REPUBLIC OF TURKEY
ERCIYES UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF AGRICULTURAL SCIENCES AND TECHNOLOGIES

THE USE OF ENTOMOPATHOGENIC NEMATODES TO CONTROL CITRUS MEALYBUG, *PLANOCOCCUS CITRI* (HEMIPTERA: PSEUDOCOCCIDAE) IN LABORATORY CONDITIONS

Prepared By
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Supervısıor
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M.Sc. Thesis

August 2018
KAYSERI

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SCIENTIFIC ETHICS SUITABILITY

I am, Essa Saab Najm, hereby declare that this piece of work is the result of my research and that this work has never been submitted anywhere for any degree. All the sources of information used were obtained in accordance with academic ethics and have been duly acknowledged.



Essa Saab NAJM

SUITABILITY FOR GUIDE

The M.Sc. thesis entitled "**The use of entomopathogenic nematodes to control citrus mealybug, *Planococcus citri* (Hemiptera: Pseudococcidae) in the laboratory conditions"** has been prepared in accordance with Erciyes University Graduate Education and Teaching Institute Thesis Preparation and Writing Guide.

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ACCEPTANCE AND APPROVAL PAGE

This study entitled "**The use of entomopathogenic nematodes to control citrus mealybug, *Planococcus citri* (Hemiptera: Pseudococcidae) in the laboratory conditions"** prepared by ESSA SAAB NAJM under the supervision of Prof. Dr. Ramazan CANHİLAL was accepted by the jury as M.Sc. Department of Agricultural Sciences and Technologies; Graduate School of Natural and Applied Sciences, Erciyes University.

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APPROVAL

That the acceptance of this thesis has been approved by the decision of the Institute's Board of Directors with the 11/09/2018 date and 2018/42-33. numbered decision.

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Prof. Dr. Mehmet AKKURT

Director of the Institute

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Essa Saab NAJM

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ABSTRACT

Planococcus citri is worthy of attention and identified as one of destructive insect that attacks of citrus in diverse regions around the world with the showing the capacity of invading a high rate of fruit under certain ecological circumstances. Their enigmatic lifestyle and potential to improve endurance to chemicals forces researchers towards improving and find alternative techniques. Entomopathogenic nematodes (EPNs), which are deadly pathogens against a large group of hosts, are identified as a reliable biological control agents for a diversity of destructive insect pests that attacks the agricultural crops. Besides, chemical substances exhaust natural enemy groups that regularly set control over mealybug populations well prior citrus harvest in (March-May). The disability of chemical substances to dominate *P. citri* in a successful way and the increasing awareness globally of the harmful ecological impacts and health risks, which are linked with pesticides, are urging citrus farmers to discover an alternative approach to chemical control. Entomopathogenic nematodes of the order Rhabditida relating to both families of Heterorhabditidae and Steinernematidae, have demonstrated to be helpful biocontrol factors of a diversity of destructive insect species that attack of citrus. Many laboratory bioassays were carried out to explore the potential of EPNs to dominate *P. citri*. Adult female *P. citri* were chosen for sensitivity to 5 indigenous EPNs. *Planococcus citri* was pronounced to be most sensitive to *Steinernema carpocapsae* and *Heterorhabditis indica* with 62% and 68% mortality, respectively. Nematode efficacy was not significantly influenced when subjected to 20°C, 25°C and 30°C. The results showed that all nematodes were capable of infecting the insect in a short period and mortality increased by increasing the time and concentration.

Keywords: Biological control, entomopathogenic nematodes, *Steinernema*, *Heterorhabditis*

LABORATUVAR KOŞULLARINDA TURUNÇGİL UNLUBİTİ, *Plancoccus citri* (HEMİPTERA: PSEUDOCOCCİDAE)'NİN MÜCADELESİNDE ENTOMOPATOJEN NEMATODLARIN KULLANIMI

Essa Saab NAJM
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Yüksek Lisans Tezi, August 2018
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ÖZET

Turunçgil unlubiti**,** *Plancoccus citri* dünyanın birçok yerinde narenciyeye zarar veren tahribkar bir böcektir ve belirli ekolojik koşullar altında yüksek oranda verim kaybı oluşturur. Esrarengiz yaşam döngüleri ve kimyasallara karşı dayanıklılık oluşturma potansiyelleri, araştırmacıları alternatif mücadele teknikleri geliştirmeye yönlendirmektedir. Önemli bir zararlı grubuna karşı entomopatojen nematodlar (EPN), tarımsal ürünlerde zarar oluşturan geniş bir böcek grubu için güvenilir doğal düşmanlar olarak bilinirler. Ayrıca, kimyasal insektisitler, turunçgil unlubiti polulasyonunu turunçgil hasatı öncesi (Mart-Mayıs) iyi bir şekilde baskı altında tutan doğal düşman gruplarına önemli ölçüde zarar vermektedir. Kimyasal maddelerin *P. citri*'yi başarılı bir şekilde baskı altına almadaki başarısızlığı ve pestisitlerle ilgili zararlı ekolojik etkiler ve sağlık riskleri, çiftçileri kimyasallara alternatif yaklaşımlar bulmaya teşvik etmektedir. Rhabditida takımı Heterorhabditidae ve Steinernematidae familyasına ait entomopatojen nematodlar, turunçgillere zarar yapan bir çok zararlı böceğe karşı biyolojik mücadele etmeni olabileceğini göstermiştir. Turunçgil unlubitinin mücedelesinde başarılı olabilecek EPN'leri araştırmak amacıyla birçok laboratuvar çalışması gerçekleştirilmiştir. Bu çalışmada, *P. citri* dişilerinin, 5 endemik EPN'a laboratuvar koşullarında reaksiyonu belirlenmiştir. *Plancoccus citri, Steinernema carpocapsae* ve *Heterorhabditis indica*'ya, sırasıyla %62 ve %68 ölüm oranıyla en hassas bulunmuştur. Nematod etkinliği, 20°C, 25°C ve 30°C'deki sıcaklık artışlarından önemli derecede etkilenmemiştir. Sonuçlar, tüm nematodların turunçgil unlubitini kısa bir sürede enfekte ettiğini ve ölüm oranının zamana ve doza bağlı olarak arttığını göstermiştir.

Anahtar kelimeler: Biyolojik mücadele, entomopatojen nematodlar, *Steinernema, Heterorhabditis*

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CHAPTER 1

INTRODUCTION

Mealybug (Hemiptera: Pseudococcidae) is a tiny, soft-bodied insect. Mealybug feeds by a penetration and sucking mouthparts used to extract juice from the plant [1]. The name mealybug is used to express a huge family, which is called Pseudococcidae. The reasons behind calling this insect "mealybug" because of the mealy wax which covers family's body and commonly secreted white powdery [2, 3]. During feeding, this insect causes direct and indirect damages such as deformation, the death of the plant, with some species being able to transmit plant virus diseases [4]. Extensive populations contaminate foliage with sticky honeydew excretions, leads to the growth of sooty mould, resulting in fruit infested with mealybugs, and consequent loss of market value [5, 6].

Planococcus citri (Risso), generally indicated to as the citrus mealybug, was originally described by Risso in 1813 from samples discovered on *Citrus* spp. in south France [7]. The citrus mealybug is universally distributed [8-11], highly polyphagous and commonly the most devastating sorts of its family [8, 12]. Commercially produced hosts include each of coffee, vineyards, citrus and a variety of ornamental plants [9, 12].

Globally, Spain considered the largest exporter of fresh citrus, following South Africa. Around 60% of entire citrus which is produced in South Africa exported to Europe and the Middle East [6]. *Planococcus* citri has been recorded as a serious pest of citrus in Africa [5, 6] [6], Australia [10, 13], the Mediterranean Basin [14, 15], as well as North, Central and South America [16, 17]. When ecological conditions are ideal, citrus and oleander mealybug population concentrations can grow quickly, and have the possibility to infect up to 100% of fruit, even if early spring invasion levels could hardly be detected [6]. The citrus mealybug, *P. citri* is a major kind of pests that happen or occur on ornamental crops which planted in the greenhouses [18]. Chemical control such a

measure is only designed to correct, mealybugs are most regularly controlled with chemical substances [15].

Early chemical control identifying with mealybugs can be problematic due to their indefinite performance and their tendency to be hidden in protected regions where the chemical substances cannot enter and are coated with waxes layers which capable of protecting it from the chemicals [19]. Mealybugs also have the capability to promote its resistance quickly against insecticides [8, 20, 21]. The continuous applications of broad-spectrum pesticides, such as organophosphates, are also partially responsible for citrus mealybug outbreaks [22]. Also because of environmental and regulatory concerns associated with chemical use [23-27], there is much interest in biorational approaches [28, 29].

Entomopathogenic nematodes (EPNs) (Steinernematidae and Heterorhabditidae) are obligate parasites of insects [27, 30, 31]. They are mutualistically associated with bacteria (*Xenorhabdus* spp. and *Photorhabdus* spp. for steinernematids and heterorhabditids, respectively). Infective juveniles (IJs), the only free-living stage, enter hosts through natural openings (mouth, anus, and spiracles), or in some cases, through the cuticle. After entering the host's hemocoel, nematodes release their symbiotic bacteria, which are primarily responsible for killing the host, defending against secondary invaders, and providing the nematodes with nutrition [32]. The nematodes molt and complete up to three generations within the host after which IJs exit the cadaver to search out new hosts [27, 33].

These nematodes are effective bio-control agents of a variety of economically important insect pests [27, 34] and they have been used in controlling insect pest for about 25 years, extending their usage from high value markets to large area crops, including forestry [27]. A number of studies indicate applications of EPNs can result in high levels of control for a variety of pests. Despite the progress that has been made in the use of EPNs [35], knowledge about their natural host range and their efficacy on insect populations as biological control agents is still limited [36]. Our overall goal was to determine the potential use of several EPNs for *P. citri* suppression. A crucial element to be successful in any biological control program with EPN is the pairing the most suitable nematode with the defined host, and relative pathogenicity among various

nematodes is one of the important factors to consider in determining suitability [27, 37, 38]. Therefore, this research aimed to determine the potential of EPNs (*Heterorhabditis indica*=216-H, *H. Bacteriaphora*=FLH, *Steinernema feltiae*=KCS, *S. Carpocapsae*=E-76, and *S. Bicornutum*=MGZ) in the laboratory conditions to be used in the biological control of citrus mealybug, *P. citri*.

CHAPTER 2

GENERAL INFORMATION AND LITERATURE REVIEW

2.1. Mealybug (Hemiptera: Pseudococcidae)

Mealybug (Hemiptera: Pseudococcidae) is a tiny, soft-bodied insect. Mealybug feeds by a penetration and sucking mouthparts used to extract juice from the plant [1]. The name mealybug is used to express a huge family, which is called Pseudococcidae. The reasons behind calling this insect "mealybug" because of the mealy wax which covers family's body and commonly secreted white powdery [2, 3]. During feeding, this insect causes direct and indirect damages such as deformation, the death of the plant, with some species being able to transmit plant virus diseases [4]. Extensive populations contaminate foliage with sticky honeydew excretions, leads to the growth of sooty mould, resulting in fruit infested with mealybugs, and consequent loss of market value [5, 6].

2.1.1. Origin, host range and geographical distribution

Planococcus citri (Risso), generally indicated to as the citrus mealybug, was originally described by Risso in 1813 from samples discovered on *Citrus* spp. in south France [7]. The citrus mealybug is universally distributed [8-11], highly polyphagous and commonly the most devastating sorts of its family [8, 12]. Commercially produced hosts include each of coffee, vineyards, citrus and a variety of ornamental plants [9, 12]. Generally, *P. citri* is considered as a pest of ornamental plants in the glasshouses in the temperate region of Europe [39].

2.1.2. Morphology and identification

A female is wingless, soft, oval, clearly segmented, and coated by a waxy or mealy secretion with the head, thorax and abdomen completely combined [5, 6]. Mature male

is teeny, crisp, lives for a short time, with non-functional mouthparts, one couple of functional wings and two lengthy antennae and anal filaments [10].

2.1.3. Citrus mealybug, Planococcus citri

For many years the citrus mealybug, *P. citri* was confused with the vine mealybug, *Planococcus ficus* (Signoret) [7, 40].

2.1.4. General life cycle

Mealybugs are sexually dimorphic. Its life cycle varies from male to female. The immature stages, identified as crawlers, superficially have qualities or features, especially those of appearance look alike the adult females and gradually become bigger, maturing by every molt [10]. Although some types of mealybug are parthenogenetic, a large number of it reproduce sexually [1]. Through sexual reproduction stages, the adult females produce and produce a substance (pheromones) which leads to male attraction for mating. Adult female mealybugs produce thin, waxy threads over the dorsal length of its body, which become into a cottony mass, in which 300 to 600 eggs are produced [11]. The duration demanded to lay an egg and hatching is dependent entirely on the temperature. In the summer, laying an egg continues about (6 to 14) days and the eggs hatch after approximately (6 to 10) days [41]. During the stages of development, females subject to three nymphal (immature) instars, while males have four [40]. Following several of instars, males spin a cocoon, in which they molt a number of times, then an adult male appears. Males are short-lived, fragile, winged insects that live only to reproduce, also hold no mouthparts with which to feed [13, 20].

2.1.5. Seasonal cycle

On *Citrus* spp., mealybugs happen in most parts of the tree canopy. Although they are principally found on leaves and twigs, some females move to the main branches or trunk of their host tree to lay eggs [42]. Mealybugs move from one tree to another when crawlers are blown by the wind, or when transported by moving factors, like ants [43]. Mealybugs live through the winter in crevices as well as cracks or leaf axils throughout the tree [2, 10]. Even though the whole three life stages happen in winter, the egg phase is dominant [41]. Throughout the spring and beginning of the summer, shortly after fruit set, prime generation of nymphs transfer to the foliage and colonize young growth and

small fruit [42]. Mealybugs settle into protected areas, such the calyx of small fruit, for feeding and reproducing [6]. *P. citri* was considered the most popular type of insects on Citrus limon. The researchers have noticed that population densities of *P. citri* differ considerably from one citrus grove to another, depending on the impact of various microclimates as well as the host trees on female fertility. Citrus cultivars, like Eureka lemon produce fruit during the year. As a result, population densities of *P. citri* are significantly higher on such cultivars, in comparison with those of others, such as Clementines which just bears in short duration of the year. On Clementine trees, mealybug quantities decreased significantly to be approximately unobservable levels following harvesting through April or May. Therefore, the survival of individual insect (mealybug) appears to be considerably influenced by crop phenology. Another relevant research noticed the mealybugs prefer to crowd in fruit and to live into the fruit calyxes or another protective location, like in the middle of fruit clusters, or within bud-mite caused fruit deformations [6].

2.1.6. Economic importance of mealybugs on citrus

Globally, Spain considered the largest exporter of fresh citrus, following South Africa. Around 60% of entire citrus which is produced in South Africa exported to Europe and the Middle East [6]. *P. citri,* citrus mealybug has been recorded as a serious pest of citrus in Africa [5, 6] [6], Australia [10, 13], the Mediterranean Basin [14, 15], as well as North, Central and South America [16, 17].

When ecological conditions are ideal, citrus and oleander mealybug population concentrations can grow quickly, and have the Possibility to infect up to 100% of fruit, even if early spring invasion levels could hardly be detected [6].

2.1.7. Damage

Mealybug invasion causes a diversity of direct and indirect forms of damage to citrus [5], by feeding on whole parts of the tree, excluding the roots [44]. Damage caused by mealybugs is linked with wilting, flower drop and fruit, besides impaired fruit appearance, caused by growth deformations and sooty mould [14]. Sooty mould sediments produce a dark, superficial layer on branches, fruit as well as leaves [10],

which diminishes the possibility of the plant photosynthetic, with fruit covered in fungal growth being regarded undesirable for the export market [6].

It damages horticultural and ornamental crops that are primarily grown in greenhouses such as the oleander (Nerium oleander), garden croton (Codiaeum variegatum), lady slipper orchid (Cymbidium orchid) and chrysanthemum (Chrysanthemum morifolium) [45]. Calyx feeding leads to deform the fruit, which can be observed such as bulges and indentations on fruit [2]. Grapefruit responds to feeding damage as well as prone to promote deformations growth [6]. Young growth flush infestations can also cause severe leaf deformities [5], with such leaves being characteristically bent [6].

The oleander mealybug preference is to feed on leaves in addition to it has the potential, which leads to extensively leaves damage. In the beginning of the season feeding damage generated by mealybug can also lead to an increment fruit drop. In particular, each of grapefruit, mandarins and navel oranges are sensitive to high mealybug invasion, which leads to turn the fruit to yellow as well as to drop prematurely [46].

