

Sodium chloride reduces the viability of eggs, larvae, and pupae of *Spodoptera frugiperda*, as well as delays its development

Cloreto de sódio reduz a viabilidade dos ovos, larvas e pupa de *Spodoptera frugiperda*, bem como atrasa o seu desenvolvimento

Cloruro de sodio reduce la viabilidad de los huevos, larvas y pupas de *Spodoptera frugiperda*, así como retrasa su desarrollo

Received: 02/21/2022 | Reviewed: 03/01/2022 | Accept: 03/11/2022 | Published: 03/18/2022

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Abstract

The control of fall armyworm (*Spodoptera frugiperda*) populations involves the application of pesticides that cause several unwanted effects to humans and other animals, as well as to environment. Sodium chloride (NaCl) has been used in agriculture to potentiate the toxicity of pesticides. Here, the effects of NaCl on the viability of eggs, larvae, and pupae, as well as on development of *S. frugiperda* were evaluated. The treatment with 0.15 M NaCl caused a 35% reduction of egg hatching and the ingestion of artificial diet containing the salt caused mortality of caterpillars, pre-pupae, and pupae, as well as delayed the development of the insects. The larval, pre-pupa and pupa stages were elongated by about 2, 1 and 0.8 days, respectively. Additionally, significant reduction in pupae body weight was recorded in groups treated with NaCl at 0.62 mg/g and 3.12 mg/g. Despite these effects, NaCl ingestion did not affect the viability of adults and their reproductive performance. Food deterrent assay revealed that the salt acted as a phagostimulant agent. In conclusion, NaCl reduces the hatching of *S. frugiperda* eggs and damage the survival of caterpillars and pupae, as well interferes with insect development.

Keywords: Agricultural pest; Fall armyworm; Phagostimulant; Insecticidal activity; Saline solution.

Resumo

O controle populacional da lagarta-do-cartucho (*Spodoptera frugiperda*) envolve a aplicação de pesticidas que causam muitos efeitos indesejados para humanos e outros animais, bem como para o meio ambiente. O cloreto de sódio (NaCl) tem sido utilizado na agricultura para potencializar a toxicidade de pesticidas. Aqui, os efeitos do NaCl na viabilidade de ovos, lagartas e pupa, bem como no desenvolvimento de *S. frugiperda* foram avaliados. O tratamento com NaCl 0,15 M causou uma redução de 35% na eclosão dos ovos e a ingestão de dieta artificial contendo o sal causou mortalidade das lagartas, pré-pupas, e pupas, bem como atrasou o desenvolvimento dos insetos. Os estágios de lagarta, pré-pupa e pupa foram alargados em cerca de 2, 1 e 0,8 dias, respectivamente. Adicionalmente, uma redução significativa no peso corporal das pupas foi registrado nos grupos tratados com NaCl a 0,62 mg/g e 3,12 mg/g. Apesar desses efeitos, a ingestão de NaCl não afetou a viabilidade dos adultos e sua performance reprodutiva. O ensaio de deterrência alimentar revelou que o sal atuou como um agente fagoestimulante. Em conclusão, o NaCl reduz a eclosão de ovos de *S. frugiperda* e prejudica a sobrevivência das lagartas e pupas, bem como interfere no desenvolvimento dos insetos.

Palavras-chave: Praga agrícola; Lagarta-do-cartucho; Fagoestimulante; Atividade inseticida; Solução salina.

Resumen

El control de la población del cogollero del maíz (*Spodoptera frugiperda*) involucra la aplicación de pesticidas que causan muchos efectos no deseados en humanos y otros animales, así como en el medio ambiente. El cloruro de sodio (NaCl) se ha utilizado en la agricultura para potenciar la toxicidad de los plaguicidas. Aquí se evaluaron los efectos del NaCl sobre la viabilidad de huevos, orugas y pupas, así como sobre el desarrollo de *S. frugiperda*. El tratamiento con NaCl 0,15 M provocó una reducción del 35% en la eclosión de huevos y la ingestión de una dieta artificial que contenía sal provocó la mortalidad de orugas, prepupas y pupas, así como un retraso en el desarrollo de los insectos. Los estadios de oruga, prepupa y pupa se prolongaron alrededor de 2, 1 y 0,8 días, respectivamente. Además, se registró una reducción significativa en el peso corporal de las pupas en los grupos tratados con NaCl a 0,62 mg/g y 3,12 mg/g. A pesar de estos efectos, la ingesta de NaCl no afectó la viabilidad ni el rendimiento reproductivo de los adultos. El ensayo de disuasión alimentaria reveló que la sal actuaba como un agente fagoestimulante. En conclusión, el NaCl reduce la eclosión de los huevos de *S. frugiperda* y perjudica la supervivencia de orugas y pupas, además de interferir con el desarrollo de los insectos.

