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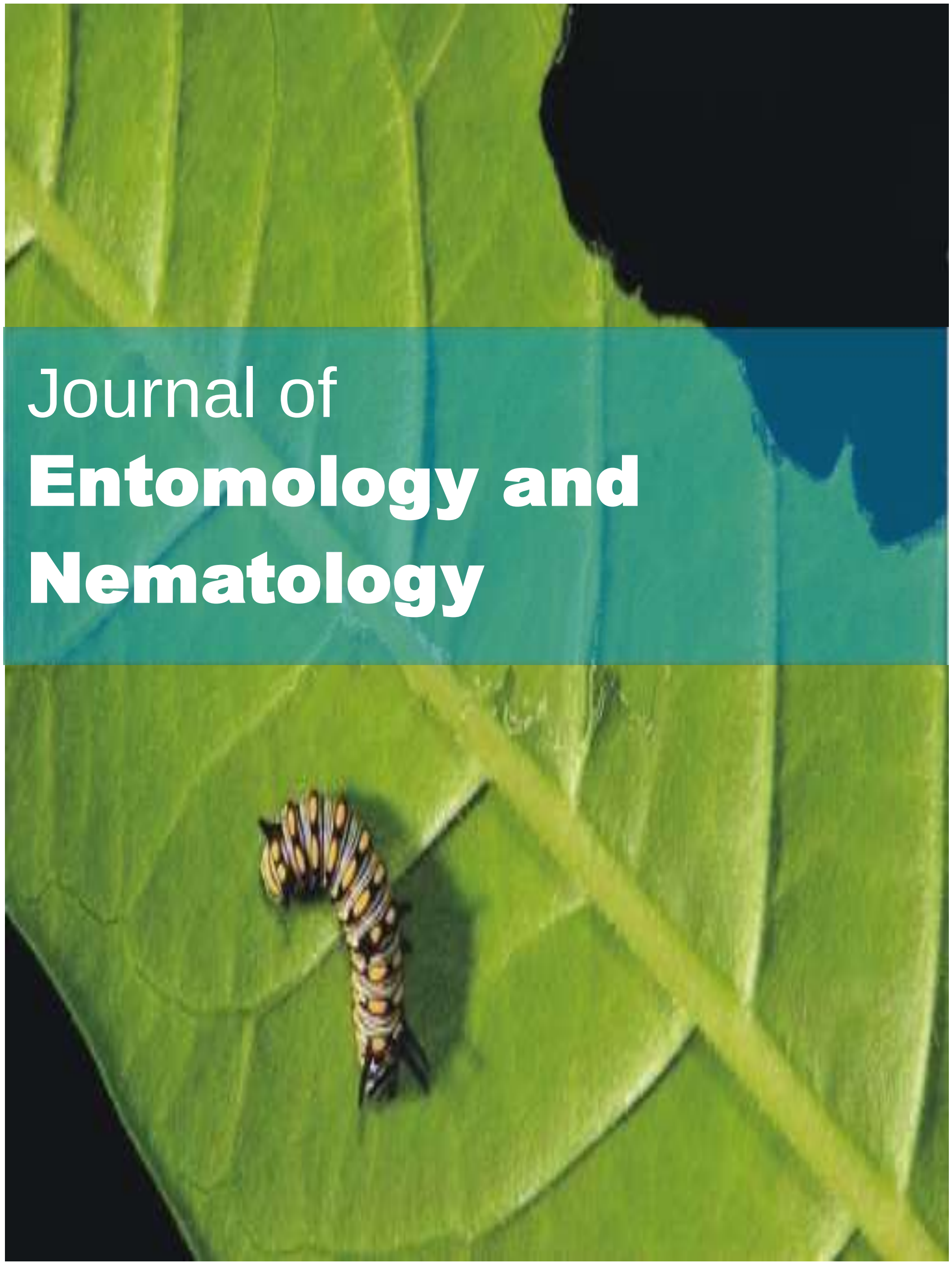

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Full Length Research Paper

Determination of the larvicidal activity of benzoyl thiosemicarbazone and its Ni(II) complex against *Aedes aegypti* and *Anopheles darlingi* larvae in Amazonas, Brazil

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Due to the resistance of some mosquitoes to pyrethroids insecticides, new synthetic compounds are of great interest for the development of new insecticides against vectors of tropical diseases, especially in the Amazon region. Our aim was to synthesize and evaluate the larvicidal potential of benzoyl thiosemicarbazone and its Ni(II) complex against larvae of *Aedes aegypti............* and *Anopheles darlingi............* The compounds were synthesized from thiosemicarbazide according to the literature, and the larvicidal potential was evaluated in triplicate at concentrations of 7 to 500 µg/mL. Benzoyl thiosemicarbazone and its Ni(II) complex showed an LC_{50} of 42.09 and 42.28 µg/mL, respectively, against *. Ae. aegypti* larvae. For *.An. darlingi* larvae, the LC_{50} values of benzoyl thiosemicarbazone (4.77 µg/mL) were lower than its Ni(II) complex (7.33 µg/mL). Benzoyl thiosemicarbazone presented satisfactory results against the larvae, and due to the insecticidal potential of this substance, the development of new chemical insecticides may be possible.

