Ciência Rural, Santa Maria, v.53:7, e20220091, 2023
http://doi.org/10.1590/0103-8478cr20220091
ISSNe 1678-4596
MICROBIOLOGY

Promising use of nanotechnology in *Pythium insidiosum*: a systematic review

Lara Baccarin Ianiski[1,2] Aline Fontanella Maciel[2] Caroline Quintana Braga[5] Carla Weiblen[3,6] Daniela Isabel Brayer Pereira[5] Luís Antônio Sangioni[3] Janio Morais Santurio[2] Luciana Pötter[4] Sônia de Avila Botton[1,3*]

[1]Programa de Pós-graduação em Ciências Farmacêuticas (PPGCF), Centro de Ciências da Saúde (CCS), Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brasil.
[2]Laboratório de Pesquisas Micológicas, Departamento de Microbiologia e Parasitologia, Centro de Ciências da Saúde (CCS), Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brasil.
[3]Programa de Pós-graduação em Medicina Veterinária (PPGMV), Departamento de Medicina Veterinária Preventiva, Centro de Ciências Rurais (CCR), Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brasil. E-mail: sabott20@gmail.com. *Corresponding author.
[4]Programa de Pós-graduação em Zootecnia, Departamento de Zootecnia, Universidade Federal de Santa Maria (UFSM), Centro de Ciências Rurais (CCR), Santa Maria, RS, Brasil.
[5]Departamento de Microbiologia e Parasitologia, Instituto de Biologia, Universidade Federal de Pelotas (UFPel), Pelotas, RS, Brasil.
[6]Universidade Regional Integrada do Alto Uruguai e das Missões (URI), Santiago, RS, Brasil.

ABSTRACT: The aquatic oomycete *Pythium insidiosum* is an emerging pathogen highly relevant in human and veterinary medicine and an etiologic agent of pythiosis, a disease of worldwide distribution mainly affecting horses, dogs, and humans, presenting cutaneous, subcutaneous, ocular, gastrointestinal, and systemic forms. The available therapeutic methods to treat this disease and its forms are not entirely effective, thus highlighting the need to investigate the forms of treatments with better efficacy, such as compounds from different pharmacological classes, compounds of natural origin, and new technological alternatives, including nanotechnology. Therefore, this study evaluated scientific publications regarding the use of nanotechnology in *P. insidiosum* treatment. For this, a systematic literature review, was carried out on articles published from 2010 to 2022 on the LILACS, MEDLINE, Google Scholar, PubMed, and SciELO databases using the descriptors '*Pythium insidiosum*,' 'pythiosis,' 'nanotechnology,' 'nanoparticles,' 'nanoemulsion,' and 'treatment.' We reported 162 articles for the researched theme; although, only four studies were included because they met the criteria established herein. A meta-analysis was used for the statistical analysis of the data obtained *in vitro* studies, and we reported the use of nanotechnology can be a promising alternative in developing antimicrobial compounds with anti-*P. insidiosum* activity. Nevertheless, additional research is needed to verify the potential use of this technology in clinical therapy against *P. insidiosum* infections.
Key words: nanotechnology, oomycete, Pythiosis, *Pythium insidiosum*, susceptibility.

Utilização promissora de nanotecnologia em *Pythium insidiosum*: uma revisão sistemática

RESUMO: O oomiceto aquático *Pythium insidiosum* é um patógeno emergente de relevância em medicina humana e veterinária. É o agente etiológico da pitiose, uma enfermidade de distribuição mundial, que acomete principalmente em equinos, caninos e seres humanos, podendo apresentar-se nas formas cutâneas, subcutâneas, oculares, gastrointestinais e sistêmicas. Considerando que os métodos terapêuticos disponíveis para o tratamento da doença não são completamente efetivos, há uma necessidade de investigar formas de tratamentos com melhor eficácia, como os compostos de diferentes classes farmacológicas, compostos de origem natural, bem como, novas alternativas tecnológicas, incluindo a nanotecnologia. Deste modo, este trabalho objetivou avaliar publicações científicas referentes a utilização de nanotecnologia em *P. insidiosum*. Para isso, realizou-se uma revisão sistemática da literatura, buscando artigos no período de 2010 a 2022, nas bases de dados LILACS, MEDLINE, Google Scholar, PubMed e SciELO, utilizando-se os descritores *Pythium insidiosum*, pitiose, nanotecnologia, nanopartículas, nanoemulsão e tratamento. Encontrou-se 162 artigos com familiaridade a temática pesquisada; no entanto, apenas quatro estudos foram incluídos, pois atendiam os critérios estabelecidos na pesquisa. Para análise estatística dos dados obtidos nos estudos *in vitro,* utilizou-se meta-análise. Demonstrou-se o promissor uso de nanotecnologia como alternativa no desenvolvimento de compostos antimicrobianos com atividade anti-*P. insidiosum*. Entretanto, constata-se que estudos adicionais se fazem necessários para verificar o potencial uso desta tecnologia na terapêutica clínica contra infecções por *P. insidiosum*.
Palavras-chave: nanotecnologia, oomiceto, pitiose, *Pythium insidiosum*, suscetibilidade.