2.1.8. Chemical control

The *P. citri*, citrus mealybug is a major kind of pests that happen or occur on ornamental crops which planted in the greenhouses [18]. Chemical control such a measure is only designed to correct, mealybugs are most regularly controlled with chemical substances [15]. Early chemical control identifying with mealybugs can be problematic due to their indefinite performance and their tendency to be hidden in protected regions where the chemical substances cannot enter and are coated with waxes layers which capable of protecting it from the chemicals [19]. Mealybugs also have the capability to promote its resistance quickly against insecticides [8, 20, 21]. The continuous applications of broad-spectrum pesticides, such as organophosphates, are also partially responsible for citrus mealybug outbreaks [22]. In addition most chemical products phased out in Turkey and European Countries due to harmfull effects to human and environment. The most effective period at which to utilize insecticides is during the spring, at that time the first crawlers appear, as the effectiveness of the chemical substances considerably diminishes mealybugs right away when settling under calyxes and other protected regions [6].

2.1.9. Biological control

Typical biological control usually includes the release of an unfamiliar, natural enemy so as to diminish and dominate population quantities of an introduced pest kind, with the intended long-lasting establishing of the biological control factor [47, 48].

In contradiction with, initiative biological control is when high quantities of the biological control factor are released with the purpose of diminishing the pest group in the nonexistence of the establishment and lasting impacts of the biological factor [48]. The most familiar actual enemies of *P. citri* includes parasitic wasps, predators, and ants. It is unfortunate that, parasitoids can just attack mealybugs when they are located on uncovered locations, consequently they are incapable to reach *P. citri* when it is buried or sneaking beneath bark or in deep fissures [49].

To exacerbate the problem, ants interfere with parasitism and diminish parasitoid amounts straight by causing individuals death. Biological control has been the main option for chemicals and that is indicated one of the bases in integrated pest management (IPM) plans [50]. Hymenopteran parasitoids are available in large quantities as a biological dominant factor of mealybugs in large parts of world [5] and are consequently applied to dominate mealybugs by augmentative releases [51]. The predator, *Cryptolaemus montrouzieri* and parasitoid, *Anagyrus pseudococci* are widely spread throughout the world [6].

- *Anagyrus pseudococci* (Girault) (Hymenoptera: Encrytidae) is familiar as biological dominant factor of mealybugs (*P. citri*) [52] and is economically superior as a vital organic control factor for farming and glasshouse pest mealybugs and to restrain the *P. citri* [53]. Nowadays, it is generally settled in California also applied for biological control toward the invasive (*P. ficus*) [54, 55]. *A. pseudococci* is a local parasitoid of vine mealybug in several countries including South Africa, Turkey, Argentina and Italy [53, 56]. Evolution is quicker than other parasitoids applied to manage mealybugs in whole temperatures [57]. Anagyrus pseudococci was the most usually raised parasitoid on vine mealybugs in California [58]. A. pseudococci are classified as an important natural enemy against the citrus mealybug [6].

- *Cryptolaemus montrouzieri* (Mulsant) (Coleoptera: Coccinellidae) has agreed satisfactory control, but most success has been achieved in the glasshouse using numerous hymenopterous parasitoids in combination with this predator [57]

- *Coccidoxenoides peregrinus* is large-scale produced and commercially available; that parasitoid is not more effective in dominating the oleander mealybug than the citrus mealybug [6].

- *Leptomastix dactylopii* (Hymenoptera: Encyrtidae) can be applied in different situations; in floriculture, greenhouses, indoor gardens, botanical gardens. The wasp prefers higher temperatures. Its effectiveness is optimal in the summer but also in spring and early autumn, they may well be active and effective. Day length and humidity are not very critical for this species. *Leptomastix dactylopii* is very sensitive to side effects of chemical control products, particularly at the stage where a population is being built up.

In situations with severe degradation or deterioration in hot spots we recommend to combine the product with larvae of the predatory beetle *Cryptolaemus montrouzieri*. *Leptomastix dactylopii* can also be used successfully in combination with the parasitic wasp *Leptomastidea abnormis*.

2.2. Entomopathogenic nematodes

Nematode parasites of insects have been identified for (1601s-1700s), but it was just that significant attention was paid to using a nematode to dominate an insect. Now, it is known that nematodes carry a symbiotic bacterium, which presents required food for the nematodes, which are affecting a host [59].

Entomopathogenic nematodes (EPNs) have been utilized for dominating insect since the 1930s [59, 60]. These nematodes have been utilized in a successful way against insects, which inhabit the soil and also the insects on the ground insects in cryptic habitats [61-63]. They have numerous perfect features to be biological domination factor; causing the death of host in 48 h, easy production economically in vivo or in vitro, seeking to find the effective host, long-term efficiency, easy to use, has the adaptability with most chemicals, and safe to environmental [59].

In the late 1970s, there has been a tremendous investigation and economic interest in entomopathogenic nematodes and their related bacteria. This interest was raised, particularly by (1) a lack of enough tools to dominate insect pests which residing at the soil in an efficient, ecologically acceptable method and (2) the ability of generating those nematodes monoxenically in the laboratory which was followed by scale-up to commercial reproduction levels [6].

Insect pests are traditionally controlled by applying chemical pesticides. These pesticides have detrimental effects on the environment, specifically through contaminated groundwater, causing food chain contamination, which, in turn, threatens human and animal health Insects also develop resistance to insecticides, which is proving to be a major driving force for seeking alternative control methods [64]. Entomopathogenic nematodes (EPNs) include two sorts, which are (Steinernema and Heterorhabditis) [65, 66]. It is considered a significant bio-control factors used for insect pests, which are residing in the soil in several valuable cropping systems [65]. The high cost of nematodes related to chemical insecticides was the reason behind the utilization of nematodes primarily against insect pests which are residing in the soil in high-value crops. EPNs have reflected an antagonistic attitude against to the plant-parasitic nematodes nevertheless that mechanisms are not quite understood. It has been newly found that soil utilization of EPNs can induce elements of systemic resistance in ornamental plants either of Arabidopsis and also hosta [6]. The widespread use of chemical pesticides has negative effects on the environment and beneficial insects which give impetus to explore alternative approaches in order to plant protection. The bio-logical control which includes the utilization of natural enemies handling pests and minimizing their negative influences on plant health is considered an optimal alternative. Amongst the diverse range of natural enemies, entomopathogenic nematodes (EPNs), Steinernematidae as well as Heterorhabditidae, serve as significant bio-control factors for insect pests which are residing in the soil. EPNs have a mutual benefit connected with entomopathogenic bacteria: *Photorhabdus* and *Heterorhabditis*, and *Xenorhabdus* with Steinernema [67]. The partnership with the bacteria gives the ability to the EPNs to use a various collection of insects as hosts. In addition, perform as an attractive bio-control factor against to targeted insect pests, EPNs may offer further advantages for cultivation. Earlier investigations have reported declines in populations

of a diversity of plant-parasitic nematodes following the utilization of EPNs into the soil [67-69]. Novel investigations have exhibited another fascinating potential advantage of EPN treatments. EPNs can promote plant immunity. It was observed that soil utilization of Steinernema carpocapsae diminished reproduction of the foliar nematode Aphelenchoides fragariae in hosta leaves which indicate the potentiality of induced systemic resistance [70]. Further studies revealed that soil application of *S. carpocapsae* infective juveniles and their symbiotic bacteria *Xenorhabdus nematophilus* activated the production of key defense enzymes and hormones in hosta and Arabidopsis thaliana leaves, and induced the expression of a plant defense protein promoter PR1 gene in A. thaliana [71]. They proposed that the difficult antagonistic impact of EPNs on plant-parasitic nematodes may be at least slightly referred to this EPN induced plant resistance response [71].

2.2.1. Life cycle

Either of steinernematids and heterorhabditids proceeds through 4 immature levels before becoming matured [72]. The third level, in which they are known as infective juveniles (IJs) or dauer, is the only free-living, non-feeding stage within the life cycle, and happens naturally into the soil.

Once a host is found, nematodes penetrate the host via natural openings such as (mouth, spiracles and anus), or by entering via thin outer layer in its cuticle. Directly entering the host's outer cuticle generally happens in heterorhabditids, which are provided with a dorsal tooth. Once inside the host's haemocoel, the nematodes function is to release a symbiotic bacterium that reproduces swiftly, causing hosts death by septicaemia during (24 - 48) hour [6]. Symbiotic bacteria linked with each of steinernematids and heterorhabditids fall in the gender, *Xenorhabdus*, and *Photorhabdus*, sequentially [71]. Nematodes feed on each of host tissue and bacteria cells, broken down by growing bacteria, and then proceed to the 4th and adult level. According to the host size, nematodes moult and accomplish one to three generations inside the host [33]. The bacteria also release antibiotic items that assist in maintaining the insect body and that defend it against opportunist organisms [73].

When the source of food is exhausted, infective juveniles are produced that leave the host for seeking a new host. Infective juveniles can remain alive in soil for numerous

months via entering a nearer to anhydrobiotic status [30]. Either of steinernematids and heterorhabditids have several method regarding for reproduction. First-generation of heterorhabditids are bisexual while self-fertilization following generations composed of cross-fertilizing females and males [74]. In steinernematids, cross-fertilizing males and females happen in all generation [75].

2.2.2. Safety and impact on non-target organisms

On studying the possible negative impact of EPNs and their symbiotic bacteria [76], observed no persuasive of any sharp or chronic toxicity to humans or other vertebrates, or any meaningful long-term impact on non-target invertebrate groups. There are not any safety worries that should prevent EPNs from being applied as biological control factors have been specified [77].

Even though EPNs are mainly aimed for controlling insect pests which residing at the soil, these biological control factors have confirmed to dominate some of the foliar pests [78], however, their broad host range is a concern, as they could probably have an adverse effect on beneficial organisms [79].

2.2.3. Compatibility of EPNs with agrochemicals

Employment of EPNs in a successful way in an integrated pest management program, it is necessary to examine their compatibility with agrochemicals. Each of time and labor expenses should be saved, it is regularly desired to tank mix one or more agrochemicals or biopesticides. Nematodes may also be used shortly after another chemical materials, or together with another control factors in order to reach preferable dominance of a single pest [80].

2.2.4. Foliar application

Commonly, nematodes are utilized to foliage as a hydrous suspension, utilizing conventional agrochemical spray tools [81, 82]. Nematode considered as a living organism, the water which is used for applying should not be densely treated with chlorine, and the temperature should be in the scope of (4 to 30) °C [83]. Managing foliar pests in a successful way with the assist of nematodes is a quite challenging, while conditions on the ground are not optimum for nematode to be alive [84, 85].

To progress post-application nematode survival rates, high relative moisture is required to retard desiccation, as nematodes require a water film to assure their survival and to keep its mobility [83]. On foliage, the nematode survival percentages progresses in each of tropical circumstances and rainy times as well [84]. As wind also advances the evaporation, it is convenient not to utilize nematodes on the windy days [86]. Applied materials can be advanced to retard desiccation [87]. Water- retention factors have been utilized successfully to restrain the evaporation of application suspensions at foliage, therefore expanding the term of nematode survival [88-90]. Schroer and [77] examined the impact of a formulation including 0.3% Rimulgan® (surfactant) and 0.3% of the polymer xanthan (antidesiccant) on post-application nematode survival and infection in foliage bioassays.

The results showed that nematodes enter into hosts in the first hour after utilization. The researchers exhibited that foliar treatment formulas should attempt to provide ideal ecological conditions for nematode host infection, instead of enhancing nematode survival. Hence, useful to choose a nematode or species that has the capability to infect hosts and energetically locate. When utilizing nematodes, the temperature should be recognized. The infectivity of most EPN species is extremely diminished at temperatures overtake 32°C or below 15°C [91]. Exposing nematodes to sunlight is also problematic, as they are receptive to the ultraviolet (UV) radiation of certain wavelengths [92, 93]. Such exposure can be prevented by applying nematodes at dusk [94].

2.3. Previous works on controlling mealybugs by entomopathogenic nematodes

In the literature, numerous studies have concentrated on the use of entomopathogenic nematodes to control mealybugs.

Stuart et al. **[95]** studied the sensitivity of the mealybug, to infect by several species and strains of EPNs. Four types of nematode were tested at the doses of 10, 50,100 as well as 500 IJ. EPNs was examined in laboratory's assay (sand-dish assay). The results revealed that no one of the EPNs species generated a considerable level of mealybug mortality in a comparison with the control after a 48-hr in the duration of exposing. *H. bacteriophora* gave a considerable level of mortality percent reach up 93.8 % after a duration of 120 hours at the highest dose which is 500 IJs. *H. bacteriophora* did not

give a considerable level of mortality percent at the dose of 100 IJs. Although it gave a considerable level of mortality percent reach up 93.8 % at the dose of 500 IJs. *H. indica* gave a mortality level reaching up 56% at a dose of 100 IJs and a mortality level reaching up 100% at a dose of 500 IJs.

Stokwe et al. **[96]** carried out various bio-assays to identify the potential of EPN in order to dominate the obscure mealybug, *P.viburni*. This investigation concerned revealed that *P. viburni* varies considerably in sensitivity from one nematode type to another. Commonly, *P. viburni* presented higher sensitivity to Heterorhabditis in comparison with Steinernema sorts.

De Waal et al. **[97]** examined the potential of 3 South African sorts (*H. bacteriophora, H. zealandica and S. khoisanae*) in order for infecting the adult female stage of *P.ficus*. The results showed that *P. ficus* was more responsive to Heterorhabditids (with mortality percent reach in the range of 70% - 100%), in a comparison with *S. khoisanae* which gave mortality percent 54%. Also perceived that in response to the infection stage, the mealybugs released a yellowy fluid as a potential defense attitude, and its color altered and converted to darkness following the infection. The results, together with those taken from the sand-dish bio-assay showing that nematodes have a favorable potential by applying against *P. ficus*.

Alves et al. **[98]** examined the potential of several Heterorhabditis in order to control the coffee root mealybug. Laboratory experiments revealed that most of the strains tested were highly virulent against the target host. The EPN strain JPM3 gave 65% mortality, and was the only treatment that caused significantly higher mealybug mortality than did the control treatment.

Niekerk et al **[99]** reported that the *P. ficus* is the most critical kinds of mealybug identified to infest citrus in South Africa. Numerous laboratory bioassays were carried out to determine the possibility of entomopathogenic nematodes to dominate *P. ficus*. Grown female *P. ficus* was chosen for sensitivity to six indigenous kinds of the nematode. *P. ficus* was observed to be most sensitive to *S. yirgalemense and H. zealandica*, leading to 96% and 65% mortality, sequentially. The process of developing of nematodes after infecting grown female *P. ficus* revealed that both *H. zealandica and S. yirgalemense* were capable to perform their lifecycles inside the host. This study

explained *P. ficus* to be a proper host for each of *H. zealandica and S. yirgalemense*, with both nematode species showing having significant capacity for future use in the range of controlling the *P. ficus*.

Niekerk et al **[100]** reported that the *P. citri* is the most critical kinds of mealybug identified to infest citrus in South Africa. Numerous laboratory bioassays were carried out to determine the possibility of entomopathogenic nematodes to dominate *P. citri.* Grown female *P. citri* were chosen for sensitivity to six indigenous kinds of the nematode. *P. citri* was observed to be most sensitive to *S. yirgalemense* and *H. zealandica*, leading to 97% also 91% mortality, sequentially. The process of developing of nematodes after infecting grown female *P. citri* revealed that both *H. zealandica* and *S. yirgalemense* were capable to perform their lifecycles inside the host.

2.4. Previous works on entomopathogenic nematodes

Hazir et al. **[79]** explored the possibility of nematodes, which are generally found in Turkey, as vital control factors against pests there. Various entomopathogenic nematodes beneath each of the genera, *Steinernema* and *Heterorhabditis* are being generated in a commercial process and utilized as biological control agents against common soil in several territories of the world. These nematodes, which are linked mutually with bacterias such as *Xenorhabdus* bacteria and *Photorhabdus* bacteria. Propose a number of features because they have a broad host area, ruin their hosts in 2 days, could be readily produced in each of (vivo and vitro), can be utilized with conventional spray apparatus, are harmless to humans, have no adverse impacts on the atmosphere are perceived.

