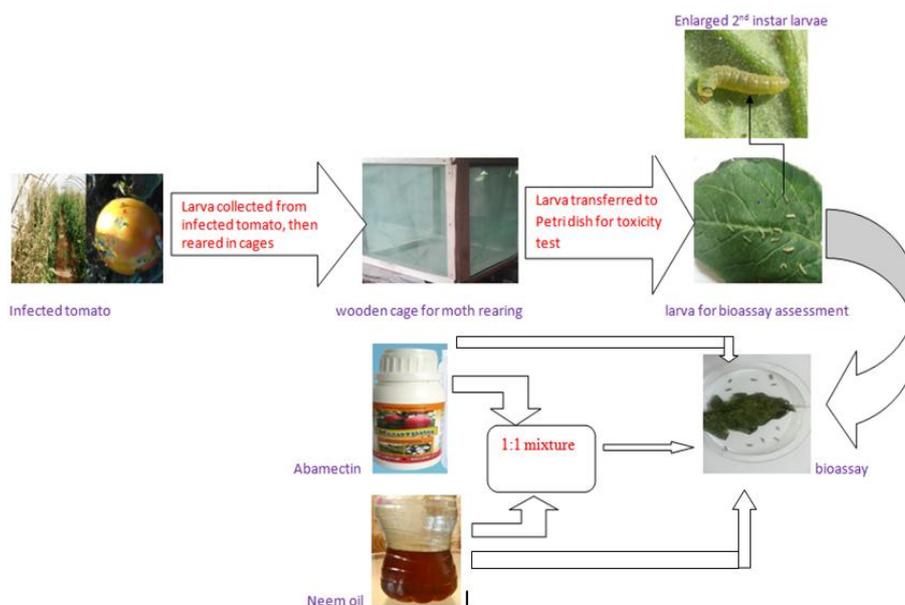


Improving the Efficacy of Abamectin Using Neem Oil in Controlling Tomato Leaf miners, *Tuta absoluta* (Meyrick)

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ARTICLE INFO	ABSTRACT
<p>Received: 1 January 2019 Revised: 26 January 2019 Accepted: 13 February 2019 Available online: 5 March 2019</p> <p>DOI: 10.29088/SAMI/AJCA.2019.2.216224</p>	<p>Tomato leaf miners, <i>Tuta absoluta</i> pose a control challenge against dozens of chemical pesticides including abamectin as a result of increase in resistance with time. This study aimed at improving the field performance of abamectin using crude neem oil. The oil was obtained from the healthy neem seeds by cold pressing method. A total of 10 larvae were used in each bioassay experiment. Abamectin at 1000 ppm caused corrected mortality of 79% and its concentration that kills half of the experimental larvae (LC50) was 363 ppm. The crude neem oil at concentration of 2500 ppm caused corrected mortality of 61% with LC50 at 1089 ppm. The 1:1 mixture of abamectin and crude neem oil (without heating) at concentration of 1000 ppm gave 83% as highest corrected mortality and LC50 at 251 ppm. The findings from bioassay experiments revealed that, both neem oil and abamectin are potential pesticides; however their performance was even high after mixing. It was the synergism effect that gave the mixture high performance.</p>
<p>KEYWORDS</p> <p>Biopotency Pesticide Bioassay Tomato leaf miners Pest control</p>	

GRAPHICAL ABSTRACT



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Introduction

In Tanzania, tomato (*Lycopersicon esculentum* Mill. or *Solanum lycopersicum* L) is grown by small scaled farmers in open field and less in green houses. It is grown for domestic consumption and as source of income [1]. It is an integral part of diet throughout Tanzania and can be eaten as processed or raw. Its production has been on a decreasing trend due to many factors including pests and diseases [2, 3]. Tomato crop is damaged by different types of pests in both open and green houses; of these, tomato leafminers, *Tuta absoluta* is a trouble shooter.

The pest was reported for the first time in Tanzania in 2014 [3] and thought to be introduced through Kenya borders. Lack of strong quarantine regulations or poor enforcement of available regulations and lack of screening at the borders are the factors behind their spread African countries including Tanzania [4, 5].