INTRODUCTION

The genus *Pythium* belongs to the class Oomycetes and contains numerous saprobic and pathogenic species (DICK et al., 2001; KAGEYAMA, 2014; MCCARTHY et al., 2017). *Pythium insidiosum*, the oomycete that causes pythiosis, is commonly reported in temperate climate regions and swampy environments, where it reproduces asexually and produces infective zoospores that seek a host to settle

Received 02.17.22 Approved 08.22.22 Returned by the author 10.07.22
CR-2022-0091.R2
Editor: Rudi Weiblen

and develop its biological cycle (GAASTRA et al., 2010; MENDOZA et al., 2013).

Although, various countries have reported cases of pythiosis (MENDOZA et al., 1986; CARDONA-ALVARÉZ et al., 2010; LUIS-LEÓN et al., 2011; PERMPALUNG et al., 2015; ROMERO et al., 2019; PERMPALUNG et al., 2019; TARTOR et al., 2020; HTUN et al., 2021), Thailand has the highest occurrence of human pythiosis (KRAJAEJUN et al., 2006; PERMPALUNG et al., 2019). Nevertheless, there are still a concerning number of reports in Brazil for animals (especially Equidae), more notably in the western-central Brazilian region of Mato Grosso (SANTURIO et al., 1998; LEAL et al., 2001; SANTOS et al., 2014), Rio Grande do Sul (MARCOLONGO-PEREIRA et al., 2012; WEIBLEN et al., 2016; REIS-GOMES et al., 2018) and northeastern Brazil (SOUTO et al., 2021). The Brazilian equine industry is an important activity connecting agribusiness, sports and leisure sectors with significant socio-economic influence. Given this context, pythiosis is a highly relevant disease that impacts the health and economy of the Brazilian equine sector (WEIBLEN et al., 2016).

Due to the morpho-physiological differences between the oomycete *P. insidiosum* and members of the kingdom Fungi; the main components of the fungal cell wall and plasma membrane are chitin and ergosterol, respectively, in addition to oomycetes having cellulose, β-glucans, and hydroxyproline as constituents of the cell wall and an incomplete pathway of ergosterol biosynthesis, being ergosterol the main target of action of antifungal drugs (GAASTRA et al., 2010; LERKSUTHIRAT et al., 2017). Thus, pythiosis is a complex disease to treat because the commercially available drugs have limitations, making it essential to improve the therapeutic alternatives to control this disease that affects human and animals.

Nanotechnology is the area of science that studies materials at the nanometer scale and is a multidisciplinary innovation that has gained worldwide notoriety in recent decades. In the health field, nanotechnology has shown significant advances, primarily in therapeutic applicability, as nanostructured systems have been used to improve drug delivery, increase bioavailability and therapeutic index, control the release of the active ingredient, and reduce toxic effects (ERDOĞAR et al., 2018; IANISKI et al., 2022). In fact, new drug delivery systems (e.g., nanostructures) have been researched to develop alternative methods against oomycete infections (ZABRIESKI et al., 2015; VALENTE et al., 2016b), and further research is pivotal for advancing pythiosis treatment.

This carried out a systematic literature review seeking articles from 2010 to 2022 that describe the use of compounds developed in nanotechnology and with anti-*P. insidiosum* activity.

MATERIALS AND METHODS

This study was composed of a systematic literature review that was carried out according to the PRISMA flow chart, as suggested by PAGE et al. (2021). A bibliographic search was performed in the following databases: Latin American and Caribbean Literature on Health Sciences (LILACS), Online Literature and Recovery System (MEDLINE), Google Scholar , PubMed and Scientific Electronic Library Online (SciELO). In addition, we used combinations of Health Science Descriptors (DeCS) and Medical Subject Headings (MeSH) to assist in the search: 'Pythium insidiosum', 'pythiosis', 'nanotechnology', 'nanoparticles', 'nanoemulsion' and 'treatment'. The inclusion criteria included articles available in the aforementioned databases in Portuguese, English and/or Spanish. The period from 2010 to 2022 was used as a time frame. Articles that did not provide information on the use of nanotechnology in the treatment of pythiosis, that contained duplicate information or that were out of the selected period were excluded.

Data analysis was performed by first logit transformation of proportion data. Data from *Pythium insidiosum* isolates were obtained by meta-analysis under a random-effects model. The graphical representation of the meta-analysis was done using forest plots. The meta-analysis was performed in the "Metaprop" package of the R 3.4.2 software®.

RESULTS AND DISCUSSION

After searching the databases, 162 articles were obtained. After reading the titles and abstracts, 68 articles did not meet the inclusion criteria and were excluded, and 94 articles remained. These articles after reading the titles and abstracts, four of them were selected and read in full (Figure 1). The studies involving nanotechnology applied to the oomycete *P. insidiosum* are summarized in table 1.

