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SUPPRESSION OF SYMBIOTIC PROTOZOA AND SURVIVORSHIP OF THE TERMITE *CRYPTOTERMES BREVIS* (WALKER) BY *BACILLUS SPHAERICUS* (MEYER AND NEIDE) AND STARVATION.

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Abstract

Efficacy of the bacterium *Bacillus sphaericus* concentrations as well as starvation, on the symbiont protozoa and survivorship of the termite *Cryptotermes brevis* was evaluated. The occurred protozoa belong to *Calonympha* sp., *Devescovina* spp., *Foaina* spp., *Hexamastix* spp. Oxymonas sp. and *Tricercomitus* spp., showed highly significant differences after tested treatments. Total mean of the protozoa reached 17626. 67, relative percentages of species to the control varied between 12.71 to 21.63 of *Hexamastix* spp. and *Devescovina* spp., respectively. The *Tricercomitus* spp. was the only protozoa, significantly affected by the-lowest concentration ($21x10^7$ v.s./ml), while no significant variation was found between means of other species at the low or intermediate concentrations 21×10^7, $42x10^7$ and $62.16x10^7$ v.s./ml, respectively. All protozoan species distinctly decreased due to the starvation. Great loss of total protozao was observed at the highest concentration of *B.sphaericus* (84×10^7 v.s./ml). Significant variation occurred between total protozoan mean after starvation and the highest concentration treatments (1413.33 and 6426.67, respectively), while survivorship of both treatments were relatively similar (82.00 and 84.00%, respectively). It could be claimed that decrease in protozoan numbers, may not be the only factor limiting the survivorship of the termites, Pathogenicity of the *B.sphaericus* would be other critical affecting factor. Intermediate concentration $42x10^7$ and $62.16x10^7$ v.s./ml gave insignificant variation between their survival percentages. For ideal slow acting termiticide, on field application, the higher concentration of the intermediate ones (62.16×10^7 v.s./ml) is preferred to compensate loss of the bacterium, due to suppression of virulence, diffusion or penetration within wooden surfaces.

INTRODUCTION

The gut microorganisms of lower termites, mainly protozoa, are responsible for the cellulose digestion and survival of the termites (Cleveland, 1925; Hungate, 1939). Furthermore, the most important role of the gut microbes of the termites, including the protozoa, is production of acetate in the hind-gut from glucose or other intermediates of cellulose degradation, because termites lack pyruvate dehydrogenase or any other enzyme capable of converting pyruvate to acetate. The digested cellulose was found, by the radio activity, to be incorporated into triglycerids, therefore, protozoa seem to be involved in the ability to synthesize lipids, (Mauldin, 1977). Eliminating only one species of protozoa of the termites *Coptotermes formosanus* Shiraki, or *Reticulitermes flavipes* (Kollar), prevents normal lipid synthesis and survival (Mauldin *et al.*, 1972; Mauldin, 1977). Further demonstration has been obtained by Lenz and Backer (1972) and Da Costa *et al.*, (1976), showing that some toxic chemicals eliminated some or all protozoa from several species of termites including *R.flavipes*, *C.acinaciformis* and *N.exitiosus*.

The protozoan species are in many cases specifically related with the genus or even the species of termites that posses them. Some of the unique intestinal fauna, found to harbor in the hind-gut of the dry-wood termite *Cryptotermes brevis* (Walker), are namely: *Calonympha* sp., *Devescovina* spp., *Foaina* spp., *Hexamastix* spp., *Oxymonas* spp. and *Tricercomitus* spp. (Kudo, 1971; Yamin, 1979).

The microbiological agent *Bacillus sphaericus* (Meyer and Neide) was found to be a pathogen to the dry-wood termites *Cryptotermes brevis* and *Kalotermes flavicollis* (Nasr and Moein, 1992). Since the protozoa are involved in the digestion of the cellulose in the hind-gut of the termites, eleminating the protozoa might suppress the survival of the termites. However, any control of the termites should affect its symbiotic protozoa. The present study aimed to determine the efficacy of *B.sphaericus*, in comparison to starvation treatment, on the symbiotic protozoa as well as survivorship of the dry-wood termite *Cryptotermes brevis*.

