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Deanship of Graduate Studies
Department of Applied Earth and Environmental Studies

Thesis Approval

Laboratory Investigations on the Tolerance of Entomopathogenic Nematodes *Heterorhabditis* and *Steinernema* Species to Desiccation and Hypoxia

Prepared By: Amani Jubra'al Awad Abu Sa'da
Registration No.: 20411669

Supervisor: Dr. Sameer Barghouthi
Co-Supervisor: Dr. Naim Iraki

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The names and signature of the examining committee members are as follows:

1.	Head of Committee:	Dr. Sameer Barghouthi	Signature----------
2.	Internal Examiner :	Dr. Azzam Saleh	Signature----------
3.	External Examiner:	Prof. Adnan Shqueir	Signature----------
4.	Co-Supervisor:	Dr. Naim Iraki	Signature----------

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Abstract

This study evaluated the effect of desiccation and hypoxia on the dauer juveniles (DJs) survival rate of eight *Heterorhabditis* and seven *Steinernema* strains collected from diverse habitats.

Tolerance to desiccation was assessed after incubation of DJs in 25% glycerol at 25°C for 24, 48 and 72 hr. Hypoxia was tested by sealing the DJs in 300 µl tubes at 25°C for 24, 48 and 72 hr.

Percent survival was determined microscopically by prodding each DJ. The local isolate *S.abbasi*-09 (Gaza isolate) was the most tolerant strain to desiccation stress which showed 93% survival rate after 72 hr of incubation, followed by *S.abbasi* (Oman isolate), however this isolate was the most tolerant to hypoxia stress (survival rate was 86% after 72hr of treatment). Tolerance to desiccation correlated poorly with hypoxia tolerance.

Differences in tolerance to hypoxia within and among isolates cannot be explained since mechanisms of tolerance to hypoxia are poorly understood. One hypothesis to be investigated is that nematode may enter into a quiescent stage where low level of metabolism reduces oxygen consumption. On the other hand, nematodes may have oxygen storage or regeneration capability to overcome the low oxygen concentration

H.tayseerae showed the least level of tolerance to desiccation while *H.tayseerae*-06 was the least tolerant isolate to hypoxic conditions. These two strains are considered to be weak bio-control agents in areas which have low humidity and oxygen respectively. The three *S.abbasi* isolates (*S.abbasi*-08, 09 and the reference isolate *S.abbasi*) and *H.bacteriophora*-05 are recommended for further studies, experimentation, and field test application in the area of bio-control especially in semi-arid and arid climates.

بحث مخبري لمدى تحمل بعض أصناف النيماتودا الممرضة للحشرات من الجنسين *Heterorhabditis* و*Steinernema* لكل من الجفاف ونقص الأكسجين

ملخص

إن دراسة أهمية تأثير العوامل البيئية وغير البيئية خاصة الجفاف ونقص الأكسجين في التربة على فعالية النيماتودات يساعد في تحديد الموسم والمناخ والطبيعة الجغرافية الملائمة لاستخدام هذه الديدان من أجناس النيماتودا.

إن التغييرات الموسمية للعوامل غير الحيوية، خاصة الجفاف ونقص الأكسجين، تنعكس سلبا على فعالية الطور المعدي لحشرات التربة كعامل هام في المكافحة الحيوية.

يهدف البحث لدراسة تأثير الجفاف ونقص الأكسجين على الطور المعدي لثمانية أطياف من النيماتودات التي تنتمي إلى جنس *Heterorhabditis* وسبعة أطياف تنتمي إلى جنس *Steinernema* والتي عُزِلت من مناطق بيئية مختلفة.

ولمعرفة مدى مقاومة النيماتودات للجفاف قمنا بوضع الطور المعدي من النيماتودات في بيئة تحتوي على 25% جليسرول على درجة 25 مئوية لثلاث فترات زمنية 24، 48 و 72 ساعة. أما قياس فعالية النيماتودات لنقص الأكسجين فقد تمت عن طريق وضعها داخل أنبوب محكم الإغلاق بحجم 300 مايكرولتر على درجة حرارة 25 مئوية لثلاث فترات زمنية 24، 48 و 72 ساعة. إن تحديد نسبة مقاومة النيماتودات بعد التعرض للصدمة يتم بواسطة وخز كل نيماتودا باستخدام رأس مدبب أثناء المشاهدة بالميكروسكوب.

