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Efficacy of certain chemicals against leech, *Glossiphonia complanata* (L) on freshwater pearl mussel *Lamellidens marginalis* (L)

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Abstract
The study was conducted to control leech infestation in the freshwater pearl mussel by applying different concentration of chemicals. Among these chemicals, it was observed that efficacy of ivermectin (0.15 ppm) and malachite green (5 ppm) were significantly effective ($P< 0.05$) in reducing the leech infestation from the body cavity and shell surface followed by deltamethrin (0.30 ppm) as compared to other treatments. Further the survivability of the mussel was recorded for the period of 10 days and 100% survivability was found in case of potassium permanganate, malachite green, deltamethrin and albendazole as compared to ivermectin (30%) and other treatments. However, the efficacy of malachite green in killing the leech from the mussel body was significantly better ($P< 0.05$) than deltamethrin. To conclude it was found that malachite green @ 5ppm is the most effective to kill leeches with good survivability of mussel and useful for health management of mussel.

Keywords: *Lamellidens marginalis*, *Glossiphonia complanata*, malachite green, ivermectin

Introduction
Globally, freshwater pearl farming is one of the most lucrative businesses in aquaculture sector. It is alluring the stake holders as it is one of the largest aquaculture activities in term of value [1] and trade. Over 98% of pearls produced worldwide are freshwater pearls [2]. In India, there are more than 52 species of freshwater mussels, but only three species *Lamellidens marginalis*, *Lamellidens corrianus* and *Parreysia corrugata* are employed for freshwater pearl production. Among them, *L. marginalis* is prime mussel species used for good quality of pearl production and is easily available to different parts of the country and easy to culture with less farming expenditure. Further, the potential for development of successful pearl cottage industry using this species is promising and bright. Being a bottom dwelling organism, they feed on detritus and dead organic matter and are known to harbor a wide variety of bacterial pathogens and parasites that include protozoan, flukes (trematodes), annelids (oligochaetes), leeches, copepods, unionicolid mites and insects [3]. Leeches are the worm like creature with a membered body which has a large sucking disc at each end. The main concern about the parasite is their negative impacts on mussels health like sucking body fluid from the body causing inflammation, interference with their growth, competing for food, predation on mussel eggs by ones that filter-feed, and fouling by settling on the external shell [4, 5]. Leech infection also often transmits microbes and hemoparasites during feeding and causes chronic anemia in aquatic animals [6]. So, it is critically important to eradicate these parasites from mussels' body as they affect the growth and survival of mussels. Besides these issues pearl mussel growers face problem due to leech infestation while doing implantation of nuclear beads inside the body cavity, a prerequisite for inducing nacre secretion in captivity. Harboring any pathogens including leeches reduces success of pearl formation and may lead to mortality of implanted mussel due to secondary infection. Heavy infestation and mortality of mussel (*L. marginalis*) were observed in the ponds of ICAR-Central Institute of Freshwater Aquaculture (ICAR-CIFA), Bhubaneswar, causing huge production loss. Live mussels were collected and checked for disease causing agent. All mussels were found to be infested with leech, at various density of infection (5-20 no mussel^{-1}) [7]. The key lesion caused by the parasite are ulceration, hemorrhage and inflammation near attachment site causing sabotage of the host health status and may pose an opportunity hosts to bacterial infections [8]. Further, the sub-lethal or lethal

effects of leech infestation on the mussels and its removal procedure in captivity have not been adequately studied in recent time. Moreover, it is also important to prevent spreading of leech infestation to other cultured aquatic organisms by applying suitable method. In this backdrop, a study was conducted to find out the efficacy of laboratory chemicals in killing the leech present on the shell and body cavity of the mussel.

Materials and Methods

Sample collection

The infected mussel samples were collected from the pond ecosystem of pearl mussel unit of ICAR-Central Institute of Freshwater Aquaculture, Bhubaneswar (Lat. 20º 11′ 06″-20º 11′ 45″N; Long. 85º 50′ 52″-85º 51′ 35″E). After stocking in 35 litre tank, the mussels were examined carefully and found that leeches were present both on outside of shell as well as internal body cavity of mussels. The infected leech was identified as *Glossiphonia complanata* in our laboratory by 28S rDNA PCR followed by sequencing and phylogenetic analysis [7].

