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Research Application Summary

Efficacy of Neem *(Azadirachtin indica)* biopesticide against tomato leaf miner (*Tuta absoluta*) in greenhouse conditions

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Abstract

The *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is one of the most devastating invasive new pest affecting tomato crops in Kenya. This study evaluated the insecticidal effect of neem (*Azadirachtin indica*) against *Tuta absoluta* at various dose rates namely; 20 ml/20L, 40 ml/20L, 60 ml/20L and control where only water was sprayed in greenhouse conditions. Neem was compared to Coragen®, a synthetic chemical recommended for lepidopteran pests. The experiment was laid out in a complete randomized design with four replications. The results revealed that neem significantly ($p < 0.05$) lowered *Tuta absoluta* populations on treated plants compared to the untreated. The mean Tuta absoluta population reduction at 40ml/20L and 60 ml/20L was significantly ($p < 0.05$) higher than that at 20 ml/20L, the lowest dose rate. Mean population reduction at 40 ml/20L and 60 ml/20L dose rates did not differ. Corgen® treatment had the lowest *Tuta absoluta* population in the greenhouse compared to in other treatments. High fruit damage of over 90% was recorded in control treatments compared to 30.0%, 23.4% and 20.0% for 20 ml/20L, 40 ml/20L and 60 ml/20L dose rates, respectively. The results demonstrate the potential of neem in reducing *Tuta absoluta* population which can be exploited in the pest control mitigation program for sustainable management.

Keywords: Biopesticide, neem, tomato, *Tuta absoluta*

Résumé

Le *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) est l'un des nouveaux ravageurs envahissants les plus dévastateurs affectant les cultures de tomates au Kenya. Cette étude a évalué l'effet insecticide du neem (Azadirachtin indica) contre Tuta absoluta à divers débits de dose, à savoir; 20 ml / 20L, 40 ml / 20L, 60 ml / 20L et témoin où seule de l'eau a été pulvérisée dans des conditions de serre. Le neem a été comparé au Coragen®, un produit chimique synthétique recommandé pour les lépidoptères nuisibles. L'expérience a été présentée dans une conception randomisée complète avec quatre répétitions. Les résultats ont révélé que le neem a significativement ($p <0,05$) abaissé les populations de Tuta absoluta sur les plantes traitées par rapport aux plantes non traitées. La réduction moyenne de la population de Tuta absoluta à 40 ml / 20 L et 60 ml / 20 L était significativement ($p <0,05$) supérieure à celle à 20 ml / 20 L, le débit de dose le plus faible. La réduction moyenne de la population à des débits de 40 ml / 20L et de 60 ml / 20L ne différait pas. Le traitement par

Corgen® avait la plus faible population de *Tuta absoluta* dans la serre par rapport à d'autres traitements. Des dommages importants aux fruits de plus de 90% ont été enregistrés dans les traitements témoins, contre 30,0%, 23,4% et 20,0% pour des débits de 20 ml / 20L, 40 ml / 20L et 60 ml / 20L, respectivement. Les résultats démontrent le potentiel du neem dans la réduction de la population de Tuta absoluta qui peut être exploité dans le cadre du programme d'atténuation de la lutte antiparasitaire pour une gestion durable.

Mots-clés: Biopesticide, neem, tomate, *Tuta absoluta*

Introduction

The tomato leaf miner *Tuta absoluta* (Meyrick) is a key pest in Kenya since 2014 and is adversely affecting tomato production in open field and in greenhouses. *Tuta absoluta* is a serious pest primarily in tomatoes but, is also reported in Irish potatoes and even solanaceous weeds (CABI, 2015). Potential yield loss in tomato quality and quantity can reach up to 100% if the pest is not managed. Many control programs have been investigated in many countries affected by *Tuta absoluta*, with chemical control as first line of treatment (Siqueira *et al.,* 2000; Lietti *et al.,* 2005). Various studies have reported the use of biological control agents in greenhouse tomatoes in Europe and South America where this pest was reported earlier (Desneux *et al.,* 2010). However, due to the development of resistance, chemical control has shown limited efficacy even after using different types of pesticides and increasing the application frequencies (Siqueira *et al.,* 2000; Lietti *et al.,* 2005). Increasing concern about the risks from synthetic insecticides to the environment and human health has given way for a search of less hazardous chemicals or biologically based products (Isman, 2006). Due to increasing resistance to chemical pesticides, there is need for emphasis on the importance of Intergrated Pest Management (IPM) programme in a resistant management strategy.