Nanotechnology is the science that refers to manipulating matter at the nanometer scale and producing structures between 1 and 100 nm, thus enabling innovation in the areas of biotechnology, industry, electronics, agriculture, disease diagnosis, and pharmacological therapies (PELAZ et al.,

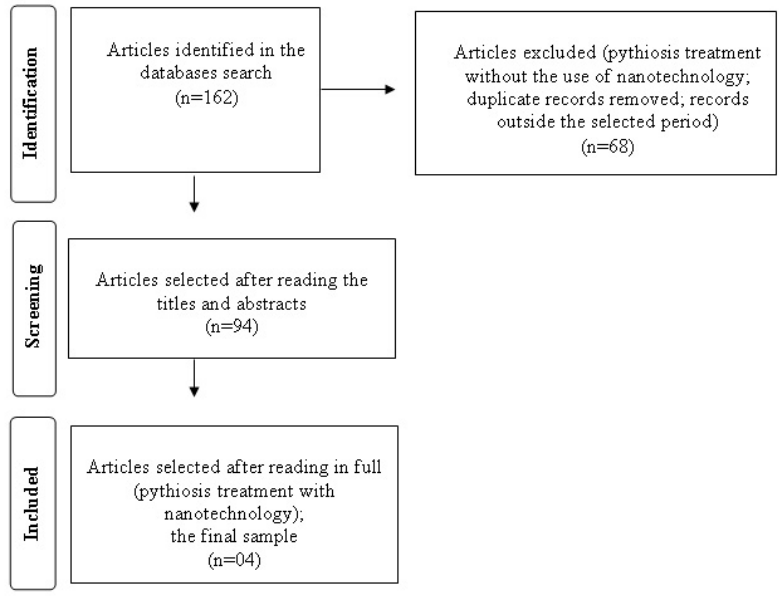

Figure 1 - Flowchart of the articles published from 2010 to 2022 and selected in the systematic literature review on nanotechnology and *Pythium insidiosum*.

2017; IANISKI et al., 2022). Drug delivery systems using nanoparticles have numerous advantages over conventional drugs because they enable the delivery of unstable and insoluble drugs, maintain active ingredient concentrations at the expected site of action, and reduce systemic toxicity. Hence, nanoparticle formulations are used at lower therapeutic doses, making it possible to minimize adverse effects and reduce treatment costs (PARVEEN et al., 2012).

Successfully treating infections by *P. insidiosum* poses numerous variables, such as size and time of lesion, patient age, and the nutritional status of the affected individual (GAATRA et al., 2010). Additionally, there are limitations when using chemical treatments, which are mainly due to the difference in cellular constituents of the oomycete compared to members of the kingdom Fungi (SANTURIO et al., 2006; LERKSUTHIRAT et al., 2017).

The currently available protocols for treating pythiosis include surgical incision, immunotherapy, and antimicrobial compounds. Among the arsenal of antimicrobial compounds evaluated include antifungals (CAVALHEIRO et al., 2009; ARGENTA et a., 2012; PEREIRA et al., 2013; CARVALHO et al., 2019; IANISKI et al., 2021), antibacterials (LORETO et al., 2011; JESUS et al., 2015b; LORETO et al., 2018; WORASILCHAI et al., 2020), and substances of natural origin (FONSECA et al., 2015; JESUS et al., 2015a; VALENTE et al., 2016a). However, given the known challenges, research must be constant to develop more efficient treatments for pythiosis.

In this regard, VALENTE et al. (2016b) described *in vitro* anti-*P. insidiosum* activity of *Melaleuca alternifolia* essential oil in the free oil and nanoemulsion forms. In another study, VALENTE et al. (2019) demonstrated *in vitro* susceptibility of biogenic silver nanoparticles (Bio-AgNP) against *P. insidiosum* isolates. The authors, using scanning electron microscopy (SEM) and transmittance microscopy (MET), verified that Bio-AgNP affected the ultrastructures and cells of the oomycete. Additionally, VALENTE et al. (2020) and SILVEIRA et al. (2022) assessed *in vivo* anti-*P. insidiosum* activity of Bio-AgNP and nanoemulsion of *M. alternifolia*, respectively, using an experimental model in rabbits.

The meta-analysis showed that the total number of *P. insidiosum* isolates collected *in vitro* studies was 412, 130 of which were treated with *M. alternifolia* free oil, 130 with *M. alternifolia* nanoemulsion and 152 with Bio-AgNP. Forest plot analysis (Figure 2) revealed that Bio-AgNP-treated isolates had the best results, with a 22% growth

Table 1 - Studies including nanotechnology applied to the oomycete *Pythium insidiosum*.