Treatment trial

Two hundred seventy infected mussels (average length (81.78 ±4.12 mm) and average weight 66.36± 4.74 g) were stocked in 27 tanks (35 litre) @ 10 no. of mussel per tank. The infected mussels were subjected to a control (C) and 8 chemical treatments process for 24 hours; potassium permanganate- 10 ppm (T_1), sodium chloride-5% (T_2), malachite green (T_3)- 5ppm, Di–decyldimethyl Ammonium Chloride-100 ppm (T_4), formalin (T_5)- 100 ppm, deltamethrin- 0.30 ppm (T_6), ivermectin- 0.15 ppm (T_7) and albendazole -20 ppm (T_8). The behavior of the mussels and efficacy of different chemicals were monitored for 24 h. It is also important to mention that mussels immediately close both their valves when it comes in contact with chemicals however after sometime they open it, therefore, the exposure time was prolonged for 24 h to provide sufficient time for chemical to penetrate inside the body cavity of mussel.

Quantification of leeches

The dead leeches from each treatment tank were collected manually with the help of sterile forceps after 24 h and counted. The leech *Glossiphonia complanata* present in the body cavity was also collected by opening the valve with the help of shell speculum. Further, the behaviour of treated mussels was monitored up to 10 days to check the health condition like normal or stress stage and survivability of mussel.

Statistical analysis

The experiment on efficacy of different treatments on dead parasite count per mussel was conducted using completely randomized design. To identify the best treatment, the data were analyzed by one-way classified analysis using PROC GLM of SAS 9.3 package (SAS Institute, Cary, NC) followed by Tukey HSD test and Duncan's multiple range test (DMRT) to construct box plot to determine significance difference at 5% ($P< 0.05$) level.

Results

The results of the comparative efficacy of eight chemicals against leech *Glossiphonia complanata* infestation in *Lamellidens marginalis* are presented in Table 1 & 2. The number of dead parasite affected was quantified to characterize the antiparasitic effectiveness of chemicals. The leech was assumed to be dead when there was no visible movement and/or it was autolysed. In the present study efficacy of different treatments were significantly ($P< 0.05$) different in terms of causing death of leech present in/on the mussel body as compared to control. In control condition, death of leech was nil because no antiparasitic chemical was used, resulting only 5% survival of mussel. We observed that *G. complanata* was significantly affected when exposed to malachite green (T_3) and ivermectin (T_7). In this case, the parasite movement was slowed within 3 h and the entire parasite was dead within 24 h. However, the mussels receiving ivermectin treatment was in stressed condition but in case of malachite green the mussels was in healthy condition and showing normal behaviour. Further, dead parasite count was not significantly ($P > 0.05$) different in case of T_1, T_2, T_4 and T_8, however, reduction of leech infestation was noticed in these treatments (Table 1). The survival of mussel in T_1, T_2, T_4 and T_8 was 100%, 10%, 90% and 100% respectively (Table 2). The dead parasite count was not significantly ($P > 0.05$) different in T_3 (malachite green) and T_7 (ivermectin) but dead parasite count in T_3 and T_7 was significantly ($P< 0.05$) higher as compared to other treatments. The dead parasite count in T_6 (deltamethrin) was significantly ($P< 0.05$) higher than T_1, T_2, T_4, T_5 and T_8 but significantly lower that T_3 and T_7. The survival of mussel in T_3 & T_6 and T_7 was 100%, 100% and 30% respectively (Table 2). Leech exposed to Treatment T_5 recorded least effect and dead parasite count was minimum among all the treatments. Overall, the susceptibility of *G. complanata* against different chemicals was summarized as 1) high effect: malachite green and ivermectin; 2) moderate effect: deltamethrin; 3) low effect: potassium permanganate, sodium chloride, di-decyldimethyl ammonium chloride, albendazole and formalin.

Discussion

Evaluating the efficacy of different chemicals to reduce leech infestation from mussel is a challenging task as selection of chemicals should be in such a way that it doesn't affect the mussel health. The present study revealed that among all the eight chemical used, ivermectin and malachite green was most effective causing death of leeches from the host body followed by deltamethrin (Figure 1). Ivermectin is an avermectin parasiticidal agent that has been widely used as a treatment against sea lice infections [9-10] and also against freshwater lice *Argulus siamensis* [11]. The general mode of action of the avermectins is to interrupt the transmission of signals in the nervous system and interact with many ligand-gated chloride channels, causes increase the permeability of the cell membranes in the nervous system to chloride, resulting in dysfunction of the nervous system [9, 12]. In one study Bahmani *et al.* (2012) [13] reported that ivermectin was very effective in killing of leech. In another experiment, Murwantoko *et al.* (2017) [14] reported that ivermectin at a concentration of 62.5 ppm was able to kill the marine leech after immersion for 30 min.