MATERIALS AND METHODS

Termites: Termites used were undifferentiated nymphs of *C.brevis* beyond the fourth instar. Individuals were taken from colonies, collected from infested furniture in Alexandria, Egypt, maintained at standerized conditions 26±1°C and 75±1% R.H., feeding on *Picea* sp. wood chips and filter paper.

Pathogenicity test: *Bacillus sphaericus** (Meyer and Neide) was isolated from nymphs of the dry-wood termites, *Kalotermes flavicollis* Faber. (Nasr and Moein, 1992). The Bacterium *B.sphaericus* was maintained at room temperature (28°C) in

* Isolated bacterium was identified by Prof. Dr. H. De Barjac of the biological control, Pasteur Institute; a collaborating center for Entomopathogenic *Bacillus* in Paris, France

slant tubes containing Glycerol Agar Medium (G.A.M.) (Mortignoni and Steinhaus, 1961). Plate count technique by Kiraly *et al.*, (1970) was used to estimate the number of viable bacterial spores per ml to determine the various concentrations. Bioassay tests were carried out in petri-dishes (5 cm in diameter). The nymphs subjected to infectivity tests, were starved for 6 h. before treatment, standered pices of filter paper (15.90 cm2) were used as a source of feeding, after dipping in tested concentration for 30 seconds. Filter paper dipped in distilled water was used for control treatment. Four concentrations $21x10^7$, $42x10^7$, $62.16x10^7$ and $84x10^7$ spores/ml were used. Each concentration was replicated three times, where ten nymphs were used for each replicate. After five days of treatment, the tested termites were fed on untreated filter paper in other petri-dishes, where number of the survived individuals and contents of protozoa were counted through 24 hours.

Starvation test : Groups of termites (10 nymphs), for each of three replicates, were kept in petri-dishes for the same period without diet, then provided with untreated filter paper. Survivorship and protozoa were counted within 24 hours.

Evaluation of the protozoa: The hind-gut was carefully pulled out from the posterior end of the nymph by a dissecting needle and gently macerated in a concave of a slide glass filled with 40 µl of 0.8% saline soft water, mixed thoroughly, the pieces of tissue were removed. A 20 µl sample of the suspension was transported by a microsyring into a haemocytometer slide, where the number of protozoa in each 16 square at random was counted. This process was replicated with five nymphs. The total number of protozoa was calculated by multiplication on the basis of the volume tested as in Mannesmann (1970. 1972, 1974).

Data analysis : Effects of the tested concentrations of *B.sphaericus* and starvation teatment, on the protozoan species or survival percentages of the termites were analyzed and differentiated using the complete random design (ANOVA). Separation of means was fulfilled according to L.S.D. test. Relative percentages of means of protozoa to the control were also calculated.

RESULTS AND DISCUSSION

Morphological characteristics of the monitored protozoa, Fig. 1, could be summarized as follows (Kudo, 1971 and Yamin, 1979).

A. *Tricercomitus* spp.: Three anterior flagella; a long trailing flagellum, adhering to body; nucleus anterior, without endosome; small; blepharoplost large, with a parabasal body and an axial filament; small; parasitic. The species *T.brasiliensis* De Mello and *T.divergens* Kirby were found to belong this genus.

B. ***Hexamastix* spp. :** Six anterior flagella and one trailing flagellum which may be partially attached to body or free; no undulating membrane; parabasal body prominent; axostyle after conspicuous. This genus was represented with two species *H.conclaviger* Kirby and *H.disclaviger* Kirby.

C. ***Devescovina* spp. :** Three anterior flagella about the body length; trailing flagellum, slender to band-Form, about 1-1.5 times the body length; cresta medium to long; parabasal body spiraled around axostyle or nucleus; body elongated and usually pointed posteriorly. Species found belonging to this genus are *D.cometoides* de Mello and de Brito, *D.lemniscata* Kirby, *D.striata* Foa.

D. ***Foaina* spp. :** Small to medium large; three anterior flagella; trailing flagellum two or three the body length; cresta slender; 2.5-17 μ long; single, in some with rami. Three species of this genus are existed, namely: *F.nana* Kirby, *F.minuta* De Mello and *F.reflexa* Kirby.

