J Pest Control & Environ Sci Vol 4 No 2 pp 91 - 99 (1992)

Bacillus sphaericus (Meyer and Neide) AS A NEW DRY WOOD TERMITES PATHOGEN ON *Kalotermes flavicollis* (Fabr.) AND *Cryptotermes brevis* (Walker) (ISOPTERA : KALOTERMITIDAE)

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Received 22/7/1992 Accepted 15/9/1992.

ABSTRACT

The pathogenicity of the bacterium *Bacillus sphaericus* (Meyer and Neide) against the dry wood termites *Kalotermes flavicollis* (Fabr.) and *Cryptotermes brevis* (Walker) (Isoptera:Kalotermitidae) has been studied. This is the first record of this microbial agent in Egypt, and was isolated from the termite *K. flavicollis*.

The results indicated that the nymphs of *K. flavicollis* were more susceptible to *B. sphaericus* than the nymphs of *C. brevis*. The percentages of mortality were 87.5% and 65% for both species, respectively , after fourteen days using the highest tested concentration of *B. sphaericus*.

INTRODUCTION

The dry-wood termites in Egypt include three species belonging to the family Kalotermitidae, Isoptera. The first is *Kalotermes flavicollis*

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(Fabr.) which is found in almost all Governorates in Egypt. It attacks live trees which had been previously infested with wood borers, such as Casuarina , Ficus , Egyptian Willow and Mulberry(Hafez,1980). The second is Cryptotermes brevis (Walker) that causes serious damage to furniture and wooden works within the buildings. Kalotermes sinaicus (Kemner) is the third dry-wood termite which mainly attacks trees.

The means of control of the dry wood termites are mainly based on chemical insecticides. The use of microbial control offers a promising trend for the control of insect pests (De Bach and Mc Omic,1939;Steinhaus,1963;Davidson et al., 1975; Burges,1981; Menon et al.,1982 and Hussein et al.,1991).

During these observations on the termite K. flavicollis in Alexandria,Egypt, it was observed that the mortality among this termite reached as high as 100% in the control, with obvious discoloration in the whole body. Bacterial isolations were obtained from the dead individuals.

The present study aims to offer some information on the identification and pathogenicity of this microbial agent against the two species of the dry wood termites in Egypt, C. brevis and K. flavicollis. Such studies may be significant for the progress of biological methods of control.

MATERIALS AND METHODS

The bacterium:

Bacillus sphaericus(Meyer and Neide) was isolated from dead nymphs of Kalotermes flavicollis (Fabr.) obtained from an infested tree of Casuarina glauca. This bacterial strain was cultured on Glycerol Agar Medium (3 gm beef extract ,5 gm pepton, 20 gm agar, 20 ml glycerol and 1 L water). Plate count technique (Kiraly et al.,

1970) was used to determine the number of viable spores of B. sphaericus.

The termites:

Nymphs of K. flavicollis were obtained from an infested tree of C. glauca cultivated at El-Sabahia Research Station, Alexandria. A severely infested table, made of Fagus sylvatica wood, was the source of the termite C. brevis. Individuals of both species were kept at 86±1% R.H. and 27± 1°C for about one month before experimentation.

Pathogenicity test:

Pathogenicity tests were carried out under laboratory conditions of 27±1°C and 86±1% R.H. in plastic covered cups (5.5 cm in diameter and 6.5 cm in height). Nymphs of both species were subjected to the infectivity tests. All tested individuals were starved for about 24 hours, then introduced to the cups provided with pieces of the wood C. glauca (4 x 2.5 x 0.2) as a source of feeding and dipped for five minutes in the bacterial suspension. Six concentrations were used namely , 21 x 10^7 , 31.5 x 10^7 , 42 x 10^7, 50.4 x 10^7 , 62.16 x 10^7 and 84 x 10^7 spores/ml. Each concentration was replicated four times and 40 nymphs were used for each replicate. Only distilled water was used in the control.

Infected and uninfected (checks) cups were examined daily for fourteen days to determine the mortality percentages. Lc-P lines were drawn to estimate the L_{c50} values (Litchfield and Wilcoxon, 1949).

