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DIAGNOSIS AND COUNT DENSITY DETERMINATION OF SYMBIOTIC PROTOZOA INTO HINDGUT OF *MICROCEROTERMES DIVERSUS* (SILVESTRI)

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Abstract

The symbiotic microorganisms of the microbiota of hindgut play a key role in nutrient supply, digestion process, and absorption. Protozoa are a microorganism that can be free-living or parasitic. This study was conducted to investigate and diagnose the types of symbiotic protozoa and its numerical density in the hindgut of the *Microcerotermes diversus* (Silvestri) insect. The results of the microscopic examination based on the morphological form presented four types of protozoa (Monocercomonodidae) *Hexamastix termopsidis*, (Trichomonadidae) *Trichonympha campanula*, (Trichomonadidae) *Trichonympha sphaerica* and (Pyrosnymphidae) *Dinenympha gracilis*. Their existence density in the hindgut of the termite insect differed significantly. The existence number of *Dinenympha gracilis* is highest (3959) Protozoa/hindgut followed by *Hexamastix termopsidis* (918.33) Protozoa/hindgut. *Trichonympha campanula* and *Trichonympha sphaerica* had lower individuals with a density of (626.11) and (542.23) Protozoa/hindgut, respectively. Protozoa have a significant role in the survival of the Microcerotermes diversus (Silvestri) insect as it is the main producer of cellulase enzyme that digested cellulose.

Key words: Protozoa, termite, *Microcerotermes diversus* (Silvestri), symbiotic microbiota

Introduction

Symbiotic organisms are found in the digestive system of insects that feed on wood or other plant Lignin-rich substances. One of the most prominent insects is *Microcerotermes diversus* (Silvestri). It is an English name that is a termite. Symbiotic organisms play a prominent role in digesting lignocellulose in a complex chain of processes between the symbiotic organisms that excited in the digestive tract and the host. Cleveland (1924) conducted a large-scale study on the hindgut and its living organisms and indicated the presence of protozoa and bacteria. They are anaerobic organisms, some of which have gaps that encroach on wood fragments in the back digestive canal (Cleveland, 1925). Some also have hydrogenosomes, a structure found in protozoa that functionally rewards the mitochondria in organisms and is responsible for energy-generating by electron transportation, Smit-Cavalier, (1993), Wafaa Alawsy (2018). Most of the digestion processes take place in the frontal and central gastrointestinal tract and the active role is for the symbiotic microorganisms of protozoa and bacteria in the hindgut, Breznak (2000); Brune (2003); Khaeim Hussein (2013). This adds to its role in hydrogen and carbon dioxide metabolism, acetogenesis oxygen-reducing, nitrogen fixation, and amino acid synthesis, Warnecke *et al.,* (2007); Ohkuma (2008); Xie *et al.,* (2012). The importance of the protozoa is because it occupies the largest space in the hindgut. It forms more than one-third of body weight, and protozoa are sensitive to oxygen and the process of digestion of wood is not done without it, since it is the main source of cellulose enzymes, Honigberg (1970); Yamin (1979); Inoue *et al.,* (1997). Kirby (1932) diagnoses Protozoa in six species of termite belonging to the species of Amiterme. He collected five species from Californians state and the sixth one from Panama in the United States. The presence of *Trichomonas light* in all termite species. *Amitermes beaumonti* and *Entamoeba beaumont* were found in five species. Nyctotherus silvestrianus existed in three types of those collected from California. A. coccidian species was found in *Amitermes minimus*. The different types of protozoa found in different termite species and

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different regions. Yamin (1979) isolates and diagnoses more than 430 types of protozoa belonging to 205 different species of termite belonging to the Kalotermitidae and Rhinotermitidae families, Hussein Khaeim (2019). The presence of protozoa species belonging to the Devescovinidae and Calonymphidae families in the Kalotermitidae family was recorded. He also recorded the presence of the oxymonad that belongs to Pyrsonymphidae family in the termite family of Rhinotermitidae.