سجل الصنف المحلي المعزول من غزة 09-*S. abbasi* أعلى قدرة تحمل للجفاف بعد 72 ساعة وهي 93% يليه صنف *S. abbasi* المعزول من عُمان وقد أظهرت النتائج قدرته العالية على تحمل نقص الأكسجين وسجل 86% بعد 72 ساعة من المعالجة.

أظهرت التحاليل الإحصائية ارتباطاً ضعيفاً بين مقاومة مختلف أطياف النيماتودا للجفاف وبين مقاومتها لنقص الأكسجين.

إن الفروقات بين مقاومة أطياف النيماتودا من كلا الجنسين لنقص الأكسجين غير واضحة وما زالت بحاجة إلى دراسة أعمق ولكن هناك فرضيات قد تفسر هذه الظاهرة وهي أن النيماتودات تدخل في مرحلة سكون تحت تأثير هذا النقص وبالتالي تحتاج إلى كمية أكسجين قليلة لتقوم بعملياتها الحيوية أو ربما باستطاعة النيماتودا خزن كمية معينة من الأكسجين لتقوم باستخدامها تحت الظروف الصعبة خاصة نقص الأكسجين.

سجل الصنف *H. tayseerae* الذي ينتمي إلى جنس *Heterorhabditis* أقل نسبة مقاومة للجفاف بينما سجل الصنف *H. tayseerae*–06 أقل نسبة مقاومة لنقص الأكسجين. يعتبر هذان الصنفان الأقل فاعلية من حيث إستخدامهما في التربة كوسيلة مكافحة حيوية خاصةً في المناطق الجافة أو شبه الجافة أوالتي تعاني من نقص الأكسجين.

صنف *S. abbasi*، والذي يتضمن الأطياف التالية: *S. abbasi*–08، 09 وَ *S. abbasi* والصنف *H. bacteriophora*-05 من الجنس *Heterorhabditis* تعد من الأطياف التي تستحق دراسات مستقبلية خاصة في مجال إستخدامها في الحقل، ويوصى بها للإستخدام كوسيلة للمكافحة الحيوية خاصة في المناطق شبه الجافة والتي تتميز بمناخ ذي درجات حرارة مرتفعة.

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Chapter One

Introduction

1.1. Overview

Chemical pesticides are commonly used for the control of many agricultural pests. However, many problems are associated with using pesticides, one major problem is the failure to control pests, this is due to the appearance of new pest strains that are resistant to applied pesticides. The lack of specificity and the harming of beneficial insects is another major problem (Brent, 1987).

Environmental contamination is yet a third problem related to pesticide application. As the applied pesticide or its breakdown products may persist in soil and water causing groundwater contamination, poisoning and destroying habitats of a large number of life forms (Dempster, 1987).

Therefore, to avoid environmental risks of chemical pesticides, efforts have been directed to the use of alternative methods in controlling pest populations. One of these alternatives is the application of biological control. Before commercializing nematodes as a safe alternative to chemical pesticide, it is important to determine their biological properties, such as heat tolerance, infectivity and tolerance to desiccation and hypoxia. Persistence of EPNs in soil, infectivity, development, maturation and reproduction of Entomopathogenic Nematodes (EPNs) are influenced by many abiotic factors. Therefore to overcome environmental obstacles and achieve successful biocontrol application, research has been conducted to select improved nematodes that withstand environmental stresses (Van Driesche and Bellows, 1996).

1.2. Statement of the study Problem

EPNs mainly from the genera *Heterorhabditis* and *Steinernema* lose fitness and capacity to act as an ideal bio-control agent in infecting and destroying agricultural insect hosts in response to seasonal fluctuations of the environmental conditions, especially desiccation (low humidity) and hypoxia (low soil oxygen).

1.3. Aim of the study

The aim of this study is to answer the following question:
To what extent desiccation and hypoxia will affect the dauer juveniles survival rate of eight *Heterorhabditis* and seven *Steinernema* species?