Neem (*Azadirachta indica*) as a botanical product can be used for management of *Tuta absoluta*. Neem (Azadirachta indica) has insecticidal properties which have been put into use for several thousands of years since it is biodegradable and effective against pests without harming beneficial insects (Haseeb *et al.,* 2004). Its biological properties are mediated by compounds such as limonoids and particularly azadirachtin mainly present in the neem seeds. The limonoids are considered the most active components responsible of both anti-feedant and insecticidal effects on insect pests (Isman, 2006). Neem compounds present various effects ranging from repellency to toxicity against a wide spectrum of insect pests (Shannag *et al.,* 2014). Neem based insecticides have low negative environmental impact because of its rapid degradation in plants and in the soil and low effects on beneficial insects (Haseeb *et al.*, 2004; Defago *et al.,* 2011). According to Mordue (2004) and Isman (2006) azadirachtin is non-toxic to vertebrates. Therefore, neem botanical pesticide represents a valuable tool to control population outbreaks in integrated pest management programmes. The aim of this study was to determine the insecticidal effects of neem biopesticide in the management of tomato leaf miner (*Tuta absoluta*) in greenhouse conditions.

Materials and Methods

Study site selection. This experiment was carried out in a greenhouse at Kabete Campus Field Station, University of Nairobi which is located 15km North West of Nairobi City Centre and lies at latitude 10 15'S and longitude 360 44'E and at altitude 1941 meters above sea level.

Neem biopesticide experiment under greenhouse conditions. Tomato seedlings were raised in a nursery bed measuring 1 x 2 M and after one month they were transplanted onto polythene pots measuring 20cm diameter and 26cm height containing 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 (6 M width x16 M length), with a temperature range of 15-34 ^{0}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 was repeated twice. The treatments consisted of different concentrations of Nembicidine® at 20 ml/20L, 40 ml/20L, 60 ml/20L and a control where only water was applied, which were then compared with Coragen® a synthetic insecticide recommended by the Kenya Ministry of Agriculture, Livestock and Fisheries for controlling this pest. Nembicidine® (Azadirachtin 0.03%) and Coragen® are commercial pesticide products in Kenya. Larval count was done on four leaves per plant on 12 tomato plants randomly selected per treatment on weekly basis. Treatment application was done using a knapsack sprayer on a weekly basis. Initiation of treatments was based on economic threshold of three *Tuta absoluta* adults caught on a sticky delta Tuta trap hanged at the centre of the greenhouse. *Tuta absoluta* larvae attacks fruits by making galleries, penetrating mainly at the base or near the peduncle insertion zone. 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.

Statistical Analysis. The larval count data collected were square-root transformed before analysis. The data were then subjected to analysis of variance (ANOVA) to assess treatment effects while the Fischer's protected least significance difference (LSD) test was used to compare treatment means at $p \leq 0.05$. Similar analysis was done for tomato fruits yield data. The analysis was done using GenStat- PC v.14.1, 14th Edition.

Results

Efficacy of neem biopesticide in the management of tomato leaf miner in greenhouse conditions. During the experimental period, *Tuta absoluta* naturally affected the tomatoes established in the greenhouse. Tuta populations generally decreased after neem applications began up to the fourth week before the populations started to increase in the fifth week. The populations in control plots continuously increased to reach a mean high of (7.2). Neem at different dose rates tested significantly reduced Tuta absoluta populations compared to control. Neem (Azadirachtin 0.03%) application significantly ($p < 0.05$) lowered *Tuta absoluta* populations on treated plants compared to the untreated. In season one neem significantly ($p < 0.05$) reduced *Tuta absoluta* larvae populations on tomato plants. The control had the highest mean Tuta absoluta population (3.9) while the mean count achieved by the highest dose rate (60 ml/20L) was low (1.4) and that achieved by Coragen® being the lowest (1.1). The mean population count in the 60 ml/20L and 40 ml/20L neem dose rates were significantly lower and that counted in the Coragen® treatment. Further, the mean *Tuta absoluta* population in 40 ml/20L and 60 ml/20L treatments were significantly ($p < 0.05$) lower than that counted on plants treated with 20 ml/20L dose (Table 1).