Title	Objective	Result	Conclusion	Country of origin	Reference
In vitro activity of *Melaleuca alternifolia* (Tea Tree) in its free oil and nanoemulsion formulations against *Pythium insidiosum*	To analyze *in vitro* antimicrobial activity of *Melaleuca alternifolia* in its free oil and nanoemulsion forms against Brazilian isolates of *P. insidiosum*. Broth microdilution technique was used according to CSLI document M38-A2.	MIC free oil: 531.5–2125 µg/mL MIC nanoemulsion: 132.7–2125 µg/mL	The nanoemulsion formulation inhibited *P. insidiosum* growth at significantly lower concentrations than the free oil formulation.	Brazil	VALENTE et al. (2016b)
In vitro anti-*Pythium insidiosum* activity of biogenic silver nanoparticles	To analyze *in vitro* antimicrobial activity of biogenic silver nanoparticles (bio-AgNP) against *P. insidiosum* isolates. Broth microdilution technique was used according to CSLI document M38-A2. The damage to the ultrastructure of *P. insidiosum* hyphae was evaluated by scanning and transmission electron microscopy.	MIC Bio-AgNP: 0.06–0.47 µg/mL. SEM: hyphae presence with rough surfaces and areas with increased electronic density, retracted cell walls, loss of continuity, presence of scaling, and broken areas TEM: cell wall with wrinkled and partially ruptured borders; observation of homogeneous intracytoplasmic elements, making it impossible to differentiate the structures and organelles.	*In vitro* results showed the anti-*P. insidiosum* potential of the Bio-AgNP.	Brazil	VALENTE et al. (2019)
Biogenic silver nanoparticles in the treatment of experimental pythiosis Bio-AgNP in pythiosis therapy	To analyze the activity of biogenic silver nanoparticles (bio-AgNP) *in vivo* pythiosis treatment.	Gel formulation administered topically and aqueous formulation administered intralesionally, both containing 1 µg/mL of Bio-AgNP, significantly reduced the size of pityriasis skin lesions in the study animals. One animal treated with the topical formulation showed a clinical cure.	*In vivo* experiment proved the anti-*P. insidiosum* potential of the Bio-AgNP.	Brazil	VALENTE et al. (2020)
Melaleuca alternifolia formulations in the treatment of experimental pythiosis	To analyze the activity of *M. alternifolia* nanoemulsion *in vivo* pythiosis treatment.	Daily topical application of 5 g of non-ionizable gel-based formulation containing 5 mg/mL of *M. alternifolia* nanoemulsion was ineffective in regressing the size of lesions. Intralesional application every 48 h and containing 1 mL of *M. alternifolia* and 5 mg/mL nanoemulsion in aqueous solution reduced the area of pityriasis skin lesions in the study animals.	*In vivo* experiment demonstrated the ineffectiveness of the topical use of nanoemulsion of *M. alternifolia* in the regression of cutaneous lesions of pythiosis. However, it demonstrates effect with the use of the intralesional formulation.	Brazil	SILVEIRA et al. (2022)

MIC: minimum inhibitory concentration; Bio-AgNP: Biogenic silver nanoparticles; SEM: scanning electron microscopy; TEM: transmission electron microscopy.

inhibition of *P. insidiosum* isolates, followed by the treatment with *M. alternifolia* nanoemulsion with a 16% reduction in *P. insidiosum* isolates. Nonetheless, *M. alternifolia* free oil treatment only inhibited 8% of the isolates. These results highlighted that the use of nanostructured compounds has better anti-*P. insidiosum* activity, suggesting that developing antimicrobial compounds using nanotechnology