Malachite green has also been reported widely as the most effective agent known for treating water mold infections of fish and its egg. It is also effective against protozoan ectoparasites and some myxozoan parasites [6, 15]. The biocidal effects of malachite green are due to its capacity to intercalate nucleic acids and to induction of radical-mediated redox changes [16]. In the present study malachite green was

significantly effective in killing of leech with no detrimental effect on host mussel. Hence, it is inferred that malachite green is an ideal chemical for successfully killing the leech present on the surface and body cavity of mussel. The present study revealed that exposure to 10 ppm potassium permanganate was less effective against leech parasite without any deleterious effect on health of mussel. Similar results were reported by Woods *et al.* (1990) [17] who also found that potassium permanganate at 10 ppm have no effect on detachment of leech from fish body. Hence, it is inferred that potassium permanganate at 10 ppm is not effective chemical to control this leech infestation in freshwater bivalve species.

Similarly, the current study also revealed that efficacy of sodium chloride and albendazole in terms of killing of leeches was significantly better than Di –decyldimethyl Ammonium Chloride and formalin treatment. Kabata (1985) [18] also reported that treatment with 2% sodium chloride for 1 h caused detachment of leeches from the fish body but they were not killed. In this study, we had found poor survivability of mussels against sodium chloride treatment. Due to inability of mussel to tolerate the salt concentration, the application of sodium chloride to control leech infection in mussel is not advisable.

Formalin is an aqueous solution of 37-40% formaldehyde gas which equals 100% formalin having moderate to weak antibacterial activity which cross links proteins, resulting in cell death [19]. It is effective parasiticide for bath treatment of most ectoparasitic protozoa and monogeneans. Cruz-Lacierda *et al.* (2000) [20] reported 50 ppm formalin bath for 1 h was effective in controlling marine leech *Zeylanicobdella arugamensis* in grouper. Similarly, Murwantoko *et al.* [14] found that formalin with a dose of 500 ppm and 250 ppm able to kill marine leech harvested from hybrid grouper. In contrast our study revealed that formalin was less effective in reducing leech infestation and survival of mussel was only 45%. Hence, it is inferred that formalin is not suitable chemical to control the leech infection in the mussel.

There were no reports available on the efficacy of ammonium chloride against the leech infestation. However, the present study revealed that this chemical was effective in killing of leeches at a lesser extent as compared to other treatments however the survivability of mussel was good (90%) as compared to formalin and sodium chloride.

Albendazole is a broad-spectrum antihelminthic agent of the benzimidazole type used for treatment of variety of parasitic worm infestations. In the present study, albendazole was effective in removing the leech infestation from the mussel and the medicine was also safe for host organisms. Similarly, Bahmani *et al.* (2012) [13] also reported anti-leech effect of albendazol. In contrast, Murwantoko *et al.* (2017) [14] reported that albendazole at a concentration of 1000 ppm and for 4 h of immersion could not kill the leech.

Based on the current study, it was found that all the chemicals used for this experiment were effective in removing the leech infestation with varying degree of success. However, it was found that ivermectin and malachite green were significantly more effective in killing of leeches from the host body, followed by deltamethrin, sodium chloride, albendazole, potassium permanganate, di-decyldimethyl ammonium chloride and formalin respectively (malachite green = ivermectin > deltamethrin > sodium chloride > albendazole > potassium permanganate > di-decyldimethyl ammonium chloride > formalin). The survivability of the mussel was recorded for a period of 10 days and it was found that 100% mussels survived in potassium permanganate, malachite green, deltamethrin and albendazole whereas the survivability of mussels were 10%, 30%, 45%, and 90% in case of sodium chloride, ivermectin, formalin, and di-decyldimethyl ammonium chloride respectively (Table 2). Keeping leech eradication and survival of mussels in view, it was found that malachite green @ 5 ppm was the most effective chemical in terms of removing leeches from the host body with 100% survivability of mussels, which is one of the precious organisms for freshwater pearl production.

Table 1: Comparative efficacy of different chemicals against leech, *Glossiphonia complanata* infestation in freshwater pearl mussel *L. marginalis*

Treatment	Dosage (ppm)	Dead Parasite count (Mean ± S.D.)
C: Control	-	0±0
T_1: Potassium permanganate	10	5.33^{c}±1.53
T_2: Sodium chloride	5%	7.33^{cd}± 1.53
T_3: Malachite green	5	19.33^{a}±0.58
T_4: Di-decyldimethyl ammonium chloride	100	5.00^{cd}±1.0
T_5: Formalin	100	4.00^{d}±1.0
T_6: Deltamethrin	0.30	15.00^{b}±1.0
T_7: Ivermectin	0.15	19.33^{a}±0.58
T_8: Albendazole	20	6.33^{cd}±0.58

Means bearing different superscripts in a column are significantly different ($P<0.05$)

Table 2: Survivability of treated freshwater mussel, *L. marginalis* against different chemical treatment

