PROPOSED MEASUREMENTS FOR THE EFFICIENCY OF ENTOMOPATHOGENIC NEMATODES

By

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B.Sc. Agric. Sc. (Arid Agric.), Alexandria University, ٢٠٠٦

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Approval Sheet

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ABSTRACT

Mokhtar Abd El Raouf Abd El Aty Abo Naeem: Proposed Measurements for the Efficiency of Entomopathogenic Nematodes. Unpublished M.Sc. Thesis, Department of Plant Protection, Faculty of Agriculture, Ain Shams University, ٢٠١٣.

Accurate, quick and economic measurements of the quality of entomopathogenic nematodes (EPNs) were proposed and applied on two Egyptian nematode isolates, namely *Steinernema carpocapsae* (Weiser) BA٢ and *Heterorhabditis bacteriophora* Poinar S١. Proposed measurements on infective juveniles (IJs) included assays of the image analysis, dry weight, heat tolerance and heat shock. For testing their validity, results of proposed measurements were compared with those of the viability assay, traditional laboratory bioassay and semi-field assay of the nematode efficiency using larvae of *Agrotis ipsilon* (Huf.) and/or *Galleria mellonella* (L.). Five levels of the nematode quality were induced by storing IJs at ٢٨^{o}C for different durations and the resulting nematodes were used for detailed comparisons. The nematodes were finally sprayed in a corn field for assessing their field performance against larvae of *A. ipsilon* and *Spodoptera littoralis* (Boisd.). Results on nematode species showed a strong positive correlation (r > ٠,٩) between the image analysis, mean dry weight (MDW) and traditional bioassays of the nematode quality in laboratory and semi-field scales. Mean gray level (MGL) had a strong correlation with all other measurements of the quality of *S. carpocapsae* BA٢ except with the heat tolerance (HT) and heat shock (HS). The correlation was positive with all measurements except with the storage period (SP). MDW had also a significant strong correlation with all other measurements of the quality of *S. carpocapsae* BA٢ and *H. bacteriophora* S١ except with HT and HS. The heat assays (HT and HS) looked independent in their relation to other quality measurements of *S. carpocapsae* BA٢ and *H.*

bacteriophora S١. These two assays were also independent in relation to each other. *S. carpocapsae* BA٢ caused ١٠٠٪ larval mortality for *A. ipsilon* and *S. littoralis* in the field, while *H. bacteriophora* S١ induced ٨٣,٨١ and ٣٣,٥٧٪ mortality for the two species, respectively. For testing the potential of an EPN species, the image analysis is recommended as an accurate, quick and economic quality measurement instead of the traditional laboratory bioassay and semi-field assay.

Key words: *Steinernema carpocapsae*, *Heterorhabditis bacteriophora*, image analysis, dry weight, heat tolerance, heat shock, nematode efficiency.

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I- INTRODUCTION

The agricultural production still depends on the use of chemical pesticides. In ٢٠٠٠-٢٠٠١, an average of ٢,٣٦ × ١٠^٦ tons of active ingredients of pesticides was used in the world (**Kiely *et al.*, ٢٠٠٤**). The use of insecticides has caused adverse consequences such as soil and water pollution, impacts on food chains (**Wood and Ehui, ٢٠٠٥**) and developing resistance in insects (**Van Bortel *et al.*, ٢٠٠٨**). The biological control methods appear to be feasible options for pest management (**Lacey *et al.*, ٢٠٠٦**). Entomopathogenic nematodes (EPNs) have been effective in many agroecosystems (**Jenkins *et al.*, ٢٠٠٧**). EPNs of Steinernematidae and Heterorhabditidae have been known for decades (**Poinar, ١٩٩٠**). Both steinernematids and heterorhabditids pass through four juvenile stages before the maturing. Only the third-stage infective juvenile can survive outside the insect host and move from one insect to another. Infective juveniles (IJs) carry symbiotic bacteria (*Xenorhabdus* spp. for steinernematids and *Photorhabdus* spp. for heterorhabditids) in their intestines and release them in the insect haemolymph **(Akhurst and Boemare, ١٩٩٠)**. Nematodes invade through natural openings (spiracles, mouth and anus) or the cuticle of certain insects **(Bedding and Molyneux, ١٩٨٢ & Peters and Ehlers, ١٩٩٤)**. The nematode secretes proteinaceous substances, which inhibit the activity of the insect immune system (**Simoes *et al.*, ١٩٩٢**) and paralyse its nervous system (**Burman, ١٩٨٢**) to provide initial conditions for developing a bacterial colony. Bacterial cells proliferate and eventually kill the insect host within ٧٢ h. The bacterial species breaks down the haemolymph and provides a suitable diet for the nematode development. It also releases high antibiotic substances that protect the cadaver from the invasion of opportunistic organisms to allow undisturbed nematode development (**Kondo and Ishibashi, ١٩٨٦ & Akhurst, ١٩٩٠**). At ١٨-٢٨°C, the life cycle lasted ٦-١٨ days depending on the insect host and the nematode

species (**Poinar, ١٩٩٠ and Zioni *et al*., ١٩٩٢**). Invading IJs belonging to *Steinernema* develop into females or males and those of *Heterorhabditis* develop into hermaphrodites. One-three progeny generations develop inside one host and the reproduction continues until host nutrients are depleted. Nematodes become third-stage IJs that leave the cadaver to search for new hosts.

EPNs have a broad host range, search actively for hosts and present no hazard to mammals (**Gaugler and Boush, ١٩٧٩**). Although EPNs are listed among the important microbial control agents, no standard universal assays for evaluation of the nematode quality or efficiency exist **(Kaya *et al*., ١٩٩٣)**. The lack of fundamental knowledge on the nematode-bacterium-host interaction has blocked the development of such assays. The susceptibility of important insect pests to EPNs has been tested in laboratory bioassays. The most commonly used bioassay consists of the insect exposure to the IJ stage in filter paper arenas (**Kaya and Hara, ١٩٨٠ and Glazer, ١٩٩٢**). Assuming a positive correlation between the nematode dose and the host mortality, the probit analysis has been used to analyze data from dose-response tests and calculate the $LD_{٥٠}$. (**Morris *et al*., ١٩٩٠ and Glazer, ١٩٩٢**). However, when a pathogen is highly virulent, the probit analysis is not useful, since the bioassay results are likely biased by large errors in the dosage (**Burges and Thomson, ١٩٧١ and Huber and Hughes, ١٩٨٤**). The estimation of the nematode virulence by $LD_{٥٠}$ values is questionable, since a single steinernematid or heterorhabditid IJ is capable of killing an insect. The efficacy of field applications is affected by the nematode (invasion rate and bacterial release), the bacterium (establishment and multiplication rate), the host (behavior and immune response) and the environment (insect location, temperature, moisture, pH, soil composition and texture). **Molyneux (١٩٨٦), Fan and Hominick (١٩٩١), Mannion and Jansson (١٩٩٢) and Westerman (١٩٩٤)** developed a series of sand- or soil-based assays to simulate closely the effect of these factors on the