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Treatment of gregarines sporozoites in shrimp with allicin an herbal preparation and control intermediate hosts with copper sulphate

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Abstract

Purpose: The purpose of study is to evaluate Allicin supplementation to tackle Gregarines Sporozoites infection in shrimp. Parasitic infections and associated diseases are a significant risk factor in aquaculture productivity, marine, and freshwater. Apart from bacterial and viral infections, aquatic animals are prone to protozoa infection. As the most common parasitic protozoa infection in marine shrimp, Gregarines reported a significantly high infection frequency, i.e., up to 90%. Penaeid shrimp are infected by at least three genera of gregarine (Protozoa, Apicomplexa).

Method: Here, in this study, allicin an organo-sulfur compound used to treat gregarines infections in shrimps. Here, garlic paste containing allicin was incorporated in the feed for 10 days at 10, 20, 30, 40, and 50 gm/Kg concentration twice in a day. Microscopic analysis was carried out to evaluate effect of Allicin with copper sulphate supplemented in shrimp feed.

Results: Study reports here, shrimps fed with garlic paste as the major source of allicin shown a significant reduction in gregarines. As a control, we used turmeric in shrimp feed given at 5, 10, 15 and 20 g/Kg in two times a day for 10 days. Here, we also incorporated a second dose of allicin along with copper sulphate at 0.1, 0.2, 0.3, 0.4 and 0.5 ppm concentrations to minimize secondary infection.

Conclusion: We report here, administration of garlic paste to shrimp significantly reduces gregarines in shrimps.

Keywords: Sporozoites, allicin, shrimps, protozoa infection, aquaculture and midgut

Introduction

Gregarines are a common group of parasitic protozoa infections to aquatic animals and reported significantly higher in shrimps with an infection frequency of up to 90% (Budiardi *et al.*, 2005) [1]. Penaeid shrimp are infected by at least three genera of gregarine (Protozoa, Apicomplexa). These are: *Nematopsis* spp., *Paraophioidina* spp., *Cephalolobus* spp. In many invertebrate groups, especially arthropods, annelids, and mollusks, gregarines (Protozoa, Apicomplexa) are common parasites. These infections were described in the post-larvae of *Litopenaeus Vannamei* under cultivation conditions. Gregarines have an indirect biological cycle, where the intermediate hosts are polychaete worms and bivalve mollusks. The parasite grows inside these invertebrates until the stage of gymnosperm, the shrimp infecting process. In the shrimp, the gymnosperm germinates, which results in sporozoites invading the intestinal epithelium (Chaweeapak *et al.*, 2015) [2]. There were also records of direct transmission of the parasite by infected fecal products. The ingestion of an infected intermediate host containing gregarine spores leads to infection in shrimps. Ingested spores germinate in the shrimp to develop as sporozoites that bind to the chitinous lining of the gastric filter walls, and terminal lappets invade or connect with their special epimerase (holdfast) system to a midgut or anterior midgut caecum epithelial cell. A maximum of three trophoons can occur in syzygy (Durai *et al.*, 2015) [3]. They are released in the stomach or midgut from their attachment, become the intermittent parasite stage, and moves to the hindgut where they lodge in that organ's folds. Each sporadic cell develops into a gametocyte in the hindgut, with some microgametes forming cells and others forming macrogametes. Gametes intermingle and combine to form zygotes, which are released into the external world when the gametocytes burst (Hasanah 2016) [4].

Zygospores, including *Polydora cirrhosa*, a very common polychaete worm that lives in burrows in shrimp pond bottoms, are eaten by a bivalve mollusc or an annelid worm. In the infected epithelial cells, the mollusc or polychaete's intestine becomes infected by gregarine, and sporogony occurs. Sporocysts are either released or swallowed by a shrimp in the Mollusca pseudo faeces, or a shrimp eats the infected polychaete. Sporozoites are released into the shrimp's gastrointestinal tract and infect the posterior stomach or midgut by connecting to the posterior stomach cuticle or attaching or penetrating the host cell membrane (Johnson 1989) [5]. Sporozoites progress into trophozoites in the midgut (*Nematopsis spp.* and *Paraophioidina spp.*) or posterior stomach (*Cephalolobus spp.*). Gregarine-infected shrimp are tough to distinguish from uninfected shrimp unless they are heavily infected. However, shrimp with severe infections may exhibit yellowish midgut coloration, extreme midgut lesions, and physical lumen blockage due to parasite masses (by *Nematopsis spp.*; maybe >100 trophozoites per cm of midgut) (Jones *et al.*, 1994) [6]. Damage to the midgut mucosa can provide a route of entry for potentially lethal bacteremia for opportunistic *Vibrio spp.* Other numbers of this protozoan may interfere with particles' filtration through the hepatopancreatic duct. In pond-grown prawns, the infection rate has been reported to reach 94% (Jones *et al.*, 1994) [6]. The study was aimed to explore the therapeutic potential of Allicin in Gregarines Sporozoites treatment and control.