Key words: Benzoyl thiosemicarbazone, nickel (II), larvicidal activity; *Aedes aegypti*, *Anopheles darlingi.*

INTRODUCTION

Many pathogens can be transmitted to humans from infected mosquitoes such as *Aedes aegypti* (Linnaeus, 1762), a species responsible for the transmission of dengue fever, yellow fever, Chikungunya and Zika. In Brazil, this vector is one of the main public health problems, since it is extremely urban, has high population growth rates and is difficult to control (Simon et al., 2008; Puccioni-Sohler et al., 2017).

Anopheles darlingi (Root, 1926) is of great medical relevance, as it is the vector responsible for the transmission of Malaria in Brazil, where it is basically confined to the Amazon region. Among the anopheline mosquitoes found in the region, *A. darlingi* is the species that most benefits from human modifications to the

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environment. It is highly anthropophilic and endophagic (Deane, 1986; Tadei et al., 1998; Maciel-de-Freitas et al., 2012; Sinka et al., 2012). In this case, the control of mosquito populations is performed with insecticides, which, despite numerous records of resistance and high toxicity to nontarget organisms, still provide one of the most effective methods for combatting mosquitoes in endemic areas (Rivero et al., 2010; WHO, 2017).

Thiosemicarbazones belong to the thiourea group, an important class of N and S donor compounds that have high pharmacological potential, and in many cases, the mechanism of action of thiosemicarbazone is associated with complexed transition metals. From a biological point of view, metal complexes are more bioactive than free ligands, particularly thiosemicarbazones, which are active only when complexed with transition metals (Wasi and Singh, 1987; Rosu et al., 2010; Viñuelas-Zahínos et al., 2011; Netalkar et al., 2015).

The pharmacological applications of the different structural derivatives of thiosemicarbazones and their metal complexes include antifungals (Parrilha et al., 2011), cytotoxics (Rebolledo et al., 2005; Braga et al., 2016), antibacterials (Despaigne et al., 2010), antimalarials (Greenbaum et al., 2004; Chellan et al., 2010; Nandal and Deep, 2017) and insecticides (Rayms-Keller et al., 1998; Wang et al., 2010; Silva et al., 2015). Metal complexes or even free metal ions are toxic to aquatic organisms and may be found available at low levels in the environment (Arnold et al., 2005). Thus, due to the high incidence of vector mosquitoes in the region, the permanent and semi-permanent expressions of *Aedes* and *Anopheles* mosquitoes in the urban areas of cities and control actions on immature forms represent a functional alternative for the control of insects (WHO, 2014).

The synthesis and biological activity of the 1-benzoyl analogue have been reported in the literature (Xue et al., 2007; Pingaew et al., 2010). In the present study, we examined the larvicidal activity of benzoyl thiosemicarbazone and its nickel (II) complex in bioassays against *A. aegypti* and *A. darlingi*, for the control of tropical diseases in the Amazon. Our study addresses the process for obtaining the substance and its nickel complex as well as their larvicidal activity against vectors of dengue and malaria in the Amazon region not yet described in the literature.

MATERIALS AND METHODS

Synthesis of Benzoyl thiosemicarbazone (HBzS) and the nickel metal complex [Ni(BzS)2]

All reagents were purchased from the Sigma-Aldrich and used without further purification. The compounds were synthesized (Beraldo et al., 1997 and Pingaew et al., 2010). The benzoyl thiosemicarbazone was prepared using 11 mmols of thiosemicarbazide for 11 mmol of benzaldeyde. The mixture was heated under reflux for 8 h and ethanol as a solvent. The nickel metal complex was prepared using 2 mmols of Benzoyl thiosemicarbazone for nikel chloride II heated under reflux and drops of amonium hidroxide in ethanol. The FT-IR spectra (KBr) were recorded on Perkin Elmer 283B (4000–400 cm^{-1}) spectrometer. The 1H NMR and 13C NMR were obtained on a Unity Inova 500 Varian spectrometer, em DMSO-d6.

Synthesis of compounds

HBzS: yellow crystals; yield (%): 70; melting point (°C): 205; IR (KBr, cm-1): v (C=N): 1575; v (C=S):857; v (N-H): 3176; v (N-H2): 3052. 1H NMR (500 MHz, DMSO-d6) (δ): 8.71 (s, 1H), 7.89 – 7.87 (dd, 2H, J: 2 Hz), 7.52 – 7.48 (m, 2H J: 5 Hz), 7.45 – 7.40 (m, 1H, J: 7.5Hz), 6.85 (s, 3H), 3.40 (s, 2H). 13C NMR (500 MHz, DMSO-d6) (δ):184.14; 161.44; 133.78; 131.37; 128.92; 128.36; 125.76; 76.95.

