The reaction was stopped by adding 200 µL of 10% (v/v) trichloroacetic acid (TCA), and the assay was incubated at 4 °C for 30 min. Each assay was accompanied by a blank (identical to the test except that TCA was added before the addition of azocasein). Next, it was centrifuged at 9000×*g* for 10 min, and the absorbance of the supernatant was determined at 366 nm using a spectrophotometer. One unit of protease activity was defined as the amount of enzyme that yielded an increase of 0.01 in the absorbance. A control assay containing OfiL without the gut extract was also performed. The assays were performed in triplicate.

α-Amylase activity assay was carried out based on the method described by Bernfeld [29]. Briefly, the gut extract in acetate buffer (100 µL; 600 µg of protein) was previously incubated (15 min, 28 °C) with 100 µL of OfiL (1.5–100 µg) or distilled water (100% activity control). Next, the samples were incubated at 50 °C for 10 min with 400 µL of 1% (w/v) soluble starch solution in acetate buffer. The reaction was stopped by adding 500 µL of 3,5-dinitrosalicylic acid (DNS) reagent. Then, the assays were heated at 100 °C in boiling water for 6 min and immediately cooled on ice for 15 min. The absorbance was measured at 540 nm using a spectrophotometer, and the amount of reducing sugars was calculated using a standard curve of the reaction between glucose and DNS reagent. One unit of the α-amylase activity was defined as the amount of enzyme required to generate 1 µmol of glucose per minute. Reaction blanks were performed without starch. The assays were performed in triplicate.

### Statistical analysis

The data were expressed as the mean of replicates ± standard deviations (SD). Significant differences between the treatment groups were analyzed by one-way analysis of variance (ANOVA), followed by Tukey's test, with a significance level at $p < 0.05$. The analyses were performed using the Action 2.4.163.322 software (Estatcamp, São Carlos, Brazil).

## Results

The ingestion of OfiL did not result in significant difference ($p > 0.05$) in the mortality rates in comparison with the control group after 7 and 15 days of the assay (Table 1). The relative consumption rate was also similar to that of the control ($p > 0.05$) in all the treatments, showing that the presence of OfiL did not affect the intake of diet (Fig. 1A). Indeed, the calculation of FDI revealed no deterrent effect.

Although OfiL did not cause insect death, the relative biomass variation was negative for the treatments at 60 and 95 mg/g (Fig. 1B), showing that lectin ingestion resulted in the decrease in body weight. The data on the efficiency of the conversion of the ingested food are in agreement with the loss of biomass of the insects, because the feed conversion values were also negative for the treatments at 60 and 95 mg/g (Fig. 1C).

OfiL exhibited a stimulatory effect on the protease activity from *S. zeamais* gut extract (Fig. 2). Concerning the α-amylase activity, OfiL was not able to promote significant reduction in starch hydrolysis (Fig. 2B), when *S. zeamais* gut extract was previously incubated with it.

**Table 1** Mortality of *Sitophilus zeamais* adults maintained for 7 and 15 days on artificial diets containing the lectin OfiL

| Treatment | Mortality rate (%) | |
|---|---|---|
| | 7 days | 15 days |
| Control | 2.5 ± 2.8 a | 16.2 ± 4.8 a |
| OfiL (mg/g) | | |
| 15 | 2.5 ± 2.8 a | 23.2 ± 5.5 a |
| 60 | 7.5 ± 2.8 a | 21.0 ± 5.3 a |
| 95 | 1.2 ± 2.5 a | 20.0 ± 7.1 a |

Significant differences were not observed between the treatments ($p > 0.05$)