RESULTS AND DISCUSSION

Symptoms of the disease were easily distinguished by prohibition of feeding, slowing down until prevention of motion, tending to leave away

the group and discoloration to brown which was obviously observed on the head. The bacterial diseases of the termite Zootermopsis sp., caused by Serratia sp. and Bacterium sp.,as recorded by De Bach and Mc Omic (1939) were characterized by such common symptoms,similar its early stages to these caused by B. sphaericus , but differed in discoloration,which was red or black in Serratia and Bacterium, respectively. The discoloration occurred due to the effect of bacterial end products on the exoskeleton, while tending to leave the group was reported to be as a result of the hypersensitivity of the diseased termites to contact, and hence move away from the group.

The present data indicated that the numphs of K. flavicollis were more susceptible to B. sphaericus than those of C. brevis (Figs. 1-2). Mortality usually occurred 2-14 days after infection. The percentages of mortality increased according to the increase of the bacterial concentrations for both species of termites. These percentages at the tested concentrations (21 x 10^7, 31.5 x 10^7 , 42 x 10^7 , 50.4 x 10^7, 62.16 x 10^7 and 48 x 10^7 s/ml), were 15, 25, 30, 35, 35 and 40% for K. flavicollis after 48 hours from treatment, the corresponding records for C. brevis were 10, 15, 15, 20, 20 and 25%. The percentages of mortality after seven days from treatment were 35, 45, 50, 75, 80 and 82.5% for K. flavicollis while they were 15, 30, 35, 40, 55 and 60% for C. brevis. After fourteen days from treatment the percentages of mortality rose to 35, 50, 55, 77.5, 82.5 and 87.5% for K. flavicollis while they were 15, 30, 35, 50, 57.5 and 65% for C. brevis. The values of L_{c50} and slope for K. flavicollis and C. brevis are shown in Table (1). It was found by De Bach and Mc Omic (1939) that the bacterial species Serratia marcescens and Bacterium sp. were pathogenic causing 50% and 22% mortality, respectively, to the termite Zootermopsis angusticollis Hagen after two weeks from treated individuals. The

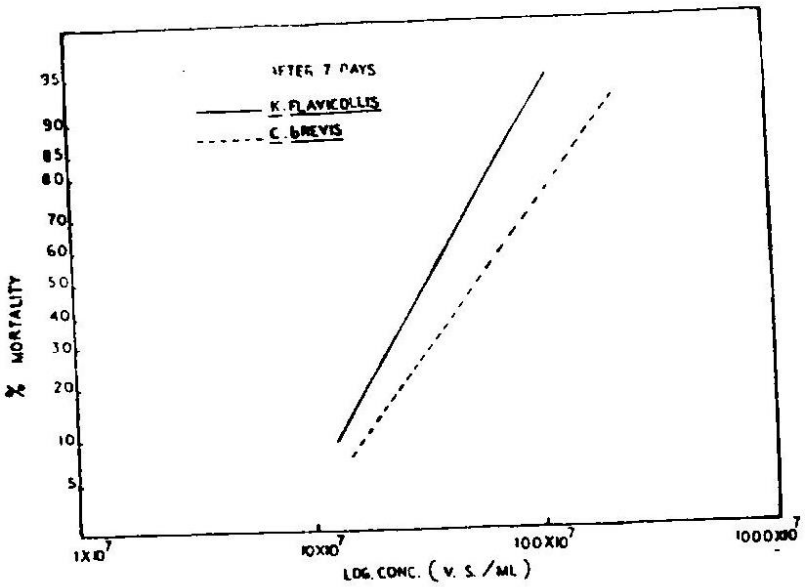

Fig.(1): Lc-P lines showing susceptibility of K. flavicollis and C. brevis nymphs after 7 days from treatment by B. sphaericus.

