



Integrated use of Kenyan Entomopathogenic Nematodes (*Steinernema* Species) and Neem Against *Tuta Absoluta* on Tomato

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Abstract – Tomato leaf miner, *Tuta absoluta* (Meyrick) is one of the most devastating pests affecting tomato crop in Kenya. This study was carried out to evaluate management options for sustainable management of *Tuta absoluta* with the aim of reducing over reliance on synthetic pesticides used for tomato production in greenhouse conditions. The management options evaluated included; EPNs alone and EPNs combined with neem. The treatments consisted of EPNs alone and EPNs combined with neem compared to Coragen® SC (20% Chlorantraniliprole) as a standard synthetic pesticide and control where only water was applied. A sticky pheromone trap was used for monitoring *Tuta absoluta* adults to guide the initiation of treatments. The experiment was laid out in a complete randomized design with four replicates and repeated twice. The results showed that all the treatment used significantly ($p < 0.05$) reduced *Tuta* populations on tomato plants compared to control. *Steinernema kari* combined with neem significantly ($p < 0.05$) reduced populations of *Tuta absoluta* and, the mean population was different from that of Coragen® treated tomato plants. EPNs alone significantly ($p < 0.05$) reduced *Tuta* populations compared to control but the mean population reduction was not comparable to that of EPNs and neem or Coragen®. Tomato fruit damage was highest in control treatments with 91.7% compared to 10.2%, 7.4% and 2.9% for EPNs alone, EPNs combined with neem and Coragen®, respectively. The results demonstrate the potential of EPNs alone and EPNs combined with neem (Azadirachtin 0.03%) as alternative tomato leaf miner management options for the sustainable management of *Tuta absoluta* in greenhouse conditions.

Keywords – Neem, *Steinernema Karii*, Tomato, *Tuta Absoluta*.

I. INTRODUCTION

The tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) which is native to South America was recently introduced to Kenya, and has become a major pest of both field and greenhouse tomatoes [1]. The larvae of *Tuta absoluta* is the most destructive stage by causing damage on the stem, fruits and mining on leaves producing wide irregular mines [2]. The larvae can destroy up to 100% of the leaf surface and damage 50-100% of fruits in severely attacked fields [3]. The extensive insecticide use can cause on the one hand several undesired side-effects on human and environment safety and on the other hand resistance development in *Tuta absoluta* [4]. In this view, there is need for environmentally-friendly strategies availability in order to reduce the use of synthetic pesticides and consequently, improve food safety and environment quality [5].

Botanical products, biophysical products and biological methods fit within the environment friendly strategies, being biodegradable and effective against pests without harming beneficial insects [4]. The application of EPNs is widespread in most parts of the world and can be multiplied experimentally in large quantities at low costs [6]. They are also potent and effective against target insect pests which make them worthy of attention by researchers, especially those interested in the preservation of the environment, particularly after demanding calls for preservation of the environment from wide use of chemical pesticides [6], [7] and [8]. Extensive studies have been undertaken in the field for biological control of insect pests using several bio-control agents such as entomopathogenic nematodes [9]. Entomopathogenic nematodes are safe to the environment and do not cause any harmful effects, either to humans or farm animals and beneficial insects [10]. Entomopathogenic nematodes can be combined with other compatible agricultural chemicals and control agents for various purposes [11]. First, nematodes and other control agents may be applied simultaneously or within a short time at least one hour interval of each other to control different pest species or stages of a pest. For convenience, nematodes may also be tank-mixed with other compatible control agents that is, combined in the tank of the application equipment, thus increasing the chances of interactions due to the higher concentration of both agents [12]. Entomopathogenic nematodes may be combined with other compatible control agents to achieve better control of a single pest through additive or, preferably synergistic effects on pest mortality [13]. Furthermore, in an IPM (Integrated Pest Management) programme, pesticide treatments using either chemical or biologically-based insecticides may not be compatible with biological control agents such as parasitoids and/ or predators, since some active ingredients are harmful to some bio-control agents of *Tuta absoluta* [14]. The *Tuta absoluta* management should be geared towards an IPM programme in a resistance management strategy through provision of alternative measures. The use of conventional selective pesticides is also advisable. This may be achieved by using eco-friendly plant extracts with bio-insecticide properties like neem [15], mass trapping using tomato leaf miner's sex pheromone which combined may provide environmentally safe and adequate control of this pest [16], [17]. This study was conducted to evaluate the effectiveness of EPNs alone and EPNs combined with neem as a sustainable management option