Palabras clave: Plaga agrícola; Gusano cogollero; Fagoestimulante; Actividad insecticida; Solución salina.

1. Introduction

Spodoptera frugiperda (Smith, 1797) (Lepidoptera: Noctuidae), popularly known as “lagarta-do-cartucho” in Portuguese and “fall armyworm” in English, is a cosmopolitan pest that preferentially attacks corn crops, but also harms other cultures including pumpkin, cotton, peanuts, rice, sugar cane, beans, sorghum, and soybean (Silva et al., 2017). Its biological cycle can last approximately 30 days depending on relative humidity, photoperiod, temperature, and food availability and comprises the phases of eggs, larvae (five instars), prepupae and pupae, from which the adults emerge (Gallo et al., 2002, Sarmiento et al., 2002).

The application of synthetic insecticides is still the main strategy for controlling populations of *S. frugiperda*, being frequently used the active ingredients Cyfluthrin, Cypermethrin 200 CE, Esfenvalerate 250 CE, Fenitrothion 500 CE, Fenvalerate 200CE, Lambda-cyhalothrin 50 CE, Malathion methyl 600 CE, Permethrin 384 CE, and Trichlorfon 500 CE (Wan et al., 2021). However, these compounds may show low effectiveness and cause unwanted effects such as intoxication of producers and consumers, toxicity to non-target organisms, eventually reducing populations of natural enemies, and environmental persistence. The selection of resistant insect populations and the increase of costs in crop production are also critical consequences of the unplanned use of insecticides (Sarmiento et al. 2002; Goedel et al., 2021). This context has stimulated the search for new strategies to control *S. frugiperda*.

Sodium chloride (NaCl) is an essential mineral for human and animal physiology whose use in agriculture as a phage arresting agent to potentiate the insecticidal action of chemicals is widespread (Corso and Gazzoni, 1998; Bonoan et al., 2016). Ramiro et al. (2005) showed that the insecticide Actara Mix 110 + 220 EC (thiamethoxan + cypermethrin) had its efficiency against *Nezara viridula* increased in the presence of NaCl solutions. In addition, the treatment of *Nicotiana tabacum* with saturated NaCl solutions reduced the consumption of the leaves by *Helicoverpa armigera* caterpillars in a dose-dependent way

(Yang et al., 2013). In this way, we describe here the investigations on the effects of NaCl on the viability of eggs, larvae, and pupae as well as on development of *S. frugiperda*.

2. Methodology

2.1 Rearing of insects

All experiments were carried out at the *Laboratório de Biologia e Resistência de Plantas a Insetos* from the *Departamento de Agronomia* of the Universidade Federal Rural de Pernambuco (UFRPE), Recife, Pernambuco, Brazil. The insects were kept under controlled conditions [27 ± 1 °C, $70\pm 10\%$ relative humidity (RH), and 12 h of photophase]. Newly hatched *S. frugiperda* caterpillars were transferred to transparent plastic pots (25×25 cm), covered with filter paper, and an artificial diet prepared according to Greene et al. (1976) was offered daily until the insects reached the pupal stage. Next, the pupae were transferred to plastic pots (16×10 cm) covered with filter paper moistened with tap water and incubated for about 12 days. When the adults emerged, 30 couples were separated in PVC cages (20×25 cm) covered with bond paper. Each cage received a plastic plate (25×25 cm), covered with filter paper to serve as a substrate for oviposition and the cages were closed with PVC plastic film. The couples were fed with a 10% (v/v) honey solution offered in a hydrophilic cotton swab inside 10 mL plastic tubes. The eggs were collected daily and transferred to transparent plastic pots (16×10 cm) lined with filter paper until hatching. The caterpillars were fed as described above.

2.2 Effect of sodium chloride on egg hatching

To evaluate the effect of NaCl on the hatching of *S. frugiperda* eggs, a paper containing the eggs was removed from the oviposition cages and cut into sections measuring about 10 cm² containing 50 ± 5 intact eggs, which were selected under a microscope (Bel Photonics WF 10). Then, the pieces of paper containing the eggs were immersed in 0.03 M or 0.15 M NaCl solution for 30 s and placed in Petri dishes (9.5×1.5 cm) lined with filter paper moistened with distilled water. Distilled water was used as a control solution. Next, the plates were kept under controlled conditions (27 ± 1 °C, $70\pm 10\%$ RH and 12 h of photophase), and the viability of the eggs as well as the time required for hatching were recorded daily, until the complete hatching in control group (ca. 3 days). Six experiments were performed in triplicate. The data were submitted to analysis of variance and the means compared by Tukey's test (significance level at $P < 0.05$). All the statistical analyzes in this paper were performed using the SAS Statistical Program (SAS Institute 2002).

