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ORIGINAL ARTICLE

SEASONAL PREVALENCE OF ASEPTATE GREGARINE PARASITES OF INDIAN EARTHWORM

Beauty Kundu[1], Saikat Saha[1], Mandira Saha[1, 2], Bidisha Bhattacharya Ghosh[1], Snehasree Majumder[1] and Sabir Hossen Molla[1*]

[1]Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani-741235, West Bengal, India
[2]Department of Zoology, Chakdaha College, University of Kalyani, Kalyani-741235, West Bengal, India
* Correspondence: hossensabir.zoo@gmail.com

Abstract: *An investigation was conducted to examine the prevalence, mean intensity, abundance and seasonality of acephaline gregarine parasites of earthworms in soil of different districts of West Bengal, India. A total 1011 of Metaphire posthuma earthworm have been collected from soil of three different districts of West Bengal, India and examined for acephaline gregarine parasites during pre-monsoon, monsoon and post-monsoon season of June 2021 to August 2023. Four genera of acephaline gregarine parasites have been found viz. Monocystis, Stomatophora, Nematocystis and Apolocystis. The highest gregarine infection has been observed during monsoon season, i.e. from July to October, followed by the pre-monsoon period while the lowest infection was observed during post-monsoon, i.e. from November to February.*
Keywords: Earthworm, gregarine, aseptate, mean intensity, mean abundance

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1. INTRODUCTION

Earthworms are important members of the invertebrate population in most soils, both in terms of gross belowground biomass and influence on soil biogeochemical cycles [1-3]. According to Sankar and Patnaik [4], temperature, pH, soil texture and water content of soil have an impact on earthworm dispersal. In last decades many researchers have found that in India, the seminal vesicles of earthworms were heavily infested with aseptate gregarine parasites belonging to phylum Apicomplexa [5-7]. There is a cause of concern that extensive infection with gregarines might result in economic losses due to host

infertility and a reduction in the population of earthworms in soil [8]. The gregarines are a widespread group of apicomplexan parasites that infect two invertebrates and are common among insects and annelids, often reaching 100% frequency in a host population [9 -10]. Gregarines show significant seasonal variation [11]. Earlier researchers reported various gregarine species belonging to genera *Aikinetocystis*, *Apolocystis*, *Dirhynchocytis*, *Monocystis*, *Nematocystis*, *Stomatophora*, *Zygocystis*, *Enterocystis* and *Stomatocystis* [5-7, 12-18] were found in the seminal vesicles of earthworms in India.

Using this information as a background, a survey of the aseptate gregarine parasites in earthworms of different districts of West Bengal, India, was conducted to investigate the seasonal prevalence, mean intensity and mean abundance of aseptate gregarines. A total 1011 *Metaphire posthuma* earthworm specimens were collected from the soil of different districts of West Bengal, India and examined for acephaline gregarine parasites during pre-monsoon, monsoon and post-monsoon season of June 2021 to August 2023.

Four genera of acephaline gregarine parasites have been found viz. *Monocystis* sp., *Apolocystis* sp., *Nematocystis* sp. and *Stomatophora* sp. The highest gregarine infection has been observed during the monsoon season, i.e. from July to October, followed by the pre-monsoon season while the lowest infection was observed during the post-monsoon, i.e. from November to February.

2. MATERIALS AND METHOD

Collection of earthworms

A total 1011 of*Metaphire posthuma* earthworm specimens have been collected from soil of Bankura (23.2593^0N, 87.0587^0E), Purulia (23.3698^0N, 86.3045^0E) and East Bardhaman (23.2461^0N, 87.8286^0E) brought alive to the Parasitology Laboratory for examination. The survey work was carried out during the period June 2021 to August 2023. The survey period was divided into three seasons, namely pre-monsoon, i.e. March to June, monsoon, i.e. July to October and post-monsoon, i.e. November to February.

Parasitological Examination

The seminal vesicles of earthworms were thoroughly examined. After, dissection of the earthworm seminal vesicles was carefully removed and immersed in 0.65 % NaCl solution. A thin film of seminal fluid made on a slide covered with a coverslip. The slides were examined under an Olympus (BX43) phase-contrast microscope to identify the parasites in unstained condition. Then the content of the seminal vesicles was semi-dried and fixed in Schaudin's fluid for 20 minutes. The smears were stored in 70% alcohol for removal of mercuric chloride. The slides were then passed through 100%→ 90%→ 70%→ 50% alcohol (5 minutes each) and placed in distilled water. These were kept in 3% iron alum solution (throughout the night) and stained with Heidenhain's hematoxylin solution for 20 minutes. 1% iron alum solution was used for differentiation. The slides were then washed in distilled water and dehydrated in an ascending series of alcohol. They were then cleared in xylene and mounted in Canada balsam as described by Bandyopadhyay *et al*. [7]. The photomicrographs have been taken using BX43 Olympus phase-contrast microscope and Labomed 500 phase contrast microscope.