for tomato leaf miner (*Tuta absoluta*) in greenhouse conditions.

II. MATERIALS AND METHODS

1.1. Study Site Selection

This experiment was carried out in a greenhouse in Kabete Campus Field Station, University of Nairobi which is located 15km North West of Nairobi City Centre and lies at latitude 1° 15'S and longitude 36° 44'E and at an altitude 1941 meters above sea level.

1.2. Greenhouse Experiment to Evaluate Effectiveness of EPNs Combined with Neem in the Sustainable Management of *Tuta Absoluta*.

Experiments were conducted to evaluate the effectiveness of *Steinernema karii* alone, *S. karii* combined with neem (Azadirachtin 0.03%), compared to Coragen® (Chlorantraniliprole) in an integrated management of tomato leaf miner (*Tuta absoluta*) in greenhouse conditions. Tomato seedlings were raised in a nursery bed measuring 1 x 2M and after a month of care the seedlings were transplanted into polythene pots filled with three kilograms of soil, where normal agronomic practices were carried out to nurture the plants till harvesting. The plants were nurtured in a greenhouse measuring (6M width x 16M length), with a temperature range of 16.5-34 °C and a relative humidity (RH) range of 75.4-85.8%. Infestation of the crop by *Tuta absoluta* occurred naturally. The experiment was laid out in a complete randomized design with four replicates and repeated twice. The experiment had four treatments namely; EPNs alone applied at a rate of 1000 Ijs/ml of water with oil adjuvant Addit® (Koppert) as wetting agent, EPNs with oil adjuvant in water followed by Nembicidine® (Azadirachtin 0.03%) application at a rate of 60ml/ 20L after a short duration of 1 hour. Synthetic pesticide Coragen® SC (20% Chlorantraniliprole) applied at the rate of 3ml/ 20L and control where only water was applied. A delta sticky trap supplied with *Tuta absoluta* pheromone was hanged at the centre of the greenhouse at a height of one meter for monitoring *Tuta* populations to enable the initiation of treatments. Coragen® was used for comparison purposes with other treatments in this experiment. All the treatments started when three *Tuta absoluta* adults were caught in the trap. The action threshold used was based on occurrence of the three *Tuta absoluta* adult moths on the trap as reported by Bajonero [33]. The number of three caught adults was selected to prove the pest presence. Treatment was carried out using a knapsack sprayer on a weekly basis. *Tuta* larval count was done on four leaves per plant on twelve tomato plants randomly selected from each treatment. According to damage descriptor by Fernandez [18], the *Tuta absoluta* larvae attacks tomato fruits by making galleries, penetrating mainly at the base or near the peduncle insertion zone and indiscriminate, wide irregular mines on leaves. The damage observed included: puncture marks where the larvae entered the fruit, exit holes and dried frass produced by last larvae as they pupate especially under the calyx. Therefore, fruits harvested with *Tuta*

absoluta marks were recorded as damaged fruits, while those without were recorded as undamaged fruits. Harvesting was done per plot.

1.3. Statistical Analysis

The data collected was square-root transformed before analysis. The transformed larval count data was subjected to analysis of variance (ANOVA) to assess treatment effects while the Fisher's protected least significance difference (LSD) test was used to compare treatment means. Similar analysis was done on tomato fruits yield data. The data was analyzed using GenStat- PC v.14.1, 14th Edition [19].