According to [6, 7, 8] and references therein, *T. absoluta* is a micro lepidopteron moth with high reproduction potential. A single life cycle is completed within 30-35 days under suitable conditions and the pest is capable of producing ten to twelve generation per annum. Once eggs are hatched to young larvae, they immediately start feeding on leaves, stem, flowers, buds

and there- by making mines and galleries and hence capable of causing 100 percent production loses. Due to its endophytic feeding habit, it is quite challenging to control leaf miners, as they feed inside the leaves, fruits or stems [9].

Tomato leaf miners were reported to be resistant to dozens of insecticides including diamide insecticides, chlorantraniliprole, abamectin, deltamethrin, methamidophos, permethrin, cartap [10, 11]. One study conducted in Brazil in 2014 by [12], showed *T. absoluta* resistance to spinosad. Another research at South-East Sicily (Italy) by [13], confirmed the first case of diamide-resistant to *T. absoluta*. Diamide pesticides include chlorantraniliprole, flubendiamide. Cartap was once used as registered pest for control of leaf miners but according to [14], the pesticide demonstrated control failure against leaf miners. Resistance to abamectin, cartap, methamidophos and permethrin was also reported in Brazil [14] and that on abamectin, deltamethrin and methamidophos was reported in Argentina [11]. The field failure of many pesticides against leafminers is of great concern.

Nem oil is one of the future promising natural pesticides. The oil comes from seed of neem tree, *Azadirachta indica* [10, 15]. It contains biopotent compounds with microbial and insecticidal properties. In addition to fatty acid, sterol and protein

content, it contains triterpenoids or limonoids with some bioactivity. Important neem oil limonoids are azadirachtin, salannin, nimbin, azadiradione, azadirone, nimbolin, nimbinin, melianone and melianol. However, it was only azadirachtin which has been commercially exploited as bio-pesticide effective to over 600 species of pests including insects, nematodes, bacteria, fungi and viruses. Azadirachtin works as natural Anti-feedant, growth regulator and ovi-positional repellent for several insects. **Nimbin** is another triterpenoid which has been credited with some of neem oil's properties as an antiseptic, antifungal, antipyretic and antihistamine while **Salannin** is insect anti-feedant and growth regulator [15].

In Tanzania, abamectin is one of the pesticide recommended for *T. absoluta* control but has proved field failure due to pest resistance. As the result, over application of abamectin was in due increase. Application of botanical pesticide as greenery approach was not a common practice among farmers. What many farmers in Tanzania are not aware of is that, pests like *T. absoluta* which have a short generation time and high reproductive rate, are at an increased risk of developing resistance to pesticides. To avoid a similar predicament, a shift in current pest management practices in Tanzania is

necessary. To control the pest effectively it is critical to combine all available control measures and alternate the use of active substances with different modes of action. Neem oil can be used alone or formulated with other synthetic pesticides. Formulations made of neem oil also find wide usage as a bio-pesticide for organic farming as repellent to many pests. Therefore, formulation of abamectin using crude neem oil served for the similar purpose. The advantages of using bio-pesticides as control agents are their efficiency and safety to human and other non-target organisms, reduction of pesticide residues in food, preservation of other pest natural enemies, and increased biodiversity in ecosystem [16].

Methodology

Study area

The study was conducted at Mto wa Mbu, an administrative ward in Monduli district Arusha region-Tanzania. It is situated at 3.35° south and 35.85° east. The area is located along the East Africa rift valley near Lake Manyara where the local irrigation system is practiced. The place have perfect weather conditions required for maximum growth of tomato and *T. absoluta*, as it is fertile, warm, and humid [17].

Plant materials

Matured and healthy seeds from neem trees

(*Azadirachta indica*) were identified and collected. The neem seeds were collected at Karatu district (Latitude: 3° 19' S Longitude: 35° 39' E) in Arusha region-Tanzania through October to December, 2016. Collected seeds were washed under running tap water and shade dried for 10 days and then sun dried for 6 days. The seeds were cold pressed using hand operated sunflower compressing machine to extract oil.

Pest sample collection and rearing

A stock rearing of *T. absoluta* was established using larvae collected in commercial tomato fields, in Arusha municipality-Tanzania. The tomato fruits with the sign of infection (mines) were collected from the field and then dissected to extract larvae. Larvae were reared in 2 wire-meshed wooden cages with dimension (50×50×60 cm); fed with untreated tomato leaves. The stocks were maintained in normal environmental conditions at the study site. Adult moths were kept in cages for laying eggs and were feed on 10% honey solution. The larvae obtained at this stage were used in bioassays.