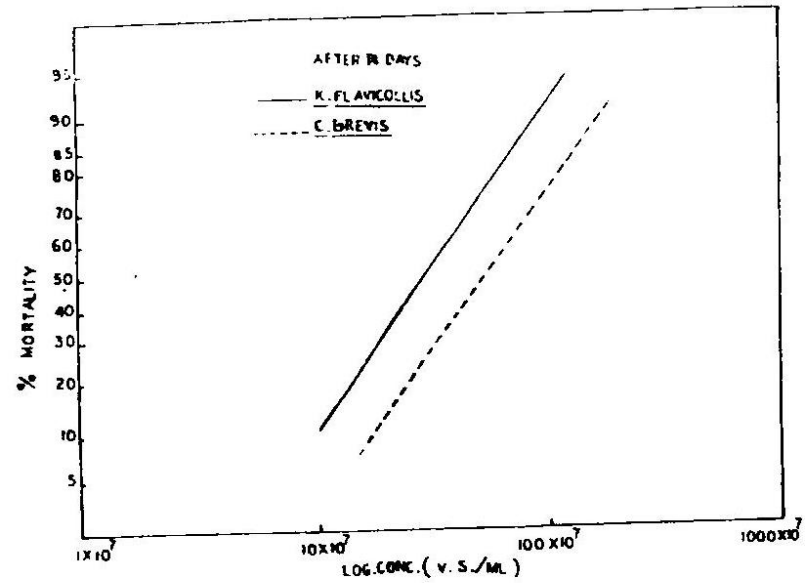

Fig.(2): Lc-P lines showing susceptibility of K. flavicollis and C. brevis nymphs after 14 days from treatment by B. sphaericus.

Table(1): Parameters determined according to the statistical method of analysis adopted by (Litchfield and Wilcoxon , 1949) for susceptibility of the nymphs of Kalotermes flavicollis and Cryptotermes brevis to different concentrations of Bacillus sphaericus.

Time (days)	L_{c50} viable spores per ml	Slope (S)	Confidence limits of L_{c50}
K. flavicollis			
After 7 days	33 x 10^7	2.40	38.28x10^7 : 28.44x10^7
After 14 days	30 x 10^7	2.35	35.70x10^7 : 25.21x10^7
C. brevis			
After 7 days	58 x 10^7	2.52	69.60x10^7 : 48.33x10^7
After 14 days	50.6 x 10^7	2.48	60.21x10^7 : 42.52x10^7

inoculation experiments gave two to three-fold increase in mortality more than by ingestion, and caused death much more quickly. On the other hand, the inhibitory action of B. sphaericus was investigated on the larvae of the mosquitoes Culex pipines L. and Aedes caspius Pal. A positive correlation was found between post treatment time andATPase inhibition (Hussein et al., 1991). B. sphaericus possessed a lethal cell-bound toxin which was released when the bacterial cells were digested by mosquito larvae (Davidson et al.,1975).

However , this is the first report of B. sphaericus as a dry wood termite pathogen in Alexandria, Egypt. The pathogenicity of B. sphaericus proved to be successful as microbial control agent against the examined termites. Nevertheless further experiments in this field including mode of action and field trials are required to throw some light on the full potential of B. sphaericus as a biological control agent.

ACKNOWLEDGEMENT

The authors wish to thank Prof. Dr. H. De Barjac of the biological control , Pasteur Institute , WHO Collaborating Centre for Entomopathogenic Bacillus in Paris, for helping in identifying the bacterium B. sphaericus.

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J P C & E S. Vol:4 No:2 (1992).

الملخص العربي

فعالية المسبب المرضي Bacillus sphaericus ضد نوعين من النمل الابيض Kalotermes flavicollis و Cryptotermes brevis

تم في هذا البحث عزل البكتريا B. sphaericus لاول مرة في مصر من حوريات النمل الابيض K. flavicollis، وكذلك اجريت دراسة تاثير المسبب المرضي B. sphaericus على الحوريات لكل من K. flavicollis و C. brevis.

ايضا اظهرت النتائج ان حوريات K. flavicollis اكثر حساسية للمرض البكتيري عن C. brevis حيث كانت نسبة الموت ٥ر٨٧% في حوريات النوع الاول و ٦٥% في حوريات النوع الثاني وذلك بعد ١٤ يوما من المعاملة باعلى تركيز تم استخدامه.