[Ni(BzS)2]: green solid; yield (%): 65; melting point (°C): > 300; IR (KBr, cm-1): v (C=N): 1575; v (C=S): 751; v (Ni-N): 497; v (Ni-S): 448; v (N-H): 2950; v (N-H2): 3052. 1H NMR (500 MHz, DMSO-d6) (δ): 8.71 (s, 1H), 7.89 - 7.86 (dd, 3H, J: 2 Hz), 7.52 – 7.48 (m, 2H, J: 5Hz), 3.34 (s, 2H). 13C NMR (500 MHz, DMSO-d6) (δ): 162.12; 134.49; 132.05; 129.61; 129.06; 127.98.

The formation of all compounds was confirmed by IR spectroscopy, 1H NMR and 13C NMR. The IR spectrum of Benzoyl thiosemicarbazone showed absorption bands at 3052, 1575 and 857 cm^{-1}, corresponding to the NH, C=N and C=S groups, respectively. The IR spectrum of the nickel metal complex showed absorption bands at 3052-2950, 1625, 751, 497 and 448 cm^{-1}, corresponding to the NH, C=N, C=S, Ni-N and Ni-S groups, respectively. Compounds showed a sharp singlet observed at δ 8.70 and 8.71, which confirmed the presence of the NH to HBzS and [Ni(HBzS)2], H-aromatic rings at δ 7.42 – 7.87 and δ 7.50-7.89, respectively. The 13C NMR spectra of compounds showed peaks at δ 193.24 and δ 161.33, corresponding to C=S carbon to HBzS and [Ni(HBzS)2], respectively. The above values are evident for formation of compounds (Figure 1).

Study site and period

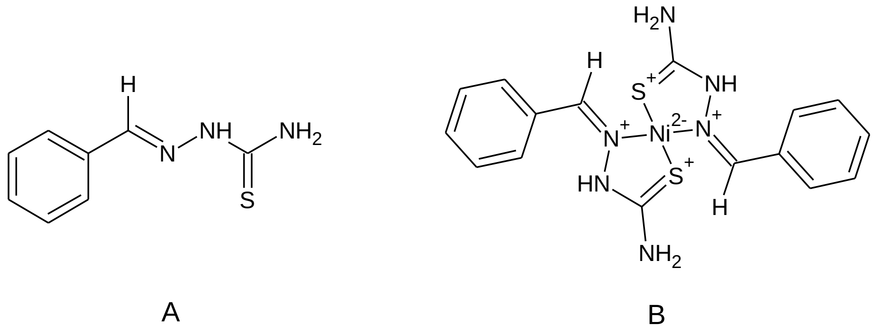
The study was conducted in 2011 in the city of Manaus, Amazonas State, Brazil (-3.096240 latitude, -59.986194 longitude), located in the northern region of the country, which comprises the Brazilian Amazon region. Manaus has a population of 1,802,014 people according to the last census of 2010.

Mosquito collection and maintenance

The larvae were obtained from the insectarium of the Laboratory of Malaria and Dengue, National Institute of Amazonian Research (Instituto Nacional de Pesquisas da Amazônia - INPA), located in Manaus. The larvae remained in trays and were fed with TetraMin® (fish feed); the adult population was kept in cages with cotton soaked in 10% sucrose solution, whereas the females were also fed blood every other day for egg development. Plastic cups with moistened filter paper strips were provided for oviposition by pregnant females. The population was maintained under laboratory conditions of 26 ± 2°C and 70-80% relative humidity.

Larvicidal activity assays

Benzoyl thiosemicarbazone and its Ni(II) complex were synthesized and characterized according to Beraldo et al. (1997) and Pingaew et al. (2010). In the assays, the compounds were dissolved in



A

B

Figure 1. Benzoyl thiosemicarbazones (A) and Nickel complex II (B) structure.

Table 1. Larvicidal bioassays of benzoyl thiosemicarbazone (1) and nickel (II) complex (2) against *A. aegypti* larvae.

A. aegypti				
Substances	**Regression equation**	**LC_{50} µg/mL (95% CI) 24 h**	**Number of larvae**	**χ^2**
Benzoyl thiosemicarbazone	y = -1.73 + 4.14 * log x	42.09 (25.94, 66.05)	50	5.97 s
Nickel complex (II)	y = 0.54 + 2.74 * log x	42.28 (23.83, 66.48)	50	4.91 s
Substances	**Regression equation**	**LC_{50} µg/mL (95% CI) 48 h**	**Number of larvae**	**χ^2**
Benzoyl thiosemicarbazone	y = -1.73 + 4.24 * log x	38.49 (24.02, 60.08)	50	5.82 s
Nickel complex (II)	y = 0.28 + 3.03 * log x	35.84 (28.52, 44.02)	50	2.16 s

x: concentration; y: probability of mortality; s = significant; χ^2 = chi-square; CI: confidence interval.

dimethyl sulfoxide (DMSO) and evaluated at concentrations of 15.6 to 500 µg/mL against *A. aegypti* larvae and from 7.8 to 250 g/L against *A. darlingi* larvae. The assays were performed in triplicate in plastic cups containing 10 mL of distilled water, 10 larvae, feed and 100 µL of the evaluated concentration. Control was performed with DMSO. After 24 and 48 h, the number of dead larvae was recorded, and the median lethal concentration (LC_{50}) was calculated (Dulmage et al., 1990; WHO, 2005).

