REPRODUCTION OF ENTOMOPATHOGENIC NEMATODES *Steinernema carpocapsae* AND *Heterorhabditis bacteriophora* ON THE GERMAN COCKROACH *Blatilla germanica* AT DIFFERENT TEMPERATURES.

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Abstract

Adult and nymph stages of German cockroaches *Blatilla germanica* were infested with two entomopathogenic nematodes, *Steinernema carpocapsae* and *Heterorhabditis bacteriophora in vitro* at 15, 20, 25° C. Results showed that *S. carpocapsae* was more virulent than *H. bacteriophora* at all temperatures over the exposure times of 24, 48 and 72 h. Infection of nymphs by *S. carpocapsae* at 20° C caused 100% mortality after 72h and 100% mortality of adults at 25 ° C after 72h. Reproduction of *S. carpocapsae* significantly increased at 25° C after 72 h compared to *H. bacteriophora.*

Key words: Entomopathogenic Nematodes, German cockroach

النشاط التكاثري للديدان الخيطية المتطفلة *Steinernema carpocapsae* و *Heterorhabditis bacteriophora* على الصرصر الالماني *Blatilla germanica* عند درجات حرارية مختلفة.

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الخلاصة

عُرضت بالغات وحوريات الصرصر الالماني *Blatilla germanica* للاصابة باثنين من الديدان الخيطية *Heterorhabditis bacteriophora* و *Steinernema carpocapsae* خارجياً ضمن درجات حرارية ١٥، ٢٠، ٢٥ م. اظهرت النتائج ضراوة اكثر للنوع *S. carpocapsae* مقارنةمع النوع *H. bacteriophora* لكل الدرجات الحرارية المستخدمة وعلى فترات تعرض ٢٤، ٤٨، ٧٢ ساعة . اصابة الحوريات بالنوع *S. carpocapsae* في درجة حرارة ٢٠ م سبب هلاكات بنسبة١٠٠٪ بعد فترة ٧٢ ساعة ، و١٠٠٪هلاكات ضمن البالغات بدرجة حرارة ٢٥ م بعد نفس الفترة.كما اظهرالنوع *S. carpocapsae* زيادة نوعية في التكاثر بدرجة حرارة ٢٥ م وبعد فترة ٧٢ ساعة مقارنة مع النوع *H. bacteriophora*

الكلمات المفتاحية:- الديدان الخيطية الطفيلية، الصرصر الالماني

Introduction

German cockroach (*Blatella germanica*) is a household pest throughout the world. Cockroaches adulterate food or food products with their feces and defensive secretions, physically transport and often harbor pathogenic organisms and may cause severe allergic responses [1]. The chemical approach to cockroach control has become increasingly less popular because of increased public concern about pesticide exposure in a domestic environment but also because of decline in pesticide efficacy due to the development of multi – chemical resistance [2, 3, 4, 5]. Biological control could play an important role in managing cockroach populations. Studies with the entomopathogenic nematodes. Nematodes in the families Heterorhabditi-dae and Steinernematidae had much interest in their use as biological control agents [6, 7, 8, 9 The species *S. carpocapsae* have shown that nematodes can be used successfully in biological control programmes [9, 10, 11, 12, 13, 14, 15]. These nematodes are obligate parasites of a Wide range of insect species including cockroaches [7]. Insect hosts are parasitized by a third stage infective Juvenile (IJ) nematode that locates the insect, either by following co_2 or temperature gradients [8]. In a laboratory *Steinernema* sp. successfully infected adult and nymph stages of *B. germanica* and significant reductions in natural infestations have been reported [16 , 17]. Appel and Benson [18] found 100% mortality of *B. germanica* continuously exposed to *S. carpocapsae* after 7days. Temperature can have an effect on the infectivity of different nematode species [19, 20, 21]. This experiment was conducted at Baghdad University, College of Science, Department of Biology, to examine the effect infectivity of two entompathogenic nematode on the German cockroach (nymph and adult stages) at different temperatures.