Material and Methods

Sample collection

White shrimp, *L. Vannamei* (Gregarine-infected), with an average wet weight of 12-15 mm in the case of PL and 4 gms in case of cultured pond was obtained from farming area Nellore and used for the current research. From the identified hatchery PL 12 to 15 mm size was reared in nursery rearing ponds of four batch was collected of the sample size of 250 to 400 shrimp juvenile with an average weight of 5gm was collected in oxygenated water sourced from the nursery rearing pond with minimum water of 800 to 1000 L based on the sample size collected maintaining salinity to 30%. Followed for acclimation for about 12 hours before test is performed.

Sample feed preparation

The experimental sample was given feed was pellet with 40% protein content. It was combined with turmeric powder, garlic paste and allicin through feed at different dosages against treatment. The commercial pellet feed, which was generally used, was mixed by increasing dosage and No. of days, depending on the treatment.

Physical observation

The shrimp sample is examined for physical behaviour, general condition, appearances, change in coloration, size variation, floating of fecal strings etc.

Observation of post larvae PL under direct microscopic

The samples for microscopic examination are processed as per the procedures described by Lightner 1993, 1996 [7, 8]. The following aspects were studied using bright field microscopy. A routine microscopic observation was performed for its assessment by removing the midgut and stripped midgut. The contents were transferred onto a sterile glass slide with forceps and the edge of the cover slip. The gut contents are

spread isolated place, and a wet mount is prepared. In the present study, the wet mounts were observed by direct microscopy using 4x and 40x objectives for the possible occurrence of sporozoites, trophozoites, and gametocytes in baggage, and some specific control methods were implemented. Wet mount of pleopod for Chromatophore stage and protozoan infestation, gills, exoskeleton in case of unusual fouling, white fecal matter.

Wet-mount diagnostic procedures for gregarines

For wet mount diagnostic preparation, the midgut was removed. Onto a clean glass slide strip, using forceps, the midgut material and the edge of a coverslip. A wet mount is prepared by spreading the gut contents. The wet-mounts are examined directly under microscopy (by reduced light, bright field, or phase) for trophozoites, gregarine sporozoites or gametocytes with 10x and 20x objectives.

Results and Discussions

Development of lethargy due to the presence of gregarines sporozoites

It was observed that the weak shrimps coming to the sides and appears to be lethargic. And it was found that very few shrimps were observed to be dead, and pigmentation was dark. The affected shrimps were observed to be relatively small in size. The feed conversion ratio in affected shrimp was observed to be poor (Chaweeack *et al.*, 2015) [2]. These symptoms were attributed due to the prevalence of gregarine sporozoites in the affected shrimp. During the present investigation, the cultured shrimps with poor growth and yellow colour gut contents were selected, and their wet mounts of gut contents were observed under the microscope, which has demonstrated the various stages of gregarines (Fig. 1). The mechanism in which two mature trophozoites pair before a gametocyst is formed is syzygy. Only three cells can associate to form a syzygy (Hasanah 2016; Lightner 1993, 1996) [4, 7, 8]. It was found that in the present study, more than four cells forming syzygy (Figs. 2 and 3). Unlike as reported earlier, it was clearly evident that the syzygy can be formed in any fashion (Figs. 4 and 5); the involvement of three trophozoites in syzygy and fig. 5 shows another trophozoite creating syzygy with the same on lateral side.

The disease of white gut or white faeces syndrome

During the investigation, it is observed that White Gut Disease (WGD) was found in *L. Vannamei* a cultured pond, which is referred to as White Faeces Syndrome (WFS). The occurrence of WFS was typically observed 50-60 days after the PLs have been deposited. The cultured pond is observed with white, somewhat yellowish, floating fecal strings were collected in the feeding trays. It is noticed that the gut contents or fecal strings, when studied, consists of masses of vermiform bodies that superficially resemble gregarines (Maimuna and Kilawati 2015) [9]. The junction of the midgut and the midgut are distended and packed with white to yellow-golden materials. More significant the number of gregarines, the greater the risk of lesions and infections in cultured shrimp. In contrast to average ponds, severely impacted ponds show a decline in shrimp survival of 20-30%. Reduce intake of feed, the growth rate is reduced, and average daily weight gain is also predominantly reduced.

