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Antibiotic susceptibility of fungi isolated from tiger shrimp *Penaeus monodon*.

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Abstract

The Seven Standard antibiotics such as ampotericin, clortimazole, fluconazole, griseofluvin, ketakonazole, miconazole and nystatin were tested for antibiotic susceptibility against seven pathogenic fungi *viz.*, *Aspergillus fumigates*, *Curvularia geniculata* , *Fusarium oxysporum*, *Helminthosporium oryzae*, *Penicillium janthinellum*, *Nigrospora sphaerica* and *Rhizopus spp*. isolated from *Penas monodon* in a culture pond. The antimicrobial activity were tested by using standard single disc diffusion method. The maximum Zone of inhibition was recorded for nystatin (22 mm) against *Fusarium oxysporum* and the minimum zone of inhibition was recorded for fluconnazole (8 mm) against *Penicilliun janthinellum*.

Keywords: antiobiotics, antimicrobial activity, pathogenic fungi, *Penaeus monodon*, tiger shrimp, susceptibility

INTRODUCTION

The cultivation of shrimp is a world wide economically important activity especially in developed and developing countries. However, the shrimp industry is now suffering from serious problems linked to infectious diseases, which cause a decrease in growth and thereby vast economic losses. Fungal Diseases caused by members of the genus *Aspergillus* Spp, *Curvularia* Spp *Fusarium* spp. *Helminthosporium* spp., *Penicillium* spp., *Nigrespora* spp., and *Rhizopus* spp., have often been reported in many cultured Penaeid shrimps (Arikan et al., 1999). Heavy mortality associated with massive fungal infection in the digestive organ of the shrimp has been observed among pond cultured *P.monodon* especially on the 105 days of culture (Maeda, 1994; Karunasagar, 1994; Bernan, 1997). In the present paper, we report an outbreak of fungal disease in a *Penaeus monodon* culture pond and explore the cause of the observed infection in grow out ponds of *P.monodon* at Mallippattinam, Thanjavur, District, Tamil Nadu, East Coast of India.

The addition of substantial amount of antibiotics is still the method of choice for control the proliferation of pathogenic fungal species. But the abuse of antibiotics has resulted in the development of resistant strains and antibiotic residues in shrimp products (Lightner , 1993; Jonatan et al., 2009). Although the use of antibiotics to control shrimp fungi in shrimps leads to development of drug resistant pathogenic fungal strains, the practice of using antibiotics as a part of chemotherapy in shrimp farming is still vogue in India (El-Kassas, 2008). Therefore, to make the shrimp aquaculture industry more sustainable, alternative strategies to control infections are urgently needed. (Destoumieux et al., 1999). Hence, the study of antibiotic resistance to seven antibiotics in seven pathogenic fungal species isolated from *Penaeus monodon* was also carried out in the present study.

MATERIALS AND METHODS

The fungal pathogens were obtained from infected shrimp *Penaeus monodon* cultured in semi-intensive farm located in Mallippattinam. Thanjavur District, Tamil Nadu, India, during July 2011. Shrimps were considered diseased when they showed symptoms such as lethargy, anorexia and colour changes of the body. Animals were transported in the diseased condition to the laboratory and the pathogens were isolated by standard methods (Baur et al., 1996).

The antimicrobial activity of seven antibiotics were tested against selected seven fungal pathogens. The selected fungal pathogens were pregrown in Patato dextrose against broth at 27^{0}C for 72 hours. Sterilized Potato dextrose agar medium was poured into petridishes and after solidification the fungal cultures were evenly spreaded over the appropriate media by using sterile cotton. Then, the wells were punched in the fungal spread medium with 3 mm diameter gel puncher. One hundred µl of fungal cultures were pipetted into separate wells. An empty well inoculated with sterile potato dextrose broth alone served as negative control. Plates were incubated at 27^{0}C for 72 hrs. After incubation the results were observed and

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measured as the diameter of inhibition Zone around the each well.

RESULTS AND DISCUSSION

Antibiotic susceptibility of isolated fungal strains viz., *Aspergillus fumigatus, curvularia geniculata, Fusarium oxysporum, Helminthosporium oryzae, Penicillium janthinellum, Nigrospora sphaerica* and *Rhizopus* spp., were studied against standard antibiotics viz., ampoterian, clotrimazole, fluconazole griseofluvin, ketakonazole, micnazole, and nystatin and the results are given in Table 1.

