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Activity of powdered methylxanthine applied to poultry litter on adults of *Alphitobius diaperinus* (Panzer, 1797) (Coleoptera: Tenebrionidae)

Atividade da Metilxantina em pó aplicada na cama de frango sobre adultos de Alphitobius diaperinus (Panzer, 1797) (Coleoptera: Tenebrionidae)

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Abstract

One of the pests that most affect and compromise poultry production worldwide is the insect *Alphitobius diaperinus*, known as the lesser mealworm. This insect is a vector of diseases that compromise not only chicken production but also human health. This study proposes to examine the efficacy and determine the appropriate rate of methylxanthine (MTX), a natural insecticide extracted from caffeine, for the control of an adult population of lesser mealworms in poultry litter. A total of 2,500 adult mealworms were distributed into five treatments in a completely randomized design using 10 replications with 50 insects per replication. The treatments consisted of a control group and four concentrations of MTX (14, 16, 18, and 20 g/m^2) spread in plastic boxes containing reused poultry litter and feed, allocated in a broiler shed, to simulate the farm condition. The experimental period was 18 days, and five readings were performed on days 2, 4, 6, 10, and 18. Methylxanthine affected ($P<0.05$) the mealworms' cumulative mortality rate, with the groups of insects housed in boxes treated with 16 g /m² MTX showing the highest cumulative mortality (86.6%) at the end of the experimental period. In conclusion, MTX has insecticidal action on adults of lesser mealworm and can be used on chicken litter to control the population of this insect in poultry sheds. The MTX concentration of 16 g/m^2 showed the greatest effectiveness.

Keywords: chicken bed; insecticide; lesser mealworm; methylxanthine

Resumo

Uma das pragas que mais afetam e comprometem a produção avícola no mundo é o inseto *Alphitobius diaperinus*, conhecido como cascudinho. Este inseto é vetor de doenças que comprometem não só a produção de frangos como também a saúde humana. Objetivou-se com esse trabalho avaliar a eficácia e determinar a dose adequada de Metilxantina (MTX), inseticida natural extraído da cafeína, para o controle da população adulta de cascudinhos em cama de frango. Foram utilizados 2.500 cascudinhos adultos distribuídos em delineamento inteiramente casualizado, cinco tratamentos, 10 repetições com 50 insetos por repetição. Os tratamentos consistiram de grupo controle e quatro concentrações 14 g/m^2, 16 g/m^2, 18 g/m^2, 20 g/m^2 de MTX espalhadas em recipientes plásticos contendo cama de frango reutilizada e ração, alocados em um galpão de frangos de corte a fim de simular a condição de granja. Período experimental foi de 18 dias e realizadas cinco leituras nos dias dois, quatro, seis, 10 e 18. A MTX afetou ($P<0,05$) a taxa de mortalidade acumulada de cascudinhos, grupos de insetos alojados em caixas tratadas com 16 g/m² de MTX apresentaram maior mortalidade acumulada (86,6%) ao final do período experimental. Conclui-se que MTX tem ação inseticida sobre adultos de cascudinho, podendo ser utilizada sobre a cama de frango para o controle da população deste inseto em galpões de criação de frangos, a concentração 16 g/MTX/m^2 demonstrou maior efetividade.

Palavras-chave: Metilxantina; inseticida; cama de frango; cascudinho

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Introduction

Alphitobius diaperinus, a beetle commonly known as the lesser mealworm, is one of the most abundant insects in chicken production facilities. The species stands out in the poultry industry for having the status of a pest, as it is a reservoir and vector of pathogens and is difficult to control. The lesser mealworm breeds in litter used on chicken farms and feeds on poultry waste, feed leftovers, broken eggs, dead birds, and other organic materials[1,2,3]. The greatest economic losses in poultry production establishments are due to: i) the preference of birds to consume this insect instead of feed, which reduces their weight gain[4,5]; ii) damage to the structures of the facilities, as mealworm larvae usually dig tunnels into the walls, insulating materials, and on the floor of the shed in search of places to pupate and escape from the enemies present in the litter[2, 6, 7, 8, 9, 10, 11, 12, 13, 14].

The birds' health is also compromised because *A. diaperinus* act as transmission routes for etiological agents of diseases such as: i) Salmonella [15, 16, 17], Campylobacter sp.[18], *Escherichia coli*[19,20,21]; ii) fungi[20]; iii) Marek's disease virus[22], Newcastle disease and yaws[23], enteritis[24], and Gumboro disease[25,26]; iv) chicken tapeworm[27], and *Ascaridia galli*[27]. The chemical products most widely used are pyrethroids (bifenthrin, deltamethrin, fenitrothion, pirimiphos-methyl), which are highly toxic to birds and whose accumulation in muscle tissue renders the meat of these chickens unsuitable for human consumption[1,28]. The number of reports of lesser mealworm populations resistant to these compounds is increasing[29, 30, 31], including in Brazil[32,33].