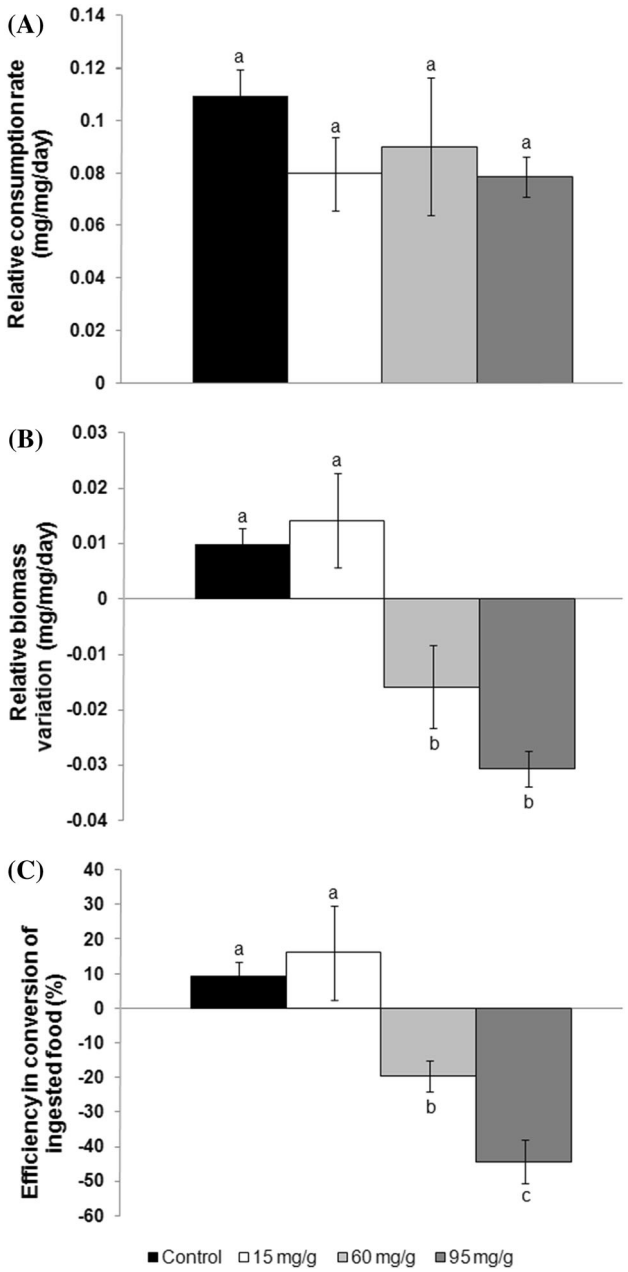


**Fig. 1** Nutritional parameters of *Sitophilus zeamais* adults reared on artificial diets consisting of wheat flour disks without (control) or with *Opuntia ficus-indica* lectin (OfiL, 15–95 mg of lectin per g of wheat flour). (**A**) The relative consumption rate indicates the mean estimative of the amount of food consumed in mg per mg of insect body weight per day. (**B**) The relative biomass variation indicates the mean estimative of the amount of biomass in mg gained or lost every day per mg of initial body weight. (**C**) The efficiency in conversion of ingested food (%) indicates the amount of ingested food incorporated by insects as biomass after 7 days from the beginning of the experiment. Each bar corresponds to the mean ± SD of four replicates. Different letters indicate significant ($p < 0.05$) differences between treatments

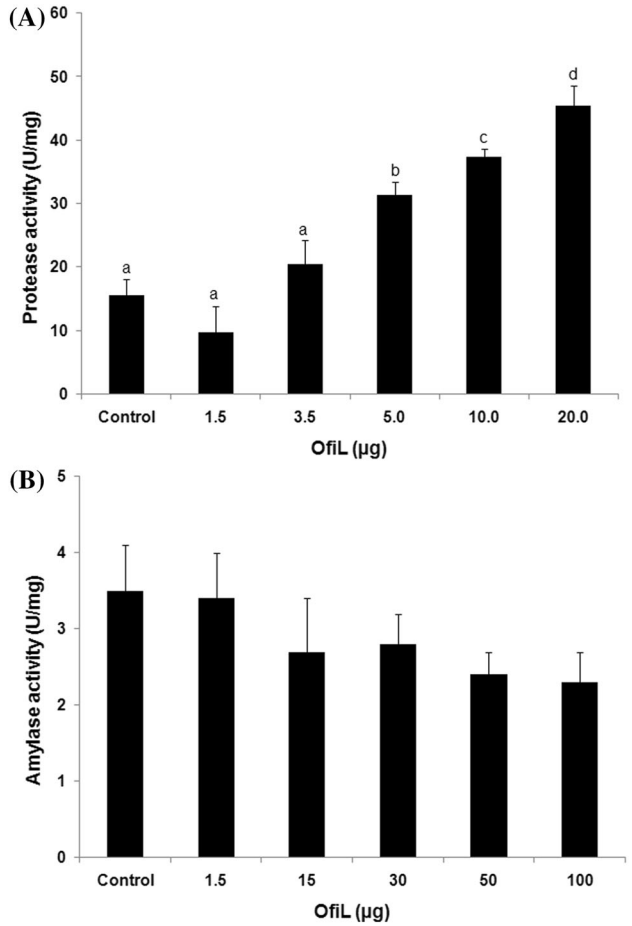


**Fig. 2** Protease (**A**) and α-amylase (**B**) activities from *Sitophilus zeamais* gut extracts incubated with or without *Opuntia ficus-indica* lectin (OfiL). Each bar corresponds to the mean ± SD of three replicates. Different letters indicate significant ($p < 0.05$) differences between the assays

## Discussion

The entomotoxic effects of lectins have been reported, and the potential of these proteins in crop protection has been indicated in several studies [12, 19, 20]. The ingestion of plant lectins might exert deleterious effects on insects at all stages, interfering with survival, feeding, morphology, and development. In this study, we investigated the effect of an artificial diet composed of wheat flour and the lectin OfiL on the survival and nutritional parameters of *S. zeamais* adults. For this, the OfiL isolation procedure was repeated about 30 times, yielding a lectin amount sufficient for the insecticidal assays at concentrations of 15, 60, and 95 mg/g.

Similar to OfiL, the lectin from *M. urundeuva* leaf (MuLL) did not promote mortality in *S. zeamais* adults after 7 days of treatment [10]. However, the authors partly attributed this result to the feeding-deterrent action of MuLL, which was not ingested by the insects and thus not

able to exert acute toxic effects. In the present study, the insects did not avoid the diet containing OfiL, and thus, the absence of mortality might not be explained as in case of MuLL. It is plausible that damage in the gut of *S. zeamais* adults was not caused by OfiL, or, if it occurred, was not sufficient to promote the death of the insects in the periods evaluated.