Larval bioassays experiment

Bioassay was performed at the study site under the normal environmental conditions; as this provides exactly real conditions in tomato field where insecticides are to be

applied. The leaf-dip method was used to test the toxicity of abamectin, neem oil and mixture of abamectin and neem oil on *T. absoluta*. Tap water was used as a control. The leaves for bioassays were taken from tomato plant, 50 days after seedlings have been transplanted. Tomato leaflets and leaves with their petiole were cut from the 4th leaf from the stem apex. The leaves were dipped for fifteen seconds in respective insecticides solution and water (control); removed and dried at room temperature for 30 minutes. After drying, tomato folioles were transferred to two-litre transparent PET bottles with petiole immersed in glass vials containing 120 ml of water. Ten larvae were used in each treatment in triplicates.

The first group was treated with abamectin, second group with neem oil, third group with neem oil + abamectin and control group with tap water. The color and the turgidity of leaves were evaluated daily. The leaves were infested with 10 larvae of 2nd instar and larvae were transferred to the leaves with help of a thin brush. Larvae used in bioassays experiment were those reared in wooden cages. Mortality rate was assessed after every 2 days for six days. The larvae were considered dead if they were unable to walk when prodded with a fine paint brush [18, 19, 20].

Larvae mortality and LC-Values calculations

Five concentrations of each pesticide were used, apart from control that consists of water only. Three replicates were used for each pesticide concentration and bioassay was carried out following a completely randomized design. Assessment of larval mortalities was expressed as corrected percentage mortality of total larvae for each treatment after every 2 days for 6 days. Corrected percentage mortality was calculated according to Abbott's (1925) formula [21], as follows:

$$\text{Corrected mortality (\%)} = (T - C) / (10 - C) \times 100.$$

Where, T = No. of dead larvae in treated replicates, C = No. of dead larvae in control replicates and LC50 = the lethal concentration that kills 50 % of larval individuals.

Concentration-mortality data were subjected to a probit analysis correcting the data for natural mortality. The 50% lethal concentrations (LC₅₀) was computed by Microsoft excel using regression equation.

Results and discussion

Results

Larval toxicity bioassay data obtained from the study site were presented using tables and graphs. Data were collected per week (six days) after every two days (48 hours), that was 2, 4, and 6th day. All data obtained were in triplicate and cumulative mean corrected mortality after six days was determined. Corrected larval mortality was determined using Abbot Formula (1925) according to [21]. The percentage mortality and LC 50 was also determined.

Table 1. Bioassay data on *T. absoluta* 2nd instar larvae with abamectin

Conc. in ppm	100	250	500	750	1000	Control
Cumulative mean mortality	2.53	3.56	4.78	6.67	7.89	0
% Mortality	25.3	35.6	47.8	66.7	78.9	0
LC50 = 363						