Protozoa classification is continuously modified using the available molecular technology power. It is divided into three main classes: Anaeromonadea, Trichomonadea and Trichonymphea. The first is the row Anaeromonadea, which contains protozoa known as Oxymonads, most of which have structures that enable them to adhere to the walls of the hindgut, which includes three species Pyrsonympha, Dinenympha, Oxymonas, Moriya and others (2003); Luma, A. *et al.,* (2018); Bushra Jeber *et al.,* (2019). The second class of Trichomonadea includes a variety of families Monocercomonadidae, Trichomonadidae, Devescovinidae and Calonymphidae. Monocercomonadidae and Trichomonadidae have small-sized protozoa that tare suspected of cellulose digestive role. It is possible to distinguish between the members of these families through the presence of this flagellum in the family of Trichomonadidae. Protozoa of Devescovinidae and Calonymphidae families have a number of flagella in, which involved in one group at the present time. The third class is Trichonymphea that consists complex and large size species that have a number of flagella and is common in various types of termite, Viscogliosi - Delgado *et al.,* (2000); Ohkuma *et al.,* (2000); Muneer Al-Baldawy *et al.,* (2019).

Materials and Methods

Diagnosis of protozoa species

A group of living termites insects fed on non-treated filtration papers was sampled for preliminary screening and identification of protozoa species in the hindgut. They were sterilized by (95) % alcohol and was dissected using a pair of tiny tweezers by holding the back of termite insect with one of the tweezers and the head with another one. The head was pulled out so that the digestive system was fully withdrawn and placed in a saline solution. Each hindgut was cut off and placed in the same solution. Special glass slides were prepared to examine the protozoa. Each hindgut was transferred and placed on a single glass slide and then dissected in saline solution. After complete drying of these slides, the samples were fixed with (95)% alcohol by placing drops of it on the surface of the slides. After evaporation of alcohol, the (10)% Gamsa pigment drops were applied with and left for 30 minutes. Slides were then washed with distilled water and left to dry. Finally, Canada dye was applied to these slides and the examination was performed under the optical microscope compound magnification with a power of (100 X). The diagnosis of protozoa was based on morphology form based on Castle (1934), Yamin (1979), Lewis and Forschler (2006) and different species of protozoa were identified.

Calculation the number of symbiotic protozoa

In order to calculate the number of symbiotic protozoa in the hindgut of termites insects, their gastrointestinal tracts were cut off and stored in saline solution. They were then placed in a (1.5) centrifuge tube and 40 microliters of saline solution were added to. The saline water contains a neutral red dye. This red pigment was prepared by dissolving (1) gm of it in distilled water and the size was complete to (100) mL. After that, (0.5) ml of this solution was taken and added to (10) ml of saline solution. These tubes were transferred to a centrifuge for full mixing for (15) minutes. The protozoa counts were calculated using the Hemacytometer. (0.4) μl of this mixed solution that contains protozoa mixed was added on each side of the hemocyte count. Individuals were calculated in the middle square that is divided into 5 squares. Each of which is divided into 16 squares. Protozoa numbers are calculated according to the followed equation, Maistrello *et al.,* (2002):

$$XF = \frac{GXn}{VX_3}$$

Where:

XF: Count of protozoa

G: volume (ml) of the solution containing the three hindguts.

n: mean of the two counts within hemacytometer.

V: volume (ml) of the counted Area.

Results and Discussion

The Species of Protozoa in the hindgut

The protozoa species were identified in the hindgut of the *M. diversus* according to Castle (1934); Yamin (1979); Lewis and Forschler (2004) based on the morphological form of the protozoa species, Fig. 2. Four species of protozoa were identified in M. diversus, including:

1- Hexamastix termopsidis (Monocercomonodidae)

Results characterized this species as a small protozoa

microorganism that has five flagella, Figure 2-A. This result is consistent with Gerbod *et al.,* (2000). Deigado-Viocogliosi *et al.*, (2000) reported that the Monocercomonodidae includes small size protozoa species that can be distinguished according to their presence and number of flagella.

2- Trichonympha campanula (Trichomonadidae)

3- Trichonympha sphaerica (Trichomonadidae)

The results of the morphological are similar (Fig. 2B and C) to those found by Tai *et al.,* (2013).

4- Dinenympha gracilis (Pyrosnymphidae)

The results of the microscopic test of this type are similar to the findings Lewis and Forschler (2004) diagnosed the isolated protozoa from three species of termites: Reticulitermes virginicus, Reticulitermes flavipes and Reticulitermes hageni. Eleven species of protozoa were recorded of Reticulitermes flavipes (Dinenympha fimbriata, D. gracilis, Holomastigotes elongatum, Microjoenia fallax, Monocercomonas sp., Pyrsonympha major, P. vertens, Spirotrichonympha flagellata, Spironympha kofoidi, Trichomitus trypanoides and Trichonympha agilis).

