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Note

Comparative efficacy of individual and combined anti-parasitic treatments for Argulosis control

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Abstract

Argulus species have emerged as a major challenge in aquaculture, significantly impacting fish health and farm productivity. A survey in fish farms of Ganjam District, Odisha revealed widespread infestation by *Argulus siamensis* and *Argulus bengalensis*. Among the two species, *A. siamensis* exhibited higher prevalence, abundance and infection intensity. Two anti-parasitic drugs *viz.,* Cypermethrin (immersion) and Emamectin benzoate (oral through feed) were selected for both individual and combined use to control infection by *A. siamensis*. For individual applications, Cypermethrin was administered at 0.94 µg l^{-1}, 2.84 µg l^{-1}, while Emamectin benzoate was provided through feed at 161.41 µg kg^{-1} and 484.23 µg kg^{-1}. In the combined treatment, alternating doses of both drugs were used. In fishes experimentally infected with *A. siamensis*, parasite mortality rates recorded were 74 and 87.2% against doses of Cypermethrin at 0.94 µg l^{-1} and 2.84 µg l^{-1} respectively, after 48 h of treatment. For Emamectin benzoate, parasite mortality rates observed were 81 and 88% respectively at doses of 161.41 and 484.23 µg kg^{-1} feed. Combined treatment demonstrated better efficacy, with 100% parasite mortality observed 48 h post-treatment using 2.84 µg l^{-1} Cypermethrin and 161.41 µg kg^{-1} Emamectin benzoate in feed. In a farm trial to evaluate the efficacy, this dosage was found effective for complete eradiation (100% reduction) of the parasite within seven days post-treatment.

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Disease related losses pose a major threat to the economic sustainability of fish farming. *Argulus* spp. are major ectoparasitic disease agents accounting for 19.5% of infestations (Sahoo *et al.,* 2020). Commonly known as 'fish lice', *Argulus* is the most widespread group of crustacean ectoparasites, representing 129 species globally, except in Antarctica. In India it is represented by 17 species and one sub-species, among which 10 species and one sub-species are endemic to India and 14 species are freshwater inhabitants (Dev Roy, 2015). Affected fish exhibit behavioural abnormalities like lethargy, loss of appetite, irritation with haemorrhages, fin erosion and excess mucus secretion. Several risk factors contribute to *Argulus* infestations, including algal blooms, slow stock turnover rates, low water levels (<1 m) during the summer months and the presence of infected fishes in water body (Fenton *et al.,* 2006). Despite treatment with different drugs, recurrence of Argulosis continue along with bioaccumulation of drugs in fish tissues. Further, toxicants present in some drugs adversely affect non-target species. Therefore, reducing the dosage of anti-parasitic drugs is crucial to safeguard fish safety. To achieve this, different drug combinations need to be tested to optimise efficacy while minimising adverse effects.. This study was carried out to evaluate the combined effect of two anti-parasitic drugs for controlling Argulosis in the fish farms of Ganjam District in Odisha.

Different farms (both government and private) were surveyed to know the prevalence of Argulosis in Ganjam District, Odisha. For sample collection of *Argulus* sp., a total number of 18 ponds were surveyed from 6 fish farms between

24 January to 10 September, 2019. All fish affected by Argulosis collected from the farms were placed in a water filled container. *Argulus* specimens were carefully removed from the hosts and transferred to a petridish containing clear water and thoroughly washed in the petridish (Das *et al.,* 2016). The collected specimens were then fixed in 70% ethanol (Bush, 1997) and brought to the laboratory for further microscopic observation.