Materials & methods

German cockroaches used in all experiment were collected from different houses located in three localities (Al shoala, Al Baia and Aljadirya) belongs to Baghdad province. Collected cockroaches were maintained in cage with dimensions (30×30×30 cm) at 27±2 ^{o} C. Newly emerged adult males and females were collected daily and maintained in separate groups under the same conditions. Cultures of *S. carpocapsae* and *H. bacteriophora* were obtained from Dr. Simon Gowen at Reading University, UK, were maintained by culturing in last-instar larvae of the wax moth *Galleria mellonella* [22 , 23]. Infective juveniles (IJ) of less than 3 days old, were added to10g of sterilized dry sand in 5cm Petri dishes at the dose of 400 / dish. After applying nematodes, the moisture content of the sand was calculated to be 14% as in Sumaya [23]. Four nymphs or adults of *B. germanica* from a laboratory culture were placed in Petri dishes which were sealed with laboratory tape (Parafilm) to minimize desiccation and placed in incubators at 15, 20 and 25 °C separately. Mortality of nymphs and adults was recorded daily for 3 consecutive days. Cadavers were dissected and examined for the number of developed nematodes immediately. Results were analyzed using the FREQ procedure.

Result & Discussion

Steinernema carpocapsae (Sc) was more aggressive than *H. bacteriophora* (Hb) in attacking both stages of the cockroach. These results in agreement with Locatelli and Parleaz [16] who found that Sc was more virulent than other nematodes used in their experiment. Appel and Benson [1] found 100% mortality of female German cockroaches continuously exposed to nematode *S. carpocapsae* within 7 days.

Temperature: Different percentages of mortality suggested that the nematodes have different temperature optima. Nymphs infested with (Sc) at 20 ^{o} C showed 100% mortality within 48 h (Fig.1) .At 25 ^{o} C the same nematode infested the adults causing 100% mortality after 72h (Fig. 2). This result was in agreement with a previous result by [9].

H. bacteriophora infected adults at 15 ^{o} C resulting in 20 % mortality after three days. While At 20 ^{o} C there was less than 20% mortality of nymphs (Fig. 3 , 4). The results indicated an interaction between temperatures,

nematode and host stage at the time of infestation.

Stage of the host: It was found that nymph stage of the cockroaches was more susceptible to Sc and Hb than the adult. *S. carpocapsae* caused 100% mortality of nymphs after 48 h at 20 ^{o} C (Fig. 1) but *H. bacteriophora* caused only 20 and 10% mortality of nymphs and adults at 20 ^{o} C (Fig. 3 and 4). These nematodes kill insects with aid of a mutualistic bacterium, which carried in their intestine [24]. Once inside the hosts haemocoel, the IJ release a symbiotic bacterium genera *Photorhabdus* and *Xenorhabdus* (Enterobacteriacae) that multiplies, kill the host, and renders the host interior conductive to nematode reproduction [8].

Exposure time: For Sc, 100% mortality occurred within 48h against the nymph stage and after 72h at adult stage (Figure 1). Koehler *et al.* [10] tested the susceptibility of different genera of cockroaches including German cockroaches. They found that German, brown-banded, oriental and smoky-brown cockroaches died within one day after placement in Petri dishes containing 500,000 nematodes of *S. carpocapsae* on filter paper. Although the percentage mortality reached only 20% after 72 h using *H. bacteriophora*, however those were more obvious on adult stages rather than nymph stages (Fig. 3, 4).

Progeny production: Reproduction of Sc was significantly greater at 25 ^{o} C on nymph or adult stages after 72h from other temperatures scale (Figures 5, 6). On the other hand (Hb) showed poor development at all treatments (Fig. 6 , 7). Many studies have shown that entomopathogenic nematodes are adapted to particular environments [1, 19, 20, 25, 26]. Belair *et al*. [27] found that at 30^{o} C there was 100% mortality of *Artogeia rapae* L2 (the imported cabbageworm) by the warm temperature species *Steinernema riobrave* 335 and *S. carpocapsae*, but only 95.8% from *S.feltiae* and 91.7% from *S.feltiae* 27 at 25 ^{o} C which are from cooler climates. Ratnasinghe and Hague [28] demonstrated that the optimal temperature range for the infectivity of *S. carpocapsae* against *Plutella xylostella* was between 20 ^{o} C and 30 ^{o} C with an optimum at 25 ^{o} C. Grewal *et al.* [25] found that temperature optima for reproduction of *H. bacteriophora* were lower than that of Steinernematids. The reproductive capacity and virulence of Heterorhabditids are low at temperatures above 30 ^{o} C. In conclusion, it is shown that the nymph stage of German cockroaches was more susceptible to nematode infection than adults. *Steinernema carpocapsae* could be used to control German cockroaches in Iraq during the spring or autumn seasons when temperatures range from 20-25 ^{o} C. Application of this nematode on baits could be very beneficial and might achieve a great success in controlling an annoying household pest.