Chemical control of the lesser mealworm through the application of pyrethroid and organophosphate insecticides is commonly used as a preventive measure[34], and this approach during downtime is more efficient for producers in the short term. However, research has already shown that this type of chemical control with the use of pyrethroids in litters during downtime no longer yields effective results due to the return of the presence of this pest during housing[35]. In this scenario, the use of natural products is another method that has stood out in the control of several pathogenic microorganisms and pests. These products can be an efficient and viable alternative for the control of the lesser mealworm, since this method does not depend on the absence of birds in the poultry shed. Some essential oils have already been tested and their insecticidal effect proven, e.g. *Melaleuca alternifolia* oils[1,36].

Caffeine (1,3,7 trimethylxanthine) is a fat-soluble alkaloid belonging to the class of compounds called methylxanthine[37] that acts on the human central nervous system and is also known for its antioxidant properties[38]. Together with theophylline, it is found in wild fruits, seeds, and leaves of numerous plant species including tea, coffee, cocoa, and nuts[39]. Studies have shown that caffeine causes toxic effects on *Aedes aegypti* larvae, interfering with their development and consequently preventing them from reaching the adult stage[40, 41], which leads to a decrease in oviposition rate[42].

The objective of this study was to examine the efficacy and determine the effective concentration of methylxanthine extracted from caffeine against adults of lesser mealworm (*Alphitobius diaperinus*) in poultry litter.

Material and methods

The experiment was carried out in the Experimental Poultry House in the Poultry Section of the Animal Science Department of the Veterinary and Animal Science School at the Federal University of Goiás, located in Goiânia - GO, Brazil. A total of 2,500 lesser mealworm (*Alphitobius diaperinus*) adults captured in several broiler farms in the region of Itaberaí - GO were used in a completely randomized experimental design with five treatments and 10 replications with 50 insects per experimental unit. The treatments consisted of four concentrations of methylxanthine (MTX) (14, 16, 18, and 20 g/m^2) spread in plastic boxes (41 × 27 × 12.5 cm) containing a 10-cm-high layer of reused chicken litter with a portion of chicken feed, plus a control group without the use of any product.

After the mealworms and MTX were placed, the boxes were sealed with tulle fabric to prevent the entry and exit of insects. Subsequently, the boxes were transferred to a broiler shed to simulate the farm condition. The experiment lasted 18 days and five readings were performed, on days 2, 4, 6, 10, and 18. At each elapsed period, the seals were removed to count dead and live insects with the aid of surgical tweezers and data were recorded in collection forms. Afterwards, the boxes were sealed again, and this process was repeated until the end of the experimental period. Data were evaluated by analysis of variance (ANOVA) and Tukey's test using the R computer package and adopting $\alpha=0.05$.

Results

The methylxanthine (MTX) concentration affected ($P<0.05$) the cumulative mortality rate of lesser mealworm. The groups of insects housed in boxes treated with 16 g MTX/m² showed the highest cumulative mortality (86.6%) at the end of the experimental period (Table 1).

Table 1. Cumulative mortality rate of lesser mealworm (*Alphitobius diaperinus*) in chicken litter treated with methylxanthine (MTX)

Observation day	Treatment									
	12 g MTX/m²		14 g MTX/m²		16 g MTX/m²		18 g MTX/m²		Control	
	Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead
2nd	354	146	352	148	198	302	322	178	497	3
4th	287	67	285	67	138	60	270	52	495	2
6th	269	18	255	30	117	21	241	29	493	2
10th	233	36	219	36	103	14	208	33	491	2
18th	183	50	167	52	67	36	158	50	486	5
Cumulative mortality (%)	63.4 b		66.6 b		86.6 a		68.4 b		2.8 c	
P-value	<0.0001									
SEM¹	4.288									

Means followed by distinct letters differ from each other by Tukey's test (P<0.05); ¹Standard error of the mean.

Discussion

The primary effect of methylxanthine (MTX) in the mealworm was found to be due to the inhibition of phosphodiesterase activity and intracellular increase of cyclic adenosine monophosphate (cyclic AMP). At low concentrations, they are potent synergists of other insecticides known to activate adenylate cyclase in insects. These data suggest the use of MTX in the control of arthropods as a natural insecticide, by inhibiting phosphodiesterase alone with the involvement of AMP cyclase; or in combination with other compounds(43). Nathanson(43) demonstrated the pesticidal and pestistatic activity of MTX in goliath worm (*Manduca sexta*) larvae and observed lethality within 24 h. Polo(44) showed that MTX alters the synthesis pattern of esterases, which are important in several physiological processes and are even involved in blocking insect metamorphosis. Esterases are also involved in several physiological processes, including neuronal activity(45), insect juvenile hormone metabolism(46), and insecticide resistance(47).