Sunanda et al. **[101]** reported that in vitro researches on the effect of temperature on the life cycle of *S. abbasi* and *H. indica* showed that IJs were able to enter *G. mellonella* at the range of temperature among 20° and 30°C including both of the species. The highest number of IJs of *S. abbasi* and *H. indica* appear from *G. mellonella* larvae through 30°C and also 25°C, sequentially. The duration (hours) required to the appearance including both of the 1st and 2nd generation of *S. abbsi* and *H. indica* upon *G. mellonella* were noticed to be 72-96 and 168-192 hr. and 72-96 and 144-240 hr., sequentially. The quantity of male and female in the sexual reproduction were 25IJs for both EPNs. Nevertheless the sex proportion was 1: 14.9 through 25°C, 1: 11.6 through

20°C and 1: 11.3 was lowest through 25°C (12.5: 32.5) then at 20°C (1: 14.7) and 30°C (1: 16.6) *S. abbasi* and *H. indica*, sequentially. As the temperature dropped, the appearance of IJs also reduced significantly.

***Hussein et al* [102]** in the comparison with the two nematodes, it was noticed that the storage possibility of *S. carpocapsae* juveniles was simply better to that of *H. bacteriophora* in case of the formation with Calcium alginate. The pathogenicity of the two EPNs in the three formations was examined against the wax moth, *Galleria mellonella*. It was concluded that *S. carpocapsae* was more malignant than *H. bacteriophora* to the *G. mellonella* in all experimented formulae.

***Goldasasteh et al* [103]** reported that both genders; females, as well as males, grew into adults at the range of temperature from (15-32) ºC and (18-32) ºC, sequentially. The whole of the first insect larva has died at each of (10, 12, and 37) ºC. Lower temperatures which are (10, 12, and 15ºC) leads to the higher egg death-rate than death-rate at the higher temperatures which are (32, 35, and 37) ºC. At all temperatures except 15ºC, the highest percentage of death-rate was noticed in the first insect larvae. The gender proportion was female-biased among (15 and 30) ºC, however, there was a bit high number of males at 32ºC. The highest grown longevity of both genders was obtained at (18 and 25) °C, sequentially.

***Vieux et al* [104]** wrote that *P. ficus* is the prevailing mealybug sorts in South Africa. Their growing objection to chemicals has driven to the exploration of novel control techniques. EPNs underbeneath to the families of (Heterorhabditidae and Steinernematidae) are harmful insect pathogens.

***Jaffuela et al* [105]** described that **e**ntomopathogenic nematodes abbreviated as (EPNs) which occur regularly in agricultural lands and participate in activities of controlling of insect pests which are dwelling in the soils. The systems in the biological environment alter in their soil criteria such as moisture, pH, and texture, and natural locality situations (root existence, plant cover, etc.), which have the ability to influence the EPNs soil food web assembly in an unexpected method. EPN action is to cause the death to insects. EPN activity was higher in grasslands.

Sulistyanto et al **[106]** reported that Entomopathogenic nematodes classified from beneath the genders of Heterorhabditis and Steinernema. EPNs are accessible in the market for utilization as pest controller. They are connected with bacteria of the families from Xenorhabdus and Photorhabdus. Mainly, the process of developing of EPNs as biological domination factors is aimed to cost reduction.

Ruan et al **[107]** worte that the entomopathogenic nematodes are restricted to parasites which is living on insects in the environment. The previous investigations, entomopathogenic nematodes were recorded to show a number of movements in each of existence and nonexistence of insect hosts.

Kapranas et al **[108]** reported the purpose of entomopathogenic nematodes (EPNs) implemented against the growing period for population deactivation is increasingly identified as an efficient option to plant protection by utilizing chemicals. Kapranas reported that the results from a series of experiments over 2 years by applying two sorts of EPN, *S. carpocapsae* and *H. downesi* with various foraging approaches. EPN was implemented to the stumps of felled pine trees. The results proposed that both kinds of EPN are evenly effective in defeating pine weevil inhabitants.

Guy et al **[109]** wrote that the EPNs are efficient bio-control factors for diverse insect pests. The use of nematodes is restrained by environmental conditions such as high cost and temperature. Guy investigated the capacity by applying air conditioning. By storing nematode infective juveniles at 9 °C for (3 weeks) prior to application – in order to improve efficiency, at each of moderate and also low temperatures. The conditioned *H. megidis* were beforehand explained to supply improved control of vine weevil (*Otiorhynchus sulcatus*) when implemented to plants at the steady temperature which was 9 °C. Guy et al shows similar results for *S. carpocapsae* conditioned for the duration among (3–6 weeks) at the temperature of 9 °C. Results showed that with conditioning the stored sorts for the duration of 3 weeks pre-application at 9 °C) increased the efficiency of both sorts toward vine weevil.

CHAPTER 3

MATERIALS AND METHODS

3.1. Source and rearing of *Planococcus citri*

Planococcus citri was provided us by Assoc. Prof. Dr. Murat Muştu in the Department of Plant Protection, Faculty of Agriculture, Erciyes University. The identification of *P. citri* that utilized in the current investigation was tested using morphological diagnosis and molecular techniques [110]. It was reared in the laboratory on sprouting potatoes (*Solanum tuberosum*) and pumpkin (*Cucurbita pepo*). Cultures were stored in cages (650×350×590 mm) with a ventilation system, enabling airflow efficiently. Particularly, only the adult female mealybugs were chosen for utilizing in experiments.



Fig 3.1. Rearing *P. citri* on sprouting potatoes (*Solanum tuberosum*) and pumpkin (*Cucurbita pepo*).

3.2. Source of nematodes

The EPNs, *H. indica, H. bacteriaphora, S. feltiae, S. carpocapsae,* and *S. bicornutum* (Rhabditida: Steinernematidae and Heterorhabditidae) used in this study obtained from the surveys conducted in Kayseri and Kahramanmaraş Provinces [111, 112]. Nematodes (Table 3.1) were cultured on the wax moth, *Galleria mellonella* larvae reared in 1L jars in incubator at 30±2^{o}C and 60-70% relative humidity in a nutrient medium consisting of soybean flour, corn flour, dry bread yeast, honey, glycerin, milk powder and wheat flour [113]. The IJs were collected through the first week of appearance and saved horizontally at 7-9ºC in 500-ml vented culture flasks containing 150 ml of distilled water. Nematodes were applied within three weeks following collection. Flasks were shaken weekly to increase aeration and nematode endurance. Nematode concentrations utilized in investigations were determined by using the process which is developed by [114].

Table 3.1. Species, strain, habitat, locality and GenBank accession numbers of entomopathogenic nematodes used in our study.

Entomopathogenic nematodes species	**Strain**	**Habitat**	**Locality**	**GenBank accession number**
S. feltiae	KCS-4-S	Pine-poplar	Kocasinan-Kayseri	KX462908
S. bicornutum	MGZ-4-S	Strawberry	Erciyes University Campus Area-Kayseri	KX462912
S. carpocapsae	E-76	Grassland	Erciyes University Campus Area-Kayseri	KX462907
H. indica	216-H	Olive	Karatas-Kahramanmaraş	KP970842
H. bacteriaphora	FLH-4-H	Orchard	Felahiye-Kayseri	KX462939

3.3. Biological study

The current qualitative research entails detailing and recording the transformation of both *S. carpocapsae* and *H. indica* inside adult female *P. citri* individuals. Thirty bioassay plates, each containing five evenly distributed mealybugs, were prepared for both *S. carpocapsae* and *H. indica*. Adult female mealybugs were inoculated within the multi-well bioassay, at the concentration of 100 IJs for both the nematodes species. Three days after the inoculation, the infected mealybugs of each nematode sorts were chosen and washed with purified water to get rid of the excess IJs from the outside cover of the insect. A totality of fifty insects was distributed on four moist filter papers within 13-cm diameter Petri dishes, which is sealed with PARAFILM®. Twenty-five individuals for each nematode sorts were picked randomly dissected and examined at the same time, starting at 48 h and subsequently again one to two days consequently until the life cycle was ended or desisted to continue. While the life cycle progression of the nematodes, and the color variations in the mealybug, and were reported throughout. To establish whether the two nematode sorts would end their life cycle in *P. citri*, ten infected individuals were left in White traps.

3.4. The effect of concentration, temperature and time on the citrus mealybug mortality by entomopathogenic nematodes

24-well bioassay trays (Flat bottom, Nunc™, Cat. No. 144530) were used. The bottom of each alternate well was lined with a circular (13 mm diameter) piece of filter paper. The impact of increasing concentrations of *H. indica, S. feltiae, H. bacteriaphora, S. carpocapsae, and S. bicornutum*) was determined at humidity level of 95±5 RH **[115]**. Four multiwell plates, with each containing twelve evenly distributed adult female *P. citri*, were prepared (4 replicates; 48 insects) for each of the different nematode concentrations (80, 100, 150 and 200 IJ/female mealybug in 50 µl per well). For the control four multiwell plates were used. Each mealybug in the treatment was inoculated by 50 µl of water only per well by using micropipette. Multiwell plates were lined with filter paper discs and covered with fine netting to let airflow while preventing mealybugs from escaping. After inoculation, mealybugs were incubated in a growth chamber at 20°C, mealybug mortality was assessed at 24, 48 and 72 h. Previous processes were repeated for remaining temperatures 25 and 30°C. The

experiment was repeated on a separate date. The data of both experiments were pooled for analysis.

3.5. Statistical Analyses

All statistical analyses were performed using Genstat software v12.1.0 (Genstat.VSNI international, 2009). Data were analyzed using ANOVA, with post-hoc comparison of means using Duncan method. Significant differences were determined on a 95% probability level. LC_{50} and LC_{90} values were calculated using probit analysis

CHAPTER 4

RESULTS

4.1. Biological studies

The stages of change in inoculated mealybug and nematode evolution were reported for the duration of 10 days following exposure to IJs of *H. indica* (216-H) and *S. carpocapsae* (E-76) as clarified in Table 4.3, Fig. (4.1. and 4.2.). Mealybugs overcrowded with *H. indica* (216-H) converted to a rusty-brown color in comparison to those overcrowded with *S. carpocapsae* (E-76), which diversified in color conversion from none to different shades of brown Fig. (4.3, 4.4). No color changes were recognized for mealybug eggs that continued viable. Hermaphrodites with eggs were recognized three days following inoculation of mealybugs with *H. indica* (216-H) with larvae were noted in the hermaphrodites following five days Fig. 4.1., and following eight days IJs appeared and the life period was ended. Adult males Fig. 4.2. (B) and females with eggs Fig. 4.2. (A) were noticed on the third day following inoculation of mealybugs with *S. carpocapsae* (E-76). Following five days, females with larvae within it were noticed, and following seven days IJs appeared and the life period was ended. Both nematode sorts achieved one generation before IJs appeared. The results showed that the increasing thickness of viscosity in mealybugs at the internal matrix it was due to the higher bacterial intensity levels.

Table 4.1. Nematodes growth stages of mature female *P. citri* treated with infective juveniles of *H. indica* (216-H) and *S. carpocapsae* (E-76).

Nematode types	No. of days	Stage of nematode development
H. indica (216-H)	2	Immature
	3	Hermaphrodites with eggs
	4	Hermaphrodites with eggs
	6	Hermaphrodites with larvae
	8	Infective juveniles
	10	Infective juveniles
S. carpocapsae (E-76)	2	Immature
	3	Males and females with eggs
	4	Males and females with eggs
	5	Males and females with larvae
	8	Infective juveniles
	10	Infective juveniles

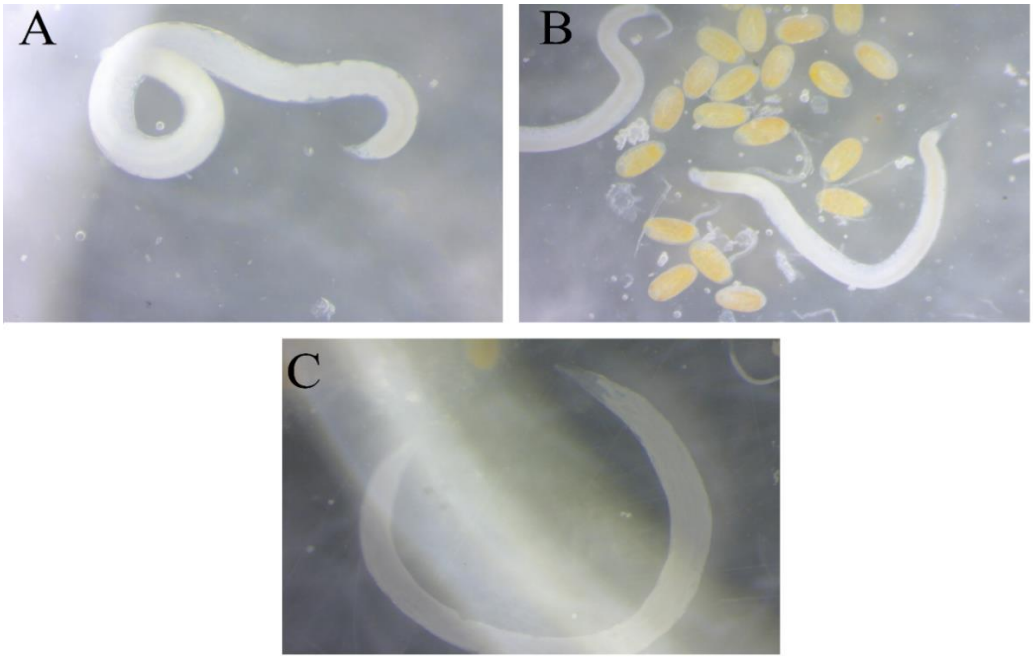


Fig. 4.1. The developing process of nematode recorded after the infection showing (A) *H. indica* with eggs displayed after three days, (B) *H. indica* also with eggs displayed after four days (C) *H. indica* with larve displayed after five days.

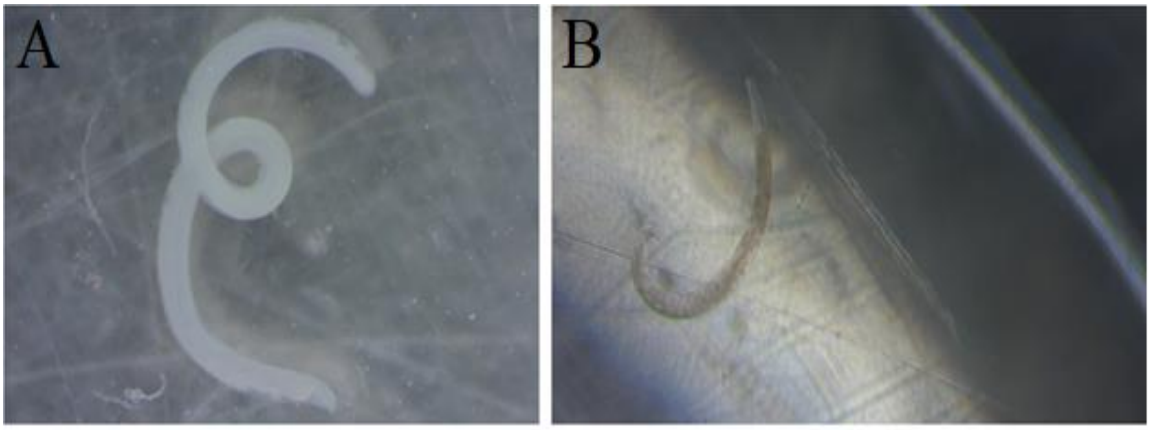


Fig. 4.2. The developing process of nematode recorded after the infection showing (A) *S. carpocapsae* female with eggs displayed after three days (B) *S. carpocapsae* male displayed after five days.