2.3 Effect of NaCl on survival and development of caterpillars

An artificial diet was prepared according to Greene et al. (1976) and NaCl solution (0.62 or 3.12 mg/g) or distilled water (control) was incorporated. After solidifying, the diet was cut into 1 cm³ cubes, which were placed in Petri dishes (9.5×1.5 cm) covered with filter paper. Each plate received a single first instar caterpillar and was sealed with PVC film. The plates were kept at a temperature of 27 ± 1 °C, RH of $70\pm 10\%$ and a photophase of 12 h. Six experiments were carried out, each one with 50 replicates per treatment. Every 24 h, the diet was replaced by another without treatment, until the caterpillars reached the pre-pupal stage. The number of dead caterpillars, the viability and duration of the larval, pre-pupal and pupal stages, the pupae weight, and the presence of malformations in the adults were recorded daily.

After the emergence of adults, up to five couples were formed from each treatment, and each couple was kept in a cage (16×10 cm) covered with filter paper and closed with PVC plastic film, being fed with a 10% (v/v) honey solution, as described in section 2.1. Adult longevity was observed daily, and the eggs were collected every 24 h for quantification and viability determination. The pre-oviposition, oviposition and post-oviposition periods of each couple were also recorded.

The data were subjected to normality and homogeneity of variance tests, and means were compared by Tukey's test

(significance level at $P < 0.05$). Some parameters were transformed into sqrt ($x+0.5$) and arcsine root ($x/100$) and those that did not meet the prerequisites for analysis of variance even after transformation were submitted to the nonparametric Kruskal-Wallis test ($P < 0.05$).

2.4 Food deterrent effect of NaCl

To evaluate the deterrent effect of NaCl, a no-choice experiment was carried out. The artificial diet was prepared, containing NaCl (0.62 or 3.12 mg/g) or distilled water (control) and cut as described above. Diet blocks were weighed and placed in the center of Petri dishes (9.5×1.5 cm) covered with filter paper. Next, one third instar *S. frugiperda* caterpillar was placed in the center of each plate, and 40 replicates were performed for each treatment. After 24 or 48 h from the beginning of the bioassay, the weight of the diet was recorded, and the food consumption was calculated by the difference between the weight of the blocks before and after they were offered to the caterpillars. To determine the loss of water suffered by evaporation, an aliquot corresponding to twenty whole pieces was weighed at the beginning and at the end of each exposure time. Thus, the average moisture loss (in %) of the twenty pieces collected at each time was deducted from the final weight of each piece of medium offered to the caterpillars. The experimental design was completely randomized, arranged in a 3×2 factorial scheme (treatments \times times) in two phases. Data were transformed into sqrt ($x+0.5$), and then submitted to analysis of variance, and the means compared by Tukey's test ($P < 0.05$).

3. Results and Discussion

Stimulated by previous reports of the deleterious effects from exposure to NaCl on insects, alone or associated with chemical insecticides (Ramiro et al., 2005; Yang et al., 2013; Bonoan et al., 2016), the experimental design described here tested the hypothesis that NaCl may interfere with the viability of eggs, larvae, and pupae of *S. frugiperda*, as well as delay its development.

The treatment with NaCl strongly reduced the viability of *S. frugiperda* eggs regarding the control group (100% viability) since a maximum hatching rate of about 35% was recorded for the group treated with 0.15 M NaCl (Table 1). Unlike our findings for *S. frugiperda* eggs, Ferreira et al. (2021) showed that 0.15 M NaCl did not affect the viability of eggs from the diamondback moth *Plutella xylostella* (Lepidoptera: Plutellidae). On the other hand, the authors showed that the extract of *Opuntia ficus-indica* cladodes prepared with 0.15 M NaCl was embryotoxic to *P. xylostella*, but when the extract was extensively dialyzed to remove the salt, this effect was neutralized, indicating an involvement of NaCl in the toxicity of the extract.

The ingestion of NaCl significantly reduced the viability of caterpillars, prepupae, and pupae in regard to control, as well as delayed the development of *S. frugiperda*, since the larval, prepupa and pupa stages were elongated by about 2, 1 and 0.8 days, respectively (Table 2). Additionally, a significant reduction of pupae body weight in 38 and 46 mg was recorded in groups treated with NaCl at 0.62 mg/g and 3.12 mg/g, respectively, in comparison with control (Table 2).