It is possible that OfiL, when present in the digestive tract of *S. zeamais* adults, interfered with the digestion and absorption process, and thus, the food was not converted into biomass. The decrease in weight was a consequence of this, because the energy spent by the insects for their physiological processes was greater than the energy that could be harnessed from feeding.

*S. zeamais* adults provided with diet containing MuLL also showed negative values of biomass variation and food conversion. However, although the final effect was similar, the mechanism involved was probably different. The insects treated with MuLL lost biomass because of the starvation process as a consequence of the deterrent effect. In the case of OfiL, the diet was ingested; however, it was not metabolically useful to the insects. Similar results obtained with OfiL have been reported in studies using lectins and other insects. Reduction in the weight and efficiency of food conversion into body mass were detected in *Anagasta kuehniella* larvae, when these insects were maintained with an artificial diet containing *M. oleifera* seed lectins [30, 31]. The ingestion of *Dioclea violacea* lectin also decreased *A. kuehniella* larval mass without affecting its survival [32].

The results instigated us to evaluate whether OfiL would be able to interfere with the activity of digestive enzymes present in the gut of *S. zeamais* adults. At the first glance, this stimulatory effect on proteases might be considered positive for the insects because such protease stimulus might facilitate digestion; however, an imbalance in the proteolysis is usually damaging because it might lead to the disruption of the intestinal tract organization. For example, it has been demonstrated that the water-soluble lectin from *M. oleifera* seeds, which shows larvicidal activity against *Aedes aegypti*, was able to stimulate the protease activity at the same time that induces strong damage to the epithelial organization of larvae gut [33, 34]. These effects could impair both digestion and nutrient absorption.

The absence of significant effect on amylase activity indicates that a direct inhibitory effect of this lectin on amylases might not be a major explanation for the reduction in the efficiency of food conversion. However, the possibility of uncontrolled digestion of enzyme molecules by proteases stimulated by OfiL, resulting in an indirect impairment in the digestion of the starch present in wheat flour, should be considered.

Chitin-binding lectins usually bind the peritrophic membrane, which is a structure composed of chitin and glycoproteins that protect the gut epithelium from insects against abrasion by plant fragments and infection by pathogens and also play a compartmentalization role in the digestive process [35]. The disruption of the peritrophic membrane may result in the deregulation of the action of enzymes and allow the access of the lectins to the gut microvillar brush border and epithelial cells. Once a lectin reaches these structures, it might interfere with several physiological processes, including the absorption of nutrients [19, 36, 37]. Powell et al. [38] showed that *Galanthus nivalis* agglutinin caused the disruption of the microvilli brush border region of *Nilaparvata lugens*.

The *D. violacea* lectin, which was not able to kill *A. kuehniella* larvae, but affected their nutrition, was able to bind to the peritrophic membrane [32]. The chitin-binding lectins from the bark, heartwood, and leaf of *M. urundeuva* were able to induce apoptosis in the digestive and enteroendocrine cells of *N. corniger* gut and consequently block the absorption of nutrients and deregulate the coordination of enzyme release into the gut lumen [39]. Alterations in the expression profile of genes in the gut of insects fed on artificial diets containing chitin-binding lectin from wheat germ have also been reported; some of these genes are linked to the expression of digestive enzymes and energy metabolism [40]. In summary, although OfiL did not induce acute lethality in *S. zeamais* adults, it caused an intoxication effect that led to the malnutrition of these insects. Some of these mechanisms already reported for other lectins might be involved, which warrants future studies on this aspect.

The nutritional impairment in the insects might lead to disturbances other than mortality that might affect their efficiency as pests. An inadequate nutrition status results in a deficient metabolic functioning, which can affect the fecundity and longevity [20, 41, 42]. A methanolic extract from *Syzygium aromaticum* flower buds had no effect on the mortality of *S. zeamais* but promoted a reduction of 37% in the $F_1$ progeny, and n-hexane extract from this same plant material showed toxicity through a possible stomach action and reduced the $F_1$ progeny in 99% [43]. This is important to reduce the impact of a pest such as *S. zeamais*, which is known for a destructive potential linked to a high reproductive fitness. In addition, compounds able to affect the insect physiology without promoting their death are important; for example, to be used as synergists or to obtain long-term effects without an increase in the selective pressure that could result in resistance establishment.

In conclusion, OfiL is a type of lectin reported to be active against *S. zeamais* adults. This lectin is able to exert anti-nutritional effects without causing a deterrent effect.

The results warrant new investigations aiming to explore the effect of OfiL on the long-term assays as well as synergists of other insecticides.

**Acknowledgments** The authors express their gratitude to the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq; 446902/2014-4; 408789/2016-6) for research grants and fellowship (PMGP). They are also grateful to the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES; AUXPE 1454/2013) and the *Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco* (FACEPE; APQ-0137-2.08/12; APQ-0108-2.08/14; APQ-0661-2.08/15) for financial support.

**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

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