Statistical analysis

The results were considered acceptable when the control mortality was less than 10%, and the number of dead larvae in the control was adjusted using the Abbott formula (Abbott, 1925). Mortality data were assessed by probit analysis (Finney, 1971). According to the regression equation, the probability of mortality value corresponds to the y-axis, whereas the tested concentration corresponds to the x-axis. To obtain the LC_{50}, we selected the concentrations that presented larval mortality above 50%. We used the Polo Plus software (Robertson et al., 2003) and a 95% confidence interval (CI); results with $p<0.05$ were considered significant.

RESULTS

The larvae of both species tested were sensitive to benzoyl thiosemicarbazone and to the metal nickel complex after 24 and 48 h of exposure. Table 1 shows that *A. aegypti* larvae were more susceptible to the benzoyl thiosemicarbazone than the nickel complex. The LC_{50} for the benzoyl thiosemicarbazone was 42.09 µg/mL, whereas for the nickel complex, it was 42.28 µg/mL. However, after 48 h, the metal complex showed the highest toxicity, with an LC_{50} of 35.84 µg/mL. In this case, the benzoyl thiosemicarbazone presented an LC_{50} of 38.49 g/L.

A. darlingi larvae were more susceptible than *A. aegypti* larvae for the compounds tested. LC_{50} values for *A. darlingi* were lower at both the 24 and 48 h intervals (Table 2). Benzoyl thiosemicarbazone showed an LC_{50} of 4.77 µg/mL, whereas the nickel complex showed an LC_{50} of 7.33 µg/mL, both after 24 h. After 48 h, the LC_{50} of the metal complex was lower (2.68 µg/mL) than the LC_{50} of benzoyl thiosemicarbazone (2.72 µg/mL). The substance benzoyl thiosemicarbazone presented higher toxicity for both species tested after 24 h. Due to the sensitivity of the larvae, *A. darlingi* was more susceptible than *A. aegypti* larvae.

DISCUSSION

The present study addresses the larvicidal activity of a thiosemicarbazone derivative and its Ni complex against *A. aegypti* and *A. darlingi*, species responsible for the

Table 2. Larvicidal bioassays of benzoyl thiosemicarbazone (1) and nickel (II) complex (2) against *A. darlingi* larvae.

A. darlingi				
Substances	**Regression equation**	**LC_{50} µg/mL (95% CI) 24 h**	**Number of larvae**	**χ^2**
Benzoyl thiosemicarbazone	y = 3.44 + 2.30 * log x	4.77 (2.76, 6.79)	30	1.55 s
Nickel complex (II)	y = 3.40 +1.85 * log x	7.33 (4.42, 10.66)	30	5.75 s
Substances	**Regression equation**	**LC_{50} µg/mL (95% CI) 48 h**	**Number of larvae**	**χ^2**
Benzoyl thiosemicarbazone	y = 3.73 +2.22 * log x	2.72 (0.61, 4.61)	30	1.78 s
Nickel complex (II)	y = 3.83 +1.78 * log x	2.68 (0.60, 4.48)	30	6.78 s

x: concentration; y: probability of mortality; s = significant; χ^2 = chi-square; CI: confidence interval.

transmission of dengue and malaria, respectively, in the Amazon region. Although the biological properties of the metal complexes of thiosemicarbazones have high toxicity associated with the free ligand (Mendes et al., 2006; Netalkar et al., 2015), we observed that after 24 h, the metal nickel complex was less toxic; that is, this complex displayed a higher lethal concentration against *A. aegypti* (LC_{50} 42.28 µg/L) and *A. darlingi* (7.33 µg/L) than benzoyl thiosemicarbazone, which displayed an LC_{50} of 42.09 and 4.77 µg/mL, respectively.

After 48 h, however, the nickel complex showed better toxicity results, with an LC_{50} of 35.84 µg/mL for *A. Aegypti* and 2.68 µg/mL for *A. darlingi*. After the same period, benzoyl thiosemicarbazone presented an LC_{50} of 38.49 µg/mL for the first species and 2.72 µg/mL for the second.

Gopinathan and Arumugham (2015) evaluated the larvicidal activity of four Cu(II) metal complexes against *Culex quinquefasciatus* (LC_{50} 0.61 to 2.09 mg/L) and *Anopheles subpictus* (LC_{50} 0.89 to 1.88 mg/L). Although all complexes showed high toxicity, urea and thiourea complexed with copper presented the best larvicidal activity results when compared to thiosemicarbazide and semicarbazide. Thiosemicarbazide derivatives showed a broad spectrum of larvicidal activity at different concentrations.