Table 1. Effect of NaCl on the viability of *Spodoptera frugiperda* eggs.

Parameter	Control	NaCl	
		0.03 M	0.15 M
Viability (%)	100.0 \pm 0.00a	68.0 \pm 5.03ab	35.3 \pm 17.13b
Time to hatch (days)	3.0 \pm 0.00a	3.0 \pm 0.00a	3.0 \pm 0.00a

Control: distilled water. Data represent means among three replicates \pm standard deviation. Different letters on the same line indicate statistical difference by Tukey's test at the 5% probability level. Source: Elaborated by the authors.

Table 2. Effect of NaCl on biological parameters of *Spodoptera frugiperda*.

Parameters	Control	NaCl (mg/g)		Statistics
		0.62	3.12	
Larval duration (days)	13.8 ± 0.15b	15.5 ± 0.26 ^a	15.9 ± 0.42 ^a	F _{2,99} =19.31; P < 0.0001
Pre-pupal duration (Days)	1.6 ± 0.08b	2.7 ± 0.19 ^a	2.5 ± 0.10 ^a	F _{2,98} = 25.24; P < 0.0001
Pupal duration (Days)	10.5 ± 0.19b	11.3 ± 0.34 ^a	11.2 ± 0.25 ^a	χ ² = 8.23; P = 0.0163
Pupal weight (mg)	274.1 ± 5.80a	236.4 ± 8.73b	228.2 ± 6.63b	F _{2,98} =14.81; P < 0.0001
Larval viability (%)	96.0 ± 2.79a	44.0 ± 7.09b	60.0 ± 6.99b	F _{2,149} =19.87; P < 0.0001
Pre-pupal viability (%)	96.0 ± 2.79a	44.0 ± 7.09b	58.0 ± 7.05b	F _{2,149} =20.14; P < 0.0001
Pupal viability (%)	86.0 ± 4.95a	34.0 ± 6.76b	46.0 ± 7.11b	F _{2,149} =18.37; P < 0.0001
Adult longevity (days)	6.1 ± 0.50a	6.9 ± 0.99 a	5.5 ± 0.79 ^a	F _{2,82} = 0.75; P = 0.4736
Malformed adults (%)	5.8 ± 7.61a	5.8 ± 12.30 ^a	6.9 ± 9.23 ^a	F _{2,85} = 0.45; P = 0.6420

Control: distilled water. Means (± SD) followed by different letters in the line differ from each other by Tukey's test at the 5% probability level or Kruskal-Wallis test at the 5% probability level. Source: Elaborated by the authors.

Sodium ions play relevant roles in osmoregulation, digestion, and neurophysiological functions. Thus, the dietary sodium content can reverberate in several aspects of insect fitness, including development. For example, higher internal concentration and availability of Na⁺ can cause hyperexcitation of the insect nervous system (Hodgkin, 1951; Rodrigues et al 2021). It has been shown that dietary sodium can be beneficial or harmful to insects depending on the content. Xiao et al. (2010) reported that *Helicoverpa armigera* larvae that fed on diet containing 0.14 mg/g of NaCl grew larger and had a shorter development period than those on diets containing higher salt content (0.3 and 0.43 mg/g). *Melanopus differentialis* nymphs raised on a high sodium diet had lower weights and reduced survival compared to control; in addition, high sodium diets generated grasshoppers with prolonged development times and reduced survival; conversely, medium sodium diets showed beneficial effects on the insects (Peterson et al., 2021).

Despite of the effects of NaCl ingestion to caterpillars, pre-pupae and pupae, the salt did not affect the longevity and the number of malformed adults, and the analysis of reproductive parameters of the emerged adults revealed no significant differences in the number of egg laying (F_{2,35} = 0.42; P = 0.6591), number of eggs (F_{2,35} = 0.42; P = 0.6585), number of eggs per laying (F_{2,35} = 0.28; P = 0.7577) or fertility (F_{2,35} = 2.31; P = 0.1156) in comparison with the control group. In addition, significant differences between the treatments were not observed for the pre-oviposition (F_{2,8} = 1.37; P = 0.3240), oviposition (F_{2,8} = 0.66; P = 0.5488) and post-oviposition (F_{2,8} = 2.10; P = 0.2037) periods (Table 3).

Table 3. Reproductive parameters of *Spodoptera frugiperda* adults from caterpillars fed an artificial diet containing NaCl.