In neuronal activity, acetylcholine is released into the synaptic cleft, binds to transmembrane receptors, and generates signal transmission. Later, acetylcholinesterase hydrolyzes acetylcholine, triggering the stimulus(48). Nishi et al.(49) confirmed that caffeine inhibits the pattern of esterase gene expression. Juvenile hormone esterase controls the concentration of juvenile hormone and, therefore, caffeine alters the gene expression pattern of esterases. Juvenile hormone is a class of sesquiterpenoids produced in the insect *Corpora Allata* and distributed throughout the hemolymph, being directly involved in several metabolic activities of insects such as metamorphosis and oogenesis. During metamorphosis, it modulates ecdysteroid activity (20E), preventing molt from occurring during the larval stage(50).

Among the different plant extracts with insecticidal effect demonstrated in laboratory studies, caffeine has been the one that acts in the intoxication of larvae, interrupting their development and leading them to death(40,42,51). Caffeine proved to be potentially effective in controlling adult red flour beetles through repellent, fumigant, and contact effects, and its action is believed to be linked to the inhibition of certain enzymes such as carboxylesterase(52). In adult insects, caffeine causes motor immobility by increasing the activity of the dopamine receptor, an antagonist of adenosine receptors, inhibiting their action(49,53,54). Caffeine acts in a dose-dependent manner, with the concentration of 1 mg/mL of water being lethal to larvae(40).

Herbal products are recognized as natural insecticides, as is the case of tea extract-based emulsion, which can be used as an insecticide to control the green peach aphid(58). Some formulations of insecticides based on medicinal herbs were effective in combating several pests, e.g. rosemary-pepper(59), neem (*Azadirachta indica*)(60, 61), garlic(62), and several species of eucalyptus(63), whose main deleterious effects in the fight against insects are related to methylxanthine.

In a similar study, Ananenka(64) tested the efficacy of a natural insecticide composed of caffeine on the house cricket (*Acheta domesticus*) and concluded that caffeine results in increased neuronal activity and consequently the death of the insect due to depolarization of neurons in the brain membrane, constituting an option to control this species. In the present study, the MTX concentration of 16 g/m^2 had positive results for cumulative mortality rate in the total observation period, which was 80% higher than in the control treatment, and ensured a consecutive increase in mortality from the 2nd to the 18th day of observation.

The control of *Alphitobius diaperinus* is essential due to its many detrimental effects on the productivity of these animals, as this species can cause superficial wounds and negatively affect the birds' growth. In addition, the ingestion of large amounts of *A. diaperinus* compromises the nutritional quality of diets, since adults and larvae can cause intestinal obstruction in broilers due to the lack of chitinase for the digestion of chitin, which is widely found in the exoskeleton of the mealworm. This results in necrotic enteritis, reduced nutrient absorption, and, ultimately, considerable economic losses for the poultry industry[65, 66].

Several studies have demonstrated that cypermethrin, dichlorvos, and triflumuron are efficient in controlling ectoparasites in poultry production in Brazil[67,68]. However, resistance of pest populations to these compounds has already been reported in various countries[69,70]. Therefore, the use of plant extracts such as citronella, caffeine, among others that have an insecticidal action on the central nervous system of insects, is encouraged. These compounds have been proven to harm the development of insects and have repellent and larvicidal activity[71,72], thereby constituting efficient ways to control the damage caused by *Alphitobius diaperinus* in poultry production.

Conclusion

Methylxanthine, extracted from caffeine, has an insecticidal action on lesser mealworm (*Alphitobius diaperinus*) adults and can be used on chicken litter to control the population of this insect in poultry sheds. For this purpose, the methylxanthine concentration of 16 g/m^2 is the most effective.

Conflicts of interest

The authors declare they have no conflicts of interest.

Author contributions

Conceptualization: M.M. Ishizuka, N.S.M.Leandro and M.B. Café. *Data curation*: J.M.S. Silva, R.R. Santos and H.F. Oliveira. *Formal Analysis*: M.B. Café. *Resources*: M.M. Ishizuka and N.S.M.Leandro. *Writing (original draft):* M.M. Ishizuka, M.B. Café and H.F. Oliveira. *Writing (review & editing):* H.F. Oliveira

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