Control of gregarines

Treatment for controlling gregarines population

The ponds with gregarine infection were treated with various

herbal products like turmeric powder, garlic paste and allicin through feed at different concentrations. Turmeric was given in feed for 10 days in different doses 5, 10, 15 and 20 g/Kg in two times a day. After the treatment, the wet mounts still showed many gregarines in the gut, envisaging that turmeric cannot control the gregarines. The garlic paste was given in feed, daily 2 times for 10 days at 10, 20, 30, 40 and 50 gm/Kg. There was a reduction in the count of gregarines in gut scrapings at 40 and 50 gm/kg of feed. It was evident that garlic could not eradicate gregarines at the dosages tried; however, it has a positive role in controlling gregarines. Our attempts with Allicin plus an herbal preparation by Zymo Nutrients Pvt Limited, Mangalore, were given in two meals for 10 days in 5, 10, 15 and 20 gm/Kg feed. No nematodes were noticed after the treatment with 20 g/Kg of feed. However, there was reoccurrence of the gregarines after three weeks of first dose of allicin plus, and this re-infection may be due to the intermediate host in the same pond. To control intermediate hosts like clams, snails and Chironomus larvae, copper sulfate was used along with a second dose of allicin plus at 0.1, 0.2, 0.3, 0.4 and 0.5 ppm concentrations. There was effective control of gregarines and no recurrence of the problem (Kilawati *et al.*, 2015; Ryan *et al.*, 2016) ^[9, 11]. Thus, the experiment clearly showed that the allicin plus application in feed at 20 g/kg of feed for 10 sulphate doses of 0.5 ppm effectively could control the gregarines in the shrimp culture ponds. This was confirmed by microscopic examination of wet mounts after the treatment and observed dead gregarine (Fig. 6).

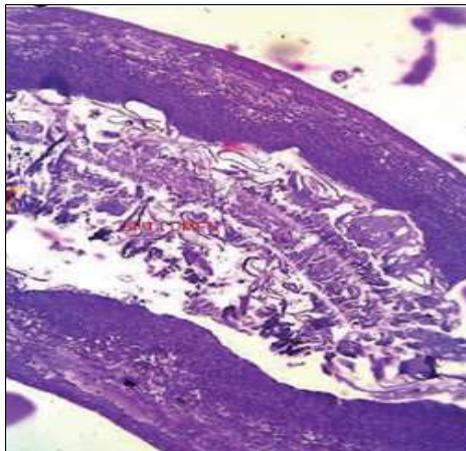


Fig 1: The figure demonstrates wet mount of mematopsis in gut of *Penaeus monodon*. Here, sporozoites, very early stage trophozoites and well developed trophozoites were seen at 400X

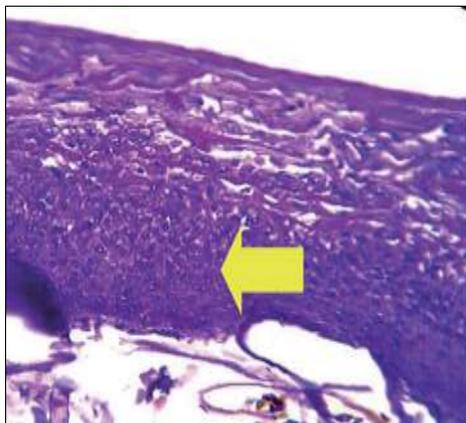


Fig 2: Figure demonstrate a microscopic picture of wet mount of gut scraping showing four cells in syzygy inline fashion (40x)



Fig 3: The figure depicts a microscopic picture of wet mount of gut scraping showing five cells in syzygy (40x)

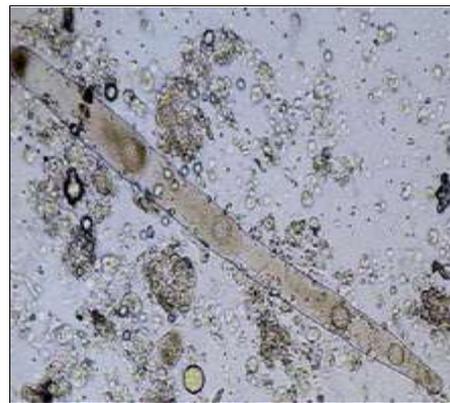


Fig 4: Figure demonstrates here three cells of gregarines in syzygy before formation of syzygy



Fig 5: Here in figure the formation of syzygy with fourth cell shows that it can be attached at any part of the body zygospores are also visible



Fig 6: A microscopic picture of the wet mount of the gut scraping showing dead gregarines

Conclusion

It was established that if the number of gregarines increases, the chances of lesions are higher and infections in cultured shrimp are high. If the number of gregarines is growing more in number, it results in nourishment for the host, leaving lesions for the infections to establish. With the help of intestinal villi, they interfere in the assimilation of nutrients. It ultimately damages the intestine, reduces the absorption capacity of nutrients from the feed given, discoloration occurs, i.e., the intestine colour is changed to yellowish colour. As a result, poor growth is seen in cultured shrimps. This is mainly caused due to sporozoites. In our attempts to effectively control this problem, successful results have been obtained using combination of allicin in feed and copper sulphate in water. The intermediate hosts can be controlled efficiently, and in turn, the severity of the problem can be reduced. Moreover, it will increase the immunity in shrimp, improving the intestinal epithelium's capacity to break down the gregarine cycle, massively preventing further infections.

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Conflict of interest

Author declares no conflict of interest.

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Availability of data and material

Not applicable

Code availability

Not applicable

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