EFFECT OF EXTRACTS OF *LUFFA CYLINDRICA*, *XANTHOSOMA SAGITTIFOLIUM* AND *MOMORDICA CHARANTIA* ON *SAPROLEGNIA* SPP. MYCELIAL GROWTH

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ABSTRACT

Among the major infectious diseases in fish, those caused by zoosporic organisms are important, especially saprolegniasis. These diseases can affect few animals or even the entire population of the production environment. The use of alternative treatments for infectious diseases in aquatic animals has proven to be effective. It prevents the mortality of part of the crop, as well as the contamination of water by chemical components. Within this scenario, the use of plants stands out, mainly unconventional food plants (UFPs). The objective of this work was to evaluate the effect of the aqueous extracts of *Luffa cylindrica*, *Xanthosoma sagittifolium* and *Momordica charantia* on the mycelial growth of *Saprolegnia* spp. The extracts obtained from the maceration of dry and ground leaves were incorporated into the potato agar dextrose (PDA), at concentrations of 0, 5, 15, 25 and 50%. Later, filter paper disks containing the mycelium of *Saprolegnia* spp. were applied on the agar and incubated at 25°C. Evaluations were performed every 24 hours, for 10 days. In the study, all concentrations of *M. charantia* extract had an inhibitory action on the mycelial growth in the order of 75% of *Saprolegnia* spp. and can be considered a promising alternative for controlling this pathogen.

Keywords: UFPs, saprolegniasis, aquaculture, alternative treatment, sustainability

EFEITO DE EXTRATOS DE *LUFFA CYLINDRICA*, *XANTHOSOMA SAGITTIFOLIUM* E *MOMORDICA CHARANTIA* SOBRE O CRESCIMENTO MYCELIAL DE *SAPROLEGNIA* SPP.

RESUMO

Entre as principais doenças infecciosas em peixes, as causadas por organismos zoospóricos são importantes, especialmente a saprolegníase. Essas doenças podem afetar poucos animais ou até mesmo toda a população do ambiente de produção. O uso de tratamentos alternativos para doenças infecciosas em animais aquáticos tem se mostrado eficaz. Evita a mortalidade de parte da cultura, bem como a contaminação da água por componentes químicos. Dentro deste cenário, destaca-se o uso de plantas, principalmente plantas alimentícias não convencionais (PANCs). O objetivo deste trabalho foi avaliar o efeito dos extratos aquosos de *Luffa cylindrica*, *Xanthosoma sagittifolium* e *Momordica charantia* sobre o crescimento micelial de *Saprolegnia* spp. Os extratos obtidos da maceração de folhas secas e moídas foram incorporados em ágar batata dextrose (BDA), nas concentrações de 0, 5, 15, 25 e 50%. Mais tarde, os discos de papel de filtro contendo o micélio de *Saprolegnia* spp. foram aplicados no ágar e incubados a 25°C. As avaliações foram realizadas a cada 24 horas, por 10 dias. No estudo, todas as concentrações

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do extrato de *M. charantia* tiveram ação inibitória sobre o crescimento micelial da ordem de 75% de *Saprolegnia* spp., podendo ser considerada uma alternativa promissora para o controle desse patógeno.

Palavras-Chave: PANCs, saprolegníase, aquicultura, tratamento alternativo, sustentabilidade

EFECTO DE EXTRACTOS DE *LUFFA CYLINDRICA*, *XANTHOSOMA SAGITTIFOLIUM* Y *MOMORDICA CHARANTIA* EN EL CRECIMIENTO MYCELIAL DE *SAPROLEGNIA* SPP.

RESUMEN

Entre las principales enfermedades infecciosas en los peces, las causadas por organismos zoospóricos son importantes, especialmente la saprolegniasis. Estas enfermedades pueden afectar a pocos animales o incluso a toda la población del entorno de producción. El uso de tratamientos alternativos para enfermedades infecciosas en animales acuáticos ha demostrado ser efectivo. Previene la mortalidad de parte del cultivo, así como la contaminación del agua por componentes químicos. Dentro de este escenario, se destaca el uso de plantas, principalmente plantas alimenticias no convencionales (PANCs). El objetivo de este trabajo fue evaluar el efecto de los extractos acuosos de *Luffa cylindrica*, *Xanthosoma sagittifolium* y *Momordica charantia* sobre el crecimiento micelial de *Saprolegnia* spp. Los extractos obtenidos de la maceración de hojas secas y molidas se incorporaron a la papa dextrose agar (PDA), a concentraciones de 0, 5, 15, 25 y 50%. Más tarde, filtre los discos de papel que contienen el micelio de *Saprolegnia* spp. se aplicaron sobre el agar y se incubaron a 25°C. Las evaluaciones se realizaron cada 24 horas, durante 10 días. En el estudio, todas las concentraciones de extracto de *M. charantia* tuvieron una acción inhibitoria sobre el crecimiento micelial del orden del 75% de *Saprolegnia* spp. y puede considerarse una alternativa prometedora para controlar este patógeno.