Parameters	Control (n=5)	NaCl (mg/g)	
		0.62 (n=3)	3.12 (n=1)
Number of laying	2.1 ± 0.37 ^a	2.1 ± 0.77 ^a	1.4 ± 0.29 ^a
Number of eggs	254.1 ± 32.50 ^a	251.6 ± 87.06 ^a	183.9 ± 52.38 ^a
Egg/laying	158.6 ± 29.19 ^a	127.8 ± 26.58 ^a	133.2 ± 35.64 ^a
Fertility (%)	66.1 ± 7.33 ^a	50.5 ± 12.01 ^a	34.6 ± 14.28 ^a
Pre-oviposition period (days)	1.4 ± 0.40 ^a	4.3 ± 2.33 ^a	3.0 ± 0.00 ^a
Oviposition period (days)	4.6 ± 1.80 ^a	3.3 ± 1.20 ^a	8.0 ± 0.00 ^a
Post-oviposition period (days)	0.2 ± 0.20 ^a	2.3 ± 1.45 ^a	0.0 ± 0.00 ^a

Control: distilled water. Means (± SD) followed by the same letter on the line, do not differ from each other by Tukey's test at the 5% probability level. n= Number of couples formed for each treatment. Source: Elaborated by the authors.

Our data indicated that, although the ingestion of NaCl was harmful to caterpillars and pupae, it was not able to impair the reproductive capacity of the adults; however, it should be noticed that the first instar caterpillars received a single dose of NaCl for 24 h. In this sense, future investigations where caterpillars receive doses of NaCl for a longer time can be conducted. Also, it is worth reporting that previous investigations using non-pest species showed that exposure to low concentrations of NaCl can even lead to an increase in insect survival and reproductive performance (Walker et al., 2015). Rodrigues et al. (2021) showed that exposure to NaCl resulted in higher fecundity of the neotropical brown stink bug *Euschistus heros* (Heteroptera: Pentatomidae), with an increase in the daily number of eggs/females and a slower reproductive decline, regardless of exposure to the insecticide imidacloprid.

The food deterrence assay revealed a greater food consumption in the group that received the diet containing NaCl at 3.12 mg/g regarding to those that received NaCl at 0.62 mg/g or the control diet (Table 4), both after 24 h ($F_{2,119} = 35.34$; $P < 0.0001$) or 48 h ($F_{2,119} = 14.63$; $P < 0.0001$). The gustatory system of insects acts in detection and processing of chemical substances in the environment, and because of these organs are distributed throughout the insect body and limbs, they are able to select their food and avoid the contact with toxic ingredients (Rajashekar et al., 2012; Rodrigues et al., 2021). *S. frugiperda* caterpillars may have detected the presence of salt through their gustatory organs, acting as a phagostimulant agent. This datum, combined with the deleterious effects of NaCl ingestion on the survival of the caterpillars, makes this salt an interesting candidate for further evaluation as a larvicidal agent, with the advantage of being environmentally clean. NaCl is not currently considered a health hazard by the 2012 OSHA Hazard Communication Standard (29 CFR 1910.1200(g)), in addition to show high LC₅₀ values for fish (4747-12946 mg/L, 96 h) and crustaceans (340.7-1000 mg/L, 48 h), indicating its low environmental toxicity [OSHA Hazard Communication Standard (29 CFR 1910.1200)].

Table 4. Food consumption (g) of *Spodoptera frugiperda* caterpillars in artificial medium containing sodium chloride (NaCl), after 24 and 48 hours of exposure in a no-choice test.

Time of treatment	Control	NaCl (mg/g)	
		0.62	3.12
24 h	0.2 ± 0.01Ab	0.2 ± 0.01Ab	0.3 ± 0.01Aa
48 h	0.3 ± 0.02Ab	0.3 ± 0.01Ab	0.4 ± 0.01Aa

Conditions: Temp.: 27 ± 1°C, RH 70 ± 10% and 12h of photophase. Control: distilled water. Means (± SD) followed by distinct letters, capital letters in the columns and lowercase in the lines, indicate significant differences between the treatments by the Tukey test at the level of 5% of probability. Source: Elaborated by the authors.

4. Conclusion

The findings reported in this paper showed NaCl as a fagostimulant agent and confirmed the hypothesis that it reduces the hatching of *S. frugiperda* eggs and damage the survival of caterpillars and pupae, as well interferes with insect

development. Future investigations can be carried out to determine the LC₅₀ value (concentrations that kills 50% of the insect population) of the salt, as well as mechanisms of toxicity such as the effect on morphology and physiology of larval midgut. In addition, the effect of NaCl under field or simulated field conditions should be assayed.

Acknowledgments

The authors express their gratitude to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; 408789/2016-6) for research grants and fellowships (PMGP and THN) as well as to the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Finance Code 001) and Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE; APQ-0108-2.08/14; APQ-0661-2.08/15) for financial support.

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