III. RESULTS

3.1. Evaluation of EPNs Alone and EPNs Combined with Neem as Management Options for *Tuta Absoluta*

In this experiment tomatoes were naturally infested by the tomato leaf miner. Results reveal that all evaluated treatments namely; EPNs alone, EPNs combined with neem and Coragen® significantly ($p < 0.05$) reduced *Tuta absoluta* populations compared to control in season one. Mean *Tuta* populations were sustained at an almost constant number within the treated plots throughout the sampling period as opposed to control where they continuously increased to reach a high of (7.9) by the eighth week of sampling. Coragen®, the standard chemical had the least mean population of *Tuta* which was not different from that of EPNs combined with neem. The mean population of *Tuta* in EPNs alone treated plants was low but significantly different ($p < 0.05$) from the mean population recorded in Coragen® and EPNs combined with neem plants. Plants sprayed with water only (control) had the highest *Tuta* mean population (5.7) which was significantly different from the other treatments (Table 1). There was a significant ($p < 0.05$) reduction in *Tuta absoluta* populations in the three treatments evaluated compared to control in season two. Mean populations of the three treatments were low during the sampling period compared to that of control which continued to increase to reach a peak of (8.0) by the eighth week of sampling. Plants sprayed with water only (control) had the highest *Tuta* mean populations (5.9) which was significantly different ($p < 0.05$) from other treatments. Coragen®, the standard chemical, had the least mean population of *Tuta* which was not different from that of EPNs combined with neem. The mean population of *Tuta* in EPNs alone treated plants was low closely following that of Coragen® and neem combined with EPNs. However, it was significantly different ($p < 0.05$) from the mean population recorded in Coragen® and Neem combined with EPNs treated plants (Table 2).



Table 1. Mean population of *Tuta absoluta* larvae recorded in four leaves per tomato plant after treatment application in season one (June to August, 2016)

Treatment	Sampling Period in Weeks								Mean
	1	2	3	4	5	6	7	8	
EPNs alone	1.8	1.8	1.6	1.7	1.5	1.4	1.3	1.3	1.6b
EPNs + Neem	1.6	1.5	1.3	1.2	1.2	1.1	1.0	1.0	1.2a
Coragen	1.6	1.4	1.2	1.1	1.1	1.0	1.0	1.0	1.1a
Control	1.8	3.9	5.6	6.0	6.4	6.7	7.4	7.9	5.7c
L.S.D.									0.1
%CV									1.1

Means followed by the same letter in the column were not significantly different at (p < 0.05)

Transformation Formula is = Square root of (X+0.5), where X is the data to be transformed.

Table 2. Mean population of *Tuta absoluta* larvae recorded in four leaves per tomato plant after treatment application in season two (September to November, 2016)

Treatment	Sampling Period								Mean
	1	2	3	4	5	6	7	8	
EPNs alone	1.8	1.7	1.5	1.5	1.4	1.3	1.3	1.2	1.5b
EPNs + Neem	1.6	1.4	1.3	1.3	1.2	1.1	1.1	1.0	1.3ab
Coragen	1.5	1.3	1.2	1.2	1.1	1.1	1.0	1.0	1.1a
Control	1.8	3.5	5.8	6.6	6.9	7.1	7.5	8.0	5.9c
L.S.D.									0.1
%CV									1.4

Means followed by the same letter in the column were not significantly different at (p < 0.05)

Transformation Formula is = Square root of (X+0.5), where X is the data to be transformed.