1.4. Objectives of the study

1. To compare variation in the tolerance to desiccation and hypoxia among local and reference isolates of different EPNs species.
2. To determine the correlation between climatic origin of different EPNs species and their survival rate under environmental stress conditions; especially in response to desiccation and hypoxia.
3. To identify and select the most tolerant nematode species to both desiccation and hypoxia to be used as an ideal bio-control agents.
4. To determine the correlation between the DJs tolerance of different EPNs species to desiccation and hypoxia.

Chapter Two

Literature Review

2.1. Introduction

Insect parasites belonging to the phylum Nematoda have been known since the 17th century. However, it was not before 1930's that serious consideration was given to using nematodes in the control of insects (Smart, 1995).

Nematodes, in general, are microscopic roundworms; the nematodes that are pathogenic to insects are called Entomopathogenic Nematodes (EPNs), only belong to the genera *Heterorhabditis* and *Steinernema* (Van Driesche and Bellows, 1996), which possess the potential to infect and control a wide range of agriculturally important pests. Such insect pests which cause disease to crops, soil and the environment. Thus EPNs are considered safe and ideal bio-control agents (Iraki *et al.*, 2000). In this study, biological control is viewed as "the use of environmentally harmless natural enemies to suppress a pest population to a minimal level rendering it less abundant and damaging" (Van Driesche and Bellows, 1996).

Biological control was used for controlling insects, mites, and weeds (Cameron *et al.*, 1989). Application of the method then broadened to include other invertebrates, plant pathogens, and even some vertebrates (Stirling, 1991). Over 1200 programs of biological control have been used to control more than 543 species of target insects. Several families of mites; which include: rust mites (Abou-Awad and El-Banhawy, 1986), tarsonemid mites (Huffaker and Kennett 1956) and the spider mites, all have been targets of biological control (Mc Murtry, 1982).

Currently, nematodes are used against soil-inhabiting insects that attack citrus, cranberries, turf, and ornamental plants. EPNs have been registered for commercial application in many countries: USA, Australia, Japan, and Europe (Caroli *et al.*, 1996). As a result, EPNs are considered a superior alternative to hazardous chemical pesticides (Jagdale, 1997).

2.2. The extent of chemical pesticides consumption in Palestine

Application of chemical pesticide is on the rise. The need to feed 11 billion humans and their farm animals, lead to intensive culturing and created a real need to control agricultural pests (Nebel and Wright, 2000). With reference to the survey conducted by the Applied Research Institute of Jerusalem (ARIJ, 1995), the total cultivated area of the West Bank is about two million dunums, one hundred thousand dunums of which are under irrigation while 1.6 million dunums are rain fed, the remaining 300 thousand dunums are fallow lands. In Gaza, nearly half the total area (about 175,000 dunums) is cultivable land (Laeremans and Sourani, 2006).

According to (Samer Farah thesis study 2007) results showed a significant increase in the number of chemical pesticides used especially during the last ten years: insecticides contribute 30.5% of applied pesticide, acaricide 7.9%, fungicides 36.2%, fertilizers 3.4%, herbicides 16.9% and others 5.1%.

As shown in Fig. (2.1), it is estimated that the total quantity of pesticide including methyl bromide used in the West Bank is around 493.83 tons per year (Saleh *et al.*, 1995).