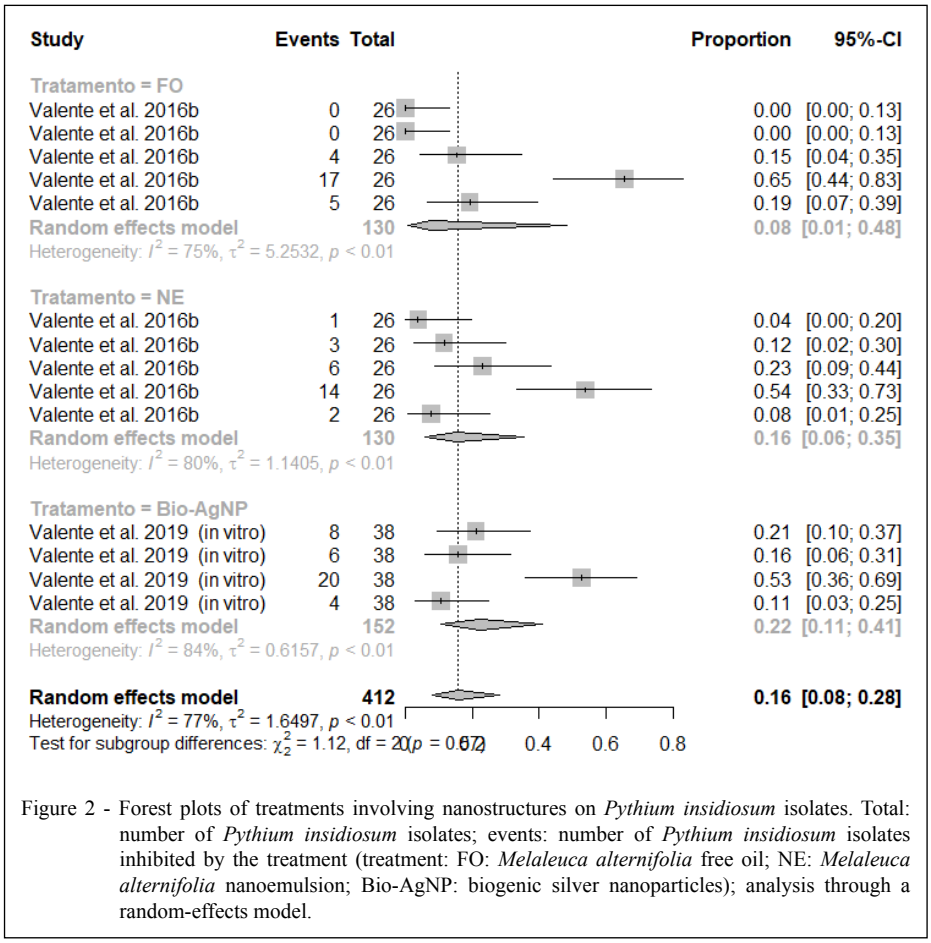

Figure 2 - Forest plots of treatments involving nanostructures on *Pythium insidiosum* isolates. Total: number of *Pythium insidiosum* isolates; events: number of *Pythium insidiosum* isolates inhibited by the treatment (treatment: FO: *Melaleuca alternifolia* free oil; NE: *Melaleuca alternifolia* nanoemulsion; Bio-AgNP: biogenic silver nanoparticles); analysis through a random-effects model.

may be a promising therapy to control *P. insidiosum* infections. It is emphasized that the advantages of using nanotechnology include improving drug delivery, increasing bioavailability and therapeutic index, controlling the release of active ingredients, and reducing the toxic effects (ERDOĞAR et al., 2018; IANISKI et al., 2022).

Given the number of experiments and similar responses, it was not possible to calculate the variance for the critical analysis of *in vivo* treatments, suggesting the need for further *in vivo* studies to compose a meta-analysis.

Notably, the studies evaluated herein were developed in Brazil, a country with the highest occurrence of equine pythiosis cases (SANTURIO et al., 1998; LEAL et al., 2001; MARCOLONGO-PEREIRA et al., 2012; SANTOS et al., 2014; WEIBLEN et al., 2016; REIS-GOMES et al., 2018; SOUTO et al., 2021), thereby demonstrating the importance of Brazilian science in developing cutting-edge research with this mammalian pathogen oomycete. Nonetheless, the development and application of nanotechnology tools in veterinary medicine in Brazil are on the rise, primarily in the therapeutic area, developing products to treat relevant diseases (IANISKI et al., 2022).

CONCLUSION

Based on the studies evaluated, nanotechnology is a promising alternative in developing nanostructured antimicrobial compounds to be explored in pythiosis treatment. Only four studies with nanotechnology and *P. insidiosum* were reported, all of which were performed by Brazilian researchers, thereby demonstrating the relevance of Brazilian science in the world context and the development of research on this important disease. Moreover, additional *in vitro* and *in vivo* studies must be developed to shed more light on the action of this new and prosperous technology against the relevant pathogenic microorganism *P. insidiosum*.

ACKNOWLEDGEMENTS

This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) [Grant no. 305451/2019-7 and 302025/2020-0]; Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul [Grant no. 17/2551-0000951-7 and 21/2551-0002260-4], and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) [Grant no.Finance code 001].

DECLARATION OF CONFLICT OF INTEREST

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

REFERENCES

ARGENTA, J. S. et al. *In vitro* and *in vivo* susceptibility of two-drug and three-drug combinations of terbinafine, itraconazole, caspofungin, ibuprofen and fluvastatin against *Pythium insidiosum*. **Veterinary Microbiology**, v.157, p.137-142, 2012. Available from: <https://pubmed.ncbi.nlm.nih.gov/22209120/>. Accessed: Oct. 10, 2021. doi: 10.1016/j.vetmic.2011.12.003.

CARVALHO, A. M. et al. The use of fluconazole associated with surgical excision in the treatment of equine cutaneous pythiosis. **Semina: Ciências Agrárias**, v.40, n.6, p.3079-3088, 2019. Available from: <https://www.uel.br/revistas/uel/index.php/semagrarias/article/view/35357>. Accessed: Oct. 10, 2021. doi: 10.5433/1679-0359.2019v40n6Supl2p3079

CAVALHEIRO, A. S. et al. *In vitro* activity of terbinafine associated to amphotericin B, fluvastatin, rifampicin, metronidazole and ibuprofen against *Pythium insidiosum*. **Veterinary Microbiology**, v.137, p.408-411, 2009. Available from: <https://pubmed.ncbi.nlm.nih.gov/19269752/>. Accessed: Oct. 10, 2021. doi: 10.1016/j.vetmic.2009.01.036.

DICK, M. W. Straminipilous Fungi: Systematics of the Peronosporomycetes Including Accounts of the Marine Straminipilous Protist, the Plasmodiophorids and Similar Organisms. **Kluwer Academic Publishers**, London, England, p.67, 2001.