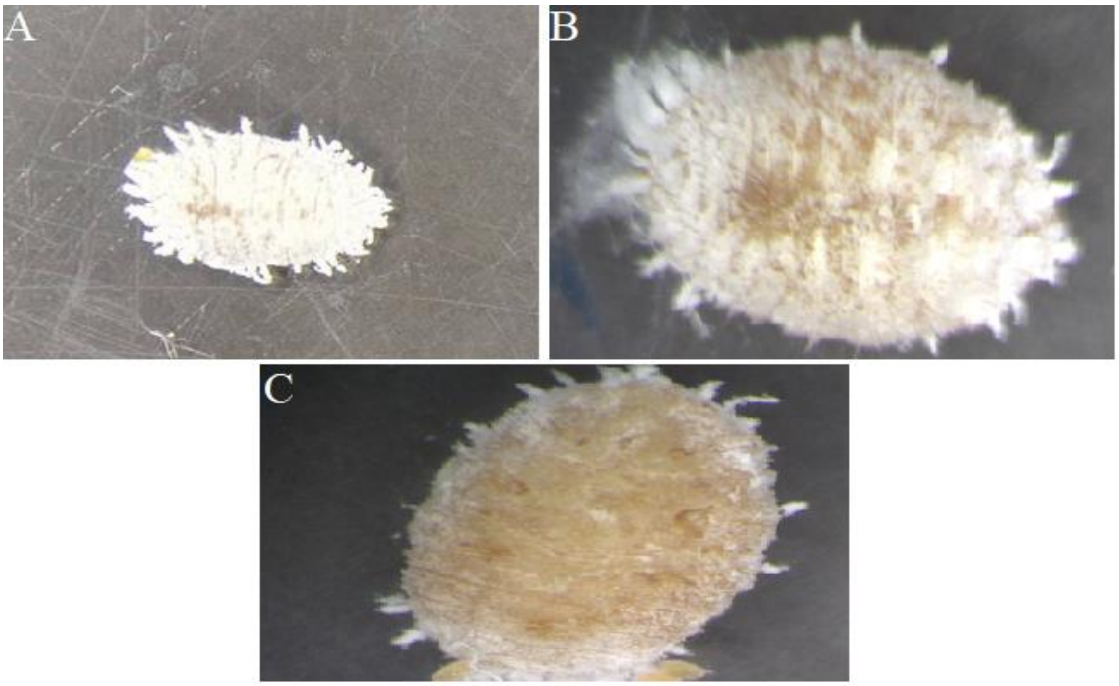


Fig. 4.3. Phases of changing in *P. citri* appearance (A) prior to infection. (B and C) *S. carpocapse*, 48 h following the infection.

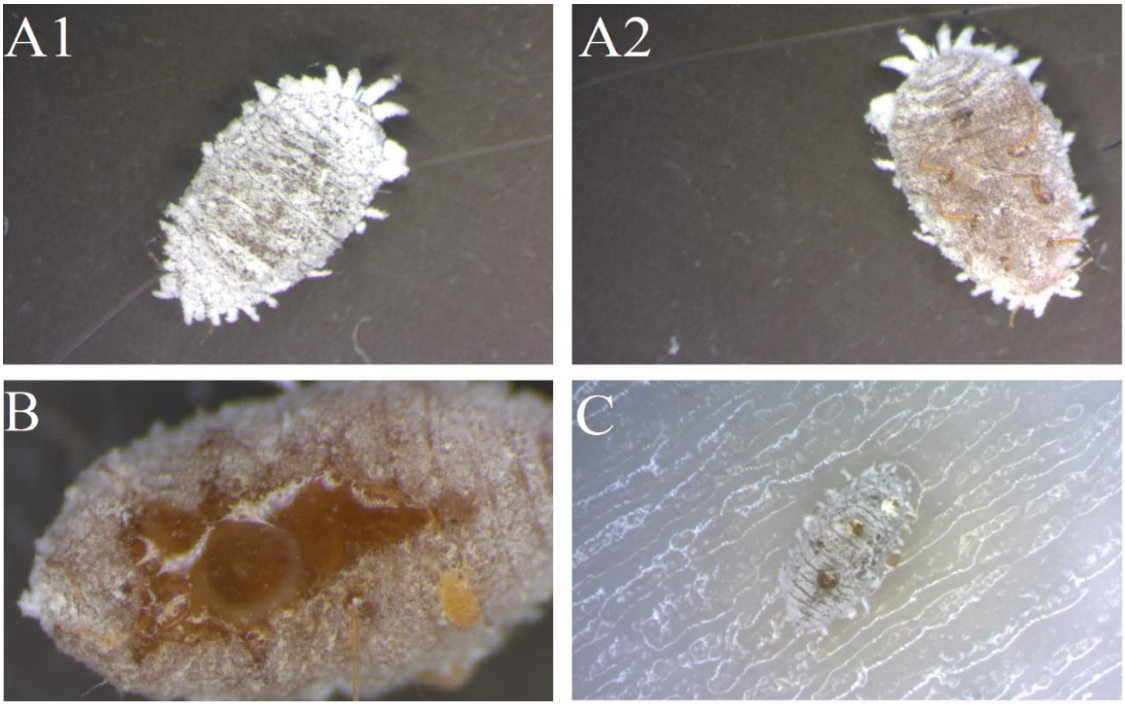


Fig. 4.4. Phases of changing in *P. citri* appearance following the infection with *H. indica* (A1, A2, B, and C) where the EPNs is obviously shown in figure B.

4.2. The effect of concentration, temperature and time on the citrus mealybug mortality by entomopathogenic nematodes

The analysis of variance of percentage mortality of *P. citri* caused by the five nematode species; *H. indica*, *S. feltiae*, *H. bacteriophora*, *S. carpocapsae* , and *S. bicornutum* as influenced by dose (80 , 100 , 150 , 200 IJ / female), and temperature (20, 25, 30^{o}C) , and time (24 , 48 , 72 hour) were analyzed using a one-way ANOVA (Table. 4.1). It showed significant differences between nematode sorts (F=294.12; df=5, 648; P=0.001) and dose (F=210.05; df=3, 648; P<0.001), temperatures (F=73.14; df=2, 648; P< 0.001), and time (F=73.14; df=2, 648; P< 0.001), beside the two way interaction of nematode x dose (P<0.001), nematode x temperature (P<0.001), dose x temperature (P<0.005), nematode x time (P<0.001), dose x time (P<0.001), and temperature x time (P<0.001). Although, the three ways and the four ways interactions were not significant (P>0.05).

The mortality rates of the nematode species tested in the female of *P. citri,* citrus mealybug generally increased as the dose and duration increased. The lowest mortality rate occurred at 20°C, while the highest mortality rate occurred at 25°C temperatures (Table 4.4. and Fig. 4.5.). The LC_{50} and LC_{90} values were 126, 202, 162, 146, and 165

IJs, and 307, 481, 375, 343, and 523 IJs for *H. indica*, *S. feltiae*, *H. bacteriophora*, *S. carpocapsae*, and *S. bicornutum*, respectively (Table 4.3).

Table 4.2. Analysis of variance of percentage mortality of *P. citri* caused by nematode species as influenced by dose, temperature and time.

Source of variation	Degrees of freedom	Mean Square	F-Calculated	P-Calculated
Nematod	5	11402,39	294,12	<.001
Dose	3	8143,25	210,05	<.001
Temp*	2	3074,3	79,3	<.001
Hour	2	39129,14	1009,33	<.001
Nematod x Dose	15	522,61	13,48	<.001
Nematod xTemp	10	664,78	17,15	<.001
Dose x Temp	6	148,35	3,83	<.001
Nematod x Hour	10	650,09	16,77	<.001
Dose x Hour	6	372,37	9,61	<.001
Temp x Hour	4	168,1	4,34	0.002
Nematod x Dose x Temp	30	28,18	0,73	0.857
Nematod x Dose x Hour	30	23,75	0,61	0,95
Nematod x Temp x Hour	20	53,12	1,37	0.129
Dose x Temp x Hour	12	6,78	0,17	0.999
Nematod x Dose x Temp xHour	60	3,36	0,09	1.000
Residual	648	38,77		
Total	863			

* Temp=Temperature

Table 4.3. LC_{50} and LC_{90} values of five entomopathogenic nematodes on *P. citri*.

Nematode species	LC_{50}	LC_{90}	Regression equation	P-Value
H. indica	126	307	Y=-0.896+ 0.0061 X	0.917
S. feltiae	202	481	Y= - 0.93 + 0.0046 X	0.723
H. bacteriophora	162	375	Y= - 0.974 + 0.0036 X	0.908
S. carpocapsae	146	343	Y= - 0.958 + 0.0065 X	0.913
S. bicornutum	165	523	Y= - 0.964 + 0.0045 X	0.996

Table 4.4. Mortality rates (percentage) of five EPNs on *P. citri* at four concentrations after 24, 48, and 72 hours at 20, 25, 30 ^{o}C (216-H=*H. indica*, KCS=*S. feltiae*, FLH=*H. bacteriaphora*, E-76=*S. carpocapsae* and MGZ=*S. bicornutum*)*.

Dose	24 h				48 h				72 h			
Time	80 IJ	100 IJ	150 IJ	200 IJ	80 IJ	100 IJ	150 IJ	200 IJ	80 IJ	100 IJ	150 IJ	200 IJ
20^{O}C												
216 H	4.0 Aa*	4.2 Aa	8.3 Aa	12.5 Aa	20.8 Ba	22.9B a	29.2 B a	41.7 A a	25.0 Ba	27.1 Bab	33.3 Bab	47.9 Aa
KCS	2.1 Ba	2.1 Ba	4.2 ABab	6.3 Aab	10.4 C b	12.5 BC b	16.7 AB b	20.8 A c	16.7 Cb	20.8 BCbc	25.0 ABb	31.3 Ab
FLH	2.1 Aa	6.3 Aa	8.3 Aa	10.4Aa	16.7 Bab	22.9 ABa	25.0A a	31.3A ab	20.8 Ca	25.0 BCab	31.3 ABab	39.6 Aab
E-76	4.2 Ba	8.3 ABa	10.4ABa	14.6Aa	18.8 Cab	25.0BC a	31.3AB a	39.6A a	27.1 Ca	31.3 BCa	39.6 Ba	47.9 Aa
MGZ	4.2 Aa	6.3Aa	8.3Aa	8.3Aab	14.6B ab	16.7B ab	22.9 ABab	29.2 Ab	20.8 Ca	25.0 BCab	29.2 ABb	37.5 Ab
Control	0.0 A a	0.0 Aa	0.0 Ab	0.0 Ab	8.3 Ab	8.3A b	8.3 Ac	8.3 Ad	14.6 Ab	14.6A c	14.6 Ac	14.6A c
25 ^{O}C												
216 H	8.3 Ca	12.5 BC a	18.8 AB a	25.0 Aa	25.0 Ca	31.3 C a	47.9 Ba	58.3 Aa	35.4 Da	43.8 Ca	58.3 Ba	68.8 Aa
KCS	2.1 Aa	4.2 Abc	6.3A bc	8.3 Abc	10.4 Cb	14.6 BCbC	20.8 ABc	25.0 Ac	18.8 Cb	22.9 BCc	27.1 ABc	33.3 Ac
FLH	4.2 Ca	8.3 BC ab	14.6 ABab	20.8 A ab	22.9 Ca	29.2 C a	39.6 Ba	47.9 Aa	29.2Ca	37.5 Cab	47.9 Bb	58.3 Ab
E-76	6.3 Ca	10.4 BCab	16.7 ABab	22.9 Aab	20.8 Ca	29.2 B a	37.5 Bab	52.1 Aa	31.3Ca	39.6 Cca	52.1 Bab	62.5 Aab
MGZ	6.3 Aa	8.3A ab	10.4A b	14.6Ab	18.8 Ba	20.8B b	27.1 Bbc	39.6 Ab	27.1 Ca	29.2 BCb	37.5AB b	45.8A bc
Control	0.0 Aa	0.0A c	0.0 Ac	0.0 Ad	4.1 Ac	4.1 Ac	4.1 Ad	4.1 Ad	10.4 b A	10.4 dA	10.4 Ad	10.4 Ad
30^{O}C												
216 H	4.2 C a	8.3 BCa	14.6 ABa	18.8 Aa	22.9Ca	27.1 Ca	39.6Ba	54.2Aa	31.3 Ca	35.4Cca	47.9Ba	60.4Aa
KCS	0.0 Aa	0.0 Aa	2.1 Abc	2.1A bc	6.3 Bb	8.3 ABCbc	12.5ABcd	16.7Ad	14.6Acd	16.7 Ad	18.8 Ad	22.9 Acd
FLH	8.3 Ba	10.4 Ba	14.6Aa	20.8A a	22.9Ca	25.0 BCab	31.3Bab	43.7Ab	27.1Cab	31.3BCab	39.6Bab	52.1Aa
E-76	2.1B Ca	6.3 BCa	12.5ABa	16.7 Aa	12.5Cb	16.7 BCb	22.9ABbc	31.3Ac	22.9 Cbc	25.0 BCbc	33.3ABbc	41.7Ab
MGZ	0.0 Aa	4.2 Aa	4.2Abc	6.3Ab	12.5Bb	14.6 ABb	18.8ABbc	22.9Ad	18.8 Bbc	20.8 ABcd	25.0 ABcd	29.2A c
Control	0.0 Aa	0.0 Aa	0.0 Ac	0.0 Ac	6.3 Ab	6.3A c	6.3A d	6.3A e	12.5 dA	12.5 dA	12.5 Ae	12.5 Ad

*Means followed by same lower-case letter within a column and upper-case letter within a line are not different according to Duncan's multiple range test ($P \leq 0.05$).

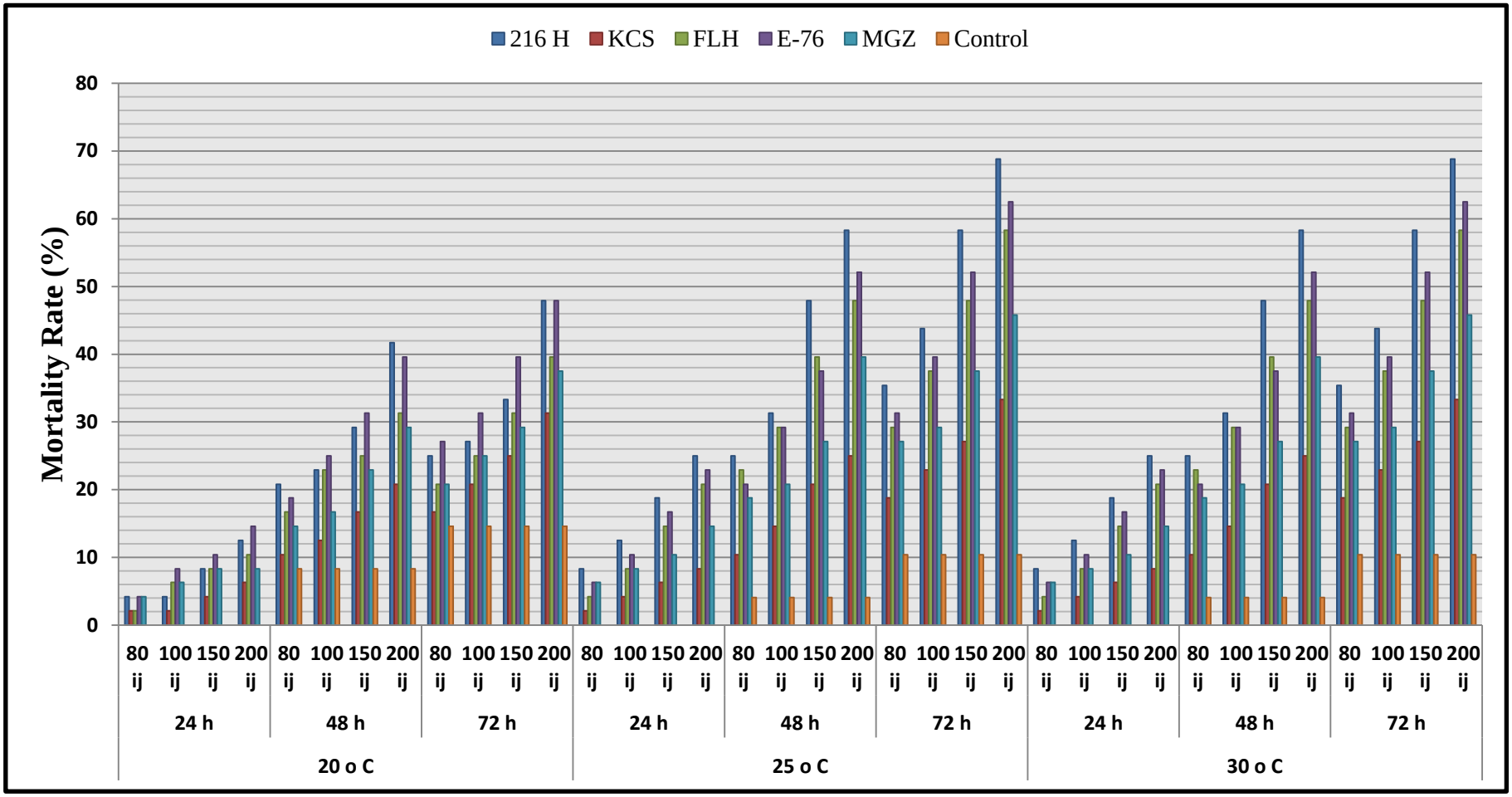


Fig. 4.5. Mortality rates (percentage) of five EPNs on *P. citri* at four concentrations at 20, 25, 30^{o}C after 24, 48, and 72 hours (216-H=*H. indica*, KCS=*S. feltiae*, FLH=*H. bacteriaphora*, E-76=*S. carpocapsae* and MGZ=*S. bicornutum*).