Calculations

The prevalence, mean intensity and mean abundance of earthworm specimens have been calculated According to Margolis *et al.* [9]:

$$\text{Prevalence \%} = \frac{\text{Total number of host infected}}{\text{Total number of hosts examined}} \times 100$$

$$\text{Mean Intensity} = \frac{\text{Total number of parasites}}{\text{Total number of hosts infected}}$$

$$\text{Abundance} = \frac{\text{Total number of parasites}}{\text{Total number of hosts examined}}$$

3. RESULTS

A total of 1011 earthworms were subjected to parasitological examination and found to be infected with different gregarine parasites namely, *Monocystis* sp., *Apolocystis* sp., *Nematocystis* sp. and *Stomatophora* sp. All these gregarine parasites have been isolated from the seminal vesicles of host earthworms.

Four types of aseptate gregarine parasites namely, *Monocystis* sp., *Nematocystis* sp., *Apolocystis* sp. and *Stomatophora* sp. have been identified up to the generic level. *Monocystis* sp. is characterized by trophozoites are either short or long in size. The shape of the gamonts is ovoid. Mucron is unmarked (Figure 1A). *Nematocystis* sp. is characterized bytrophozoites are large, cylindroid and nematoid in appearance. Trophozoites often have mucron at the anterior end (Figure 1B). *Apolocystis* sp. is characterized by trophozoites are spherical in shape without having polarity (Figure 1D). *Stomatophora* sp. is characterized by trophozoites are spherical or ovoid in shape and sucker is petaloid in appearance with radiating edges (Figure 1D).

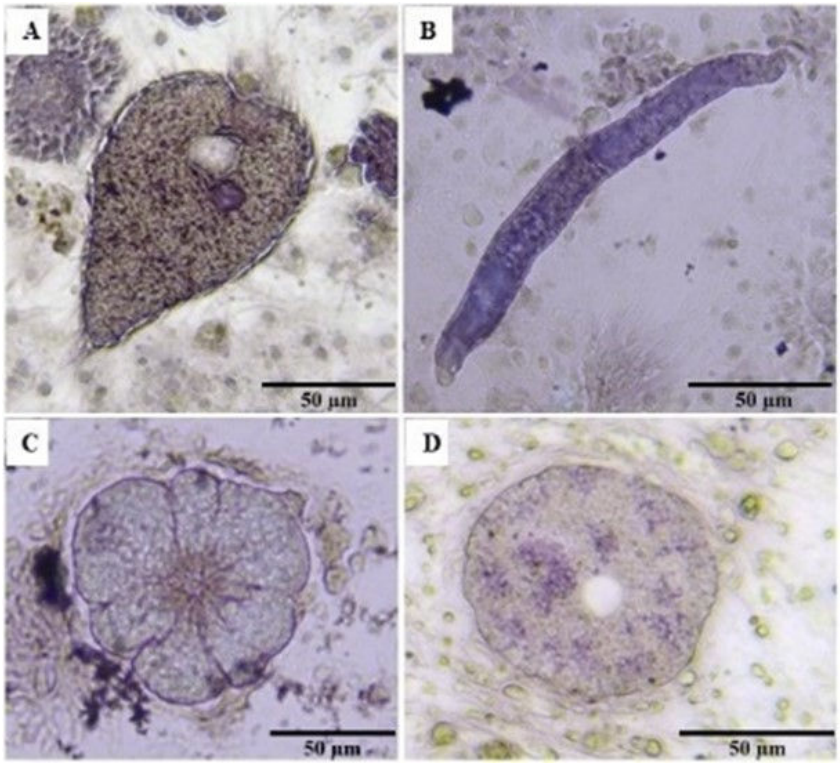

Figure 1. Light microscopic view of isolated protozoan parasites from *Metaphire posthuma.* Photomicrographs of trophozoites stage of A. *Monocystis* sp., B. *Nematocystis* sp., C. *Stomatophora* sp. and D. *Apolocystis* sp.