Rayms-Keller et al. (1998) showed that metal ions were highly toxic to *A. aegypti*. For example, copper edetate in nanostructures and chitosan microcapsules showed efficacy against *A. aegypti* larvae, with an LC_{90} of 60 and 20 mg/L, respectively, because nanostructures and microcapsules favour the slow and continuous release of the active ingredient to the environment. Thus, when complexed to the nickel, thiosemicarbazone derivative tested here against *A. aegypti* and *A. darling* showed high toxicity. However, the results of this study indicate that the metal-ligand bond did not significantly favour larvicidal activity at all reading ranges, as observed for the assays against *A. darlingi* (Table 2); that is, the metal complex did not directly affect the simultaneous ion exchange in the biological system due to the reactivity and especially the redox effect caused by transition metals in biological systems (Stohs and Bagchi, 1995; Nguyen et al., 2000).

Beraldo and Gambino (2004) and Al-Amiery et al. (2012) described a series of thiosemicarbazone derivatives and metal complexes with different chemical and biological properties and highlighted the high biological potential of the metal complex relative to the free ligand. The compounds synthesized and evaluated against mosquito larvae in the present study need to be evaluated for toxicity against nontarget insects and especially regarding the accumulation of heavy metals in the environment, which requires special treatment for their removal (Mireji et al., 2010). The search for new active compounds is challenging because of the many cases of insects resistant to the insecticides currently used in vector control campaigns (Rose, 2001). Integrated mosquito management programmes targeting larvae and mosquitoes serve as one of the most effective ways to control insect populations and consequently reduce the number of vector-borne diseases in endemic areas.

Conclusions

Benzoyl thiosemicarbazone and its Ni(II) complex showed larvicidal activity against the larvae of *A. aegypti* and *A. darlingi*, indicating that the thiosemicarbazone metal complex has insecticidal potential. However, elucidating the mode of action of these compounds in larvae and developing new compounds with pharmacological potential are necessary.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Tomato Leaf miner (*Tuta absoluta*) (Meyrick 1917) (Lepidoptera: Gelechiidae) prevalence and farmer management practices in Kirinyanga County, Kenya

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Pest invasion is one of the limiting factors affecting food production. Tomato leaf miner (*Tuta absoluta*) (Meyrick 1917) (Lepidoptera: Gelechiidae), is an invasive insect pest of tomato. However, In Kenya, there is limited information on the level of invasion of the pest in tomato producing areas in the country. We assessed the level of invasion of *T. absoluta* and farmer management practices in Kirinyaga County. Tomato farmers were interviewed using questionnaires aimed at identifying the management practices used by farmers to control *T. absoluta* and their awareness on the insect pest. Two hundred and eight tomato farmers were interviewed, 90% of the respondents rated *T. absoluta* as a major pest of tomato. Results show that 94% of the respondents use synthetic chemicals in the control of *T. absoluta* with an average frequency of 12 times per growing season of three months with the highest frequency being 16 times. Increased use of synthetic insecticides was associated with a negative impact on the natural enemies. Among the interviewed farmers, 52 and 46% of respondents stated that after chemical spraying natural enemies disappear and are killed respectively, while 2% did not know what happened to them. It is therefore imperative to design an integrated pest management program that integrates biological control and environmentally friendly chemicals for sustainable control of *T. absoluta* populations.

Key words: Synthetic insecticides, management practices, natural enemies, abundance, severity.

INTRODUCTION

The tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is an invasive pest that was first described in Peru in 1917. It was reported in Eastern Spain in late 2006 (Urbaneja et al., 2007) and has

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continually spread, throughout Europe, Middle East, Northern Africa, Sub-Saharan Africa and it is currently found in Tanzania and Uganda (Tonnang et al., 2015). In Kenya the pest was first identified in early 2014 through pest surveys conducted by research organizations including International Centre of Insect Physiology and Ecology (ICIPE), Kenya Agriculture and Livestock Research Organization (KALRO), and the National Plant Protection Organization- Kenya Plant Health Inspectorate Services (NPPO-KEPHIS).

Tuta absoluta can cause severe damage to tomato both in open field and green house production (Seplyarsky et al., 2010; Desneux et al., 2011). It can affect all parts of a plant from the leaves, leaf veins and stem margins, sepals, green and ripe fruits (Estay, 2000). After hatching, young larvae penetrate the leaves, stems and fruits of tomatoes on which they feed and develop, creating mines and galleries which affect the photosynthetic ability of the plant, thereby reducing tomato yield. The galleries formed on tomato fruits expose them to secondary infection by pathogens leading to fruit rot (Ekesi et al., 2011) and yield losses of up to 100% if not controlled (Desneux et al., 2010).