E. ***Oxymonas* spp. :** Attached phase with a conspicuous rostellum, the anterior end, forms a sucking-cup for attachment; pyriform. In motile phase, rostellum is less conspicuous; axostyle conspicuous; xylophagous. The only species of this genus, occurred in the hind-gut of *C.brevis*, is *O.brevis* Zeliff.

F. ***Calonympha* spp. :** Numerous flagella arise from anterior region; axial filaments form a bundle; round and large body with numerous nuclei. The species *C.grrassi* Foa is the only species found belonging the genus *Calonympha* in the hind-gut of *C.brevis.*

The protozoan species, at the hind-gut of the termite *C.brevis* showed highly significant differences after various tested treatments of the bacterium *B.sphaericus,* Fig. 2 and starvation.

Variations between means of the control and the lowest concentration $21x10^7$ v.s./ml treatments, were not significant for all species, unless Tricercomitus spp. was the only protozoa affected significantly, showing means of 2586.67 and 2160.00, respectively, Table 1. Also, the data indicated distinct susceptibility of the protozoa *Tricercomitus* spp., since significant difference was observed between its means affected with the lowest concentration $21x10^7$ v.s./ml and other concentrations. No significant variations occurred between means of other species, ensur-

Table. 1. Means of protozoa, its relative percentages to control and survivorship of the termite *C.brevis* exposed to concentations of the bacterium *B.sphaericus* and starvation.

Conc. (v.s./ml)	Protozoan species						Total mean	Survival %
	T	O	H	F	D	C		
21×10^7	2160.00^b (83.51)	3040.00^{ab} (87.02)	1893.33^{ab} (84.52)	2026.67^{ab} (83.52)	3466.67^{ab} (90.91)	2613.33^{ab} (85.22)	15200.00^{ab} (86.23)	a 98.00
42×10^7	1600.00^c (61.86)	2746.67^{ab} (78.63)	1573.33^{ab} (70.24)	1626.67^b (67.03)	3146.67^{cb} (82.52)	2026.67^{cb} (66.09)	12720.00^{cb} (72.16)	ab 92.00
62.16×10^7	1333.33^c (51.55)	2293.33^{cb} (65.65)	1386.67^b (61.90)	1386.67^{cb} (57.14)	2826.67^c (74.13)	1733.33^c (56.52)	10960.00^c (62.18)	ab 92.00
84×10^7	826.67^d (31.96)	1413.33^c (40.46)	586.67^c (26.19)	773.33^{cd} (31.87)	1866.67^d (48.95)	960.00^d (31.30)	6426.67^d (36.46)	b 84.00
Starvation	80.00^e (3.09)	240.00^d (6.87)	293.33^c (4.46)	213.33^d (8.79)	346.67^e (9.09)	240.00^e (7.83)	1413.33^e (8.02)	b 82.00
Control	2586.67^a (100)	3493.33^a (100)	2240.00^a (100)	2426.67^a (100)	3813.33^a (100)	3066.67^a (100)	17626.67^a (100)	a 100.00

- Means within a column followed by the same letter are not significantly different at 0.05 level of probability by L.S.D. test.

T= *Tricerocmitus* spp. O = *Oxymonas* sp. H = *Hexamastx* spp.
F = *Foaina* spp. D = *Devescovina* spp. C = *Calonympha* sp.

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ing similarity of their responeses, at the low or intermediate concentrations $21x10^7$, $42x10^7$ and $62.16x10^7$ v.s./ml.

Exposure to the highest concentration $84x10^7$ v.s./ml resulted in no significant difference than starvation, meaning similar effect of both treatments on *Hexamastix* spp. (586.67 and 293.33, respectively) and *Foaina* spp. (773.33 and 213.33, respectively).

Each of other existed protozoa *Tricercomitus* spp., *Oxymonas* sp., *Devescovina* spp. and *Calonympha* sp. showed significantly different responses at the ($84x10^7$ v.s./ml) highest concentration, giving means of 826.67, 1413.00, 1866.67 and 960.00, respectively, comparing to starvation, where means reached 80.00, 240.00, 346.67 and 240.00, respectively. No clear variances were observed between the effect of intermediate concentrations, no each of all protozoan species, where no significant difference occurred between means at the concentrations $42x10^7$ or $62.16x10^7$ v.s./ml.