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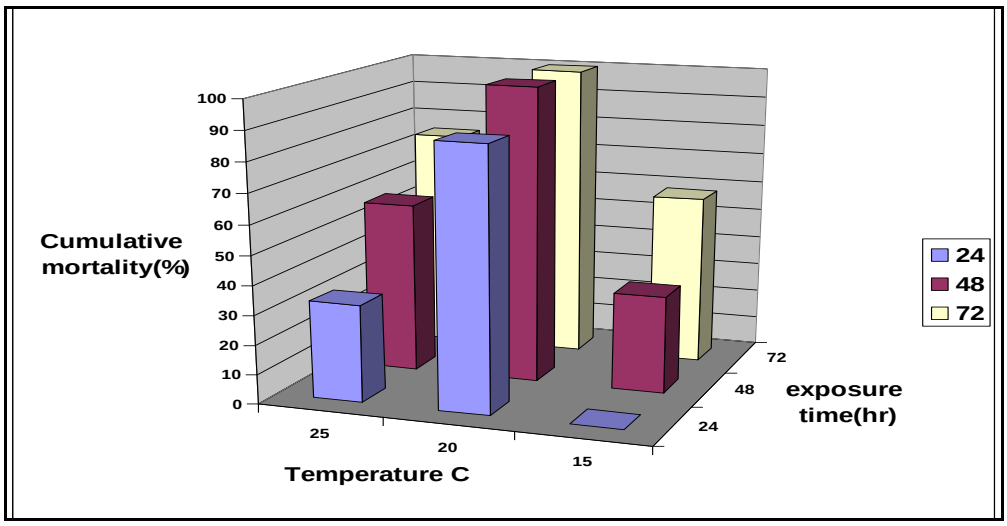

Figure 1: Percentage mortality of German cockroaches at nymph stage infested with *S. carpocapsae* at different temperatures.

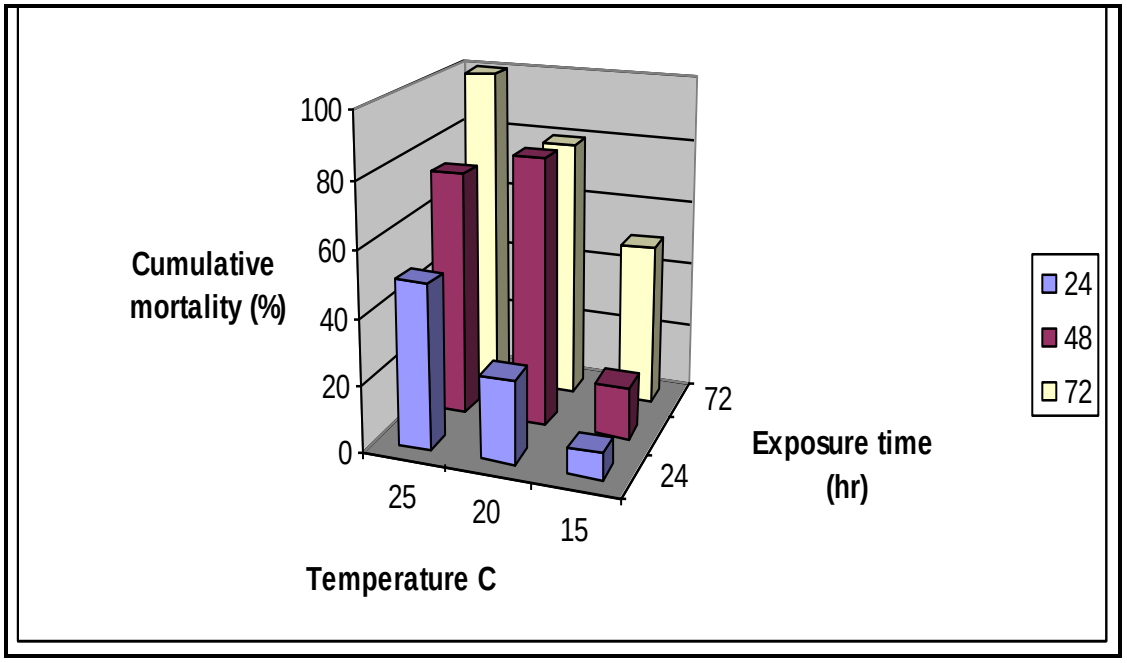

Figure 2: Percentage mortality of German cockroaches at adult stage infested with *S. carpocapsae* at different temperatures