Palabras-Clave: PANCs, saprolegniasis, acuicultura, tratamiento alternativo, sostenibilidad

INTRODUCTION

The global increase production of aquatic organisms, especially freshwater fish, requires modern management, nutrition and reproduction technologies (1). In the same context, outbreaks of parasitic, bacterial, fungal diseases caused by oomycetes, other protists, and metazoans have been frequently described in aquaculture, mainly fish farming. Hence, antimicrobials, antifungals, and disinfectants are employed to reduce mortality caused by infectious agents (2,3).

Diseases caused by fungi and pseudo-fungi, such as oomycetes, are among the main diseases in fish production. In conventional aquaculture, the oomycete *Saprolegnia* spp. is an important cause of disease in different stages of development of the cultivated animals. Saprolegniasis can affect about 10% of all salmon and 30% of fish production for consumption (4). In the confinement of aquatic organisms, both ornamental animals and those intended for consumption, including amphibians, *Saprolegnia* spp. can develop rapidly, especially when this environment presents an overload of organic matter (5).

The development of alternative treatments is proving necessary and efficient so that in addition to preventing mortality of farmed fish, it also prevents the water from being contaminated by chemical components, which are often highly toxic. Thus, the use of plants has been excelled by having active principles with antimicrobial properties and also by acting as immunostimulants (1).

The use of plants for treating fish diseases is quite old, being widespread in Asian countries (6). Focusing on Brazilian plant potential, the use of unconventional food plants (UFPs), like *Luffa cylindrica*, *Xanthosoma sagittifolium* and *Momordica charantia*, has been growing within animal husbandry systems. UFPs have not yet been fully studied by the scientific community but present a strong regional consumption although not completely inserted in the productive chain (7).

The purpose of this work was to evaluate the effect of aqueous extracts of sponge gourd (*L. cilyndrica*), tannia (*X. sagittifolium*) and bitter gourd (*M. charantia*) on the mycelial growth of *Saprolegnia* sp.

MATERIALS AND METHODS

The experiment was conducted at the Microbiology Laboratory, Federal University of Fronteira Sul, Laranjeiras do Sul - PR. *Saprolegnia* spp. (SisGen: AED8E7F), which was used in the experiment, was isolated and identified from a juvenile pacu (*Piaractus mesopotamicus*), kept in fish tanks, presenting white and greyish cotton-like structures on the skin.

The aerial parts of sponge gourd (*L. cylindrica*), tannia (*X. sagittifolium*) and bitter gourd (*M. charantium*) were collected and processed in Laranjeiras do Sul (25º24'28"S and 52°24'58"W). Authorization of access to genetic resources: SisGen – AE26D84.

The procedure for collecting and manipulating was performed according to Celoto (8). The plants were collected in the morning and then the stalks, flowers, and leaves damaged by insects or diseases were removed. Next, the leaves were washed in running water and disinfected with 10% sodium hypochlorite solution for 20 minutes in order to eliminate any microorganism. After, the material was washed again with distilled water to remove excess hypochlorite solution. Then, the aerial parts of the plants were placed on absorbent paper for 24 hours to remove excess moisture.

Subsequently, the material was packed in paper bags and dried in a forced air circulation oven for 72 hours at 40ºC. After drying, the material was ground in a knife mill and stored in a glass container. 20 g of leaves were extracted by maceration in 80 mL of water (20:80). The aqueous extract was obtained by adding boiling distilled water to the dried material and left to rest for two hours. Finally, this extract was filtrated and stored in a light-proof glass flask at 4°C until use.

Potato Dextrose Agar (PDA) medium was prepared and supplemented with 5, 15, 25 and 50% plant extracts. *Saprolegnia* spp. was inoculated on PDA medium and maintained at 25°C. After 6 days, 1 cm² of mycelial fragments were removed from the inoculum and applied on the control and test plates. The growth of *Saprolegnia* spp. on the surface of culture media was measured every 24 hours for 10 days.