4.3. The efficacy of entomopathogenic nematodes aganits citrus mealybug at 20°C for different concentrations

Mortality rates at 20^{o}C in *P. citri* ranged from 2.1% to 47.9% (Table 4.5 and Fig. 4.10). In *P. citri* female, the lowest mortality rate at 24 h was in the KSC and FLH species with 2.1% in the 80 IJ application dose (Table 4.5 and Fig. 4.7) while the highest mortality was in the E-76 species with 14.6% in 200 IJ application dose(Table 4.5 and Fig. 4.10); The lowest mortality rate on 72 h was 16.7% with KCS at 80 IJ application dose (Table 4.5 and Fig. 4.7), while the highest mortality 47.9% occurred with 216-H and E-76 at 200 IJ administration dose (Table 4.5 and Fig. 4.10). There was no significant between nematode species after 24 h for 80 and 100 IJ even with control, but at the dose of 150 and 200 IJ nematode species showed significant difference than control (Table 4.5).

After 48 hours, the difference with control was more pronounced especially at 200 IJ dose. In addition, the heterorhabditid species (*H. indica* and *H. bacteriaphora*) showed significant more mortality rate than steinernematid species (*S. feltiae*, *S. carpocapsae* and *S. bicornutum*) (Table 4.5 and Fig. 4.6).

After 72 hours exposure of *P. citri* to nematode species, the differences between doses were more pronounced with the doses of 150 and 200 IJ which caused a significant higher mortality than 80 and 100 IJ doses. The species of *Heterorhabditis* also showed significant higher mortality than the species of *Steinernema* (except *S. carpocapsae*), and both species were significantly higher than control as shown in (Table. 4.5 and Fig. 4.6).

Table 4.5. Mortality rates (percentage) of five EPN species on *P. citri* at four concentrations after 24, 48, and 72 hours at 20°C*.

20°C				
Time	**24 h**			
Dose	**80 IJ**	**100 IJ**	**150 IJ**	**200 IJ**
H. indica	4.2 Aa	4.2 Aa	8.3 Aa	12.5 Aa
S. feltiae	2.1 Ba	2.1 Ba	4.2 ABab	6.3 Aab
H. bacteriophora	2.1 Aa	6.3 Aa	8.3 Aa	10.4 Aa
S. carpocapsae	4.2 Ba	8.3 ABa	10.4 ABa	14.6 Aa
S. bicornutum	4.2 Aa	6.3 Aa	8.3 Aa	8.3 Aab
Control	0.0 A a	0.0 Aa	0.0 Ab	0.0 Ab
Time	**48 h**			
Dose	**80 IJ**	**100 IJ**	**150 IJ**	**200 IJ**
H. indica	20 Ba	22.9 Ba	22.9 Ba	41.7 Aa
S. feltiae	10.4 Cb	12.5 BCb	16.7 ABb	20.8 Ac
H. bacteriophora	16.7 Bab	22.9 ABa	25.0 Aa	31.3 Aab
S. carpocapsae	18.8 Cab	25.0 BC a	31.3 ABa	39.6 Aa
S. bicornutum	14.6 Bab	16.7 Bab	22.9 ABab	29.2 Ab
Control	8.3 Ab	8.3 Ab	8.3 Ac	8.3 Ad
Time	**72 h**			
Dose	**80 IJ**	**100 IJ**	**150 IJ**	**200 IJ**
H. indica	25.0 Ba	27.1 Bab	33.3 Bab	47.9 Aa
S. feltiae	16.7 Cb	20.8 BCbc	25.0 ABb	31.3 Ab
H. bacteriophora	20.8 Ca	25.0 BCab	31.3 ABab	39.6 Aab
S. carpocapsae	27.1 Ca	31.3 BCa	39.6 Ba	47.9 Aa
S. bicornutum	20.8 Ca	25.0 BCab	29.2 ABb	37.5 Ab
Control	14.6 Ab	14.6 Ac	14.6 Ac	14.6 Ac

*Means followed by same lower-case letter within a column and upper-case letter within a line are not different according to Duncan's multiple range test ($P \leq 0.05$).

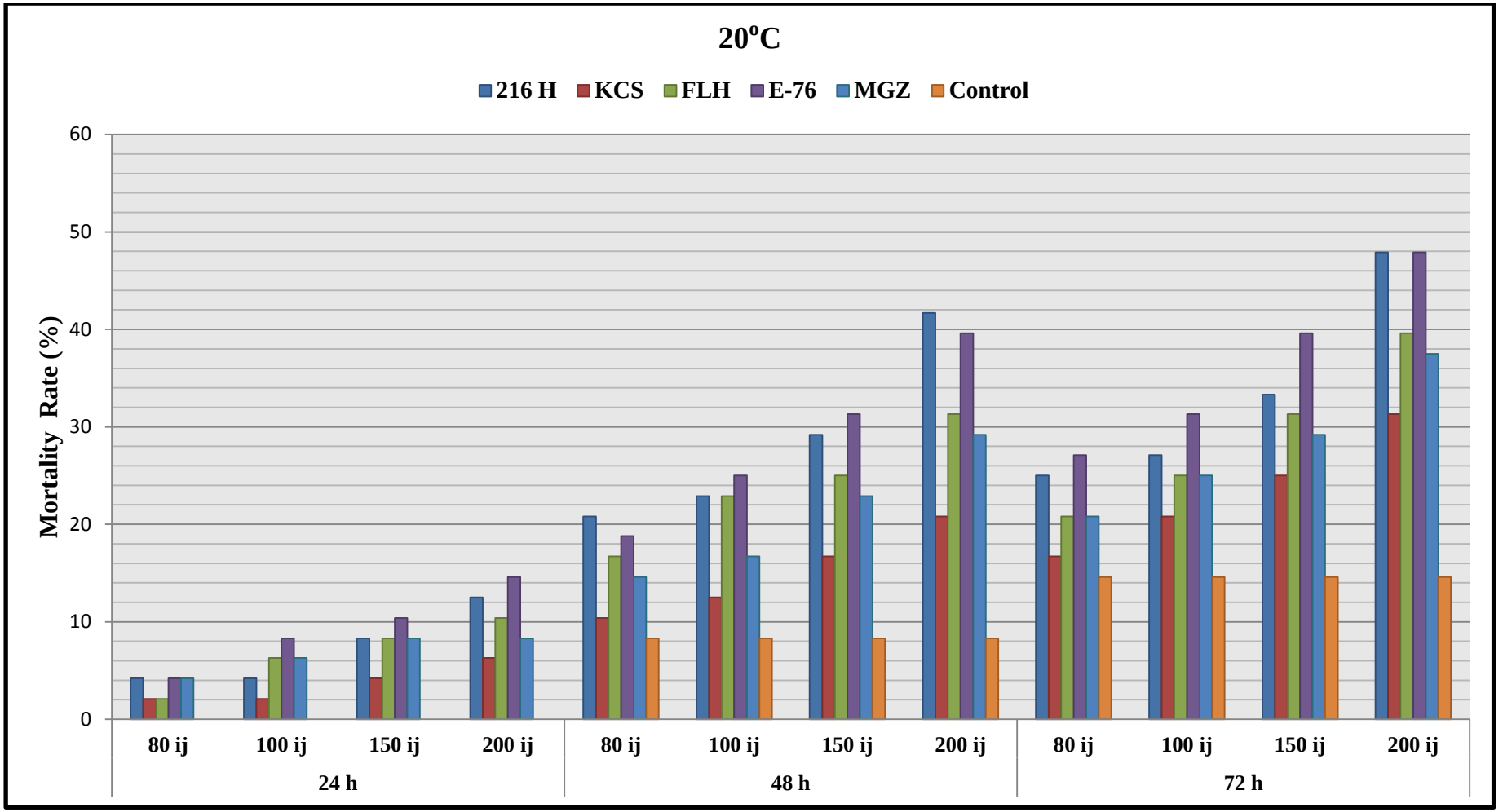


Fig. 4.6. Mortality rates (percentage) of five EPNs species on *P. citri* at four concentrations after 24, 48, and 72 hours at 20°C (216-H=*H. indica*, KCS=*S. feltiae*, FLH=*H. bacteriaphora*, E-76=*S. carpocapsae* and MGZ=*S. bicornutum*).

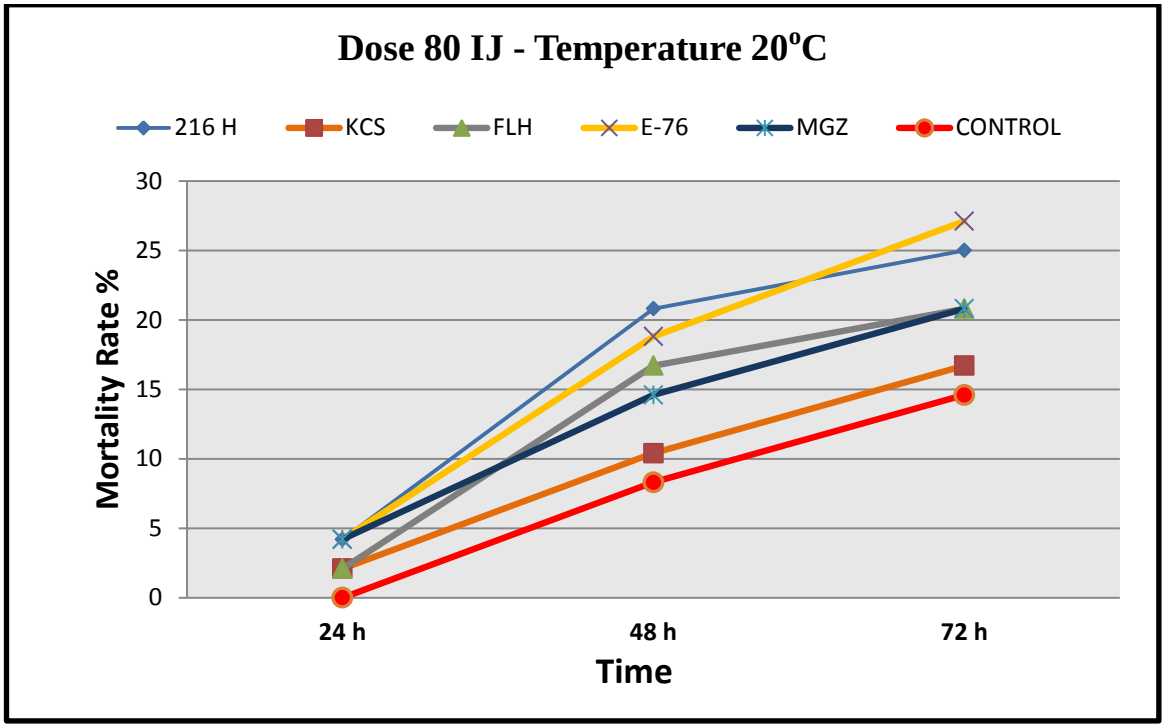


Fig. 4.7. The activity of EPNs on *P. citri* at 80 IJ through 24, 48, and 72 hours at 20°C temperature (216-H=*H. indica*, KCS=*S. feltiae*, FLH=*H. bacteriaphora*, E-76=*S. carpocapsae* and MGZ=*S. bicornutum*).

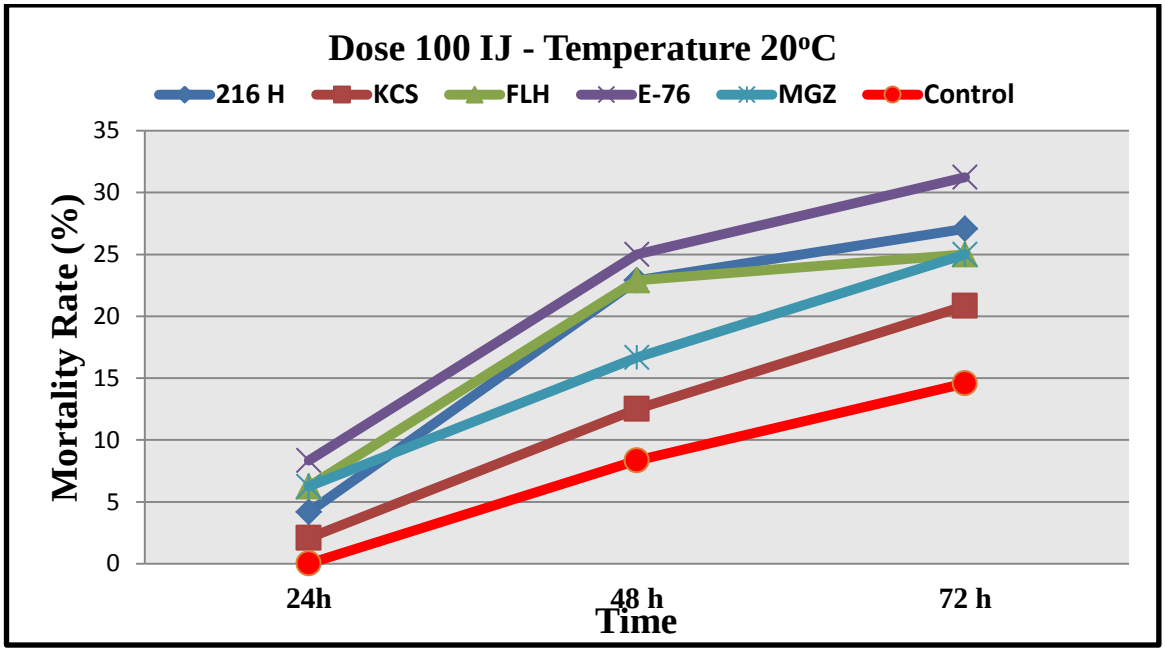


Fig. 4.8. The activity of EPNs sorts on *P. citri* at 100 IJ through 24, 48, and 72 hours at 20°C temperature (216-H=*H. indica*, KCS=*S. feltiae*, FLH=*H. bacteriaphora*, E-76=*S. carpocapsae* and MGZ=*S. bicornutum*).

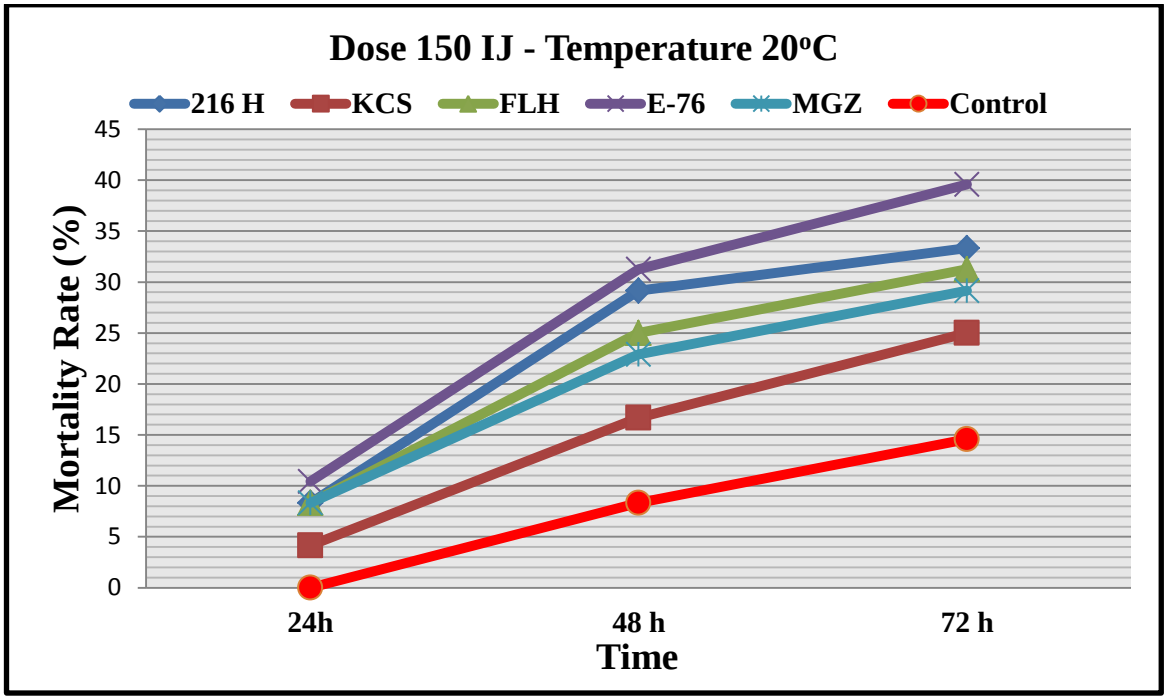


Fig. 4.9. The activity of EPNs sorts on *P. citri* at 150 IJ through 24, 48, and 72 hours at 20°C temperature (216-H=*H. indica*, KCS=*S. feltiae*, FLH=*H. bacteriaphora*, E-76=*S. carpocapsae* and MGZ=*S. bicornutum*).