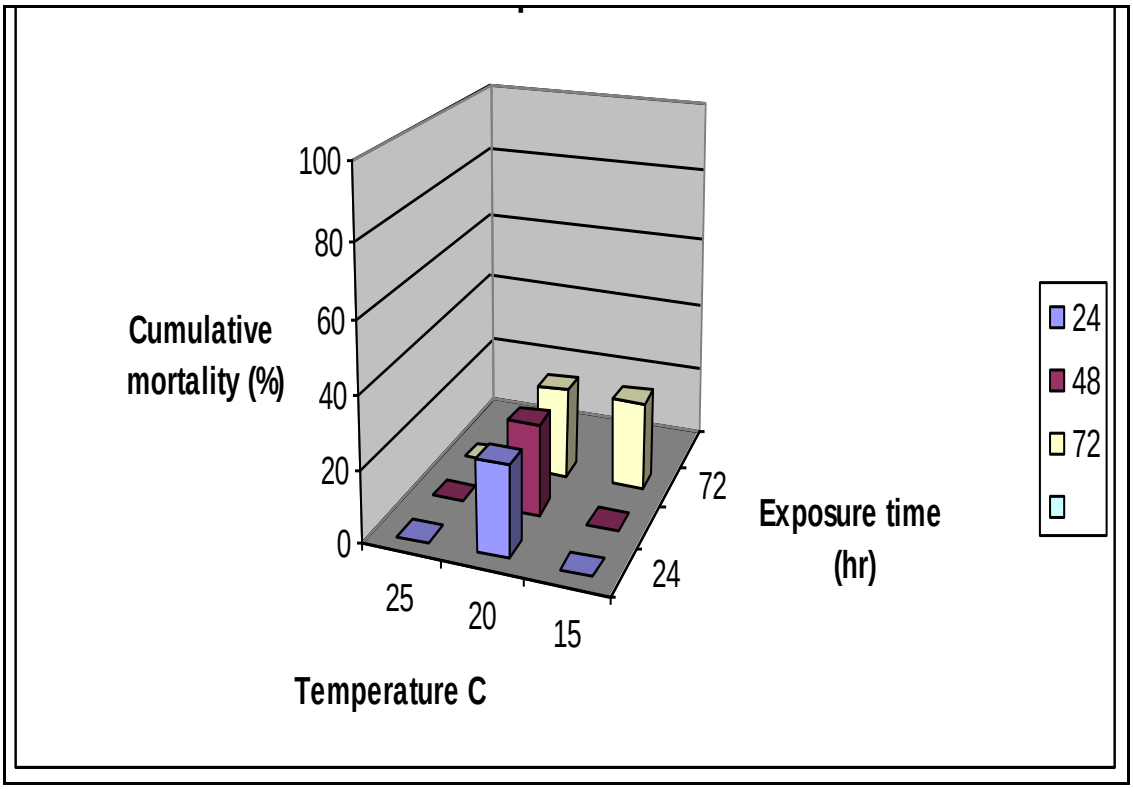

Figure 3: Percentage mortality of German cockroaches at nymph stage infested with H. bacteriophora at different temperatures.