The parameter was analyzed by determining the mean of the daily diameter of the colony, in two perpendicular positions to each other. The percentage of inhibition of mycelial growth (PIMG) (9) was determined from the results obtained, using the following formula:

$$PIMG = \left(\frac{\text{control group diameter} - \text{test group diameter}}{\text{control group diameter}} \right) .100$$

The experiment was performed in duplicate and by a completely randomized design. The data were analyzed by Tukey's test and the results expressed as mean ± SD. P values <0.05

were considered as significant. The results were processed using the software STATGRAPHICS®.

RESULTS AND DISCUSSION

The aqueous extract of tannia (*X. sagittifolium*) showed no inhibitory activity on the growth of *Saprolegnia* spp, in any concentration tested (Table 1). On the other hand, the 50% aqueous extract of sponge gourd (*L. cylindrica*) expressed a tendency to inhibit the growth of *Saprolegnia* sp. (Table 1). Tiamyiu et al. (10) added *L. cylindrica* seeds to *Clarias gariepinus* feed diet, without negative effects for fish growth. There is a growing amount of studies using plants in several countries and different fish species, especially due to its antimicrobial, antifungal, antiparasitic, as a growth promoter and immunostimulant (1,11,12,13). However, there are no reports on the plants employed in this study inhibiting the growth of *Saprolegnia* spp., neither *in vitro* or *in vivo*.

Table 1. Mycelial growth diameter (cm) of *Saprolegnia* spp. at five concentrations (0, 5, 15, 25 and 50%) against aqueous extracts of bitter gourd (BG), tannia (T) and sponge gourd (SG).

	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
BG 0%	0,75	0,90	1,40*	1,50*	2,00*	2,40*	2,80*	3,30*	3,80*	4,00*[a]
BG 5%	0,61	0,67	0,70	0,70	0,80*	0,80*	0,80*	0,80*	0,80*	0,80*[b]
BG 15%	0,67	0,77	0,77	0,77	0,77	0,77	0,77	0,77	0,77	0,77[b]
BG 25%	0,62	0,75	0,75	0,75	0,75	0,75	0,75	0,75	0,75	0,75[b]
BG 50%	0,65	0,77	0,82*	0,85*	0,85*	0,85*	0,85*	0,85*	0,85*	0,85*[b]
T 0%	0,75	0,90	1,40	1,55	2,00*	2,40*	2,85*	3,30*	3,80*	4,05*[a]
T 5%	0,75	0,95	1,11	1,65*	1,77*	2,27*	2,45*	2,87*	3,22*	3,65*[a]
T 15%	0,72	0,90	1,22	1,77*	1,85*	2,07*	2,40*	2,72*	2,07*	3,40*[a]
T 25%	0,77	1,01	1,22	1,55	1,70*	2,05*	2,55*	2,82*	3,20*	3,52*[a]
T 50%	0,78	0,94	1,30	1,42	1,70*	1,92*	2,22*	2,57*	3,02*	3,37*[a]
SG 0%	0,75	0,90	1,40	1,55	2,00*	2,40*	2,85*	3,30*	3,80*	4,05*[a]
SG 5%	0,78	1,20	1,38	1,62*	1,77*	2,17*	2,52*	2,75*	3,12*	3,47*[a]
SG 15%	0,65	1,27	1,6*	1,65*	1,65*	2,25*	2,55*	2,80*	3,10*	3,35*[a]
SG 25%	0,66	0,95	1,28	1,65*	1,82*	2,12*	2,42*	2,67*	2,97*	3,25*[a]
SG 50%	0,71	1,08	1,33	1,55	1,6*	1,95*	2,22*	2,65*	2,82*	3,02*[a]

Note. D: Day.
Values followed by (*) on the same row are significantly different in relation to time zero according to the T-test at 0.05. Values followed by the same letter, on the same column, are not different according to Tukey's test ($p > 0.05$).

The aqueous extract of bitter gourd (*M. charantia*) promoted inhibition of *Saprolegnia* spp. at all concentrations (5, 15, 25 and 50%). Since the beginning of the trial, it showed a 75% reduction (PIMG) in comparison to the control group (100%), which was statistically different. From the third day on, no growing could be noticed in any of the four concentrations analyzed

(Table 1). Several studies describe the action of *Momordica* sp. in fungi, mainly phytopathogens (14,15), indicating that α-momorcharin, a protein isolated from the extract of the plant, is effective against fungal pathogens (16), showing the viability of this UFP in the control important pathogens in agriculture. Different species of *Momordica* have been evaluated against bacteria and parasites isolated from fish with an effective inhibitory effect (17,18).