ERDOĞAR, N.; AKKIN, S.; BILENSOY, E. Nanocapsules for Drug Delivery: An Updated Review of the Last Decade. ***Recent* Patents on *Drug Delivery* & Formulation**, v.12, n.4, p.252-266, 2018. Available from: <https://pubmed.ncbi.nlm.nih.gov/30674269/>. Accessed: Oct. 11, 2021. doi: 10.2174/1872211313666190123153711.

FONSECA, A. O. S. et al. *In vitro* susceptibility of Brazilian *Pythium insidiosum* isolates to essential oils of some Lamiaceae Family species. **Mycopathologia**, v.179, p.253-258, 2015. Available from: <https://pubmed.ncbi.nlm.nih.gov/25431090/>. Accessed: Oct. 10, 2021. doi: 10.1007/s11046-014-9841-6.

GAASTRA, W. et al. *Pythium insidiosum*: an overview. **Veterinary Microbiology**, v.146, p.1-16, 2010. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/20800978>. Accessed: Oct. 11, 2021. doi: 10.1016/j.vetmic.2010.07.019.

JESUS, F. P. K. et al. *In vitro* activity of carvacrol and thymol combined with antifungals or antibacterials against *Pythium insidiosum*. **Journal de Mycologie Médicale**, v.25, e89-93, 2015a. Available from: <https://www.sciencedirect.com/science/article/pii/S115652331400287X>. Accessed: Oct 10, 2021. doi: 10.1016/j.mycmed.2014.10.023.

JESUS, F. P. K. et al. *In vitro* and *in vivo* antimicrobial activities of minocycline in combination with azithromycin, clarithromycin, or tigecycline against *Pythium insidiosum*. **Antimicrobial Agents and Chemotherapy**, v.60, p.87-91, 2015b. Available from: <https://journals.asm.org/doi/10.1128/AAC.01480-15>. Accessed: Oct. 11, 2021. doi: 10.1128/AAC.01480-15.

HTUN, Z. M. et al. An initial survey of 150 horses from Thailand for anti-*Pythium insidiosum* antibodies. **Journal of Medical Mycology**, v.31, 101085, 2021. Available from: <https://www.sciencedirect.com/science/article/pii/S1156523320302262?via%3Dihub>. Accessed: Oct. 10, 2021. doi: 10.1016/j.mycmed.2020.101085.

IANISKI, L. B. et al. *In vitro* anti-*Pythium insidiosum* activity of amorolfine hydrochloride and azithromycin, alone and in combination. **Medical Mycology**, v.59, p.67-73, 2021. Available from: <https://academic.oup.com/mmy/article-abstract/59/1/67/5836559?redirectedFrom=fulltext>. Accessed: Oct. 10, 2021. doi: 10.1093/mmy/myaa032.

IANISKI, L. B. et al. Nanotechnology in veterinary medicine: a review. **Ciência Rural**, v.52, n.6, e20210195, 2022. Available from: <https://www.scielo.br/j/cr/a/s9Mc4NZ8pkfrYRrRDhDZ4fy/?lang=em>. Accessed: Jan. 14, 2022. doi: 10.1590/0103-8478cr20210195.

KAGEYAMA, K. Molecular taxonomy and its application to ecological studies of *Pythium* species. **Journal of General Plant Pathology**, v.80, n.4, p.314-326, 2014. Available from: <https://europepmc.org/article/AGR/IND600801076>. Accessed: Oct. 12, 2021. doi: 10.1007/s10327-014-0526-2.

KRAJAEJUN, T. et al. Clinical and epidemiological analyses of human pythiosis in Thailand. **Clinical Infectious Diseases**, v.43, n.5, p.569-576, 2006. Available from: <https://pubmed.ncbi.nlm.nih.gov/16886148/>. Accessed: Oct. 10, 2021. doi: 10.1086/506353.

LEAL, A. B. M. et al. Equine pythiosis in the Brazilian Pantanal region: clinical and pathological findings of typical and atypical cases. **Pesquisa Veterinária Brasileira**, v.21, n.4, p.151-156, 2001. Available from: <https://www.scielo.br/j/pvb/a/6g4D7xYJQmWk3PsPLHBswfH/?lang=pt>. Accessed: Oct. 15, 2021. doi: 10.1590/S0100-736X2001000400005.

LERKSUTHIRAT, T. et al. Evolution of the sterol biosynthetic pathway of *Pythium insidiosum* and related oomycetes contributes to antifungal drug resistance. **Antimicrobial Agents and Chemotherapy**, v.61, e02352-16, 2017. Available from: <https://pubmed.ncbi.nlm.nih.gov/28115356/>. Accessed: Oct. 10, 2021. doi: 10.1128/aac.02352-16.

LORETO, E. S. et al. *In vitro* susceptibility of *Pythium insidiosum* to macrolides and tetracycline antibiotics. **Antimicrobial Agents and Chemotherapy**, v.55, p.3588–3590, 2011. Available from: <https://journals.asm.org/doi/10.1128/AAC.01586-10>. Accessed: Oct. 11, 2021. doi: 10.1128/AAC.01586-10.