Table 1. Mean population of *Tuta absoluta* larvae in four leaves per tomato plant after neem (Azadirachtin 0.03%) treatment in season one (December 2015 to February 2016)

		Sampling period in weeks after planting								
Treatment	Rate/20L	1	2	3	4	5	6	7	8	Mean
Low dose	20 ml	1.4	1.3	1.2	1.1	1.7	2.1	3.0	2.1	1.7c
Medium Dose	40 ml	1.4	1.2	1.1	1.0	1.3	1.9	2.5	1.9	1.5b
High dose	60 ml	1.4	1.2	1.0	1.0	1.7	1.6	2.0	1.5	1.4b
Coragen	3 ml	1.4	1.3	1.2	1.1	1.1	1.0	1.0	1.0	1.1a
Control	Water	1.4	2.1	2.3	2.6	3.2	5.6	7.2	7.1	3.9d
LSD(0.05).										0.1
CV(%)										26.4

Means followed by the same letter in the column are not significantly different at ($P < 0.05$). Transformation Formula is = Square root of (X+0.5), where X is the data to be transformed.

In season two, *Tuta absoluta* populations were higher than in season one. Nevertheless, a similar trend like that in season one was observed (Table 2). There was a general reduction of *Tuta absoluta* larvae as neem was applied up to fourth week in all the dose rates tested before the numbers started to increase in the fifth week but at a slow rate. In the p control plots the larvae population continuously increased reaching a mean high of (7.3) at the seventh week (Table 2). Neem applications significantly ($p < 0.05$) reduced *Tuta absoluta* larvae populations on tomato plants. The control had the highest mean Tuta population (4.2) while the highest neem dose rate (60 ml/20L) had low (1.4) while Coragen® had the lowest (1.0) mean *Tuta absoluta* population. The mean population count on the 60 ml/20L dose rate was however, not different from that counted on plants treated with 40 ml/20L dose (1.5). Mean *Tuta absoluta* count on 40 ml/20L and 60ml/20L dose rates treated plants was significantly lower than that counted on 20 ml/20L dose rate plants (Table 2).

Table 2. Mean population of *Tuta absoluta* larvae in four leaves per tomato plant after neem (Azadirachtin 0.03%) treatment during season two (March to May, 2016)

		Sampling period in weeks after planting								
Treatment	Rate/20L	1	2	3	4	5	6	7	8	Mean
Low dose	20 ml	1.6	1.5	1.2	1.3	1.9	2.4	2.5	2.1	1.8c
Medium Dose	40 ml	1.6	1.3	1.1	1.2	1.4	2.0	1.9	1.7	1.5b
High dose	60 ml	1.6	1.4	1.1	1.0	1.5	1.6	1.7	1.6	1.4b
Coragen	3 ml	1.6	1.2	1.2	1.1	1.1	1.0	1.0	1.0	1.0a
Control	Water	1.7	1.6 2.3	2.5	2.6	3.7	6.6	7.3	7.0	4.2d
LSD (0.05)										0.1
CV(%)										25.4

Means followed by the same letter in the column are not significantly different at ($P< 0.05$). Transformation Formula is = Square root of (X+0.5), where X is the data to be transformed.

In the first season, high tomato damage of over 90% was recorded in control compared to 30.0%, 23.4%, 20.0% and 8.3% for 20ml/20L, 40ml/20L, 60 ml/20L dose rates and Coragen®, respectively. Control had the lowest total harvested yield (Table 3).

