Butterflies are considered as an important group of ecological indicators, which are often considered as flagship taxa in many ecosystems. Danaus chrysippus (Linnaeus, 1758) is a medium sized butterfly which is commonly known as the Plain Tiger butterfly or African Monarch and considered as the commonest of butterflies. These butterflies are widely distributed in Asia and particularly in India (Fig.-1). They are endangered locally due to accidental destruction of host plants, use of pesticides in agriculture, housing, development etc. Chronic infections by microsporidian parasites have been implicated as a possible regulatory factor in controlling the populations of several naturally occurring insect pests.

Microsporidia are obligate, eukaryotic, unicellular parasites with the ability to infect both vertebrate and invertebrate hosts and have a worldwide distribution. The microsporidia identified to date belong to 187 genera and 1500 described species. Among invertebrates, microsporidian infections are most prevalent in insects. We report the presence of microsporidian parasites (designated as M-Dch) in the adult of Danaus chrysippus butterflies. In this study, investigations on the morphology and seasonal prevalence of M-Dch spores were carried out and the observations are reported herein. The study reveals that the prevalence of M-Dch spores was highest (33.84%) in rainy season followed by the winter season (25.92%) and least prevalence (16.92%) was found in the summer season. Under phase contrast microscope, the M-Dch microsporidian spores were observed as green spores with Brownian movement. The M-Dch spores were ovo-cylindrical in shape with an average (n=20) size of 3.83 ± 0.02 µm in length and 2.16 ± 0.01 µm in width.

Material and Methods

Screening of Danaus chrysippus for microsporidian infection: One hundred eighty four plain tiger butterfly Danaus chrysippus were collected from various sites in and around Lucknow city of Uttar Pradesh. The adults were sacrificed by an anesthetic (chloroform) and abdomens were homogenized individually in 0.6% K$_2$CO$_3$ solution (Fig.-2). For purification of the microsporidium, the method given by Chakrabarty et al. was followed with some modifications. The homogenate was allowed to settle for 2 minutes and filtered through double layered muslin cloth. The filtrate was centrifuged at 8000 rpm for 30 min and the pellet was suspended in sterilized distilled water. The homogenate was allowed to settle for 2 min and filtered through double layered muslin cloth. The filtrate was then transferred to 1.5 ml centrifuged tube for further purification. For the above purpose, the filtrate was centrifuged at 5000 rpm for 15 min and after centrifugation the pellet was suspended in sterilized distilled water and thoroughly mixed on cyclomixer. The obtained suspension was then centrifuged at 8000 rpm for 15 min and the process was repeated 3 times by adding sterilized distilled water to the sediment. The sediment obtained was suspended in minimal volume of sterilized distilled water and stored at 4°C.

Estimation of prevalence and spore load: Prevalence of microsporidian parasite M-Dch in the adult plain tiger butterfly Danaus chrysippus was determined by examining smear on a glass slide and examined for the presence of microsporidian spores under bright field microscope at 40X. When no spores were found, the butterflies were classified as being uninfected. Infection intensity was measured by counting M-Dch spores per infected butterfly (spore load) using a Neubaur haemocytometer (German Fine Optik).

Light Microscopy observations of fresh and stained spores: Temporary slides were prepared from samples
suspended in sterilized distilled water, and the permanent slides by staining the smear with Nigrosin stain solution and observations were made under phase contrast microscope at 40X. The size of the microsporidian spores was measured using Nikon NSBR software.

RESULTS AND DISCUSSION

Microsporidian parasites are of taxonomic importance because of their peculiar eukaryotic features and their characterization is mainly based on the ultrastructural and molecular studies. They are known to infect a wide range of vertebrate and invertebrate hosts but, apparently are most prevalent in insect hosts. Different microsporidia have been isolated from silkworm and various agricultural insect pests and described on the basis of light and ultrastructural characteristics. In the present study, light microscopy studies on M-Dch microsporidian spores isolated from plain tiger butterfly Danaus chrysippus L. were carried out. Under phase contrast microscope, the M-Dch microsporidian spores were observed as green, ovo-cylindrical spores with Brownian movement. The M-Dch spores were measured with an average (n=20) size of 3.83 ± 0.02 × 2.16 ± 0.01 µm (Fig.-3). In comparison, the size of M-Dch spores were more or less similar to the standard spore size of Nosema bombycis (3.32 ± 0.02 × 1.97 ± 0.01µm) strain, but the shape of M-Dch spores (ovo-cylindrical in shape) was different from that of Nosema bombycis strain which is oval in shape and were easily differentiated under the microscope. Based on the concentration of M-Dch spores per individual, the infection level was categorized into chronic (1×10^5 - 1×10^7 spores/ml), medium (1×10^2 - 1×10^5 spores/ml) and acute (<1×10^3 spores/ml) levels of infection. The highest spor load per individual was recorded as 3.5×10^7 spores/ml whereas lowest was recorded as 1.02×10^2 spores/ml of homogenate solution.