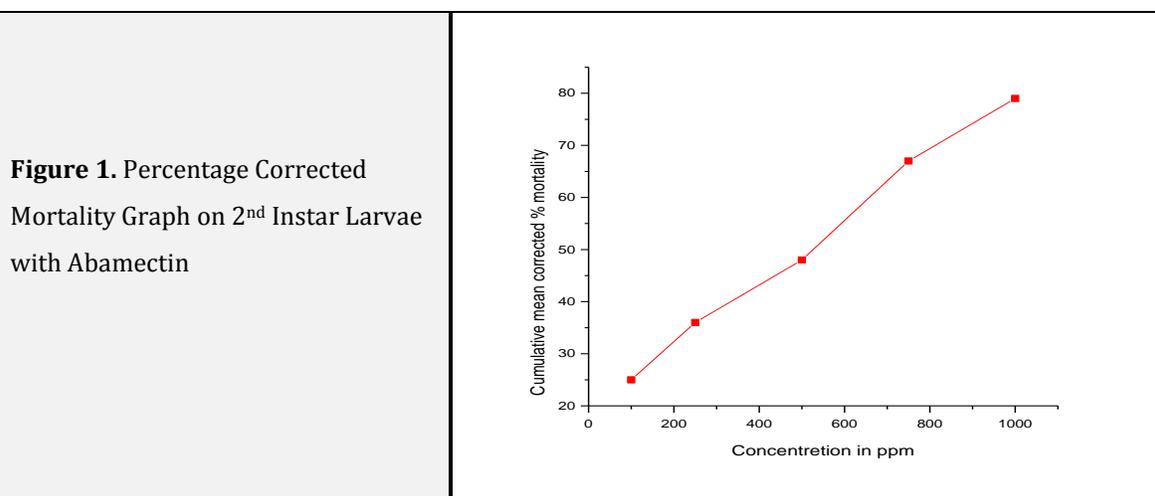


Table 2. Bioassay data on 2nd instar *T. absoluta* larvae with crude neem oil

Conc. in ppm	500	1000	1500	2000	2500	Control
Cumulative mean mortality	1.6	2.8	3.7	4.7	6.1	0
% Mortality	16	28	37	47	61	0

LC50 = 2089

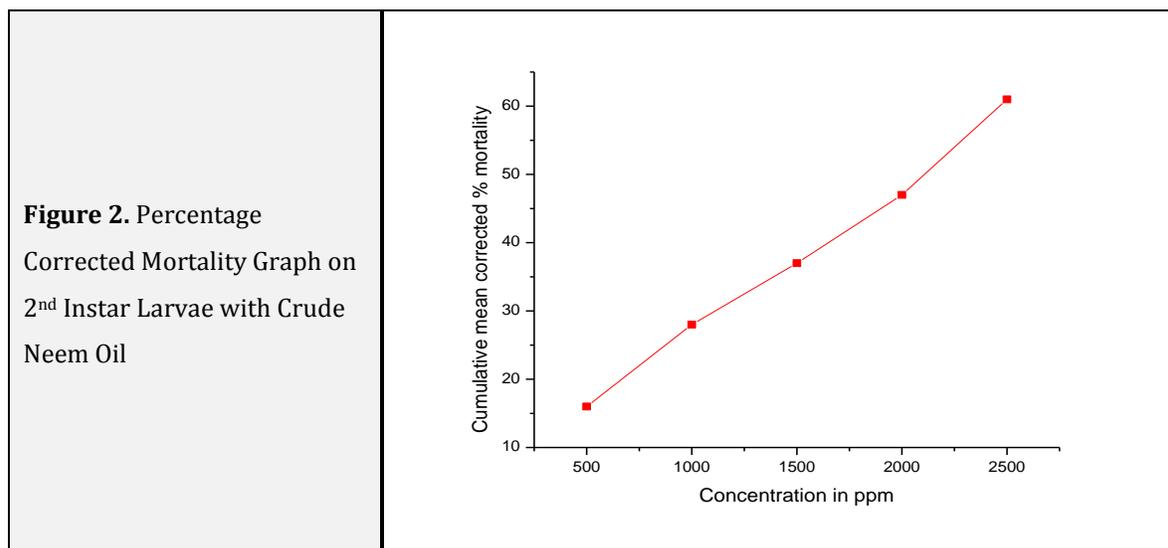
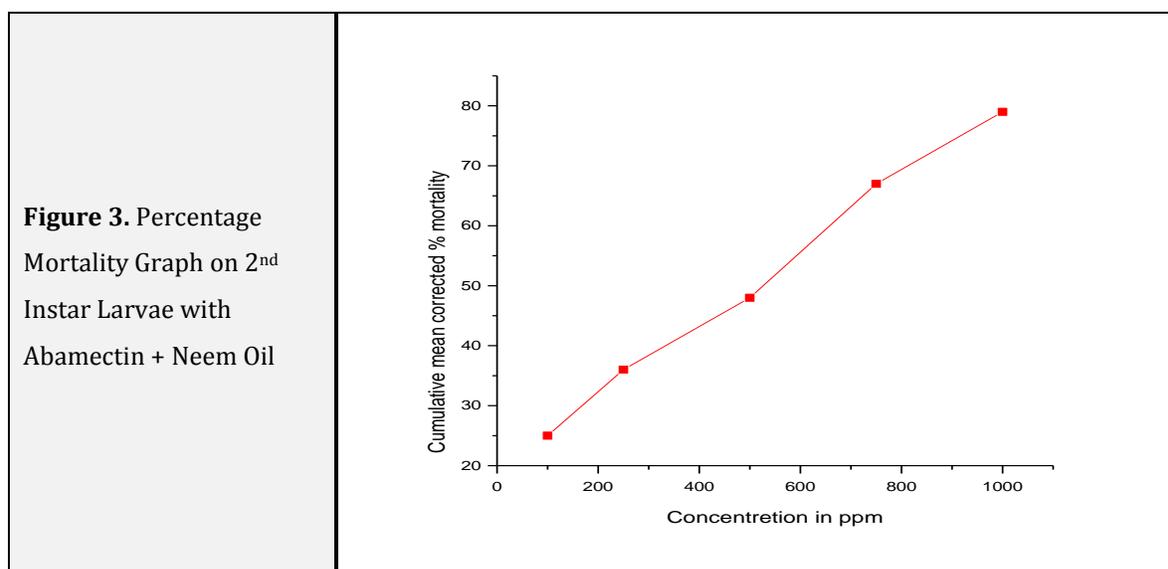