Argulus species were identified by wet mount preparation based on their morphometric criteria according to Bykhovskaya-Pavlovskaya (1962) as well as based on the figures and descriptions of Dash (2012) and Stopskopf (1993). After isolation from the host, the samples were carefully transferred onto clean grease-free glass slides with few drops of distilled water (Ramakrishna, 1951). The glass slides were examined under 40X objective of light microscope. For the long-time storage of identified parasites, they were carefully preserved in 10% formalin solution (Petchimuthu, 2018). The intensity of infection, abundance and prevalence were then estimated season-wise using the following formulae:

$$\text{Intensity of infection} = \frac{\text{Total no. of parasites examined in one host}}{\text{Total no. of infected hosts}}$$

$$\text{Abundance} = \frac{\text{Total no. of parasites in one host}}{\text{Total no. of hosts examined}}$$

$$\text{Prevalance (\%)} = \frac{\text{No. of fish infected}}{\text{Total no. of fish observed}} \times 100$$

Argulus specimens (*A. siamensis*) were collected from naturally infested fishes and transferred to a glass beaker containing clean freshwater. The gravid individulas laid egg clusters on the inner walls of the glass beaker. To stimulate hatching, the beaker was exposed to continuous light for 48 h at room temperature (Bandilla *et al.,* 2007). After hatching, the nauplii were collected and transferred to FRP tanks of 200 l capacity, containing healthy fishes. Healthy rohu fingerlings of 45-50 g size were used for challenge study. The fish were carefully monitored every 2-3 days for the presence of propagules (eggs and larvae, if any). Within a week, the infection was successfully established. To accelerate parasite multiplication, additional feed was provided to slightly deteriorate water quality, inducing stress in the fish, which facilitated proliferation of *Argulus* (Dewi *et al.,* 2018).

A survey was carried across fish farms of southern Odisha to assess the use of anti-parasitic drugs against Argulosis. Information was gathered on the types of drugs used, their concentrations, application timing, and methods of administration. This helped in selecting commonly used drugs for experimental purpose, focussing on their application methods. Based on the survey, two drugs were selected for the experiment *viz.,* Cypermethrin (administered through immersion) and Emamectin benzoate (administered orally *via* feed). Although their method of application differed, both drugs shared a similar mode of action.

Since the selected anti-parasitic drugs had same mode of action through different mode of applications, their effects were evaluated using the Joint Action Model (Schultz and Kelly, 2016). Two drugs with 25 and 75% of their respective LC_{50} values, were chosen for individual and combined use in experimental tanks. The selected doses were: 0.94 and 2.84 µg l^{-1} of Cypermethrin for immersion treatment and 161.41 and 484.23 µg kg^{-1} feed of Emamectin benzoate for oral administration. For combined treatment, the dose combinations used were: 0.94 µg l^{-1} Cypermethrin + 484.23 µg kg^{-1} feed of Emamectin benzoate and 2.84 µg l^{-1} Cypermethrin +161.41 µg kg^{-1} feed of Emamectin benzoate. For preparation of medicated feeds, the feed was coated with the drug using pre-heated rice bran oil as a binding agent. The premix was incorporated at the predetermined doses, based on the LC_{50} values (Hanson *et al.,* 2011).

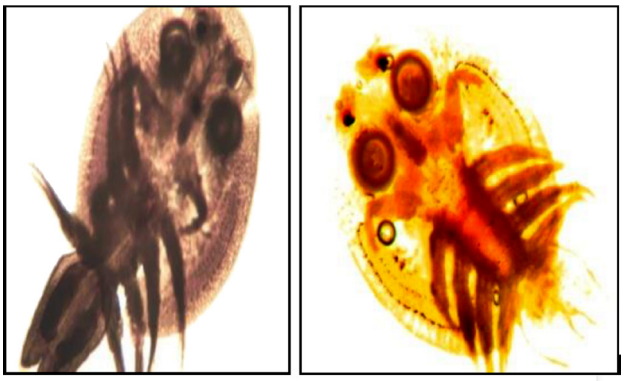
Four *Argulus* infested ponds of the Humari Fish Farm in Ganjam having an average area of 0.1-0.15 acre, were selected for farm trial to evaluate the efficacy of anti-parasitic drugs. Each pond had a stocking density of 5000 fishes per bigha. The four selected ponds were stocked with: Pond 1: Polyculture; Pond 2: Mrigal brooders, Pond 3: Indian major carps (IMCs) grow-out and Pond 4: Polyculture. The most effective dose from the challenge experiment was applied in these ponds. Sampling was conducted every alternative day to assess the parasite mortality rate, averaging parasite counts from five sampled fish per pond.