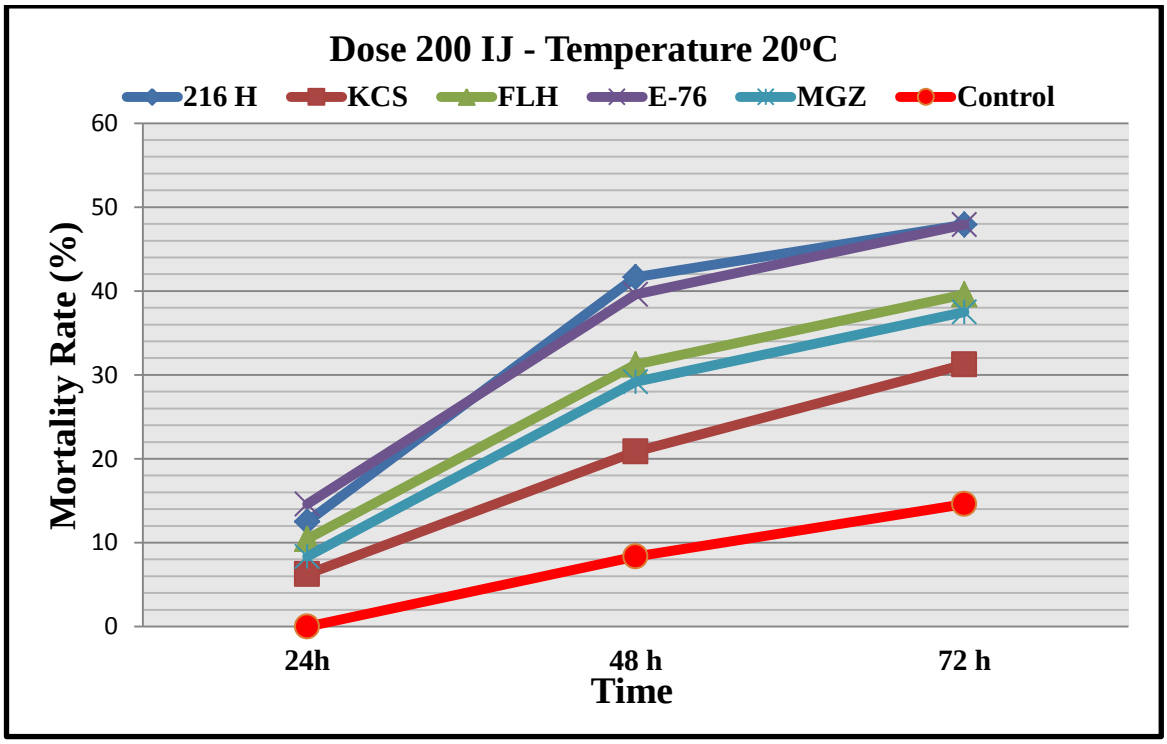


Fig. 4.10. The activity of EPNs sorts on *P. citri* at 200 IJ through 24, 48, and 72 hours at 20°C temperature (216-H=*H. indica*, KCS=*S. feltiae*, FLH=*H. bacteriaphora*, E-76=*S. carpocapsae* and MGZ=*S. bicornutum*).

4.4. The efficacy of entomopathogenic nematodes against citrus mealybug at 25°C for different concentrations

In *P. citri* females, the mortality rate at 25^{o}C ranged from 2.1% to 68.8% (Table 4.6 and Fig. 4.11). The lowest mortality rate on 24 h was 2.1% at 80 IJ administration dose for KCS species (Table 4.6 and Fig. 4.12), while the highest mortality rate was 25% for 216-H species at 200 IJ administration dose (Table 4.6 and Fig. 4.15). The lowest mortality rate at 72 h occurred in the KCS species with 18.8% at 80 IJ administration dose and the highest mortality occurred in the species of 216-H with 68.8% at 200 IJ administration dose (Table 4.6, Fig. 4.11, Fig. 4.12 and Fig. 4.15).

The difference between nematode species and control established after 24 h for 100 IJ and higher doses, but differences within nematode species were more pronounce at higher doses (150 and 200 IJ). *Heterorhabditis* species also showed significant higher mortality than *Steinernema* species (except for *S. carpocapsae*), and both species were significantly higher than control (Table 4.6, Fig. 4.11). After 48 hours the significant difference was observed between doses with 150 and 200 IJ which caused significant higher mortality than 80 and100 IJ. In addition, the *H. indica*, *H. bacteriophora*, and *S. carpocapsae* showed significant more mortality rate than *S. feltiae*, and *S. bicornutum*. A higher rate of mortality accomplished after 72 hours exposure of *P. citri* to different doses of nematode species, with *H. indica* and *H. bacteriophora* significantly cause higher mortality than *Steinernema* species (Table 4.6, Fig. 4.11).

Table 4.6. Mortality rates (percentage) of five EPNs species on *P. citri* at four concentrations after 24, 48, and 72 hours at 25°C*.

25°C				
Time	**24 h**			
Dose	**80 IJ**	**100 IJ**	**150 IJ**	**200 IJ**
H. indica	8.3 Ca	12.5 BCa	18.8 ABa	25.0 Aa
S. feltiae	2.1 Aa	4.2 Abc	6.3 Abc	8.3 Abc
H. bacteriophora	4.2 Ca	8.3 BCab	14.6 ABab	20.8 Aab
S. carpocapsae	6.3 Ca	10.4 BCab	16.7 ABab	22.9 Aab
S. bicornutum	6.3 Aa	8.3 Aab	10.4 Ab	14.6 Ab
Control	0.0 Aa	0.0 Ac	0.0 Ac	0.0 Ad
Time	**48 h**			
Dose	**80 IJ**	**100 IJ**	**150 IJ**	**200 IJ**
H. indica	25.0 Ca	31.3 Ca	47.9 Ba	58.3 Aa
S. feltiae	10.4 Cb	14.6 BCbC	20.8 ABc	25.0 Ac
H. bacteriophora	22.9 Ca	29.2 Ca	39.6 Ba	47.9 Aa
S. carpocapsae	20.8 Ca	29.2 Ba	37.5 Bab	52.1 Aa
S. bicornutum	18.8 Ba	20.8 Bb	27.1 Bbc	39.6 Ab
Control	4.1 Ac	4.1 Ac	4.1 Ad	4.1 Ad
Time	**72 h**			
Dose	**80 IJ**	**100 IJ**	**150 IJ**	**200 IJ**
H. indica	35.4 Da	43.8 Ca	58.3 Ba	68.8 Aa
S. feltiae	18.8 Cb	22.9 BCc	27.1 ABc	33.3 Ac
H. bacteriophora	29.2 Ca	37.5 Cab	47.9 Bb	58.3 Ab
S. carpocapsae	31.3 Ca	39.6 Cca	52.1 Bab	62.5 Aab
S. bicornutum	27.1 Ca	29.2 BCb	37.5 ABb	45.8 Abc
Control	10.4 Ab	10.4 Ad	10.4 Ad	10.4 Ad

*Means followed by same lower-case letter within a column and upper-case letter within a line are not different according to Duncan's multiple range test ($P \leq 0.05$).

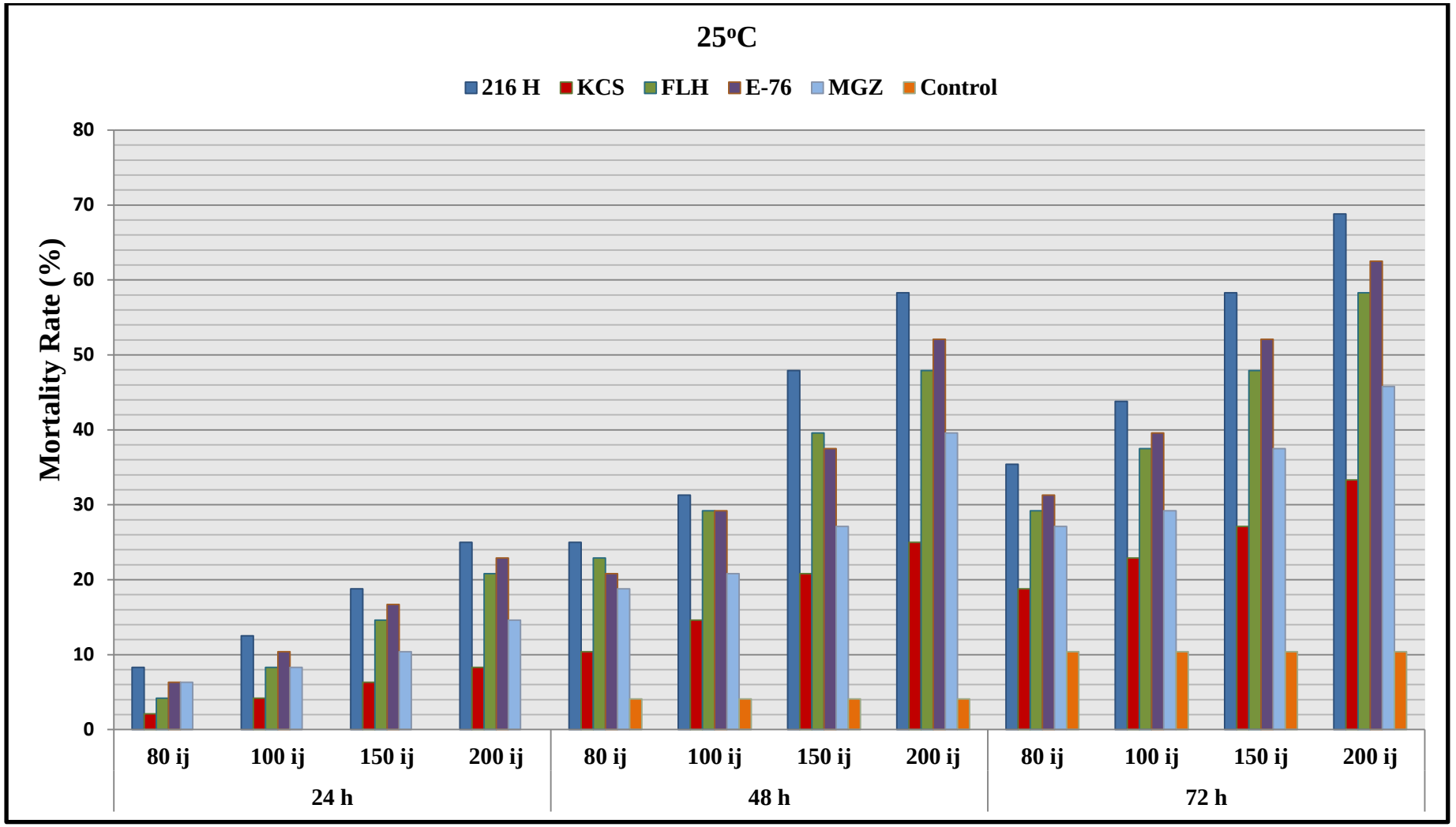


Fig. 4.11. Mortality rates (percentage) of five EPNs species on *P. citri* at four concentrations after 24, 48, and 72 hours 25°C temperature (216-H=*H. indica*, KCS=*S. feltiae*, FLH=*H. bacteriaphora*, E-76=*S. carpocapsae* and MGZ=*S. bicornutum*).

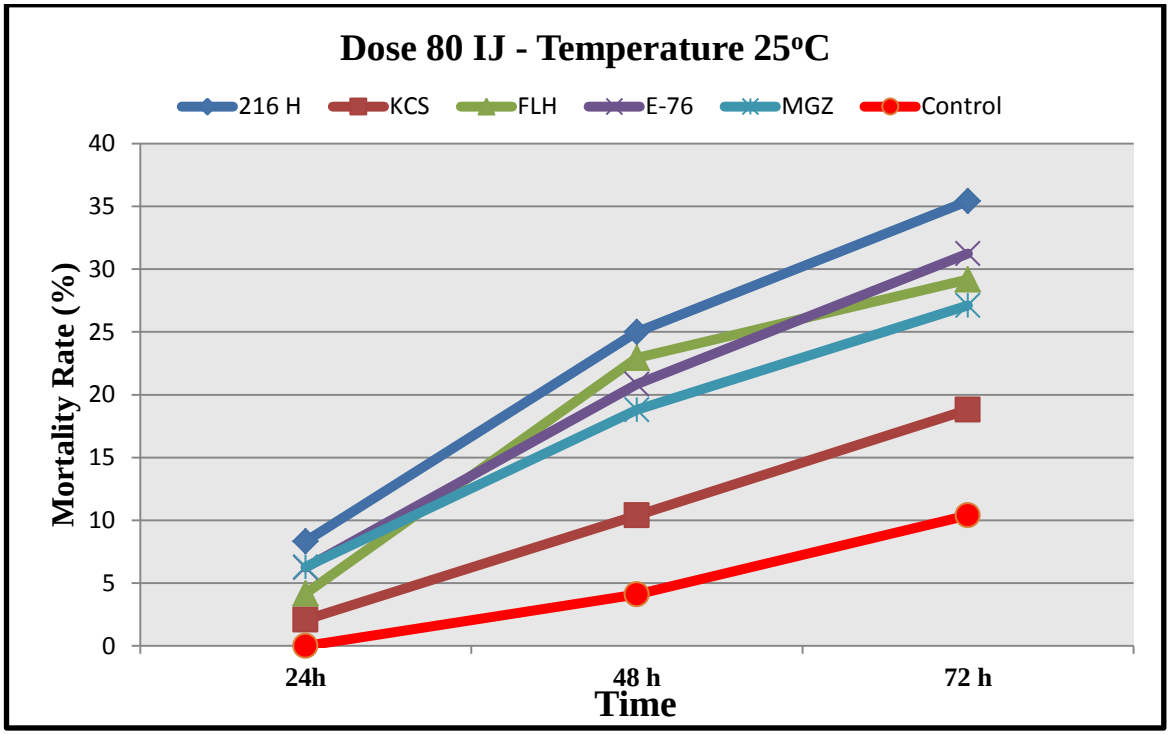


Fig. 4.12. The activity of EPNs sorts on *P. citri* at 80 IJ through 24, 48, and 72 hours at 25°C temperature (216-H=*H. indica*, KCS=*S. feltiae*, FLH=*H. bacteriaphora*, E-76=*S. carpocapsae* and MGZ=*S. bicornutum*).

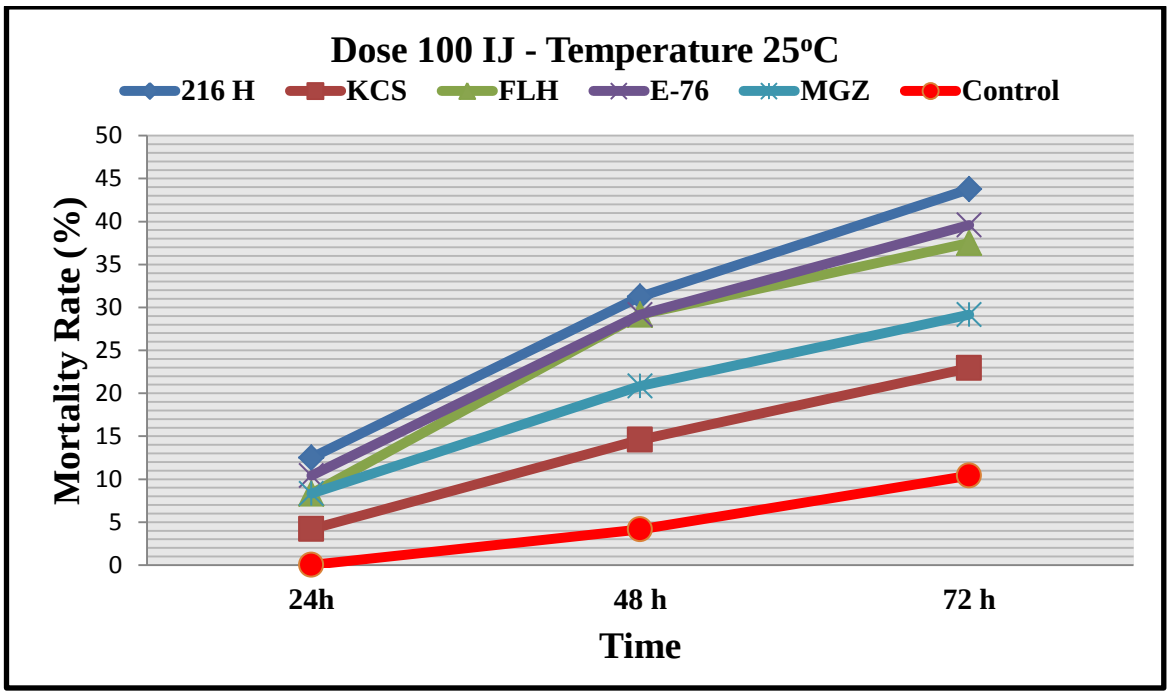


Fig. 4.13. The activity of EPNs sorts on *P. citri* at 100 IJ through 24, 48, and 72 hours at 25°C temperature (216-H=*H. indica*, KCS=*S. feltiae*, FLH=*H. bacteriaphora*, E-76=*S. carpocapsae* and MGZ=*S. bicornutum*).