Table 3. Bioassay data on 2nd instar larvae with abamectin + neem oil

	100	250	500	750	1000	Control
Cumulative mean mortality	3	4.8	6.2	7.4	8.8	0
% Mortality	30	48	62	74	88	0

LC50 = 251



Discussion

The larval toxicity of abamectin, neem oil, and abamectin + neem oil were assessed on 2nd instar *T. absoluta*. A total of 90 larvae was used in bioassay, ten per each treatment and in triplicate. The general trend was that, corrected percent mortality increases with increase in pesticide concentration (refer figure 3.1, 3.2, and 3.3). Abamectin at concentration of 1000 ppm caused corrected mortality of 79% as shown in (Table 1) and its concentration that kills half of the experimental larvae (LC50) was 363 ppm. The crude neem oil at concentration of 2500 ppm attained corrected mortality of 61% with LC50 at 1089 ppm. The findings on neem oil biopotency are in line with previous reports. The 1:1 mixture of abamectin and crude neem oil at concentration of 1000 ppm gave 83% as highest corrected mortality and LC50 at 251 ppm. From the LC50 values and corrected mortality concentration, it is clear that, both abamectin and crude neem oil are potent pesticides.

The lower LC50 value of abamectin + neem oil compared to that of abamectin or neem oil means that, the mixture of abamectin and neem oil have a better performance than when used alone. Drug synergism (drugs joint action) explains the increased abamectin/neem oil performance. Synergism is the joint action of two or more

drugs of different mode of action against one pest species. Crude neem oil does not only improve abamectin performance but also reduce its concentration.

Since abamectin and other pesticides suffer from low field performance and pests' resistance, crude neem oil can be used by the farmers to improve their performance. Interestingly the plant *Azadirachta indica* grows well in entire tropical belt and can potentially be used as a pesticide to control tomato leaf miners.

Conclusion

The findings from bioassay experiment revealed that, both neem oil and abamectin are still potential pesticides. The efficacy of both pesticides was enhanced when they are applied in 1:1 mixture ratio. The lower LC50 value and higher percentage corrected mortality of the mixture is an evidence for their improved performance. The mixing of abamectin with neem oil does not only improve their insecticidal performance but also reduces synthetic pesticide residue concentration in the environment as concentration of abamectin was halved. On the other hand, the neem oil is environmentally friendly and not hazard to beneficial insects, birds, animals and human being.

Recommendations

T. absoluta pest status in Tanzania is growing yearly without any sound action plan to halt the spread to unaffected areas [3]. We recommended that, the government through ministry of agriculture, farmers, research institutions, public and private sectors and other stakeholders to meet and come up with a plan and strategies that will help to halt the spread of *T. absoluta* to unaffected area.

In Tanzania, most farmers rely on chemical methods of pests control with little knowledge on integrated pest management, the used of botanical pesticides, biological control and biochemical pesticides [2]. In this regard, we recommend for farmer's training in integrated pest management especially the use of neem oil and other botanical oils that enhances pesticides performance.

We recommend further research study aiming at developing potential botanical pesticides that will replace the synthetic one.

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