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Artigo Completo

INFLUENCE OF CRUDE EXTRACTS OF ENDOPHYTES FROM *Luehea divaricata* (MALVALES; TILIACEAE) ON THE IN VITRO DEVELOPMENT OF *Diatraea saccharalis* (LEPIDOPTERA; CRAMBIDAE) LARVAE

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ABSTRACT

One of the main pests of sugarcane is the sugarcane borer (*Diatraea saccharalis*), that causes direct and indirect damage to this plant. Chemical defensives have been used, although the biological control of pests is the best to be used. These chemical defensives have failed to control plant diseases, generating environmental pollution and affecting human health. Thus, is observed a growing interest in biological control methods based on the use of microorganisms, among them the endophytes, which colonize the interior of plant protecting it against pests and pathogens, directly or indirectly by the production of metabolites, that may be used as an alternative method for biological control. Studies have shown that endophytic fungi isolated from the medicinal plant *Luehea divaricata* showed to be potent to control phytopathogenic fungi and human pathogenic bacteria. In the present study, the potential of crude extracts of two endophytes from *L. divaricata* was assessed in order to verify their influence in the in vitro development of larvae of sugarcane borer. To this objective, we used an explanatory and qualitative research with a completely randomized design. As results, a further unrest in the fungal-treated larvae was observed, as well as, the absence of pupal stage had occurred, in comparison with control larvae.

Keywords: *fungi; biotechnology; endophytes; diatraea.*

INFLUÊNCIA DE EXTRATOS BRUTOS DE ENDÓFITOS DE *Luehea divaricata* (MALVALES; TILIACEAE) SOBRE O DESENVOLVIMENTO IN VITRO DE LAGARTAS DE *Diatraea saccharalis* (LEPIDOPTERA; CRAMBIDAE)LARVAE

RESUMO

Uma das principais pragas da cana-de-açúcar é a broca-da-cana (*Diatraea saccharalis*), que causa danos diretos e indiretos para esta planta. Defensivos químicos ainda têm sido usados, embora o controle biológico de pragas seja o mais indicado. Esses inseticidas químicos têm falhado no controle de doenças de plantas, gerando poluição ambiental e afetando a saúde humana. Assim, é observado um aumento no interesse por métodos de controle biológico baseados no uso de microorganismos, entre eles, os endófitos, que colonizam o interior de plantas protegendo-as contra pragas e patógenos, direta ou indiretamente pela produção de metabólitos, que podem ser usados como método alternativo para o controle biológico. Estudos apontam que fungos endofíticos isolados da planta medicinal *Luehea divaricata* mostraram-se potentes para o controle de fungos fitopatogênicos e bactérias patogênicas a humanos. O presente estudo teve por objetivo avaliar o potencial de extratos brutos de dois endófitos de *L. divaricata* para verificar sua influência no desenvolvimento *in vitro* de largartas da broca-da-cana. Para tanto, utilizou-se de uma pesquisa explicativa, qualitativa, com um delineamento experimental inteiramente casualizado. Como resultado, uma maior agitação das lagartas tratadas foi observada, bem como, ocorreu a ausência de empupamento em relação às lagartas controle.

Palavras-chave: *fungos; biotecnologia; endofíticos; diatraea.*

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INTRODUCTION

Diatraea saccharalis (Fabricius) (Lepidoptera; Crambidae), the sugarcane borer, is the main sugarcane pest, also affecting other crops as sorghum, corn, and rice (1). This insect-pest may cause direct damage inside the plant, by opening galleries in their stem or reducing the sap flow, as well as, it may act indirectly, opening holes for pathogens entry (2).

Chemical defensives are failing in plant disease control, causing the pollution of soil (3), air (4) and water (5) and affecting the human health (6). Thus, the biological control of insect-pests, instead of the use of chemicals that are not recommended, is of critical importance to the ecosystem, since it maintains the balance of pest population with a low environmental impact (7).

Although *D. saccharalis* may be efficiently controlled by *Cotesia flavipes* (8), there is a residual damage of about 10% (9), that justifies the search for new sources of sugarcane borer biocontrol.

It is increasing the use of microorganisms capable of controlling plant pests and pathogens (10) and these antagonistic microorganisms assure a sustainable development and an environmental safety (11-13). Nowadays, several fungi are commercialized as a potential biological controller (14).

Endophytic fungi or endophytes colonize intra and intercellular spaces of plant tissues, without causing apparent damage (15). Furthermore, they are important for the maintenance of the host plants (16), being recognized as important for the plant protection against pests, including insects, nematodes and plant pathogens (17).