Seasonal changes of the prevalence, mean intensity and mean abundance of aseptate gregarine parasites of *Metaphire posthuma*

A total of 1011 earthworms *Metaphire posthuma* have been examined, to observe the prevalence, mean intensity and mean abundance of aseptate gregarine parasites. Out of 253 *Metaphire posthuma*, 159 (62.84%) were infected with *Monocystis* sp., 141 (55.73%) were infected with *Stomatophora* sp., 125 (49.40%) were infected with *Nematocystis* sp. and 74 (29.24%) were infected with *Apolocystis* sp. (Fig. 1) in pre-monsoon season. During monsoon season rate of infection was highest, out of 575 *Metaphire posthuma*, 90.07% were infected with *Monocystis* sp., 78.60% were infected with *Stomatophora* sp., 68% were infected with *Nematocystis* sp., and 37.73 % were infected with *Apolocystis* sp. During post-monsoon season rate of infection was lowest, out of 183 *Metaphire posthuma*, 31.69% were infected with *Monocystis* sp., 28.41% were infected with *Stomatophora* sp., 24.04% were infected with *Nematocystis* sp. and 13.11% were infected with *Apolocystis* sp. (Figure 2).

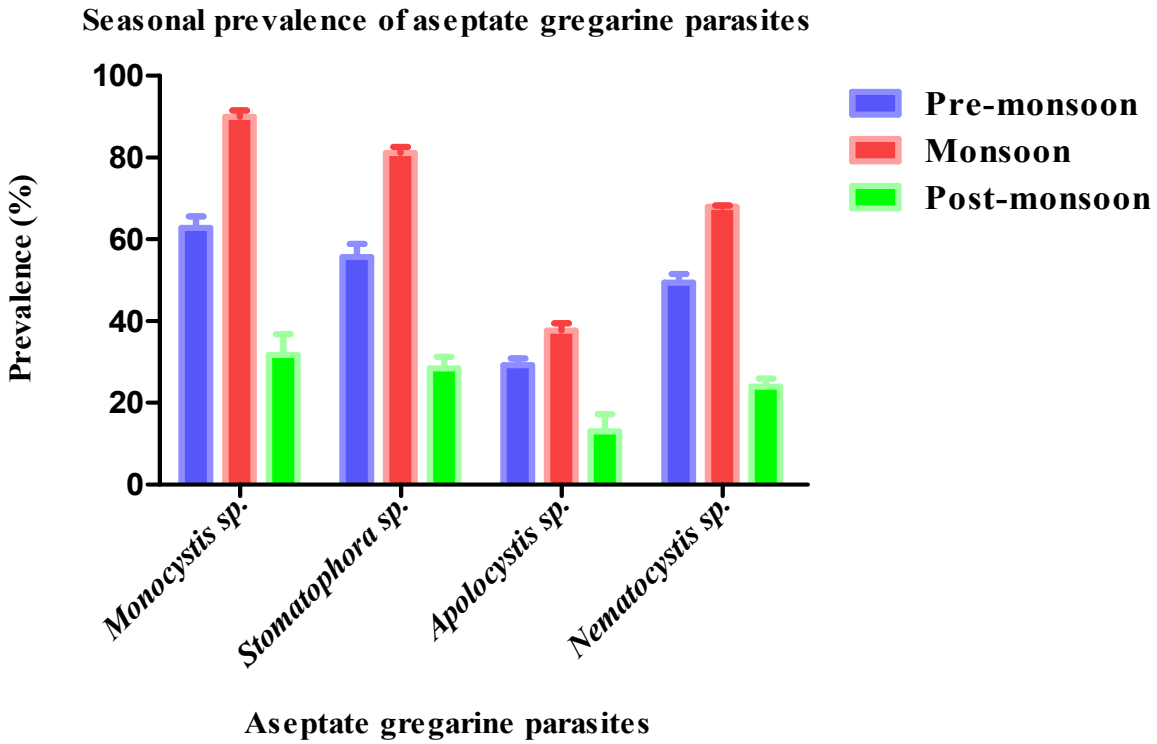

Figure 2. Graphical representation of seasonal prevalence of aseptate gregarine parasites.

In *Monocystis* sp. mean intensity increases from 20.51 in pre-monsoon and reached to its peak value 28.95 in monsoon season, then decreased to 11.99 in post-monsoon season. In *Nematocystis* sp. mean intensity increases from 12.03 in pre-monsoon and reached to its peak value 19.03 in monsoon season, then decreased to 6.21 in post-monsoon season. In *Stomatophora* sp. mean intensity increases from 16.34 in pre-monsoon and reached to its peak value 23.17 in monsoon season, then decreased to 8.99 in post-

monsoon season. In *Apolocystis* sp. mean intensity increases from 7.36 in pre-monsoon and reached to its peak value 14.38 in monsoon season, then decreased to 4.28 in post-monsoon season (Figure 3).