As shown in table 1, starvation significantly affected the protozoan populations, resulting in distinct decrease in their means. With regard to the total number of protozoa of the termite *C.brevis*, Table 1, total mean of the control treatment reached 17626.67, where relative percentage of each species to the total count varied between 12.71% and 21.63% of *Hexamatix* spp. and *Devescovina* spp., respectively. Mauldin and Co-Worker (1981) observed mean number of total protozoa of the termite *R.flavipes* around 32320.0, while numbers of each species varied between 3.1% and 82.1% of the total population. Total protozoa reached 29673-30867 for *R.flavipes* (Mauldin and Rich, 1975) and around 36490 for *R.lucifugus* (Nunes and Dickinson, 1996).

The data shown in Table 1, demonstrated the association of great loss of protozoa 6426.67 at the high concentration of *B.sphaericus*. That confirmed the results of Mauldin and Nely (1980), using antibiotic (Chlortetracycline) treated *R.flavipes*. Similar elimination of some or all protozoan species of hind-gut of the termite *R.flavipes* were claimed by Mauldin and Rich (1975) after exposure to different treatments, or as by Speck et al., (1971) and Eutick et al., (1978) with other species of termites.

Relative percentages to control, Table 1, showed that *Hexamastix* spp., followed by *Calonympha* sp., *Foaina* spp. and *Tricercomitus* spp. were the most affected protozoa, reached 26.19, 31.30, 31.87 and 31.96%, respectively at the highest concentration $84x10^7$ v.s./ml of the bacterium *B.sphaericus*. The least affected protozoa, at the highest concentration, was *Devescovina* spp., its relative percentages to control (48.95%) predominated of other species, followed by *Oxymonas* sp. 40.46%. Similar trend of the protozoan responses to *B.sphaericus* was observed at other tested concentrations (From $21x10^7$ to $62.16x10^7$ v.s./ml), where *Devescovina* spp. was the least affected protozoa (90.19 to 74.13%) while *Tricercomitus* spp. showed the highest responce (83.51 to 51.55%).

Nymphs of the termite *C.brevis* were able to survive 84.00%, even after exposure to the highest concentration $84x10^7$ v.s./ml of *B.sphaericus*, Table 1. Hence, it could be claimed that decrease in numbers of protozoan species, may not be the solely affecting factor on survivorship of the termite *C.brevis*. Also, total means of protozoa, Table 1, showed significant variation between starvation and the highest concentration (1413.33 and 6426.67, respectively), while survivorship of both treatment were relatively similar as 82.00 and 84.00%, respectively. So, existence of all species of protozoa, in spite of markedly decrease in protozoan numbers, resulted in similar survivorship. That ensured the above-mentioned phenomena, where decrease in numbers of protozoa, may not be the only critical factor limiting survivorship of the termite *C.brevis*. It could be claimed that pathogenicity of the bacterium *B.sphaericus* to the termite *C.brevis* should be other affecting factor on survivorship beside decrease of protozoan number.

Such pathogenicity, may be attributed to the role of its associated toxin. Mortality of *A.caspius* and *C.pipiens* mosquito larvae was suggested to be either due to crystal like structure or to multiplication of the ingested bacterium and its association toxin, which also led to a loss of ion control and induced histopathological effects (Menon et al., 1982). Uncontrol and irregulation of ion (Na^+, K^+ and Mg^{++}) transport are characteristics of the inhibitory action of *B.sphaericus* on the mitochondrial ATPase of mosquito larvae (Berridge, 1968).

Nunes and Dickinson (1996), reported that decrease in numbers of protozoan species of the termites *R.lucifagus* was not enough to Justify the insecticidal action of the biocide (boric acid), since the termites were able to surive even with low numbers of protozoa. Similar findings were obtained after treating *R.flavipes* with other chemicals as disodium octaborate tetrahydrate (Grace et al., 1992), where the secondary effect of the insecticide to the protozoa was admitted after its principal toxicity to the termites. Reduction of feeding and survival in *C.formosanus* Dye Sudan Red 7B treated termites, seemed, to be affected directly by toxicity of the chemical as well as indirectly via suppression of gut fauna (Delaphane and Lafage 1989).