The insect-pest control may be achieved indirectly by the production of metabolites that decrease the larval period, causing the insect death (18).

In Brazil, endophytic microorganisms (fungi and bacteria) have been isolated from several economic and medicinal plants, such as *Zea mays* (19), citrus (20), *Vigna unguiculata* (21), *Theobroma cacao* (22), Amazonian toxic plants (23), *Ilex paraguariensis* (24), *Glycine max* (25), *Manihot esculenta* (26), *Smallanthus sonchifolius* (27), *Sapindus saponaria* (28) and *Trichilia elegans* (29).

Luehea divaricata Martius et Zuccarini belongs to Malvales order and Tiliaceae family. It is a medicinal tree vulgarly known as whip-horse (“açoita-cavalo” in Brazilian Portuguese) and widely distributed across Brazil, from Rio Grande do Norte to Rio Grande do Sul states (30, 31). Some of its medicinal properties are: depurative effect, the combat of bronchitis, dysentery, leucorhea, rheumatism, blennorhea and tumors (32).

Secondary metabolites obtained from endophytic fungi isolated from *L. divaricata* leaves have been shown antimicrobial activity against phytopathogenic fungus and human pathogenic bacterium (33). However, no insecticide activity was assessed.

Therefore, this study aimed to verify the influence of crude extracts of endophytic fungi isolated from *Luehea divaricata* on the in vitro development of larvae of *Diatraea saccharalis*.

MATERIAL AND METHODS

Biological material

Two endophytic fungi from *L. divaricata* leaves, *Epicoccum nigrum* and *Guignardia vaccinii*, were selected for this study. These fungi were isolated and molecularly characterized by Bernardi-Wenzel et al. (33), belonging to the fungal culture collection of Laboratório de Biotecnologia Microbiana of the Universidade Estadual de Maringá, Paraná, Brazil.

Third-instar larvae of *D. saccharalis* were provided by the Laboratório de Morfologia e Citogenética de Insetos of the Universidade Estadual de Maringá.

Culture media

Potato Dextrose Agar (PDA) and Potato Dextrose (PD) were prepared according to Smith and Onions (34) modified by Pamphile and Azevedo (19).

Obtention of crude endophytic extracts

Crude extracts were obtained according to Souza et al. (23), slightly modified.

The endophytes were grown in 9 cm Petri dishes containing PDA medium (20 ml), in B.O.D at 28° C.

Then, Erlenmeyer flasks containing 250 ml PD medium were inoculated with three fragments (1 cm^2) of 7 days-old fungal culture and incubated at 28° C for 15 days. As negative control, PD medium was incubated without fungal inoculation.

Fungal mycelium and fermented medium were separated by filtration in sterile Whatman filter paper no. 4. Fermented medium was centrifuged at 3,600 rpm for 10 min. The supernatant was passed through a 0.22 µm Millipore membrane. The resulting liquid was the crude extract from each of the selected endophytes and it was stored at 4° C until its use.

Assessment of the influence of crude endophytic extracts on the in vitro development of *D. saccharalis* larvae

Tests were made in triplicate for the treatments (crude extract of each endophyte) and control.

Four larvae of sugarcane borer were placed on Petri dishes. The crude extracts of *E. nigrum* and *G. vaccinii* were dripped on the dorsal surface of the prothorax of each larva, in agreement with Debonsi et al. (35). As negative control, only non inoculated medium was dripped on dorsal surface.

Food was offered *ad libitum* and dishes were incubated in B.O.D. at 25° C and observed daily throughout 7 days.

RESULTS AND DISCUSSION

Influence of crude extracts on the in vitro development of *D. saccharalis* larvae

Throughout the incubation period, the larvae treated with crude extracts of the endophytic isolates showed great excitement, unrest behavior. This anomalous behavior compared to control larvae (treated with a non-inoculated medium) would result from the action of fungal secondary metabolites present in the fermented medium.

After 7 days of incubation (see Figure 1), the absence of pupal stage was presented by fungal-treated larvae in comparison with non-treated larvae, taking into account that it was possible to observe the pupal stage in the most of the larvae.