Growth and survival indices were analysed statistically, with results expressed as mean±SD (Zar, 2009). Abott's formula was used to determine the percentage efficacy of the treatment for each sampling period. The efficacy was calculated as the difference between the mean number of carp lice per fish in test and control groups, divided by the mean number of carp lice per fish in the control group as percentage (Abott, 1925). One way ANOVA was performed to determine the statistical significance of the experimental drug doses.

A total of 762 live fish samples were thoroughly examined, and *Argulus* spp. were collected from infected fish across six fish farms in Ganjam District *viz.*, OPDC Hatchery, Bhanjanagar; SP Agro Pvt. Ltd., Duba; Humari Fish Farm, Chhatrapur; Venktesh Fish Farm, Rangailunda; Ishwar Rao and Others Farm, Solabindha and Pramila Sahu Farm, B. D. Pur.

Based on morphometric characteristics, two species of *Argulus* were identified in the fish farms of Ganjam District. The most prevalent was *Argulus siamensis* (n=211), followed by *Argulus bengalensis* (n=106) (Fig. 1a, b). *A. siamensis* showed a higher prevalence, (27.6%) compared to *A. bengalensis* 13.9%) during the study period from January 2019 to October 2019. Sahoo *et al.* (2013), reported *A. siamensis* as the most prevalent *Argulus* species in India. The details on prevalence, abundance and intensity of infection of *A. bengalensis* and *A. siamensis* recorded during the survey are given in Tables 1 and 2 respectively. Fig. 2 presents the prevalence of Argulosis across the summer, rainy, autumn and winter seasons. The prevalence of parasites was higher during winter compared to other seasons. Among the surveyed species, rohu (*Labeo rohita*) was observed to be the most affected species followed by common carp (*Cyprinus carpio*), mrigal (*Cirrhinus mrigala*), grass carp (*Ctenopharyngodon idella*) and catla (*Labeo catla*), These findings based on the survey of six fish farms in Ganjam District, are consistent with the findings of Bakshi *et al.* (2006).

Cypermethrin demonstrated high effecacy in immersion treatment, reducing *Argulus* infestation by over 90% within 48 h (Table 3).



(a) (b)

Fig. 1. Photomicrograph of (a) *A. siamensis* and (b) *A. bengalensis* (x400)

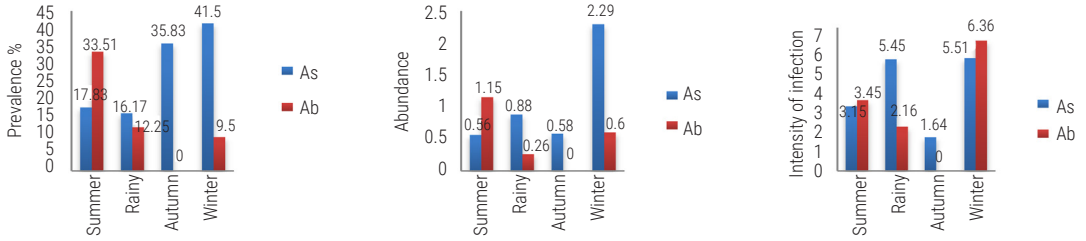


Fig. 2. Prevalence, abundance and intensity of Argulosis in Ganjam District, Odisha in different seasons of a year

Table 1. Prevalence, abundance and intensity of infection of *A. bengalensis*

Month	No. of fish observed	No. of fish infected	No. of parasites found	Prevalence (%) 5=(3/2)x100	Abundance 6=(4/2)	Intensity of infection 7=(4/3)
1	2	3	4	5	6	7
January	42	19	121	45.23	2.88	6.36
February	158	0	0	0	0	0
March	99	39	117	39.39	1.18	3
April	42	0	0	0	0	0
May	44	23	97	52.27	2.20	4.21
June	0	0	0	0	0	0
July	158	25	54	15.82	0.34	2.16
August	46	0	0	0	0	0
September	137	0	0	0	0	0
October	36	0	0	0	0	0