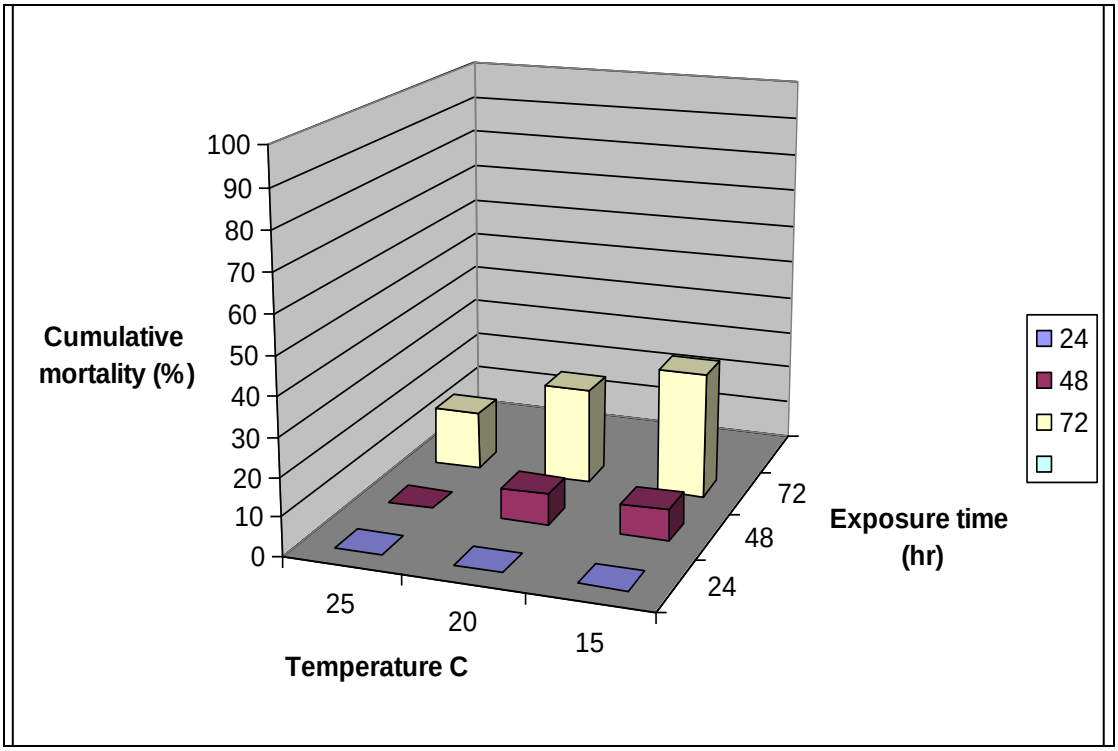

Figure 4: Percentage mortality of German cockroaches at adult stage infested with H. bacteriophora at different temperatures.

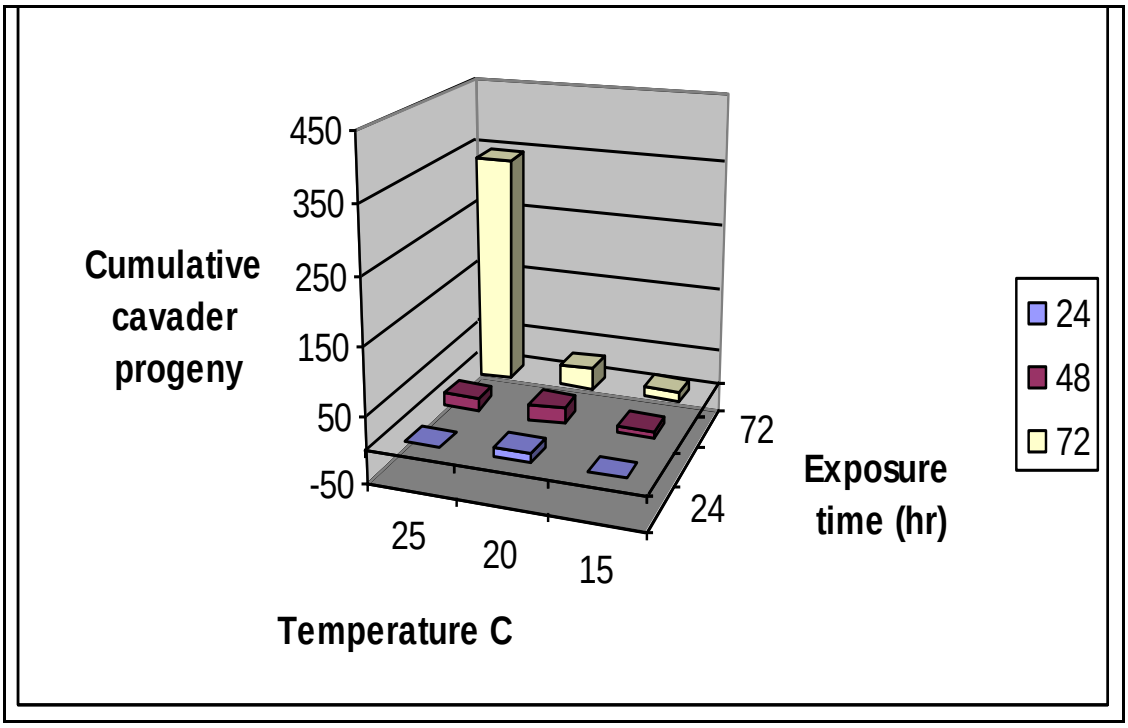

Figure 5:Influence of temperature and exposure time on S. carpocapsae progeny infected German cockroaches at nymph stage