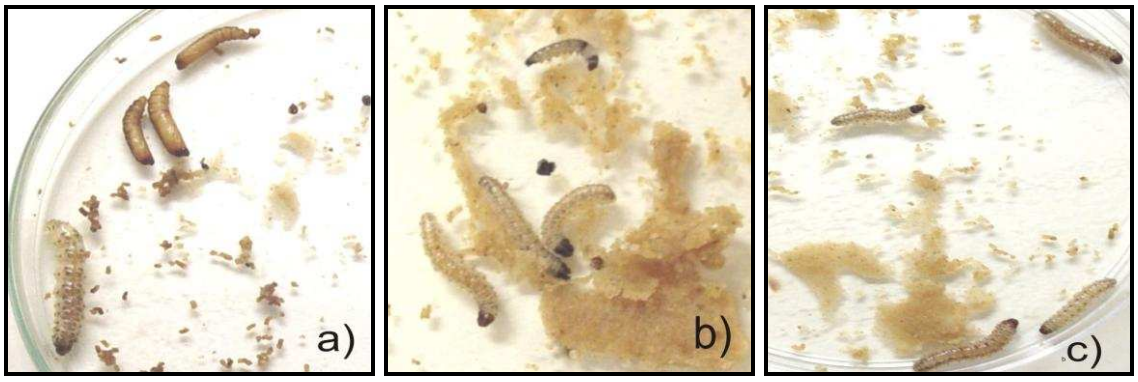

Figure 1. Development of sugarcane borer larvae in the presence of crude endophytic extracts. A) Control: most of larvae in pupal stage. B) Treatment with *E. nigrum* extract and C) Treatment with *G. vaccinii* extract: both of them showed the absence of pupal stage.

Similarly, Torres et al. (36) analyzed the effects of 13 plant aqueous extracts on the development of larvae of *Plutella xylostella*. Larvae treated with *Melia azedarach* and *Cissampelos* aff. *glaberrima* delayed their development, while *Aspidosperma pyrifolium*, *Azadirachta indica* and *A. indica* oil formulation caused 100% larval mortality. Extracts of *M. azedarach*, *C.* aff. *glaberrima*, *Laurus nobillis*, *Prosopis juliflora*, *Croton* sp. and *Eugenia uniflora* caused larval mortality ranging from 60 to 96.7%.

Also, the influence of crude extracts and fractions from seeds of *M. azedarach* on development of *Musca domestica* was assayed by Cabral et al. (37) and a reduction of the post-embryonic development of the flies was

observed and the delay in their development from newly hatched larvae to adults significantly increased.

Elbanna et al. (38) observed the larvaecidal effects of *Eucalyptus globulus* extract on the larvae of the *Culex pipiens* mosquito, reporting that seed and leaf extracts caused 100 and 80% mortality, respectively. This indicates that these extracts contain compounds there are toxic to larvae and may be developed for biocontrol of mosquitoes.

Barreto et al. (39) reported the larvaecidal activity of crude extract of *Sapindus saponaria* bark and the morpho-histological changes in larvae of *Aedes aegypti*. As result, several cellular changes were observed, indicating the action of this plant's extract on insect.

The biolarvicidal activity of extracellular metabolites of the fungus *Trichophyton mentagrophytes* against *A. aegypti* larvae was evaluated by Murugesan et al. (40) and the fungal culture filtrate produced L3 mortality of up to 90%, proving that these metabolites may be an efficient alternative to synthetic insecticides.

Endophytic bacteria have also the ability to the biological control of pests. Naves et al. (41) evaluated the action of filtrates of endophytic bacteria on motility, mortality and hatching of second stage juveniles (J_2) of nematode *Meloidogyne javanica*. As results, seven of 40 bacterial isolates immobilized the juveniles within 24 h, and they could not recover even in water, similarly to the aldicarb treatment used as control. The filtrates also inhibited egg hatching. Two isolates killed more than 90% of J_2 after 48 h of exposure, showing their potent action on nematode behaviors.

Oliveira et al. (42) investigated the effects of different concentrations of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* on biological characteristics of third-instar larvae of sugarcane borer, concluding that both of them caused the larval and pupal mortality. When they were applied in sublethal doses, biological characteristics that determine the pest's successful *D. saccharalis*, such as longevity, fertility, and eggs viability, were affected.

A similar research to ours was made by Debonsi et al. (35), who applied the seed extract of medicinal plant *Piper tuberculatum* and solution of pure amides obtained from it on the dorsal surface of prothorax of larvae of *D. saccharalis*, in order to verify the influence of the compounds on the larval development. As result, the amides showed to be a potential candidate for controlling the sugarcane borer.

In conclusion, although no larval mortality has occurred, the crude endophytic extracts altered the physiology of the *D. saccharalis* larvae, influencing their life cycle.

Our results suggest that *Epicoccum nigrum* crude extract and *Guignardia vaccinii* crude extract have the potential to inhibit *Diatraea saccharalis* development and they demonstrated to be potent for a future use in biological control of sugarcane borer.

Also, it suggests that the insecticide potential of other fungal endophytes from *Luehea divaricata* can be assessed.

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