LORETO, E. S. et al. *In vitro* activities of miltefosine and antibacterial agents from the macrolide, oxazolidinone, and pleuromutilin classes against *Pythium insidiosum* and *Pythium aphanidermatum*. **Antimicrobial Agents and Chemotherapy**, v.62, e01678-17, 2018. Available from: <https://journals.asm.org/doi/10.1128/AAC.01678-17>. Accessed: Oct. 11, 2021. doi: 10.1128/AAC.01678-17.

LUIS-LEÓN, J.; PÉREZ, R. Pythiosis: Una patología emergente en Venezuela. **Salus online**, v.15, n.1, p.79-94, 2011. Available from: <http://ve.scielo.org/pdf/s/v15n1/art09.pdf>. Accessed: Oct. 11, 2021.

MCCARTHY, C. G. P.; FITZPATRICK, D. A. Phylogenomic reconstruction of the oomycete phylogeny derived from 37 genomes. **MSphere**, v.2, e00095-17, 2017. Available from: <https://doi.org/10.1128/msphere.00095-17>. Accessed: Oct. 15, 2021. doi: 10.1128/msphere.00095-17

MARCOLONGO-PEREIRA C. et al. Epidemiology of equine pythiosis in southern of Rio Grande do Sul State, Brazil. **Pesquisa Veterinária Brasileira**, v.32, n.9, p.865-868, 2012. Available from: <https://www.scielo.br/j/pvb/a/X4pbHVYyJGnPWDCDDNV5YSS/?lang=pt>. Accessed: Dec. 17, 2021. doi: 10.1590/S0100-736X2012000900009.

MENDOZA, L.; ALFARO, A. A. Equine pythiosis in Costa Rica: Report of 39 cases. **Mycopathologia**, v.94, p.123-129, 1986.

MENDOZA, L.; VILELA, R. The Mammalian Pathogenic Oomycetes. **Current Fungal Infection Reports**, v.7, p.98–208, 2013. Available from: <https://link.springer.com/article/10.1007%2Fs12281-013-0144-z>. Accessed: Oct. 20, 2021. doi: 10.1007%2Fs12281-013-0144-z.

PAGE, M. J. et al. PRISMA 2020 explanation and elaboration: updated guidance and exemplars for reporting systematic reviews. **BMJ**, v.372, n.160, 2021. Available from: <https://www.bmj.com/content/bmj/372/bmj.n160.full.pdf>. Accessed: Aug. 17, 2022. doi: 10.1136/bmj.n160.

PARVEEN, S. et al. Nanoparticles: a boon to drug delivery, therapeutics, diagnostics and imaging. **Nanomedicine: Nanotechnology, Biology and Medicine**, v.08, p.147-166, 2012. Available from: <https://pubmed.ncbi.nlm.nih.gov/21703993/>. Accessed: Nov. 15, 2021. doi: 10.1016/j.nano.2011.05.016.

PELAZ, B. et al. Diverse applications of nanomedicine. **ACS Nano**, v.11, p.2313-2381, 2017. Available from: <https://pubmed.ncbi.nlm.nih.gov/28290206/>. Accessed: Nov. 15, 2021. doi: 10.1021/acsnano.6b06040.

PEREIRA, D. I. B. et al. Canine gastrointestinal pythiosis treatment by combined antifungal and immunotherapy and review of published studies. **Mycopathologia**, v.176, p.309–315, 2013. Available from: <https://pubmed.ncbi.nlm.nih.gov/23918089/>. Accessed: Oct. 11, 2021. doi: 10.1007/s11046-013-9683-7.

PERMPALUNG, N. et al. Treatment outcomes of surgery, antifungal therapy and immunotherapy in ocular and vascular human pythiosis: a retrospective study of 18 patients. **Journal of Antimicrobial Chemotherapy**, v.70, p.1885-1892, 2015. Available from: <https://academic.oup.com/jac/article/70/6/1885/728325>. Accessed: Oct. 10, 2021. doi: 10.1093/jac/dkv008.

PERMPALUNG, N. et al. Human Pythiosis: Emergence of Fungal-Like Organism. **Mycopathologia**, v.185, p.801-812, 2019. Available from: <https://link.springer.com/article/10.1007/s11046-019-00412-0>. Accessed: Oct. 10, 2021. doi: 10.1007/s11046-019-00412-0.

REIS-GOMES, A. et al. Epidemiology of mycoses, pitiosis and micotoxicosis in horses in southeastern Rio Grande do Sul, Brazil. **Pesquisa Veterinária Brasileira**, v.38, n.6, p.1110-1116, 2018. Available from: <https://www.scielo.br/j/pvb/a/ThvMnndYVPQJGYxzYw4cyYF/?format=pdf&lang=pt>. Accessed: Oct. 11, 2021. doi: 10.1590/1678-5150-PVB-5182.

ROMERO, A. et al. Equine pythiosis in the eastern wetlands of Uruguay. **Pesquisa Veterinária Brasileira**, v.39, n.7, p.469-475, 2019. Available from: <https://www.scielo.br/j/pvb/a/WPw3NKscPBKc34rg7yXtbJ/?lang=en&format=pdf>. Accessed: Oct. 11, 2021. doi: 10.1590/1678-5150-PVB-6256.