Survival percentages showed significant variations, Table 1, after treatment with tested concentrations of *B.sphaericus* and starvation, while survivorship at the least concentration 21 x 10^7 v.s./ml showed no distinct variation than control.

No difference occurred between survival percentages at intermediate concentrations of *B.sphaericus* (62.16x10^7 and 24x10^7 v.s./ml), indicating that the concentration 42x10^7 v.s./ml is adequate to give the same mortality as the higher one 62.16x10^7 v.s./ml.

The present results should be field practiced, mainly as slow acting toxic termiticide. Since the ideal slow termiticide, should keep the existence of exposed individuals, for transportation and exchange of toxic agent during social behaviour among the colony, the highest concentration should be excluded, and one of the intermediate tested concentrations of *B.sphaericus* could be recommonded for field application.

In addition, dilution, breaking out or leaching are among the hazards affecting termiticides on field application. It was suggested by Mauldin and Nely (1980) applying the relatively high concentration of the tested agent (Chlortetracycline) against the termite *R.flavips*, to minimize the effect of dilution on field treatment. Therefore, it could be recommended to apply the high concentration 62.16x10^7 v.s./ml of the intermediate group against the dry-wood termite *C.brevis*, to compensate the probability of loss of the bacterium due to suppression of virulence, or reduction in amount for the diffusion or penetration within wooden surfaces.

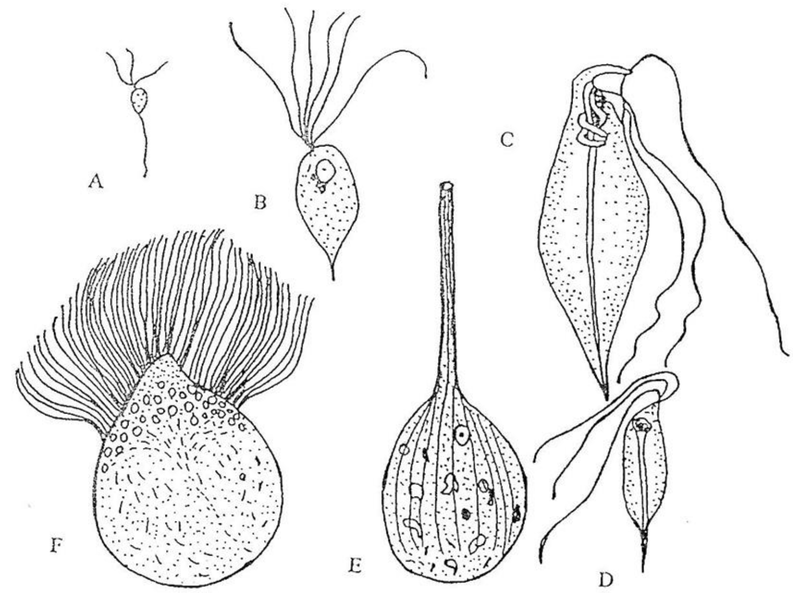

Fig. 1. (A-F): *A.Tricercomitus* spp., x 700 *T.divergens* Kirby) *B.Hexamastix* spp. x 2700 (*H.conclaviger* Kirby) *C.Devescovina* spp. x 1600 (*D.lemniscata* Kirby) *D.Foaina* spp. x 1200 (*F.nana* Kirby) *E.Oxymonas brevis* Zeliff x 920 *F.calonympha grassii* Foa x 900.

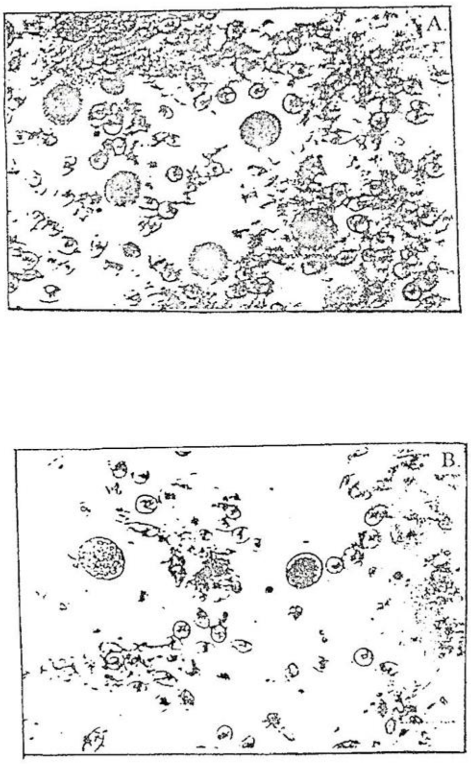

Fig. 2. (A-B): Protozoa of the termite *Cryptotermes brevis* Walker:

A. Untreated B. Treated with the bacterium *B.sphaericus*.

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تدهور البروتوزوا التكافلية ونسب حياة النمل الأبيض *Cryptotermes brevis* (Waker) عند المعاملة بالبكتيريا *Bacillus sphaericus* والتجويع

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تناول البحث تقدير فعالية أربعة تركيزات ٢١ x $١٠^{٧}$، ٤٢ x $١٠^{٧}$ ، ٦٢,١٦ x $١٠^{٧}$، ٨٤ x $١٠^{٧}$ جرثومة / مل من البكتيريا *Bacillus sphaericus* وكذلك التجويع على أعداد البروتوزوا التكافلية الموجودة فى الأمعاء الخلفية للنمل الأبيض *Cryptotermes brevis* وكذلك على نسبة حياة النمل الأبيض. البروتوزوا المتواجدة تتبع *Hexamatix* spp., *Foaina* spp., *Devescovina* spp., *Calonympha* spp., *Tricercomitus* spp., *Oxymonas* spp., ولقد أظهرت النتائج فروق عالية الجوهرية فيما بينها بتأثير المعاملات المختلفة ، وقد كانت البروتوزوا. *Tricercomitus* spp. هى الوحيدة التى تأثرت بأقل تركيز (٢١ x $١٠^{٧}$ جرثومة/مل) من البكتيريا فأعطت إختلافاً معنوياً عن المقارنة. ولم تظهر أى إختلافات معنوية بين متوسطات الأنواع الأخرى عند التركيز الأقل أو التركيزات الوسطية (٢١ x $١٠^{٧}$، ٤٢ x $١٠^{٧}$ ، ٦٢,١٦ x $١٠^{٧}$، جرثومة/مل) على التوالى. وقد أنخفضت أعداد جميع أنواع البروتوزوا بوضوح كنتيجة للتجويع. كما أن العدد الكلى للبرتوزوا إنخفض معنوياً بتأثير التركيز الأعلى ٨٤ x $١٠^{٧}$ جرثومة/مل للبكتريا المختبرة. كما أدى كل من أعلى تركيز وأختبار التجويع إلى إختلافات معنوية بين العدد الكلى للبروتوزور (٦٤٢٦,٦٧ ، ١٤١٣,٣٣ على التوالى). بينما نسبة حياة الحوريات قد أظهرت تشابهاً بعد كل منهما فكانت ٨٢,٨٤٪ على الترتيب. ولذا فإنه يمكن القول أن النقص فى أعداد البروتوزوا ربما ليس هو العامل المؤثر الوحيد فى حياة حوريات النمل الأبيض، بل أن التأثير الممرض للبكتيريا على النمل يمكن أعتباره عامل آخر مؤثر وهام على حياة هذه الآفة. وقد أدى التعرض للتركيز الأقل (٢١ x $١٠^{٧}$ جرثومة/مل) إلى نسبة حياة للحوريات لاتختلف جوهرياً عن المقارنة، كما أن التركيزات الوسطية (٤٢ x $١٠^{٧}$ ، ٦٢,١٦ x $١٠^{٧}$ جرثومة/مل) أدت إلى نسب حياة لاتختلف معنوياً عن كل منهما. وللتطبيق الحقلى، وكمبيد ذو تأثير بطئ وممتد ونموذجى، فإنه يفضل إختيار التركيز الأعلى فى المجموعة الوسيطة وهو ٦٢,١٦ x $١٠^{٧}$ جرثومة/مل وذلك تعويضاً لإحتمالات فقد الحيوية أو النقصان فى البكتيريا بتأثير الإنتشار أو التغلغل خلال الأسطح الخشبية.