Table 3. Effect of selected IPM (Integrated Pest Management) options on tomato yield in season one

Treatments	Average Fruit Weight (tons/hectare)		Total	% Damaged Fruits
	Damaged	Not Damaged		
EPNS alone	0.8	6.6	7.4	10.8b
EPNS + Neem	0.7	8.8	9.5	7.9b
Coragen	0.3	9.6	9.9	3.0b
Control	2.7	0.3	3.0	91.5a
%CV				16.8
LSD				0.1

Means followed by the same letter in the column were not significantly different at (p < 0.05). Percent damaged tomato fruits is = (weight of damaged fruits/total weight of harvested fruits)*100. (*=is Multiplication sign)

Table 4. Effect of selected IPM (Integrated Pest Management) options on tomato yield in season two

Treatments	Average Fruit Weight (tons/hectare)		Total	% Damaged Fruits
	Damaged	Not Damaged		
EPNS alone	0.8	6.5	7.3	11.0b
EPNS + Neem	0.6	8.9	9.5	6.3b
Coragen	0.2	9.7	9.9	2.0b
Control	2.7	0.4	3.1	87.1a
%CV				17.1
LSD				0.1

Means followed by the same letter in the column were not significantly different at (p < 0.05). Percent damaged tomato fruits is = (weight of damaged fruits/total weight of harvested fruits)*100. (*=is Multiplication sign)



In season one, high fruit damage of about 92% was recorded in control compared to 10.8%, 7.9% and 3.0% for EPNs alone, EPNs combined with neem and Coragen® treatments, respectively. Control had the lowest total weight of the tomato fruits harvested (Table 3).

In season two, high tomato fruit damage of 87.1% was recorded in control compared to 11.0%, 6.3% and 2.0% for EPNs alone, EPNs combined with neem and Coragen® treatments, respectively. Again control had the least yield of tomato fruits harvested (Table 4).

IV. DISCUSSION

With the invasion of Kenya by *Tuta absoluta* now a real threat to tomato production, there is need for developing a holistic approach to environmentally sustainable, economically sound and effective strategies for management of this pest [20]. Integrated Pest Management is the rational application of biological, biotechnological, cultural or crop improvements and chemical measures in pest management. Use of chemical plant protection products is limited to strictly minimum necessary to keep pests below levels that may cause economically unacceptable damage or losses [3]. In this study EPNs as biological agents, neem botanical pesticide and Coragen® synthetic pesticide were evaluated as management options for *Tuta absoluta*. Results of this study revealed that it's possible to reduce *Tuta absoluta* populations by application of *Steinernema kari* nematodes and *S. kari* combined with neem in greenhouse conditions. The ability of *Steinernema kari* to greatly reduce the pest populations, demonstrate that *Tuta absoluta* would allow the survival of the infective juveniles (Ijs) long enough to find and infect the larvae on the surface of the leaf as well as penetrate the galleries through entry holes to infect the larvae. Inside the galleries Ijs are protected from adverse environmental conditions thus enhancing their effectiveness in infecting the larvae. Other researchers have reported similar results, Schroer [21] who found that nematodes invaded the diamondback moth larvae (*Plutella xylostella*) within 1 hour after foliar application of *Steinernema carpocapsae* and obtained a mean survival of 90% Ijs for greater than 3 hours. Kim [22] reported a 12 hour survival time period of Ijs of *Steinernema carpocapsae* in foliar application on Chinese cabbage leaves in greenhouse conditions. The study shows that foliar application of EPNs, pest habitat in the leaves determines the efficacy of the nematodes. In cryptic foliage (leaf mines) the nematodes are more effective than in exposed foliage (pests on leaf surface). Hence, the effectiveness of these EPNs can also be attributed to the fact that *Tuta absoluta* makes galleries in leaves which provide nematodes an excellent habitat which is cryptic to avoid harmful environmental factors (desiccation and ultraviolet light) and hence parasitize the insect target [23]. Therefore, setting up the experiment in the greenhouse and the cryptic habitat of *Tuta absoluta* in tomato plants, afforded the Ijs some protection from un-favourable environmental conditions at the target site. Another reason for the effectiveness of the EPNs used in this study is the