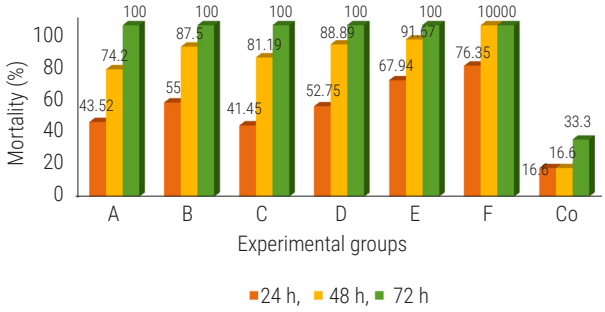
Emamectin benzoate, when administered in feed at the predetermined concentration, achieved complete eradication of *Argulus* infestation within 72 h post-treatment. In both the treatment groups, more than 90% reduction of infestation was observe within 24 h post-treatment (Fig. 3). These findings align with the results of Schultz and Kelly (2016), who reported similar efficacy of these drugs against the marine copepod *Tigriopus californicus*.

In the farm trial to evaluate the efficacy of anti-parasitic drugs, the dose of 2.84 µg l^{-1} Cypermethrin and 161.41 µg Emamectin benzoate kg^{-1} feed resulted in 63-70% mortality of *A. siamensis* by the third day post-application of drugs. Complete eradiation (100% reduction) of the parasite was achieved by the seventh day of treatment (Table 3). No recurrence of the disease occurred during the 21 days observation period.

In the present study, *A. siamensis*-infested rohu were treated with Cypermethrin and Emamectin benzoate at different doses and the most effective combined dose of 2.84 µg l^{-1} Cypermethrin and 161.41 µg kg^{-1} Emamectin benzoate in feed proved suitable for farm-level application, ensuring effective parasite control with a high survival rate. Recently organochlorines, organophosphates and chitinase inhibitors are used to treat Argulosis. Biological control using monosex tilapia and other larvivorous fish calls for attention. The combined use of anti-parasitic drugs offers a promising approach to reduce the incidence and recurrence of Argulosis in farm ponds.

Table 2. Prevalence, abundance and intensity of infection of *A. siamensis*

Month	No of fish observed	No of fish infected	No of parasites found	Prevalence (%) 5=(3/2)x100	Abundance 6=(4/2)	Intensity of infection 7=(4/3)
1	2	3	4	5	6	7
January	42	0	0	0	0	0
February	158	83	458	52.53	2.89	5.51
March	99	0	0	0	0	0
April	42	33	104	78.57	2.47	3.15
May	44	0	0	0	0	0
June	0	0	0	0	0	0
July	158	14	128	8.86	0.81	9.14
August	46	19	52	41.30	1.13	2.73
September	137	34	59	24.81	0.43	1.73
October	36	28	43	77.77	1.19	1.53



A-25% of LC_{50} value of Cypermethrin; D-75% of LC_{50} value of Emamectin benzoate
B-75% of LC_{50} value of Cypermethrin; E-(25% of LC_{50} value of Cypermethrin) + (75% of LC_{50} value of Emamectin benzoate)
C-25% of LC_{50} value of Emamectinbenzoate; F - (75% of LC_{50} value of Cypermethrin) + (25% of LC_{50} value of Emamectin benzoate); Co- Control

Fig. 3. Parasite mortality rate under challenge study

Table 3. Comparative analysis of parasite mortality rate during experimental period

Group	24 h	48 h	72 h
A	43.52 ± 1.92^{bc}	74.2 ± 7.6^{b}	100^{b}
B	55 ± 5^{c}	87.5 ± 12.5^{b}	100^{b}
C	41.45 ± 2.99^{b}	81.19 ± 3.4^{b}	100^{b}
D	52.75 ± 2.75^{bc}	88.89 ± 11.2^{b}	100^{b}
E	67.94 ± 1.28^{d}	91.67 ± 8.34^{b}	100^{b}
F	76.35 ± 3.65^{d}	100 ± 0^{b}	100^{b}
Co (Control)	16.6^{a}	16.6^{a}	33.3^{a}

Each single value in a column with a different superscript differ significantly ($p<0.05$)

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