Control of *T. absoluta* has been difficult due to its wide host range including pepper (*Capsicum annuum* L.), long-spined thorn apple (*Daturaferox* Kunth) (EPPO, 2005), Devil's trumpet (*Datura stramonium*), tobacco (*Nicotiana tabacum* L.) (Vargas, 1970) and the American nightshade (*Solanum americanum* Miller) (Fernandez and Montage, 1990). Although the tomato leaf miner prefers tomato over other solanaceous crops, in Italy it was reported on beans (*Phaseolus vulgaris*) (EPPO, 2009), *Lycium* sp. and *Malva* sp. (Caponero, 2009) which act as a further alternate host. *T. absoluta* also has a complex life cycle that involves both sexual and asexual (Parthenogenetical) reproduction leading to very rapid developmental rates at optimum temperatures (Lanzoni et al., 2002; Cocco et al., 2012).

In Kenya, there is limited information about the pest status of *T. absoluta* and the control measures applied by farmers. The dispersal rate of *T. absoluta* has increased economic losses both in field and greenhouse tomato production (New pest in Kenya: Preliminary surveillance report on *T. absoluta* by the cabinet minister (Food and Agricultural Organization of the United Nations, 2014). This study focused on determining the farmer management practices of *T. absoluta* and their awareness on the insect pest and its natural enemies if any, in Kirinyaga County, Kenya in order to effectively design management strategies for its control.

MATERIALS AND METHODS

Experimental Site

The study was conducted in Kirinyaga County, Kenya which is the leading tomato producing County in the country (Horticulture validated report, 2014). The area receives bimodal rainfall with rains typically occurring from March to May (long rains) and from October to December (short rains) with an annual rainfall range of 1100 -1250 mm. Temperature range between 12 and 26°C. The soils are volcanic loam which are deep and moderately to highly fertile (Food and Agricultural Organization of the United Nations, 2014).

Establishment of the invasion status of *Tuta absoluta*

The farmers who participated were selected from a representative sample population of the total population of tomato growing farmers in Kirinyaga County. The sample size was determined using the formula by Nassiuma (2000). A total of 208 Tomato farmers were interviewed during the 2015-2016 growing season using an open and close ended semi structured questionnaires aimed at identifying management practices used by farmers to control *T. absoluta* and their awareness of the insect pest and its natural enemies. This was carried out across the 5 sub Counties during the period of December 2015 to April 2016 namely: Mwea East, Mwea West, Kirinyaga West, Kirinyaga Central and Kirinyaga East.

Data collection procedures

Awareness of the farmers on the insect pest, its management practices and natural enemies was assessed using the questionnaire. A pilot baseline study was done prior to the main survey to test the questionnaire and remove any redundant information to refine the data collection tool.

Data analysis

Descriptive statistics was done using Excel on the survey questions to characterize the farmer and their knowledge on *T. absoluta*.

RESULTS

Field survey

Farmers' awareness on T. absoluta abundance and severity

Majority of farmers (90%) reported *T. absoluta* as one of the most severe insect pests followed by white flies (Table 1). 39% of the farmers reported higher insect attack at fruit setting stage compared to early stages of the tomato growth. Further, 27% reported to have seen high *Tuta* populations throughout the crop growing cycle (Table 2).

T. absoluta management

94% of farmers used synthetic chemicals in the control of *T. absoluta* (Table 3). A range of synthetic insecticides have been used in control of *T. absoluta*, however, 62% of farmers prefer coragen (Chlorantraniliprole 200 g/l) (Table 4). Pesticide application frequency by farmers ranged between 9-16 times per growing season (Table 5). 62% of farmers reported that 75% of *T. absoluta* are killed by pesticide application while 28% say only 50% of the pest is killed and 10% of the farmers indicated that

Table 1. Major insect pests attacking tomatoes in order of severity according to farmer respondents across Kirinyaga county.

Pests	Kirinyaga Central	Kirinyaga East	Kirinyaga West	Mwea East	Mwea West	Overall
Aphids	0	0	0	0	2	0
Ballworms	0	0	0	0	5	1
Cutworms	0	0	0	0	3	1
Mites	0	3	4	0	3	2
Nematodes	0	0	0	1	0	0
Thrips	0	0	0	3	2	1
Tuta absoluta	95	93	96	90	86	90
Whiteflies	5	3	0	6	0	3
Count	21	29	26	67	63	206

Table 2. Preferred tomato growth stage by *T. absoluta*.

plant stage	Kirinyaga Central	Kirinyaga East	Kirinyaga West	Mwea East	Mwea West	Overall
vegetative	10%	13%	6%	7%	3%	7%
flowering	33%	33%	21%	15%	16%	22%
Fruit setting	37%	27%	64%	38%	37%	39%
Before harvesting	0%	4%	3%	3%	0%	2%
Finish-harvesting	0%	0%	0%	0%	4%	1%
Through-out	13%	24%	6%	37%	35%	27%
Don’t know	7%	0%	0%	0%	4%	2%
Count	30	55	33	71	68	257

Table 3. Mechanisms used by farmers to control *T.absoluta*.