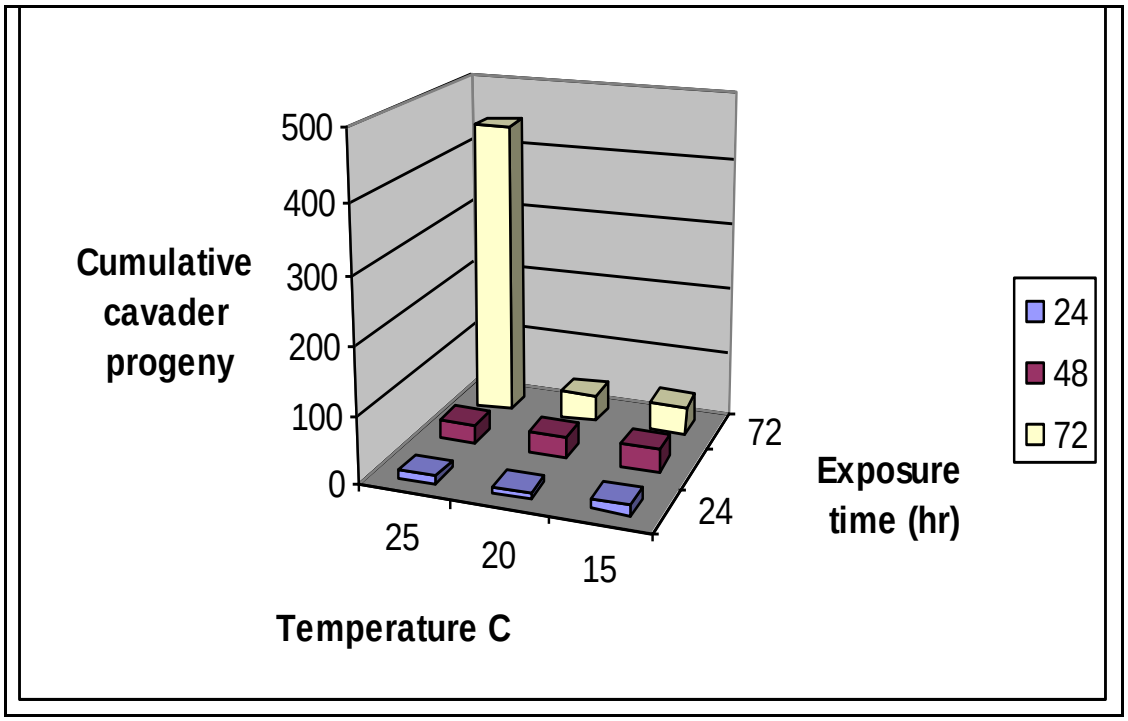

Figure 6: Influence of temperature and exposure time on S. carpocapsae progeny infected German cockroaches at adult stage.