In *Monocystis* sp. mean abundance was high 26.02 in monsoon season, decreased to 3.80 in post-monsoon season and then increased again in 14.75 in pre-monsoon season. In *Nematocystis* sp. mean abundance was high (12.94) in monsoon season, decreased to 1.48 in post-monsoon season and then increased again up to 5.95 in pre-monsoon season. In *Stomatophora* sp. mean abundance was high (18.20) in monsoon season, decreased to 2.57 in post-monsoon season and then increased again up to 9.11 in pre-monsoon season. In *Apolocystis* sp. mean abundance was high (5.42) in monsoon season, decreased to 0.57 in post-monsoon season and then increased again up to 2.16 in pre-monsoon season (Figure 4).

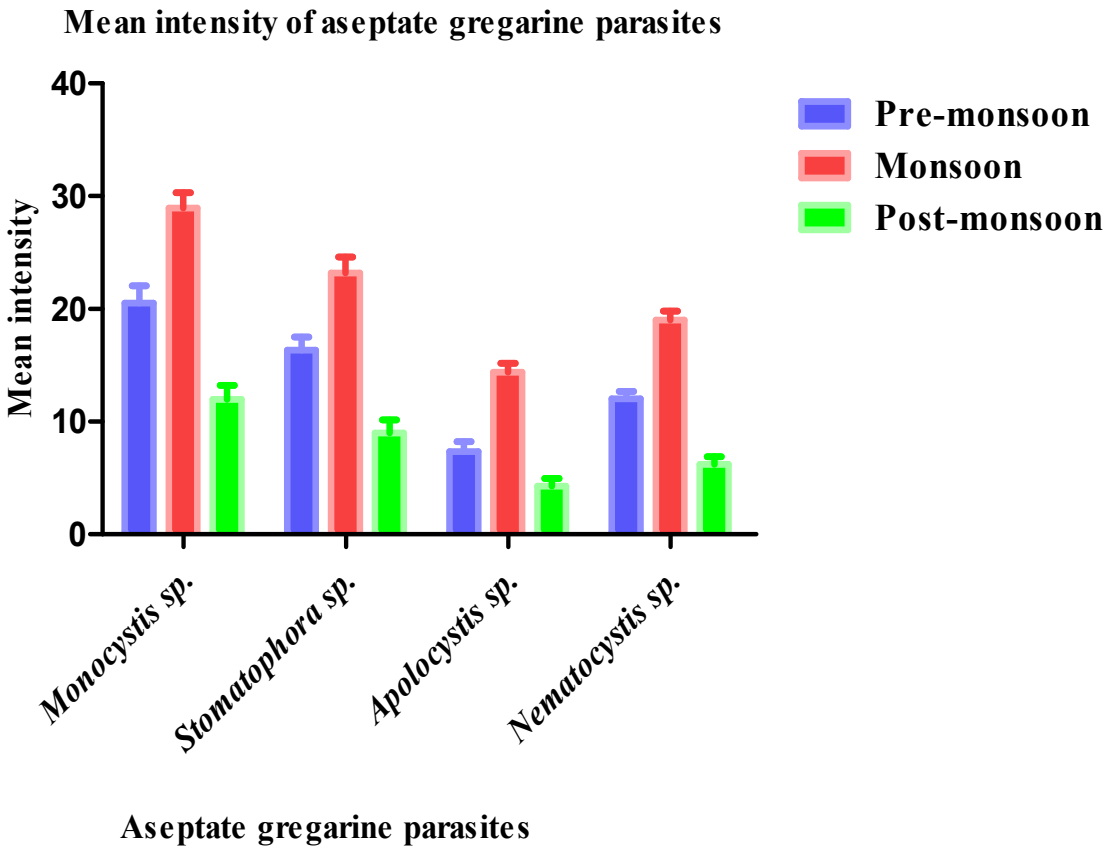

Figure 3. Graphical representation of mean intensity of aseptate gregarine parasites.