Some ribosome inactivating proteins (RIPs), such as lufacillin isolated from *L*. cylindrica, and alpha or beta-momorcarin from *M. charantia*, were described with high activity antifungal therapy against *Fusarium* sp. (19), which points to the need for extraction and isolation of substances described above, and *in vitro* and *in vivo* testing. Furthermore, in the literature it was not possible to find reports of substances responsible for the antifungal activity of *X. sagittifolium*.

The use of plants and medicinal herbs as a prophylactic measure and in the treatment of diseases is becoming eminent because it produces minimal side effects, low impact on the environment besides the favorable cost-benefit when compared to conventional treatment. Pathogens are an inherent part of any aquatic environment. In aquaculture, on account of being more difficult to control the environmental conditions, the chances of infection are increased by the stress levels to which the animals are exposed. However, there are few studies concerning UFPs, medicinal herbs as antibacterial or antiviral agents in aquaculture, as well as few studies analyzing those plants against fungal infection in fish (20).

Chemical products should be replaced by natural ones, although some of those may have a negative impact on fish farming (1). Bitter gourd has been tested on bacterial isolates from fishes due its importance as the major cause of mortality in fish production (21). Hence, *in vivo* studies are crucial to proving the inhibitory action of *M. charantia* on the growth of *Saprolegnia* spp., and if there is any toxicity of this UFP on the different stages of fish development. According to Bolognesi et al. (22) it should be emphasized that the toxicity of plant and plant-isolated substances to animals is variable, which reinforces the need for their isolation and further testing with different species of fish.

CONCLUSION

In conclusion, bitter gourd (*Momordica charantia*), at different concentrations (5, 15, 25 and 50%), can be considered a sustainable and promising alternative for controlling *Saprolegnia* spp.

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REFERENCES

1. Pereira LA, Weiss LA, Besen MA, Marengoni NG. Uso de extratos de plantas e suas propriedades profiláticas ou terapêuticas na produção de peixes. Sci Agrar Parana. 2016;15:373-80. doi: http://dx.doi.org/10.18188/1983-1471/sap.v15n4p373-380.

2. Gozlan RE, Marshall WL, Lilje O, Jessop CN, Gleason FH, Andreou D. Current ecological understanding of fungal-like pathogens of fish: what lies beneath? Front Microbiol. 2014;5:1-16. doi: https://doi.org/10.3389/fmicb.2014.00062.

3. Valladão GMR. Potencial de óleos essenciais de plantas para o tratamento de enfermidades em peixes [dissertação] [Internet]. Jaboticabal: Centro de Aquicultura, Universidade Estadual Paulista; 2014 [cited 2019 Jun 12]. Available from: https://repositorio.unesp.br/bitstream/handle/11449/110337/000777206.pdf?sequence=1&isAllowed=y

4. Earle G, Hintz W. New approaches for controlling *Saprolegnia parasitica*, the causal agent of a devastating fish disease. Trop Life Sci Res [Internet]. 2014 [cited 2019 Jun 18];25:101-9. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4814142/pdf/tlsr-25-2-101.pdf

5. Cruz LCH. Micologia veterinária. Rio de Janeiro: Revinter; 2010.

6. Inoue LAKA, Boijink CL, Chaves FCM. Plantas medicinais na prevenção e tratamento de doenças na piscicultura [Internet]. Manaus: Embrapa; 2009 [cited 2019 Jun 18]. Available from: https://www.infoteca.cnptia.embrapa.br/infoteca/handle/doc/853254

7. Gollner-Reis JP, da Silva MH, da Silva MA, Barbosa KKS, Gollner-Reis KTM. Estudo de emprego de plantas alimentícias não convencionais (PANCS): característica nutricional, propriedade funcional e emprego na alimentação humana [Internet]. In: Congresso Nacional de Pesquisa e Ensino em Ciências; 2017; Alagoas. Alagoas: Conapesc; 2017 [cited 2019 Jun 12]. Available from: https://editorarealize.com.br/revistas/conapesc/trabalhos/TRABALHO_EV058_MD4_SA80_ID1178_01052016063821.pdf

8. Celoto MIB. Atividade antifúngica de extratos de melão-são-caetano (*Momordica charantia* L.) sobre *Colletotrichum musae* (Berk. & Curtis) Arx. [dissertação] [Internet]. Ilha Solteira: Faculdade de Engenharia, Universidade Estadual Paulista; 2005 [cited 2019 Jun 12]. Available from: https://repositorio.unesp.br/bitstream/handle/11449/98859/celoto_mib_me_ilha.pdf?sequence=1&isAllowed=y

9. Nascimento JM, Serra AP, Bacchi LM, Gavassoni WL, Vieira MC. Inibição do crescimento micelial de *Cercospora calendulae* Sacc. por extratos de plantas medicinais. Rev Bras Plantas Med. 2012;15:751-6. doi: http://dx.doi.org/10.1590/S1516-05722013000500016.