All the fungal strains were more sensitive to griseofluvin compared to other antibiotics. At the same time highest inhibition (22 mm) was noted for nystatin against *Fusarium oxysporum*. The lowest inhibition (8 mm) was observed for fluconazole against *Penicillium janthinellum.*

Ampotericin showed high inhibition against *Helminthoporium oryzae* (12mm), *Pencillium janthinellum* (14 mm), *Nigrospora sphaerica* (15 mm) and *Rhizopus* spp. (17 mm), with the highest inhibition (18 mm) against *Aspergillus fumigatus* and the lowest inhibition for ampotericin (10mm) against *Fusarium oxysporum*.

Clotrimazole showed high inhibition against *Aspergillus fumigatus* (14mm), *Curvularia geniculata* (13mm), *Fusarium oxysporum* (14 mm), *Penicillium janthinellum* (11mm), with the highest inhibition against *Rhizopus* spp., (16mm) and the lowest inhibition (10 mm) against *Nigrospora sphaerica*.

Fluconazole showed high inhibition against *Aspergillus fumigatus* (14mm), *Helminthosporium oryzae* (13mm), *Nigrospora sphaerica* (15mm), *Rhizopus* spp. (17mm), The highest inhibition (18mm) for fluconazole was against *Curvularia geniculata* and the lowest inhibition (8mm) was against *Pencillium janthinellum*.

Griseofluvin showed high inhibition against *Aspergillus fumigatus* (17mm), *Curvularia geniculata* (19mm), *Fusarium oxysporum* (18mm), *Penicillium janthinellum* (12mm), and *Rhizopus* spp., (14 mm). The highest inhibition for griseoflavin was against *Helminthosporium oryzae* (20mm) and the lowest inhibition (10mm) was against *Nigrospora sphaerica*.

Ketakonazole showed high inhibition against *Curvularia geniculata* (12mm), *Helminthosporium oryzae* (14mm), *Nigrospora sphaerica* (13 mm), *Rhizopus* spp., (15mm). The highest inhibition by Ketakonazole (16 mm) was against *Pencillium janthinellum* and the lowest inhibition (10 mm) was against *Aspergillus fumigatus*.

Miconazole showed high inhibition against *Fusarium oxysporum* (13 mm), *Helminthosporium oryzae* (17 mm), *Nigrospora sphaerica* (16 mm), *Rhizopus* spp., (14mm). The highest inhibition were showed for Miconazole (21 mm) was against *Curvularia geniculata* and lowest inhibition (12 mm) was against *Penicillium janthinellum.*

Nystatin showed high inhibition against *Aspergillus fumigatus* (21mm), *Curvularia geniculata* (13mm), *Helminthosporium oryzae* (15mm), *Penicillium janthinellum* (17mm). The highest inhibition for Nystatin (22 mm) was against *Fusarium oxysporum* and the lowest inhibition (12 mm) was against *Rhizopus* spp.

Results of the present study agrees with earlier observations (Calorni, 1989; Alexopoules et al., 1996; Kawabata *et al.*, 1996; Ramasamy *et al.*, 1996; Bok *et al.*, 2004, Khoa et al., 2004; Cuthbertson *et al.*, 2006; Leslie and Summerell, 2006).

Based on the findings of the present study it can be concluded that the griseofluvin and nystatin are better antibiotics for the control the fungal growth in shrimp ponds.

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Table 1. Antibiotic Susceptibility of fungi isolated from the shrimp *Penaeus monodon*

S.No.	Antibiotics	Zone of Inhibition (dia in mm)						
		Aspergillus fumigatus	*Curvularia geniculata*	*Fusarium oxysporum*	*Helminthosporium Oryzae*	*Penicillium janthinellum*	*Nigrospora sphaerica*	*Rhizopus spp.*
1.	Ampotericin	18	-	10	12	14	15	17
2.	Clotrimazole	14	13	14	-	11	10	16
3.	Fluconazole	14	18	-	13	8	15	17
4.	Griseofluvin	17	19	18	20	12	10	14
5.	Ketakonazole	10	12	-	14	16	13	15
6.	Miconazole	-	21	13	17	12	16	14
7.	Nystatin	21	13	22	15	17	-	12

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