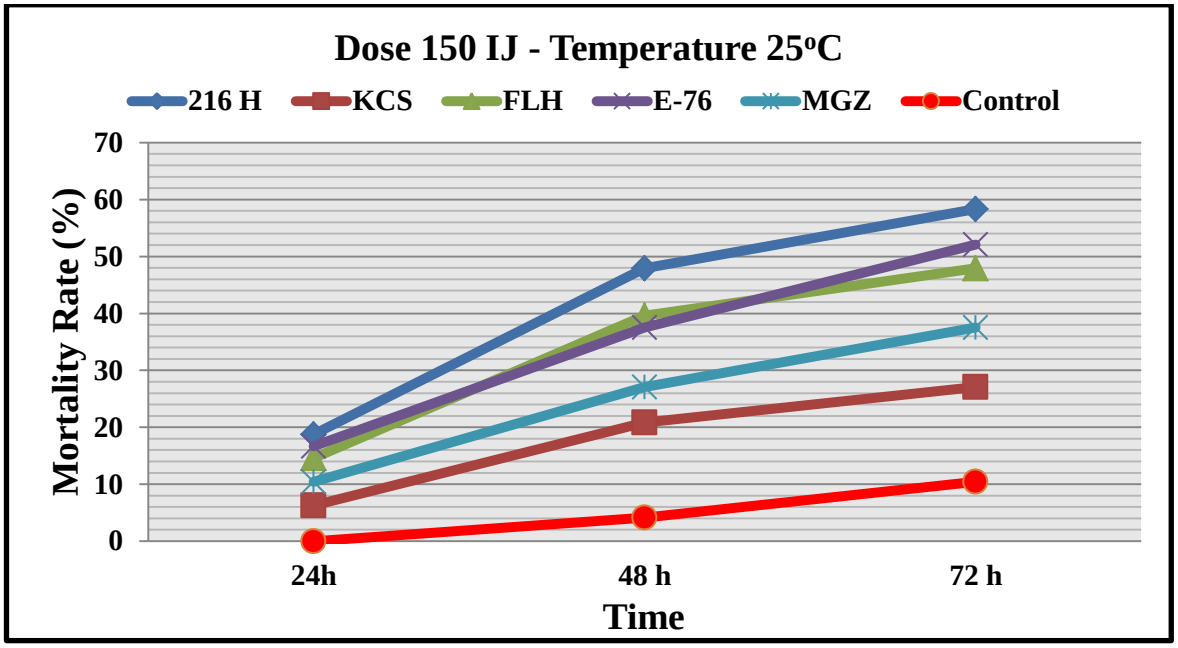


Fig. 4.14. The activity of EPNs sorts on *P. citri* at 150 IJ through 24, 48, and 72 hours at 25°C temperature (216-H=*H. indica*, KCS=*S. feltiae*, FLH=*H. bacteriaphora*, E-76=*S. carpocapsae* and MGZ=*S. bicornutum*).

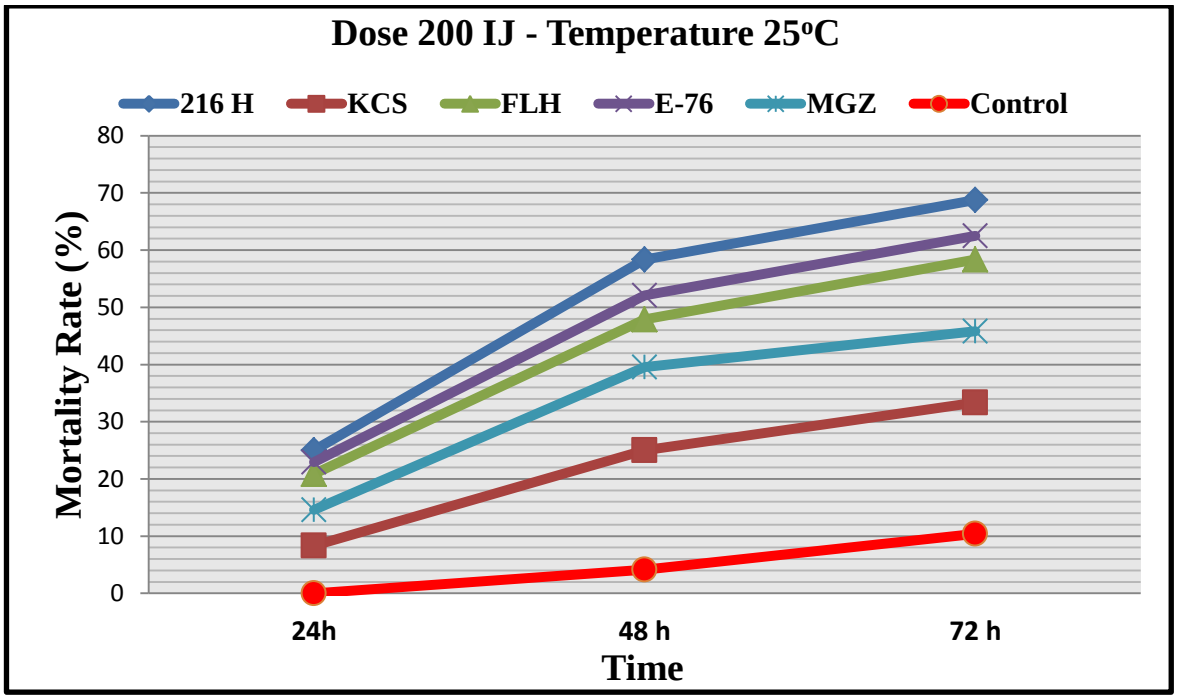


Fig. 4.15. The activity of EPNs sorts on *P. citri* at 200 IJ through 24, 48, and 72 hours at 25°C temperature (216-H=*H. indica*, KCS=*S. feltiae*, FLH=*H. bacteriaphora*, E-76=*S. carpocapsae* and MGZ=*S. bicornutum*).

4.5. The efficacy of entomopathogenic nematodes aganits citrus mealybug at 30°C for different concentrations

Mortality rates at 30^{o}C in *P. citri* female ranged from 0.0% to 60.4% (Table 4.7 and Fig. 4.16). In *P. citri female*s, the lowest mortality rate on 24 h occurred in KCS and MGZ isolates with 0.0% at 80 IJ application dose whereas the highest mortality rate was found in isolates FLH-H with 20.8% at 200 IJ on 24 h (Table 4.7, Fig. 4.17 and Fig. 4.20). The lowest mortality rate on 72 h occurred in KCS isolates with 4.2% at 80 IJ application doses, while the highest mortality occurred in isolates of 216-H with 60.4% at 200 IJ administration dose (Table 4.7. and Fig. 4.16).

After 24 hour exposure of *P. citri* to different nematode species, the differences between species pronounced at higher rate of doses (150 and 200 IJ), while after 48 hour and 72 hour the differences between nematode species appeared even at low doses (80 and 100 IJ). Same results were obtained as temperature 25^{o}C with higher mortality observed by *H. indica*, and *H. bacteriophora*, followed by *S. carpocapsae S. feltiae*, and *S. bicornutum*, and all species showed significant more mortality rate than control (Table 4.7, Fig. 4.16).

Table 4.7. Mortality rates (percentage) of five EPNs species on *P. citri* at four concentrations after 24, 48, and 72 hours at 30°C*.

30°C				
Time	**24 h**			
Dose	**80 IJ**	**100 IJ**	**150 IJ**	**200 IJ**
H. indica	4.2 Ca	8.3 BCa	14.6 ABa	18.8 Aa
S. feltiae	0.0 Aa	0.0 Aa	2.1 Abc	2.1 Abc
H. bacteriophora	8.3 Ba	10.4 Ba	14.6 Aa	20.8 Aa
S. carpocapsae	2.1 BCa	6.3 BCa	12.5 ABa	16.7 Aa
S. bicornutum	0.0 Aa	4.2 Aa	4.2 Abc	6.3 Ab
Control	0.0 Aa	0.0 Aa	0.0 Ac	0.0 Ac
Time	**48 h**			
Dose	**80 IJ**	**100 IJ**	**150 IJ**	**200 IJ**
H. indica	22.9 Ca	27.1 Ca	39.6 Ba	54.2 Aa
S. feltiae	6.3 Bb	8.3 ABCbc	12.5 ABcd	16.7 Ad
H. bacteriophora	22.9 Ca	25.0 BCab	31.3 Bab	43.7 Ab
S. carpocapsae	12.5 Cb	16.7 BCb	22.9 ABbc	31.3 Ac
S. bicornutum	12.5 Bb	14.6 ABb	18.8 ABbc	22.9 Ad
Control	6.3 Ab	6.3 Ac	6.3 Ad	6.3 Ae
Time	**72 h**			
Dose	**80 IJ**	**100 IJ**	**150 IJ**	**200 IJ**
H. indica	31.3 Ca	35.4 Cca	47.9 Ba	60.4 Aa
S. feltiae	14.6 Acd	16.7 Ad	18.8 Ad	22.9 Acd
H. bacteriophora	27.1 Cab	31.3 BCab	39.6 Bab	52.1 Aa
S. carpocapsae	22.9 Cbc	25.0 BCbc	33.3 ABbc	41.7 Ab
S. bicornutum	18.8 Bbc	20.8 ABcd	25.0 ABcd	29.2 Ac
Control	12.5 Ad	12.5 Ad	12.5 Ae	12.5 Ad

*Means followed by same lower-case letter within a column and upper-case letter within a line are not different according to Duncan's multiple range test ($P \leq 0.05$).

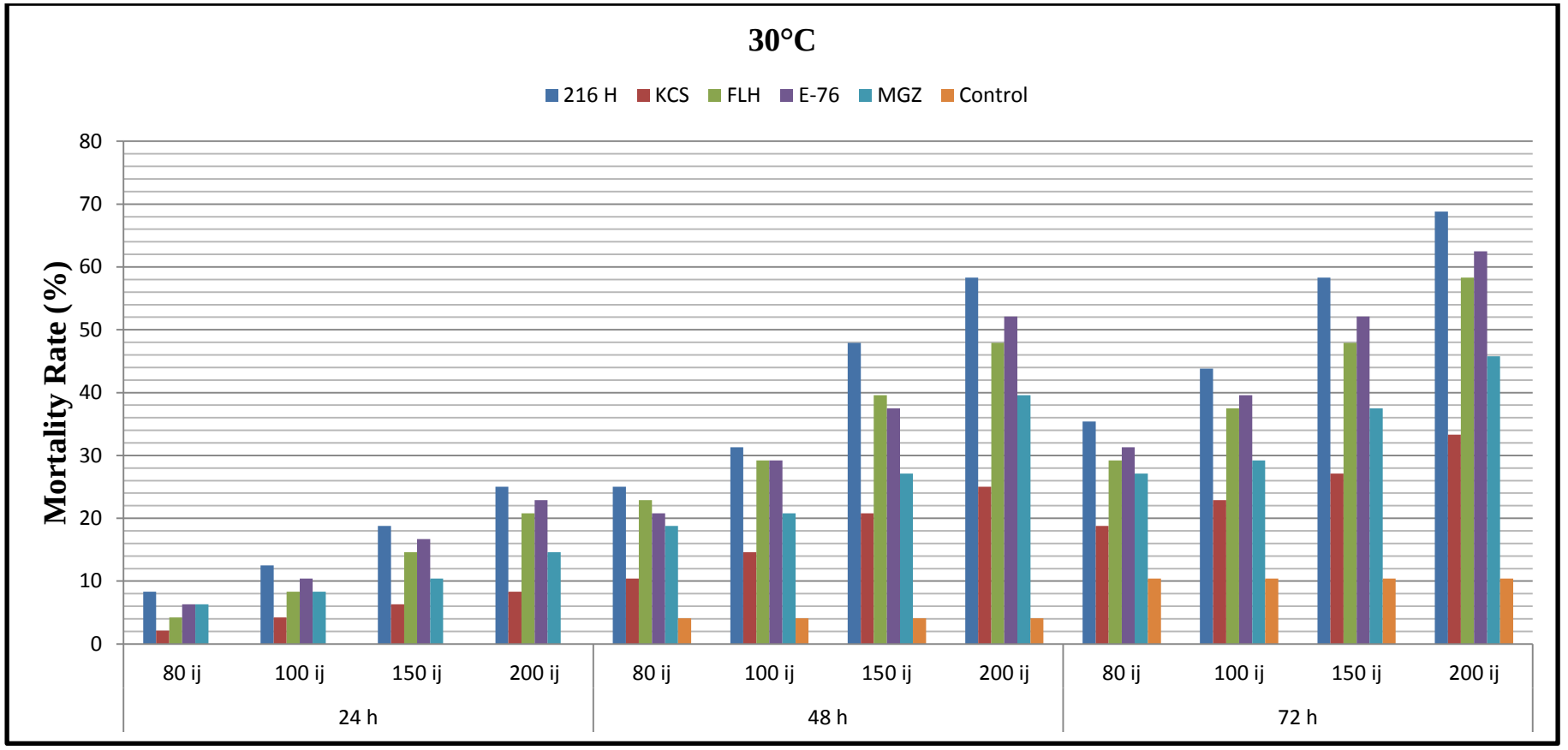


Fig. 4.16. Mortality rates (percentage) of five EPNs species on *P. citri* at four concentrations after 24, 48, and 72 hours at 30^{o}C temperature (216-H=*H. indica*, KCS=*S. feltiae*, FLH=*H. bacteriaphora*, E-76=*S. carpocapsae* and MGZ=*S. bicornutum*).

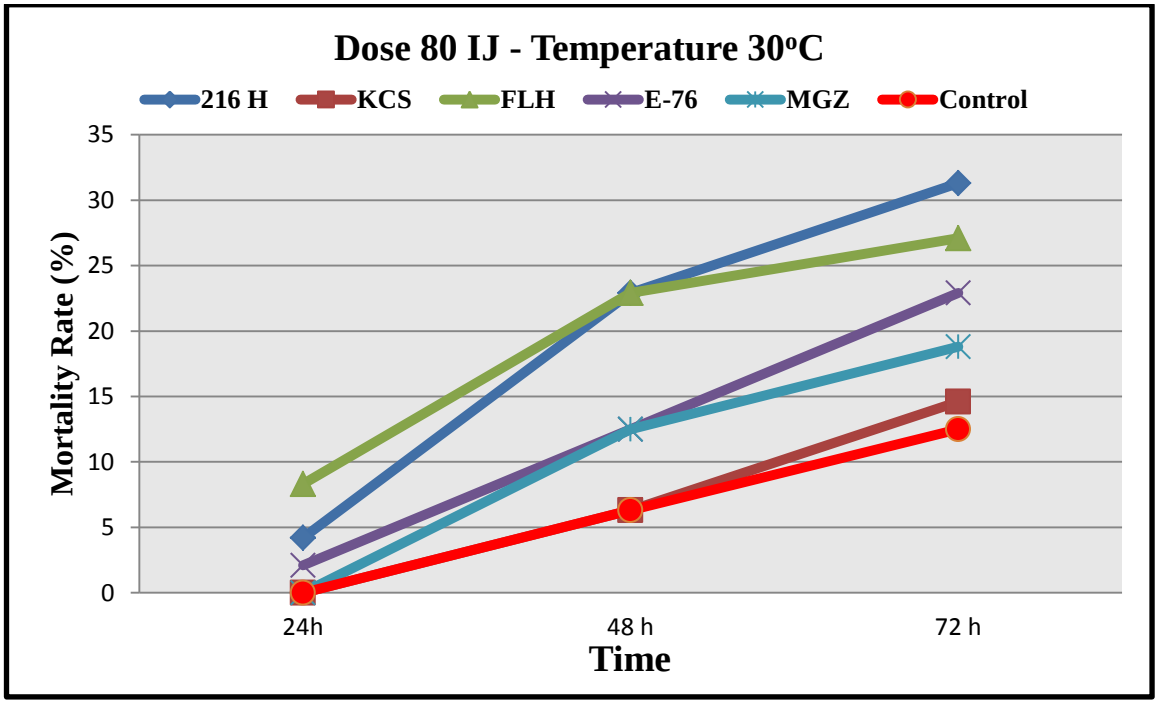


Fig. 4.17. The activity of EPNs sorts on *P. citri* at 80 IJ through 24, 48, and 72 hours at 30°C temperature (216-H=*H. indica*, KCS=*S. feltiae*, FLH=*H. bacteriaphora*, E-76=*S. carpocapsae* and MGZ=*S. bicornutum*).