***T. absoluta* control**	Kirinyaga Central (%)	Kirinyaga East (%)	Kirinyaga West (%)	Mwea East (%)	Mwea West (%)	Overall (%)
Apply pesticide	95	88	86	99	92	94
Uprooting	0	12	6	0	3	4
Baiting	5	0	4	1	3	2
Crop rotation	0	0	0	0	2	0
Others (tobacco mixer)	0	0	4	0	0	0

less than 50% of the pests actually die (Table 6). When the insecticide failed to control *T. absoluta*, 38% of farmers changed the insecticide while 34% sprayed more frequently, 13% mixed different insecticides and 11% increased the concentration (Table 7).

Knowledge on natural enemies

47% of the farmers reported to have seen other arthropods including lady birds, spiders and ants in their fields which don’t damage their crops, but attack pests. 29% of the farmers have not seen any of these arthropods in their fields while 25% have no idea at all about such arthropods (Table 8). Results also show that 74% of farmers are aware that these arthropods could be natural enemies and that they feed on other insects. 8% of the farmers say the insects dwell on leaves while 18% have no idea what this arthropods do or what they are (Table 9). After chemical control of other insect pests in the tomato field, 52% of farmers reported that most of the natural enemies disappeared while 46% reported that the natural enemies got killed in the process. 2% of the farmers did not know what happened to the natural enemies (Table 10). Results also show that 64% of farmers have no idea of what may happen when natural

Table 4. Common pesticides used to control *T. absoluta* by farmers.

Pesticides used	Active ingredient/mode of action	Kirinyaga Central	Kirinyaga East	Kirinyaga West	Mwea East	Mwea West	Overall
Agrinate	Broad spectrum	0	0	3	0	0	0
Alphatox	Alpha-cypermethrin	15	5	3	1	1	3
Avaunt	Indoxacard	0	0	0	2	7	3
Ranger	Chlorpyrifos 480 g/l	0	0	0	0	1	0
Belt	Flubendiamide	8	21	3	2	13	8
Coragen	Chlorantraniliprole 200 g/l	77	58	60	73	52	62
Cyclone	Agrinate 90sp	0	0	0	1	0	0
Dimethoate	Organophospate	0	0	3	0	0	0
Escort	Emamectin Benzoate 19 g/l	0	5	6	11	9	8
dynamec	Abamectin 1.8 g/l	0	0	0	0	2	1
Halothrin	Pyrethroid	0	5	0	0	0	0
Aster Extrim		0	0	3	0	0	0
Duduthrin	Pyrethroid	0	0	0	1	0	0
Levo	Prosuler oxymatrine 2.4%	0	0	0	0	2	1
Pegasus	Diafenthiuron 500 g/l	0	5	0	1	0	1
Prove	Emamectin Benzoate 19.2 g/l	0	0	20	6	10	9
Tobacco (local)	Botanical	0	0	0	0	1	0
Thunder	Imidaclopride Betacyfluthrine 100+45 g/l	0	0	0	0	1	0
Count		13	19	35	83	87	237

Table 5. Application frequency of pesticides per growing season.

Sub-county	mean	SD	N
Kirinyaga Central	16	17	21
Kirinyaga East	8	9	28
Kirinyaga West	13	12	26
Mwea East	9	3	66
Mwea West	15	9	59
Overall	**12**	**10**	**200**

Table 6. Percentage of *Tuta absoluta* killed by pesticide (pesticide effectiveness).

Pest killed	Kirinyaga Central (%)	Kirinyaga East (%)	Kirinyaga West (%)	Mwea East (%)	Mwea West (%)	Overall (%)
above_75	71	55	65	75	45	62
about_50	24	32	31	15	41	28
less_50	5	13	4	10	14	10

Table 7. Remedy for failed *Tuta absoluta* control.

Measures	Kirinyaga Central (%)	Kirinyaga East (%)	Kirinyaga West (%)	Mwea East (%)	Mwea West (%)	Overall (%)
mix_pesticides	48	32	4	0	10	13
increased_concentration	13	16	14	6	10	11
sprayed_frequently	22	32	7	52	33	34
changed_pesticide	17	19	57	40	45	38
Others	0	0	18	2	2	3

Table 8. Are there other arthropods that don’t cause damage to your crops.

Row Labels	Kirinyaga Central	Kirinyaga East	Kirinyaga West	Mwea East	Mwea West	Grand Total
don’t_know	14%	3%	0%	61%	11%	25%
no	14%	13%	67%	12%	42%	29%
Yes	71%	84%	33%	27%	47%	47%
Count	21	31	27	66	62	207

Table 9. Awareness by farmers that the insects could be natural enemies.

Row Labels	Kirinyaga Central	Kirinyaga East	Kirinyaga West	Mwea East	Mwea West	Overall
feed_on_insects	87%	78%	56%	94%	56%	74%
dwell_on_leaves	7%	11%	22%	6%	4%	8%
don’t_know	7%	11%	22%	0%	41%	18%
Count	15	27	9	18	27	96

Table 10. What happens to natural enemies after spraying?.