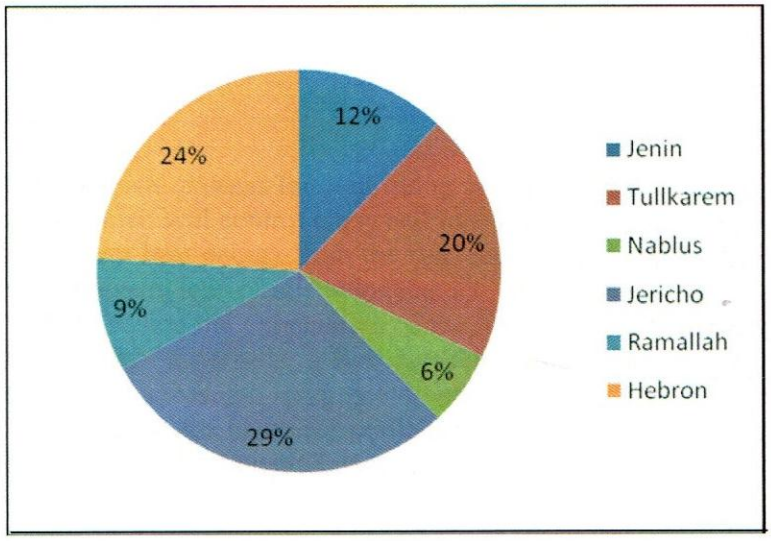

Fig.2.1: Average pesticide consumption in the West Bank according to districts (Saleh *et al.*, 1995).

In Gaza Strip 771 tons of pesticides are used these consist of: 36% as insecticides, 19% fungicides and 20% herbicides according to Palestinian National Information Center (PNIC, 1999). These different chemical pesticides can be grouped into six major chemical categories: chlorinated hydrocarbons, organophosphates, carbamates, thiocyanates, dinitrophenols, and fluoroacetates (Mastsumura, 1985).

Unfortunately, Palestinian farm workers use these pesticides extensively unaware of their risks and hazardous effects on their health and the environment as well. (Saleh *et al.*, 1995).

2.2.1. Risks of using chemical pesticides

Agriculture is the backbone of the Palestinian economy, contributing 33% and 24% of the Gross National Products in the West Bank and Gaza strip, respectively. The overuse of chemical pesticides in agriculture in recent years especially in irrigated areas has had many negative side effects (Saleh *et al*,.1995).

Evaluation of pesticide residual effect has shown that many pesticides are not readily degradable and constitute a major source of environmental contamination, especially of underground and surface water. Contaminated water resources lead to the poisoning of life forms including humans (Dempster, 1987). Furthermore, it disturbs the ecological balance as a result of the development of pesticide resistance and, in some cases, the increase in pest population.

Indeed, the number of insects that developed resistance to pesticides has increased dramatically since the 40's of the last century (Brent, 1987). In addition, the nonspecific action of chemical pesticides kills the pest's natural enemies, which are usually more sensitive to pesticides than the pest itself (Croft, 1990). Pollution of the environment and reduction of biodiversity at its current dramatic rate cannot be justified. Some countries have approved legislations to control and regulate pesticide application (Nebel and Wright, 2000).

As a result it would be useful to minimize the use of pesticides and encourage alternative methods in controlling field pest populations. Researchers are working toward finding a non-chemical, non-residual method for controlling field pests. There are two major substitutes to reduce the use of chemicals. Abiotic and biotic alternatives are efficient in controlling field pests and reducing the risks of damage to life forms, water, and environment (Bauer and Kaya, 2001). These alternatives are discussed below.

Chapter Five

Conclusions and recommendations

5.1. Conclusion

- The observed variation in hypoxia and desiccation tolerance among the tested isolates is consistent with the assumptions made in the statement of the problem and the objectives of this study.
- *Steinernema* isolates especially *Steinernema abbasi*-09 were found to have a relatively superior ability to tolerate high level of desiccation and low soil oxygen concentration.
- *H.bacteriophora*-05 isolate showed the highest level of desiccation and hypoxia tolerance compared to the eight *Heterorhabditis* spp.
- *H.tayeseerae* and *H.tayseerae*-06 isolates showed the lowest levels of tolerance compared to desiccation and hypoxia, respectively.
- No significant variation was observed in relation to tolerance to hypoxia among all stains.
- Extreme desiccation conditions (72 hr incubation in 25% glycerol) caused a significant decrease in survival rate among all strains.
- It is believed that the mechanism of desiccation tolerance in EPNs is different than that of hypoxia tolerance.

5.2. Recommendations

- *Steinernema abbasi* isolates should be the focus of field experiments addressed to a possible successful implementation of biological control in semi-arid to arid regions.

5.3. Future studies

- Field experiments should be addressed to determine a possible interaction between temperature, hypoxia and humidity and its effect on efficacy of EPNs as bio-control agents.
- Future studies must concentrate on the survival mechanisms of EPNs in hypoxia conditions.
- Hybridization through cross breeding to yield a superior EPNs hybrid with superior traits.