SANTURIO, J. M. et al. Cutaneous Pythiosis insidiosi in calves from the Pantanal region of Brazil. **Mycopathologia**, v.141, n.3, p.123-125, 1998. Available from: <https://pubmed.ncbi.nlm.nih.gov/9755503/>. Accessed: Oct. 11, 2021. doi: 10.1023/a:1006995407665.

SANTURIO, J. M. et al. Pythiosis: an emergent mycosis. **Acta Scientiae Veterinariae**, v.34, p.1-14, 2006. Available from: <http://www.redalyc.org/articulo.oa?id=289021847001>. Accessed: Oct. 11, 2021. doi: 10.22456/1679-9216.15060

SANTOS, C. E. P. et al. Epidemiological survey of equine pythiosis in the Brazilian Pantanal and nearby areas: results of 76 cases. **Journal of Equine Veterinary Science**, v.34, p.270–274, 2014. Available from: <https://www.sciencedirect.com/science/article/pii/S0737080613004152>. Accessed: Oct. 11, 2021. doi: 10.1016/j.jevs.2013.06.003.

SILVEIRA, J. S. et al. *Melaleuca alternifolia* formulations in the treatment of experimental pythiosis. **Brazilian Journal of Microbiology**, 2022. Available from: <https://europepmc.org/article/med/35239152>. Accessed: Mar. 03, 2022. doi: 10.1007/s42770-022-00720-6.

SOUTO, E. P. F. et al. Pythiosis in equidae in northeastern Brazil: 1985–2020. **Journal of Equine Veterinary Science**, v.105, 103726, 2021. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0737080621003567?via%3Dihub>. Accessed: Jan. 17, 2022. doi: 10.1016/j.jevs.2021.103726.

TARTOR, Y. H. et al. Equine pythiosis in Egypt: clinicopathological findings, detection, identification and genotyping of *Pythium insidiosum*. **Veterinary Dermatology**, v.31, p.298-e73, 2020. Available from: <https://onlinelibrary.wiley.com/doi/full/10.1111/vde.12845>. Accessed: Oct. 12, 2021. doi: 10.1111/vde.12845.

VALENTE, J. S. S. et al. *In vitro* activity of antifungals in combination with essential oils against the oomycete *Pythium* insidiosum. **Journal of Applied Microbiology**, v.4, p.998–1003, 2016a. Available from: <https://pubmed.ncbi.nlm.nih.gov/27417677/>. Accessed: Oct. 12, 2021. doi: 10.1111/jam.13234.

VALENTE, J. S. S. et al. Biogenic silver nanoparticles in the treatment of experimental pythiosis Bio-AgNP in pythiosis therapy. **Medical Mycology**, v.58, p.913-918, 2020. Available from: <https://pubmed.ncbi.nlm.nih.gov/32030424/>. Accessed: Oct. 08, 2021. doi: 10.1093/mmy/myz141.

VALENTE, J. S. S. *et al*. *In vitro* activity of *Melaleuca alternifolia* (Tea Tree) in its free oil and nanoemulsion formulations against

Pythium insidiosum. **Mycopathologia**, v.181, p.865-869, 2016b. Available from: <https://pubmed.ncbi.nlm.nih.gov/27544535/>. Accessed: Oct. 08, 2021. doi: 10.1007/s11046-016-0051-2.

VALENTE, J. S. S. et al. *In vitro* anti-*Pythium insidiosum* activity of biogenic silver Nanoparticles. **Medical Mycology**, v.57, p.858-863, 2019. Available from: <https://pubmed.ncbi.nlm.nih.gov/30597067/>. Accessed: Oct. 08, 2021. doi: 10.1093/mmy/myy147.

WEIBLEN, C. et al. Seroprevalence of *Pythium insidiosum* infection in equine in Rio Grande do Sul, Brazil. **Ciência Rural**, v.46, n.1, p.126-131, 2016. Available from: <https://www.scielo.br/j/cr/a/HxjV3ycJMXyGb46xPBJ3ngG/?format=pdf&lang=en>. Accessed: Oct. 10, 2021. doi: 10.1590/0103-8478cr20150056.

WORASILCHAI, N. et al. *In vitro* susceptibility of antibacterial agents against Thai *Pythium insidiosum* isolates. **Antimicrobial Agents and Chemotherapy**, v.64, n.4, e02099-19, 2020. Available from: <https://journals.asm.org/doi/10.1128/AAC.02099-19>. Accessed: Oct. 10, 2021. doi: 10.1128/AAC.02099-19.

ZABRIESKI, Z. et al. Pesticidal activity of metal oxide nanoparticles on plant pathogenic isolates of *Pythium*. **Ecotoxicology**, v.24, p.1305-1314, 2015. Available from: <https://pubmed.ncbi.nlm.nih.gov/26076749/>. Accessed: Oct. 12, 2021. doi: 10.1007/s10646-015-1505-x.