Row Labels	Kirinyaga Central	Kirinyaga East	Kirinyaga West	Mwea East	Mwea West	Overall
Killed	60%	47%	36%	75%	24%	46%
disappear	40%	53%	64%	25%	69%	52%
don’t_know	0%	0%	0%	0%	7%	2%
count	15	32	14	20	29	110

Table 11. Killing natural enemies by spraying chemicals can cause pest infestation.

Row Labels	Kirinyaga Central	Kirinyaga East	Kirinyaga West	Mwea East	Mwea West	Grand Total
agree	61%	55%	10%	22%	7%	25%
disagree	0%	17%	25%	9%	10%	12%
no_opinion	39%	28%	65%	69%	83%	64%
Count	18	29	20	65	59	191

enemies are killed in the process of insect pest control, 12% disagree that killing natural enemies would lead toincreased pest infestation while 25% agree that after killing natural enemies pest infestation increases (Table 11).

DISCUSSION

This study showed that Tomato was the most preferred crop for income generation and home consumption across Kirinyaga County hence an important crop to the small scale farmers. Most of the interviewed respondents were aware of the tomato leaf miner (*T. absoluta*) and its severity on the tomato crop. 90% of the farmers reported *T. absoluta* as one of the major insect pests of Tomato occurring across Kirinyaga County (Table 1).

The easy spread and survival of insect pest throughout the growing season is accelerated by intensive farming of tomatoes as a mono-crop. In addition, the packaging material used by farmers is often shared among the small holder farmers during market days. The tomato leaf miner can reduce yield and quality of tomato in newly invaded areas by 80-100% both in field and greenhouse conditions (Desneux et al., 2010). The pest was found to infest the crop at fruit setting and flowering stage, however some farmers stated that they saw the insect pest throughout the crops growth cycle (Table 2). Research shows that *T. absoluta* can affect all developmental stages of a crop (Estay, 2000).

Use of synthetic insecticides is a predominant management option for *T. Absoluta* (Table 3). Insecticide treatments were mostly calendar-based, normally 1 or 2 weeks after transplanting (Table 5). Use of pheromone lures and other monitoring tools were not at all used by farmers. Effectiveness of synthetic insecticides has been mired with a lot of challenges such as occurrence of insect resistance to the active chemical ingredient (Lietti et al., 2005; silva et al., 2011). In this study, 62% of respondents agreed that the chemicals were effective in control of *T. absoluta* while 30% believe they were not effective (Table 6). Studies show the effectiveness of insecticides alone may sometimes be impaired because of the mine-feeding behaviour of the larvae or deficient spraying technology (Lietti et al., 2005). Further, their effectiveness could be limited due to the high reproductive capacity and short generation cycle of *T. Absoluta* (Gharekhani and Salek-Ebrahimi, 2014).

Consequently, farmers increased their frequency of sprays; in our results 38% of farmers changed the insecticide, 34% sprayed the same insecticide more frequently while 13% mixed different insecticides when they failed to control the insect pest (Table 7). Ajaya et al. (2016) demonstrated similar results in India where commercial tomato farmers increased the dose of spray, mixed more than one insecticide with different modes of action. This however, leads to substantial increase of the broad spectrum insecticides which lead to economic and environmental impact, and disruption of the natural enemy complex of the insect pest (Biondi et al., 2012).

Insecticide treatments used by farmers were mainly broad spectrum ranging from organophosphates to pyrethroids targeting *T. Absoluta* and other lepidopteran pests. Coragen® (Chlorantraniliprole) was the most preferred insecticide by farmers for its effectiveness in the control of *T. Absoluta* (Table 4). Dinter et al. (2008) recorded a remarkable toxicity of Coragen® against tested larval instars of *T. Absoluta* under field conditions. It was also noted that the insecticide had low mammalian toxicity.

However, increased use of synthetic insecticides has been found to negatively impact on the natural enemies. In this study 47% of respondents claimed to have seen natural enemies in their fields while 29% had not seen any natural enemies in their fields and 25% did not know what natural enemies (Table 8) were. From our findings 52 and 46% of respondents stated that after chemical spraying natural enemies disappear and are killed respectively, while 2% did not know what happened to them (Table 10). Intensive use of synthetic insecticides has been found to cause residues on tomato fruits, destruction of the natural enemy population and compromise human health (Desneux et al., 2007; Biondi et al., 2012).

Conclusion

It is evident from the results that *T. absoluta* populations are established across Kirinyaga County. Farmers were also able to identify the insect pest whose control was inclined to synthetic chemical sprays. This however, poses a risk of occurrence of resistant biotypes of the insect pest, reduced profits from high insecticide cost and destruction of natural enemy populations.It is therefore necessary to design an integrated pest management program that is environmentally sound for sustainable management of *T. absoluta* populations.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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