Microsporidia have been described from several lepidopteran insect pests, still there are many yet to be reported. For the first time, we describe a microsporidian parasite (designated tentatively as M-Dch) isolated from plain tiger butterfly Danaus chrysippus L. (Lepidoptera, Nymphalidae) from Lucknow region. Comprehensive literature review on prevalence of microsporidia has revealed that the seasonal prevalence of microsporidian parasites was previously studied mainly in the hymenopteran insect Apis mellifera. However, very little studies have been conducted on the seasonal prevalence of microsporidia in the lepidopteran insects. It is important to study the seasonal prevalence of microsporidian parasite in insects because the parasites are used as bio-control agents of many insects in the agricultural field. In the present study, the prevalence percentage of microsporidian parasites (M-Dch) in Danaus chrysippus was recorded for the three different seasons in the year from July, 2014 to June, 2015. In the present study, prevalence of M-Dch microsporidian parasites in the host butterfly Danaus chrysippus was found highest in rainy season (33.84%) followed by winter season (25.92 %) and least prevalence were recorded in summer season (16.92%) (Table-1, Fig.- 4). Similar results were observed by Kunhamed et al., where the author reported that the infection of microsporidian parasites in butterflies was higher in rainy season 23.21% followed by winter season (16.92%) and least prevalent in the summer season (5.26%)\(^\text{15}\). Kunhamed et al. also studied the influence of weather factors on the incidence of microsporidiosis in the Silkworm Bombyx mori L. and recorded maximum

<table>
<thead>
<tr>
<th>Month (July, 2014-October, 2014)</th>
<th>Number of D. chrysippus individual with Infection screened</th>
<th>M-Dch spores</th>
<th>Prevalence Percent (%) (Infected/total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rainy</td>
<td>85</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Winter (November, 2014-February, 2015)</td>
<td>54</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Summer (March, 2015-June, 2015)</td>
<td>65</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 1. Prevalence of M-Dch microsporidian spores in plain tiger butterfly Danaus chrysippus in different seasons (spring, summer, autumn) in Lucknow region.
Seasonal prevalence of microsporidian infection in the Plain Tiger Butterfly

**Fig. 4.** Season wise prevalence of M-Dch microsporidian infection in Danaus chrysippus L.

Microsporidian infection during October-November. They also recorded highest intensity of microsporidian spores during the same period; however only one or two spores per smear was observed during summer season. The incidence of microsporidian infection in the insects may differ due to several weather factors like temperature, humidity and biotic components that influence the survival of microsporidia in an environment.

**CONCLUSION**

There are few previous reports that conducted extensive studies on the seasonal prevalence of microsporidia in insects and therefore this present study was undertaken to investigate the prevalence of microsporidian infection in a common agricultural insect, the Plain Tiger Butterfly, Danaus chrysippus. Our study has revealed that the prevalence of M-Dch microsporidia in Danaus chrysippus was highest in rainy season, followed by winter and summer season. The prevalence of microsporidian parasite in the host may vary due to the climatic conditions, food conditions, host behavior and host immunity. Our results showed that microsporidian M-Dch infection in the butterfly was found highest during rainy season. This may probably be attributed to the fact that the relative atmospheric humidity was highest during that period. It is noted that weather is one of the important parameters that determines the incidence of microsporidian infection in insects. In this study, morphological investigations were made wherein the structure of M-Dch microsporidian spores was observed by light and scanning electron microscopy. Under phase contrast microscope, the fresh and unstained M-Dch microsporidian spores were observed as green, ovo-cylindrical spores with an average size of 4.06 ± 0.13 × 2.77 ± 0.01 µm. It is concluded that this study will be helpful in providing information on the seasonal prevalence and morphology of M-Dch microsporidian parasites infecting the plain tiger butterfly Danaus chrysippus in Lucknow region of Uttar Pradesh. This study is of immense importance and will add to the existing literature. However, further studies of M-Dch microsporidia regarding their host specificity, internal structure by Transmission Electron Microscopy, and rDNA sequence analysis are needed to identify the isolated M-Dch microsporidian species and assign it to respective genus and these studies are ongoing.

**REFERENCES**

Fig. 1. Distribution of Plain Tiger Butterfly, *Danaus chrysippus* L. in India
Source: http://www.ifoundbutterflies.org/sp/744/Danaus-chrysippus#distribution

Fig. 2. Butterfly *Danaus chrysippus* L. and its abdomen used for homogenization and isolation of microsporidian spores.

Fig. 3. (A). Light micrograph of M-Dch spores suspended in Distilled water. (B). Light micrograph of M-Dch spores stained with Nigrosin solution.