use of oil adjuvant Addit® in water. The adjuvant minimized desiccation and allowed free movement of nematodes towards their hosts. Desiccation is one of the obstacles to EPNs efficacy in foliar applications as it limits their persistence [24]. The findings of this study agree with those of Bauer [25]; Mason [26] and Piggott [27] who showed that, Ijs persistence on foliage is improved by the use of adjuvants such as Silwet®L77 and Tween® 20. Similarly, improvement of EPNs in foliar/cryptic habitats has been reported with the addition of adjuvants such as glycerin and polymers [28]. A study by Piggott [27] reported a reduced desiccation rate for *Steinernema feltiae* on the surface of leaves treated with a formulation containing a polymer humectant. Foliar spray of EPNs was mainly targeted at larval stages of *Tuta absoluta* and proved to be effective in reducing pest populations. This concurs with study by Batalla-Carrera [23], which reported that larva is the most susceptible stage to the EPNs, and foliar application of these nematodes is necessary to achieve a successful control of *Tuta absoluta*. It was also observed that the application of EPNs on soil would control last instar larval stage, when they slide down from the leaves to pupate, as well as emerging adults from the buried pupae [23]. Entomopathogenic nematodes may be combined with other compatible control agents such as *Bacillus thuringiensis*, neem and other botanical insecticides or half recommended dose rates of neonicotinoids like Imidacloprid insecticide to achieve better control of a single pest through additive or, preferably synergistic effects on pest mortality [29]. Various research carried out have shown that neem does not affect the survival, virulence or infectivity of EPNs when combined together [27]. Research done by Laznic [11] on the compatibility of azadirachtin with EPNs revealed that the mortality rates for Ijs were comparable with the control (water only) treatment. Similar results were obtained by Grewal [12] which showed that *Steinernema feltiae* was compatible with azadirachtin. Moreover, a study by Kulkarni [30] confirmed that the relationships between entomopathogenic nematodes and botanical insecticides such as neem at recommended doses indicated their compatibility and allowed higher nematode survival up to 72 hours after combination where, the survival rates of *Steinernema carpocapsae* after combining with 2.0% Neemglod® (neem) was 92.4%. The present study revealed that there was no significant difference between EPNs combined with neem and Coragen® treatments in *Tuta absoluta* population reductions. Coragen® was used for comparison purposes. The effectiveness of EPNs combined with neem is attributed to pathogenicity of EPNs coupled with neem (Azadirachtin) insecticidal properties. The effectiveness of EPNs combined with neem compared to EPNs alone is attributed to joint approach in the control of *T. absoluta* with EPNs targeting the larvae, while neem (Azadirachtin) has insecticidal, insect repellent, anti-feedant and ovicidal properties [31]. The united attack by EPNs combined with neem on the pest could have brought about the synergistic effect which resulted to decreased *Tuta absoluta* population [11]. Previous studies of combined neem and a



nematode species against white grub showed that their combination was better than the use of nematodes alone [29]. The installed delta trap with sticky plate and pheromone was for monitoring to guide the commencement of treatments after the alert of the presence of *Tuta absoluta* adult moths. This is because the decision scheme of using insecticides for management of *Tuta absoluta* is largely based on adult captures in sexual pheromone traps [32]. The action threshold used was based on occurrence of the three *Tuta absoluta* adult moths on the trap reported by [33]. This study revealed that control treatments had the highest percentage of fruit damage, compared to other treatments. According to Velez [34] the larvae sometimes bore the ovary of the tomato flower promoting the fall of the buds and flowers resulting to low yields. This can be attributed to increased *Tuta absoluta* larvae populations in untreated tomato plants, while EPNs alone, EPNs combined with neem and Coragen® had reduced larvae populations in treated plants which resulted to less damage on fruits.

V. CONCLUSION

EPNs combined with neem (Azadirachtin 0.03%), applied at one hour interval are able to reduce the populations of tomato leaf miner (*Tuta absoluta*) under greenhouse conditions. EPNs with Addit® adjuvant in water were equally able to reduce *Tuta* populations on tomato plants under greenhouse condition. Further research should be carried out to evaluate the survival and virulence of Kenyan EPNs when combined with synthetic pesticides against *Tuta absoluta* in order to come up with well guided and complete strategies for IPM programs.