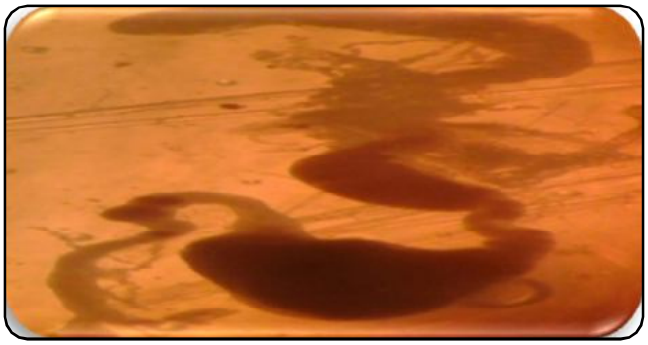

Fig. 1: The gastrointestinal of Silvestri.

The density of Protozoa in the hindgut

The results show the presence of four types of symbiotic protozoa in the hindgut of the Microcerotermes diversus (Silvestri). The species of *Hexamastix termite, Trichonympha campanula, Trichonympha sphaeric,* and *Dinenympha gracilis* were identified according to phenotypic traits. The numerical density of all species was calculated, which valued at (918.33, 626.11, 542.23 and 3959) protozoa/hindgut, respectively. *Dinenympha gracilis* species recorded the highest number density while the *Trichonympha sphaeric* species presented in the lowest density number. These species are essential for the survival of the Microcerotermes diversus (Silvestri) because they are the main source of cellulose enzyme Table 1.

Numerous studies have been conducted on the importance of coexistence and protozoa and their differences. Cook and Gold (1999) reported that there was a difference in the numerical density of coexisting protozoa among *R. flavipes*. In the study, the number of protozoa was higher in workers and wingers than in soldiers. The researchers also pointed out in 1998 that the number and types of protozoa differ between the workers themselves and the difference in the number of protozoa due to the difference in the ability of individuals to digest cellulose and the amount of food eaten,

Table 1: Count density of the symbiotic protozoa in the hindgut of *Microcerotermes diversus* (Silvestri).

Protozoa Species	Protozoa count/ 3 gastrointestinal tract
Hexamastix termites	2755
Trichonympha campanula	1878.33
Trichonympha sphaerica	1626.7
Dinenympha gracilis	11877

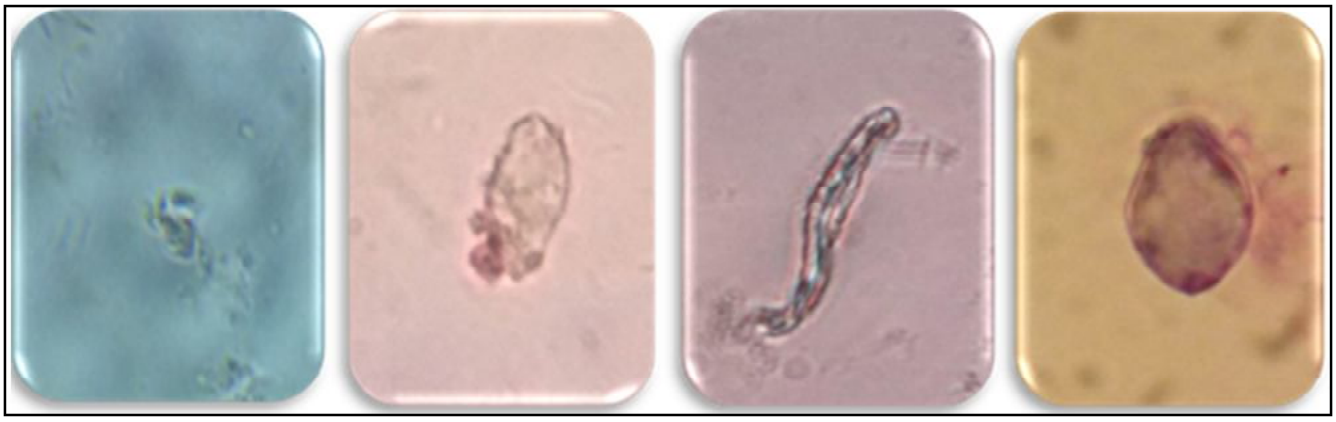

Fig. 2: The diagnosed species of Protozoa.

Hexamastix termite	*Trichonympha campanula*	*Dinenympha gracilis*	*Trichonympha sphaericas*

Mannesman (1972 a, b). The total number and species ratio of the hindgut protozoa have been influenced by sort of nutrition and different environmental conditions such as temperature, Mannesman (1972).

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