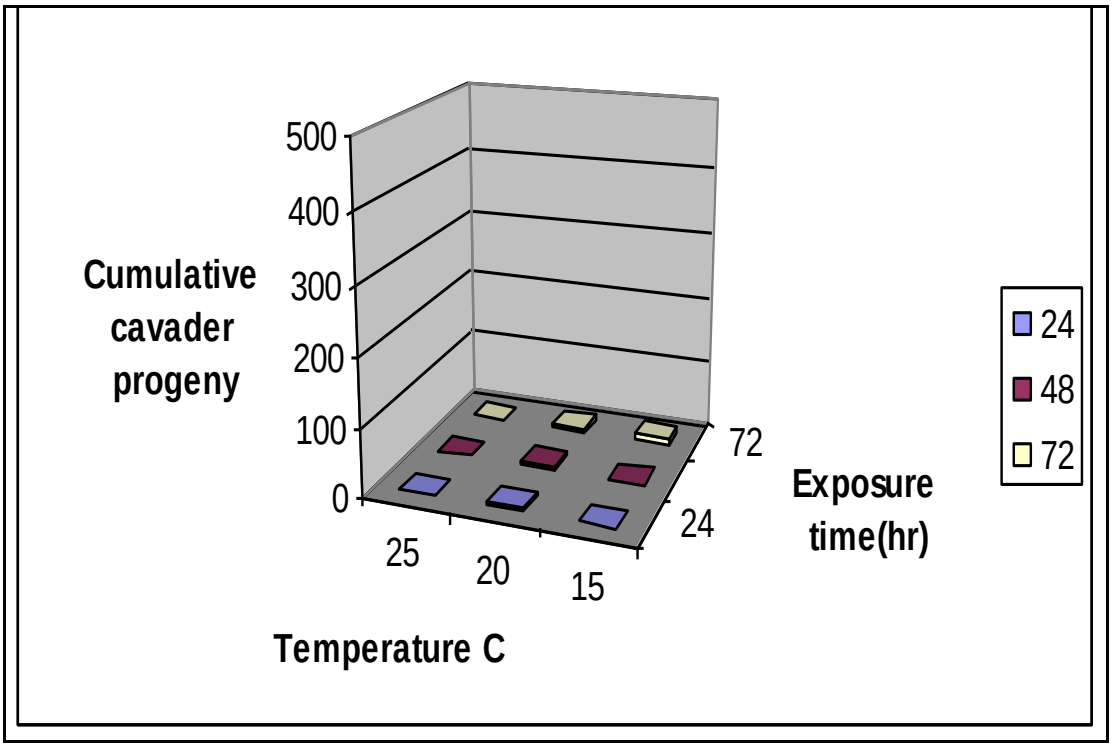

Figure 7:Influence of temperature and exposure time on H. bacteriophora progeny infected German cockroaches at nymph stage

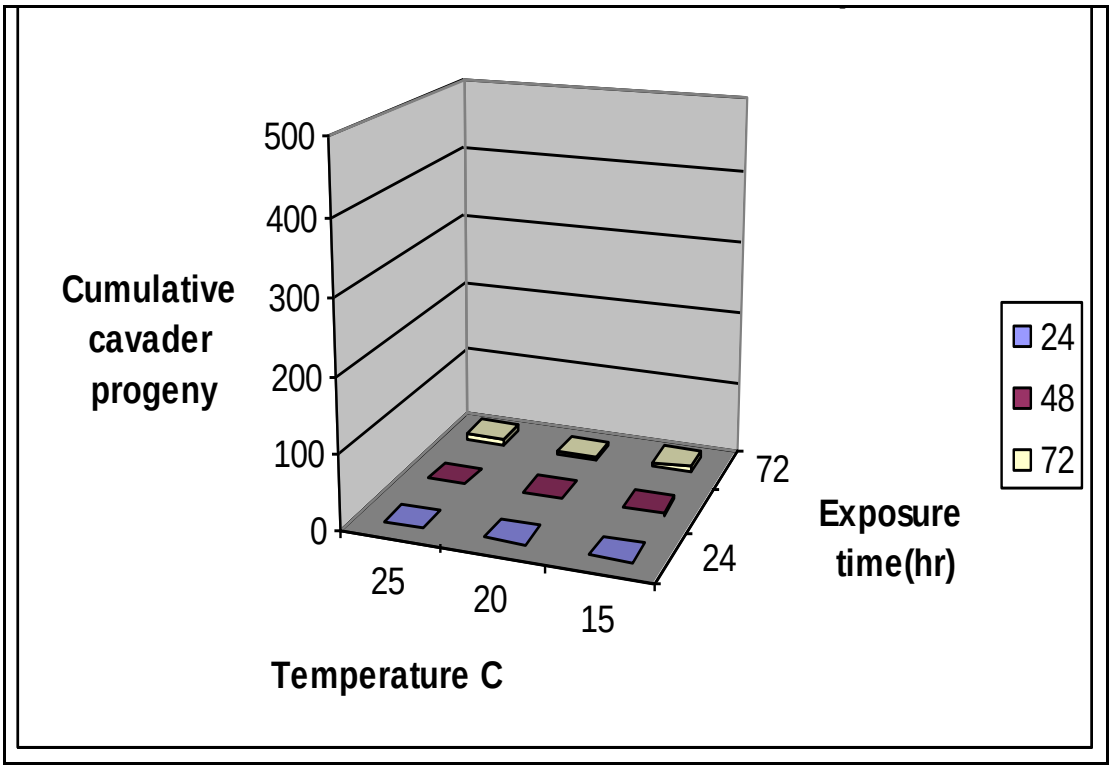

Figure 8: Influence of temperature and exposure time on H. bacteriophora progeny infected German cockroaches at adult stage.

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