Table 3. Effect of neem (Azadirachtin 0.03%) treatments on tomato yield in season one

	Average fruit weight (tons/hectare)			
Dose Rates	Damaged	Not damaged	Total	% Damaged fruits
20ml/20L	2.2	5.2	7.4	30.0b
40ml/20L	2.2	7.2	9.4	23.4b
60ml/20L	2.0	8.1	10.1	20.0b
Coragen 3ml/20L	0.8	9.6	10.4	8.3c
Control	2.6	0.2	2.8	92.9a
LSD(0.05)		0.1		
CV(%)		9		

Means followed by the same letter in the column are not significantly different at ($p < 0.05$).
Percent tomato fruits= (Weight of damaged fruits/total weight of harvested fruits)*100
(* = Is the multiplication sign).

In the second season, high fruit damage of 96% was recorded in control plots compared to 31.5%, 22.1%, 20.2% and 7.8% for 20ml/20L, 40ml/20L, 60ml/20L dose rates and Coragen®, respectively. Control had the least harvested tomato yield (Table 4).

Table 4. Effect of neem (Azadirachtin 0.03%) treatments on tomato yield in season two

	Average fruit weight (tons/hectare)			
Dose Rates	Damaged	Not damaged	Total	% Damaged fruits
20 ml/20L	2.3	5.0	7.3	31.5b
40 ml/20L	2.1	7.4	9.5	22.1b
60 ml/20L	2.0	7.9	9.9	20.2b
Coragen 3 ml/20L	0.8	9.5	10.3	7.8c
Control	2.5	0.1	2.6	96.2a
LSD (0.05)		0.1		
CV(%)		9.1		

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

Discussion

The results obtained reveal that it is possible to reduce the *Tuta absoluta* larvae populations by application of neem biopesticides. This *Tuta absoluta* population reduction can be attributed to an active ingredient in neem (Azadirachtin) which has an anti-appetizing, anti-feedant, disgusting and sterile properties that inhibit molting, growth and larval development (Patil and Goud, 2003). The evaluated neem concentrations caused significant reduction in *Tuta absoluta* populations, with the highest reduction achieved at 60 ml/20L dose rate and the lowest at 20 ml/20L dose rate. These results are not unanticipated given that the toxicity of insecticides of plant extracts has been shown to vary with dose and duration of exposure (Shannag *et al.,* 2014). Neem was effective in reducing *Tuta absoluta* population when 40 ml/20L and 60 ml/20L dose rates were applied probably because they offered a high concentration of azadirachtin. These findings are similar to those of a study by Haseeb *et al.* (2004), who reported that botanical extracts derived from *Melia azedarach* and *Azadirachta indica* reduced populations of *Plutella xylostella*. According to Patil and Goud, (2003) neem seeds extract induced high *Tuta absoluta* larval mortality under laboratory conditions. However, these results suggest that in greenhouse tomato cultivation treatment with neem alone is not enough to successfully reduce tomato leaf miner (*Tuta absoluta*) damages, as fruits in all the treatments had varying percentage damage. These results are similar to those by Mordue and Nisbet (2004), who reported that open field cultivation treatment with Azadirachtin alone was not enough to successfully reduce *Tuta absolut*a damages. Control (where only water was applied) had the highest percentage of fruit damage and a low number of fruits compared to the neem treated plants. This implies that the total number of fruits and the undamaged fruits were affected by increasing infestation densities of *Tuta absoluta*. This is probably the reason why the control had the least total yield of tomato harvested (Table 3 and Table 4). The high fruit damage percentages could have been a result of *Tuta absoluta* consumption of fruit in formation still protected by sepals. Sometimes the larvae bore the ovary of the tomato flower promoting the fall of the buds and flowers resulting into low yields. However, the reduced number of fruit produced by the plants exposed to large densities of *Tuta absoluta* in control treatments can also be in response to foliar area damage because the average number of fruit per plant is affected by the percentage of defoliation in tomato plants.

Conclusion

Among the evaluated neem (Azadirachtin 0.03%) dose rates, 40 ml/20L was effective in reducing *Tuta absoluta* population and percent fruit damage. Therefore, Neem has the potential to reduce *Tuta absoluta* population on tomato in greenhouse conditions.

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