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Statement of competing interests

The authors had no competing interests.

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List of Abbreviations

ANOVA	Analysis of Variance.
CABI	Centre for Agriculture and Biosciences International.
EPNs	Entomopathogenic Nematodes.
GOK	Government of Kenya.
ICIPE	International Centre of Insect Physiology and Ecology.
EPPO	European Plant Protection Organization.
IJs	Infective Juveniles.
IPM	Integrated Pest Management.
KALRO	Kenya Agricultural and Livestock Research Organization.
LSD	Least Significant Difference.

AUTHOR'S PROFILE



DAVID MUNYUA MUTEGEI

He is a researcher specializing in agricultural entomology. He has more than six years' experience in agronomy. He was born at Tharaka- Nithi County in Kenya, on 24th January, 1983.

Education and Credentials

Bachelor of Science in Horticulture from Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya.
Master of Science in Crop protection and Phytopathology from

University of Nairobi, Nairobi, Kenya.

Notable Achievements

Published a paper with World Journal of Agricultural Research entitled "Pathogenicity of Selected Native Entomopathogenic Nematodes against tomato leaf miner (*Tuta absoluta*) in Kenya."

Worked on a project entitled "Bio-control of Fusarium Wilt (*Fusarium oxysporum fsp. Cubense*) on banana by use of Endophytic Fusarium species."

He has worked as Precision Agriculture Agronomist and currently working as Crops Manager in Nairobi.

His research interests are developing integrated pest management programmes for sustainable management of *Tuta absoluta* and other pests in order to achieve good agricultural production. Also, developing soil health care programmes in the management of pests and diseases.

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I am an agricultural scientist specialized in Crop Protection, currently a lecturer at the University of Nairobi, College of Agriculture and Veterinary Sciences. I hold a PhD from the University of Nairobi. My field includes Entomology, Virology and Molecular biology. At the University, I teach basic and applied entomology, pest management, pesticide science and phytosanitary standards and related international trade issues. I have also worked as a civil servant with the Government of Kenya.

Key qualifications

- Research experience in various areas such as pest diagnostics, evaluation of both botanical extracts and biological control agents such as EPNs, Entomopathogenic fungi and natural enemies, crop pest management, integrated pest management and other crop protection systems as a result of own research and that with graduate students, in various crops such as passion fruits, Irish potato, beans, Brassicas, peas, citrus, maize, indigenous vegetables, tomato and post-harvest pests of grains.

Research interests:

Crop Protection in general with emphasis on the following i) Developing integrated pest management programmes for sustainable agriculture production (ii) to reliably detect and characterize viral infections in plants, particularly those that are vegetatively propagated; iii) developing tools to detect and characterize pest and diseases in economically important crops



Dr. Charles Waturu (PhD)

Current Position

Director Horticulture Research Institute (HRI)

Professional Background

- Cotton Entomologist for the last 34 years
- Principal Investigator, *Bt*-Cotton Project Carried out research on *Bt*-cotton for more than six years
- Co-investigator, *Bt*-Cotton Regional Project (Kenya, Uganda, Tanzania) on Baseline Biodiversity Impact Studies of Transgenic *Bt*-Cotton on Wild Ecosystems in the East African Region
- Principal Investigator, *GM Gypsophila* Project
- Lead Entomopathogenic Nematodes Research, Horticulture Research Institute

Work Experience

• Assistant Agricultural Officer II from 21st October 1981. Ministry of Agriculture

• Research Officer I from 14th December 1991. Kenya Agricultural Research Institute

• Senior Research Officer from 15th October 1999. Kenya Agricultural Research Institute

• Centre Director, National Fibre Research Centre-Mwea from 15th

January 1999 to 6th November 2003. The centre had a workforce of 50

research and support staff and was mandated with national obligation

for research on fibre crops

• Director, Horticulture Research Institute (HRI), 18th August 2014 to

date. In-charge of 4 centres including KALRO- Kandara, KALRO-

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