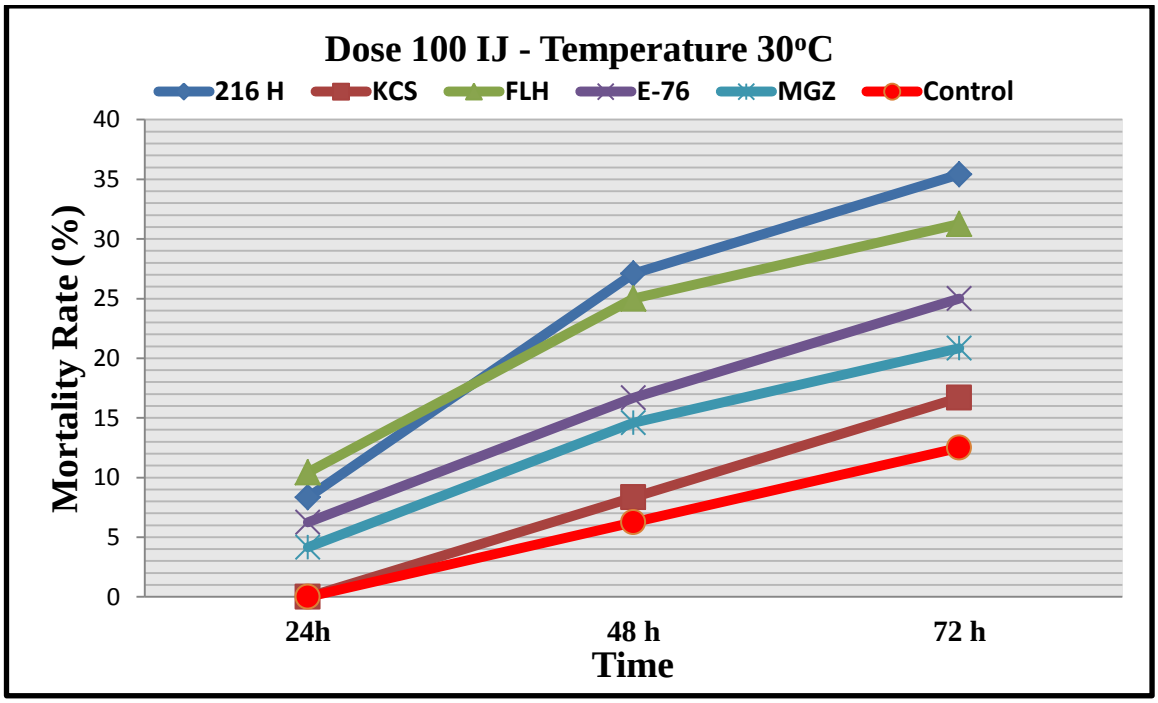


Fig. 4.18. The activity of EPNs sorts on *P. citri* at 100 IJ through 24, 48, and 72 hours at 30°C temperature (216-H=*H. indica*, KCS=*S. feltiae*, FLH=*H. bacteriaphora*, E-76=*S. carpocapsae* and MGZ=*S. bicornutum*).

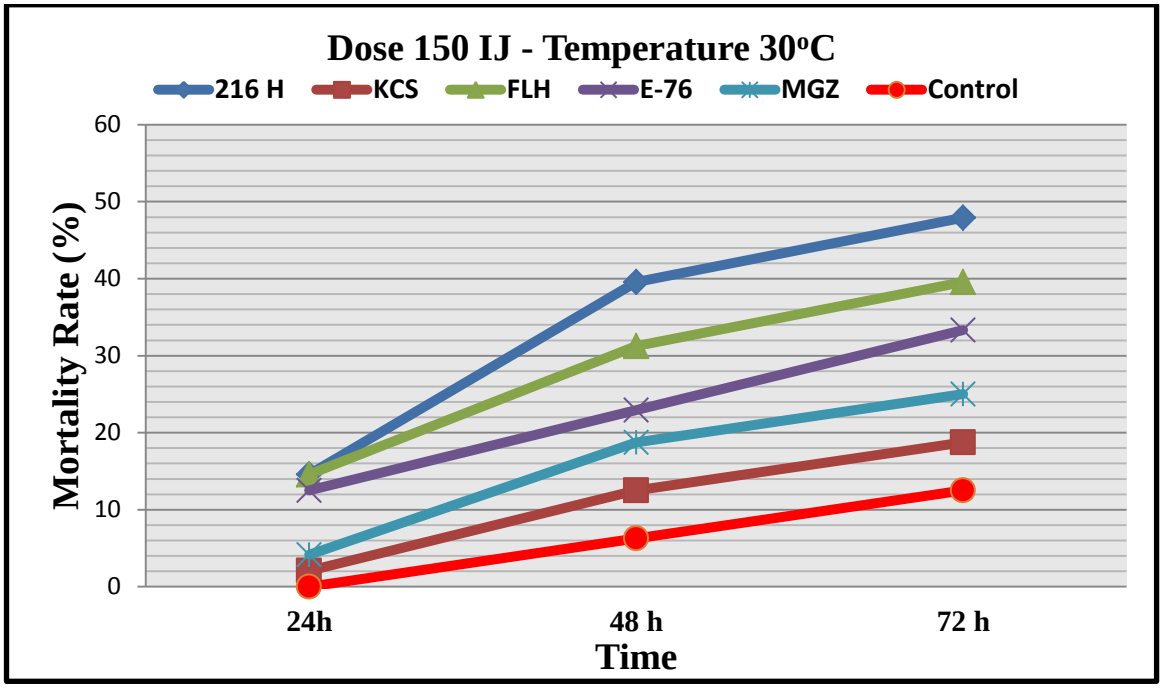


Fig. 4.19. The activity of EPNs sorts on *P. citri* at 150 IJ through 24, 48, and 72 hours at 30°C temperature (216-H=*H. indica*, KCS=*S. feltiae*, FLH=*H. bacteriaphora*, E-76=*S. carpocapsae* and MGZ=*S. bicornutum*).

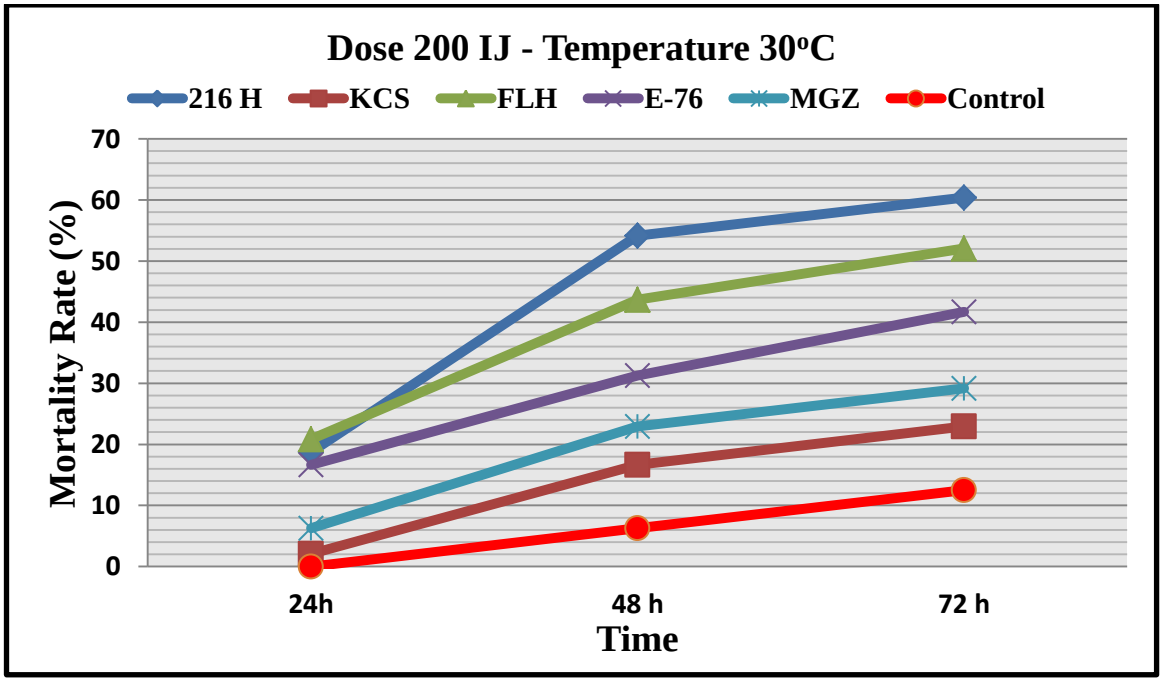


Fig. 4.20. The activity of EPNs sorts on *P. citri* at 200 IJ through 24, 48, and 72 hours at 30°C temperature (216-H=*H. indica*, KCS=*S. feltiae*, FLH=*H. bacteriaphora*, E-76=*S. carpocapsae* and MGZ=*S. bicornutum*).

CHAPTER 5

DISCUSSION

Entomopathogenic nematodes are free-living as IJs which infect an insect host. They have a big potential as biological control agents because they can be produced and used in mass numbers to attack and kill insect pests such as mealybugs. There are some natural enemies playing a part in the biological control of mealybugs. But little research on the use of entomopathogenic nematodes has been conducted, but it is assumed they can play a prominent role in regulating of mealybugs populations.

All five indigenous nematode species tested in this study were able to cause variable levels of *P. citri* mortality (33.3 - 68.8%) under optimal conditions. *Heterorhabditis indica* (216 H) caused significantly higher mortality (68.8%) compared to the other four nematode species tested. No significant difference between *H. bacteriophora* (FLH) and *S. carpocapsae* (E–76) was observed (58.3 and 62.5% mortality) and *S. feltiae* caused 33.3% mortality.

A large part of entomopathogenic nematodes can infect many insects from different groups. When looking at the host range of *S. feltiae* and *H.indica* it appears that these strains infect many species of insects belonging to different families. *S. carpocapsae* is known to infect over 250 insect species from different insect orders [116]. There are storage pests in the insect groups that EPNs can infect. *S. carpocapsae* is found in Austria, Bulgaria, Czech Republic, France, Germany, Great Britain, Italy, Norway, Poland, Portugal, Slovakia, Spain, Sweden and Switzerland in Europe, [117]. In Turkey, *S. carpocapsa* despite their having been identified in many different studies have been identified in less work than *S. feltiae* [112, 117-120]. One of the most commonly found EPN species in the World, *H. bacteriophora* can spread to worm regions, mostly in tropical regions [121].

The results are similar to those obtained by Niekerk [6] which showed that both *H. zealandica* and *S. yirgalemense* were highly pathogenic to *P. citri,* citrus mealybug, and Stokwe [96], which showed that both *H. zealandica* and *S. yirgalemense* were highly pathogenic to *P. viburni* causing 80% mortality of *P. viburni* 48-h post inoculation at 25 ºC. After 48 h, the LC_{50} and LC_{90} values were 54 and 336 IJs, respectively. The results of the studies described here confirm the length of time involved, and the dosage-dependent susceptibility to *H. indica*, *H. bacteriophora*, *S. feltiae*, *S. carpocapsae*, and *S. bicornutum* which is in accordance with the results obtained by Niekerk and Malan [100] and Le Vieux and Malan [99]. The mortality of *P. citri* was found to be highest after exposure to a concentration of 200 IJs of *H. indica* (216 H) for 72 h, and temperature of 25 ^{o}C which resulted in an LC_{50} value of 126 and an LC_{90} value of 307 IJs per insect. The lowest mortality after 72h exposed at 25°C obtained by *S. feltiae* which resulted in LC_{50} value of 202 and an 481 IJs per insect.

The results of this study clearly illustrate that *P. citri* has a nematode-concentration-dependent susceptibility to both *Steinernema* species and *Heterorhabditis* species. The mortality of both species illustrated the highest percentage mortality when citrus mealybug was inoculated with the highest concentration of 200 IJs/insect. The LC_{50} and LC_{90} values were 126, 202, 162, 146, and 165 IJs, and 307, 481, 375, 343, and 523 IJs for *H. indica*, *S. feltiae*, *H. bacteriophora*, *S. carpocapsae*, and *S. bicornutum*, respectively. These values were considerably higher than those obtained by Niekerk [6] with the concentration of 80 IJs/insect. The LC_{50} and LC_{90} values for *H. zealandica* were 11 and 162 IJs per insect, respectively, and those obtained by [96], who obtained an LC_{50} value of 54 and LC_{90} value of 330.

Planococcus citri only occur in cryptic habitats above ground on citrus trees, which is an important consideration for evaluating the potential of nematodes to control them. [122] stated that the temperature to which nematodes are exposed has a great influence on their ability to infect hosts, as nematodes proved to be active only at temperature levels of 15 to 30^{o}C. The current study demonstrated that the mortality of *P. citri* caused by the infestation of nematodes were more potent at 25^{o}C than the on at 20^{o}C and 30^{o}C. Nematode activity has proved to increase from 15°C to 32°C [91], and nematodes should be able to reach hosts within a shorter time, when they are exposed to higher temperatures depending upon the host insect.

The results of this study clearly illustrate that the exposure of *P. citri* to a nematode infestation is a time dependent susceptibility to both *Steinernema* species and *Heterorhabditis* species. The mortality of both species illustrated the highest percentage mortality when mealybugs were exposed for 72 hour (31.77% mortality) compared to 24 and 48 hour (8.23 and 24.89% mortality, respectively).

The overall results obtained during this study indicate that both *Steinernema* and *Heterorhabditis* hold great potential for the control of *P. citri.* They performed differently, with *Heterorhabditis* species showed more active than *Steinernema* species controlling *P. citri*. The *H. indica* (216 H) showed the more efficacy than other entomopathogenic nematodes used in this study. The optimum concentration for nematode infestation to *P. citri* was 200 IJ/female at 25^{o}C for 72 hour.

Entomopathogenic nematodes may be combined with other agricultural chemicals and control agents for various purposes. Based on our research, we conclude that the benefits of utilizing EPNs can offer better control management in an IPM programme. EPNs can be applied with nearly all commercially available ground or aerial spray equipment, including pressurized sprayers, mist blowers, and electrostatic sprayers [33]. This offers a cost-effective alternative to pest control, while simultaneous application of fungicide and EPNs influence as well on fungi agents of plant diseases as on insects, and it saves time and at the same time money spent for controlling pest organisms.

5.1. Conclusion

The results of many foreign studies presented in this study prove that entomopathogenic nematodes are, when applied in optimal conditions, efficient agents for suppressing harmful pests. Of course we must be aware that they are in general not as efficient as chemical insecticides, although some studies have also shown that appropriate applications of entomopathogenic nematodes can provide mortality rates of target harmful pests which are comparable with mortality rates of organisms exposed to synthetic insecticides. When deciding to use entomopathogenic nematodes or even implementing them into the systems of food production, our expectations should not be the same as when using conventional plants protection agents. We particularly should not focus solely on greater yields, but also bear in mind that using the said agents is acceptable for food, human and nature. In this case we should be prepared to tolerate

Slightly less attractive appearance of plants grown for food or decoration. At present we no longer have insecticides for suppressing many harmful insects, this means that circumstances are exceptionally favourable to increase the use of natural enemies and other forms biological control, and entomopathogenic nematodes are in this regard one of the most efficient alternatives to insecticides.

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