Treatment	Dosage (ppm)	Condition of mussels after 10 days	Survivability of mussel (%)
C: Control	-	Huge mortality of mussel	5
T_1: Potassium permanganate	10	Normal	100
T_2: Sodium chloride	5%	Inactive	10
T_3: Malachite green	5	Normal	100
T_4: Di-decyldimethyl ammonium chloride	100	Normal	90
T_5: Formalin	100	Normal	45
T_6: Deltamethrin	0.30	Normal	100
T_7: Ivermectin	0.15	Highly stress condition of mussel	30
T_8: Albendazole	20	Normal	100

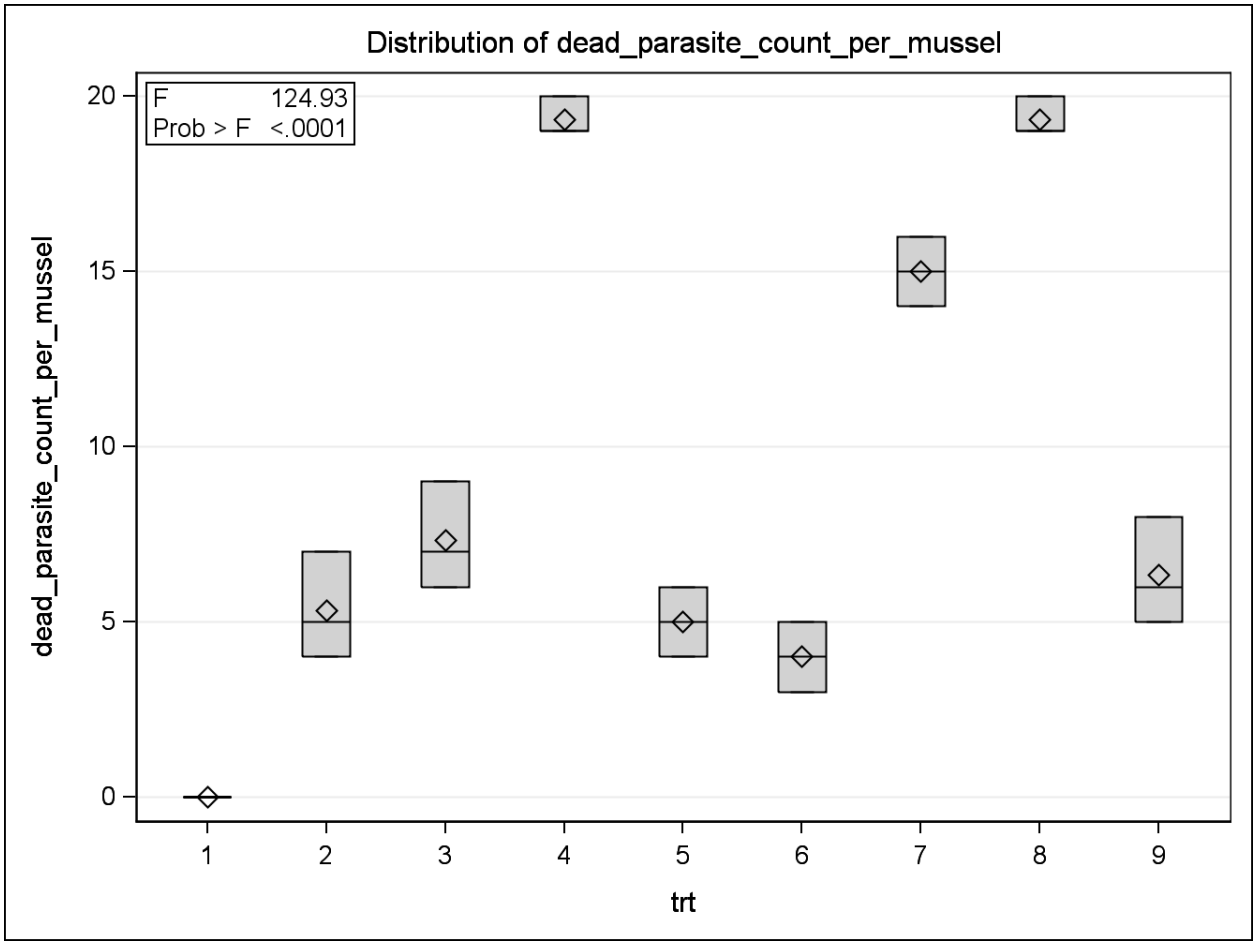

Fig 1: A box plot graph displaying the efficacy of different chemicals on leech of freshwater mussel, *L. marginalis* (X-axis: 1: Control; 2: T_1, 3:T_2, 4:T_3, 5:T_4, 6:T_5, 7:T_6, 8:T_7, 9:T_8)

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