4. DISCUSSION

During this study, we observed the seasonal prevalence, mean intensity and mean abundance of aseptate gregarines namely, *Monocystis*, *Stomatophora*, *Apolocystis* and *Nematocystis*, infecting seminal vesicles of *Metaphire posthuma*. During the study, we observed that the seminal vesicles

of earthworms are heavily infected with these parasites. These are also referred by Bandyopadhyay *et al*. [5], Sarkar *et al*. [6, 20] and Bandyopadhyay and Mitra [7]. The rate of infection of gregarine parasites in earthworms was highest in monsoon season and lowest in the post-monsoon season. These finding are showing similarities with the findings of Sinha and Modak [21]. Among them, *Monocystis* was the highest prevalent followed by *Stomatophora*, *Nematocystis* and*Apolocystis*. This observation also corroborated with the studies done by Sinha and Modak [21].

According to Forbes *et al*. [22], gregarine parasitism was seasonally unimodal in terms of both prevalence and intensity. One of the reasons for this pattern could be that, like the density of hosts which peaks mid-season, the density of infective stages of directly transmitted parasites like gregarines also peaked mid-season [22]. Forbes *et al*. [22] found that gregarine density in damselflies is highly positively correlated with host density. This finding supports the observations placed here. In our study we also observed the intensity of infection caused by aseptate gregarine parasites peaks in mid-season i.e. monsoon followed by the density of host Earthworms.

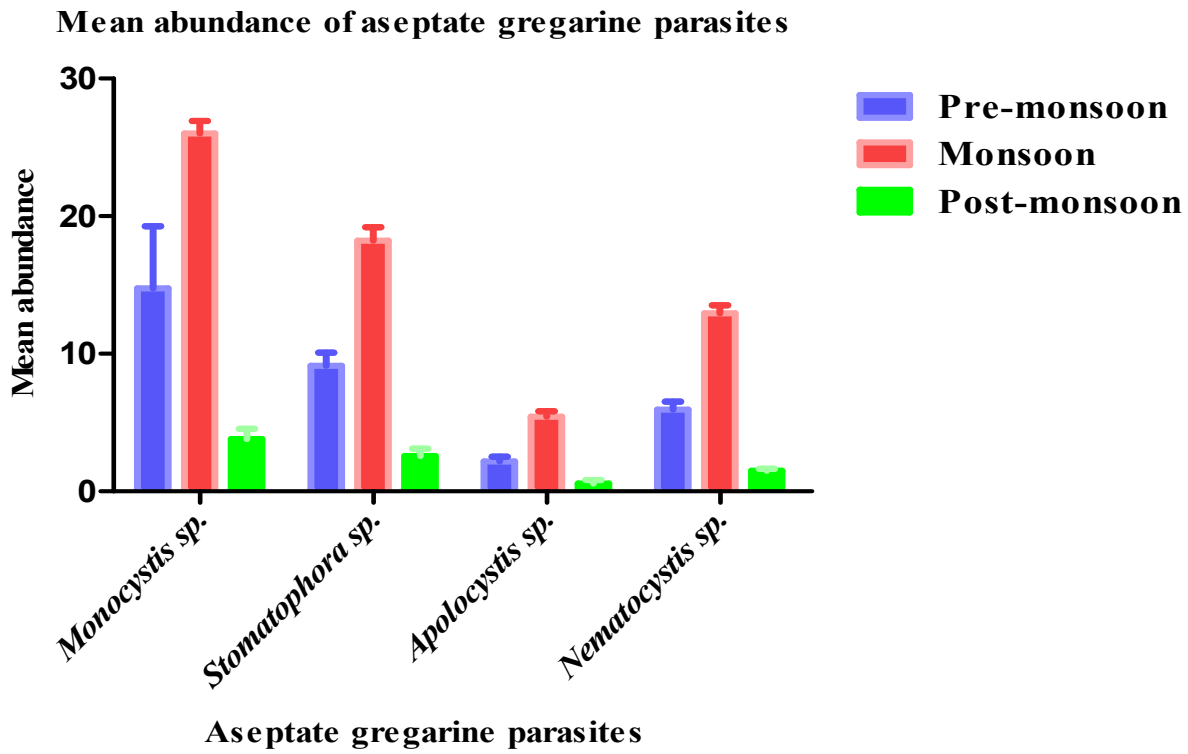

Figure 4. Graphical representation of mean abundance of aseptate gregarine parasites.

The seasonal occurrence of aseptate gregarine parasites of earthworms in India has been recognized as a significant component of the biodiversity of these parasites, and it will be

defined by conducting a thorough assessment on the seasonality of these parasites of *Metaphire posthuma*earthworm species. Thus, the present study provided some helpful information for future studies on the taxonomic diversity and host-specificity of these gregarines.

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