10. Tiamiyu LO, Solomon SG, Satimehin SPD. Growth performance of *Clarias gariepinus* fingerlings fed varying levels of the seed of *Luffa cylindrica* meal in outdoor hapas. Octa J Biosci. 2014;2:8-12. doi: http://dx.doi.org/10.5376/ija.2016.06.0020.

11. Huang XL, Liu RJ, Whyte S, Du ZJ, Chen DF, Deng YQ, et al. The in vitro antifungal activity of 30 Chinese herb extracts to *Saprolegnia* sp. J Appl Ichthyol. 2015;31:681-6. doi: https://doi.org/10.1111/jai.12773.

12. Syahidah A, Saad CR, Daud HM, Abdelhadi YM. Status of herbal applications in aquaculture: a review. Iran J Fish Sci. 2015;14:27-44. doi: http://jifro.ir/article-1-1818-en.html.

13. Valladão GMR, Gallani SU, Pilarski F. Phytotherapy as an alternative for treating fish disease. J Vet Pharmacol and Ther. 2015;38:417-28. doi: https://doi.org/10.1111/jvp.12202.

14. Ahmad B, Khan AA. Antibacterial, antifungal and phytotoxic activities of *Luffa cylindrica* and *Momordica charantia*. J Med Plants Res [Internet]. 2013 [cited 2019 Jun 12];7:1593-9. Available from: https://pdfs.semanticscholar.org/0f0b/678f5cdba70f40df5b77030b4a352fbac477.pdf

15. Gupta M, Sharma S, Bhadauria R. *In vitro* efficacy of *Momordica charantia* extracts against phytopathogenic fungi, *Fusarium oxysporum*. J Biopest [Internet]. 2016 [cited 2019 Jun 12];9:8-22. Available from: http://www.jbiopest.com/users/LW8/efiles/vol_9_1_8-22.pdf

16. Wang S, Zheng Y, Xiang F, Shiming L, Yang G. Antifungal activity of *Momordica charantia* seed extracts toward the pathogenic fungus *Fusarium solani* L. J Food Drug Anal. 2016;24:881-7. doi: https://doi.org/10.1016/j.jfda.2016.03.006.

17. Pannu R, Dahiya S, Sabhlok VP, Kumar D, Sarsar V, Gahlawat SK. Effect of probiotics, antibiotics and herbal extracts against fish bacterial pathogens. Ecotoxicol Environ Contam. 2014;9:13-20. doi: https://doi.org/10.5132/eec.2014.01.002.

18. Prieto A, Ocampo AA, Fernández A, Pérez M. El empleo de medicina natural em el control de enfermedades de organismos acuáticos y potencialidades de uso em Cuba y México. TIP Rev Espec Cienc Quim Biol [Internet]. 2005 [cited 2019 Jun 12];8:38-49. Available from: http://www.redalyc.org/articulo.oa?id=43200805

19. Zhu F, Zhou YK, Ji ZL, Chen XR. The plant ribosome-inactivating proteins play important roles in defense against pathogens and insect pest attacks. Front Plant Sci. 2018;9:1-14. doi: https://doi.org/10.3389/fpls.2018.00146.

20. Ramudu KR, Dash G. A review on herbal drugs against harmfull pathogens in aquaculture. Am J Drug Discov Devel. 2013;3:209-19. doi: https://doi.org/10.3923/ajdd.2013.209.219.

21. Ozusaglam MA, Karakoca K. Antimicrobial and antioxidante activities of *Momordica charantia* from Turkey. Afr J Biotechnol [Internet]. 2013 [cited 2019 Jun 12];12:1548-58. Available from: https://www.ajol.info/index.php/ajb/article/view/128722/118279

22. Bolognesi A, Botolotti M, Maiello S, Battelli MG, Polito L. Ribosome-inactivating proteins from plants: a historical overview. Molecules. 2016;21:1-19. doi: https://doi.org/10.3390/molecules21121627.

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