Surveying for Natural Enemies of *Tuta absoluta* (Meyrick) in Selected Parts of Zambia

ABSTRACT

Tuta absoluta (Meyrick) is a major pest of tomato for both greenhouse and field grown. In Zambia, it was first reported in 2016 and has since cause far huge damage on the crop. Several interventions were embarked on ranging from the use of neem but chemicals have been mainly used to control the pest. The purpose of our research was to survey and identify indigenous natural enemies for *T. absoluta* associated with tomato in central and Lusaka provinces of Zambia. Surveys were conducted in both in the rainy and dry seasons of 2019 in five sites and potential natural enemies collected were sent to CABI Plantwise laboratory for identification. A predatory species from the family of miridae was found and its DNA 100% matched *Nesidiocoris Tenuis* (Reuter) and later collected for rearing at the University of Zambia Insectaria. This species is reported for the first time on *T. absoluta* in Zambia. Nesidiocoris tenuis identification will provide scientific basis for biological control of *T. absoluta*.

Key words: *Tuta absoluta*, Natural enemies, Biological control, *Nesidiocoris tenuis*, Surveying

1.0 INTRODUCTION

Tomato (*Solanum lycopersicum* Mills.), is an economically important horticultural crop which belongs to the Family Solanaceae. It is an important vegetable and in addition to its fruits, leaves and vines is used in the manufacture of medicine [1]. The world largest producer of tomato is China with an annual average tomato yield of 56 tonnes per hectare. In sub Saharan Africa the leading producer is Nigeria followed by Cameroon producing an annual yield of about 2.4 million metric tonnes and 0.9 million metric tonnes per hectare respectively [2].

In Zambia, smallholder farmers grow tomatoes extensively as a high value horticultural crop for the domestic market, processing and export. However, the yield of tomato is low averaging 5 tonnes per hectare, which is lower than the world mean yield of about 34 tonnes per hectare [3]. This low yield can be attributed to both the biotic and abiotic stresses. Among the biotic stress, in the recent past an alien pest *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) causes yield losses of up to 100% in the absence of biological and other control methods [4; 5].

Lack of co-evolved natural enemies in the new invaded areas in Zambia may have contributed to rapid diffusion and spread of this moth. Chemicals have been used to control the pest but it is expensive and is an environmental hazard [6]. In this context, biological control may be an environmentally and economically sound tool for the management of *T. absoluta* [7]. In particular, conservation biological control strategies that rely on the use of indigenous biological control agents could play a key role against invasive pests [8] since introduction of exotic natural enemies may be costly and in certain cases the introduced agent may fail to get established. In this regard, it is imperative to survey and identify predators, which can prey successfully on this alien pest, *T. absoluta*. Key candidate predator (s) can further be evaluated in the laboratory to investigate the suitability of using it as natural enemy. The objective of the study was therefore to survey and identify the natural enemies of *Tuta absoluta.*

2.0 MATERIALS AND METHODS

2.1 A survey for *Tuta absoluta* and natural enemies in Zambia’s Central and Lusaka Provinces

2.1.1 Survey Sites

A survey was conducted in five sites (**Table 1, Figure 1**). These sites were chosen purposely being the highest tomato producers in the region areas. A susceptible tomato genotype to *T. absoluta* as described by [9], Tengeru select, was deliberately planted in all areas. This was done to facilitate the pull of tomato leaf miner (*T. absoluta*) to the plant and possible assessment of natural enemies.

Table 1: Five sites where surveys for *T. absoluta* and natural enemies were conducted and their position coordinates

Site	Positional Coordinates	Altitude	District	Province
CABI	15°22’14’’S, 28°26’30’’E	1256m	Chongwe	Lusaka
GART	14°58’41’’S, 28° 05’38’’E	1144m	Chisamba	Central
KATC	15°16’23’’S, 28°28’37’’E	1125m	Chongwe	Lusaka

UNZA	15°23’34’’S, 28°20’3.9’’E	1150m	Lusaka	Lusaka
ZARI	15°28’13’’S, 28°16’57’’E	1168.9m	Chilanga	Lusaka

Latitudes*;* CABI, GART, KATC, UNZA and ZARI and their respective coordinates

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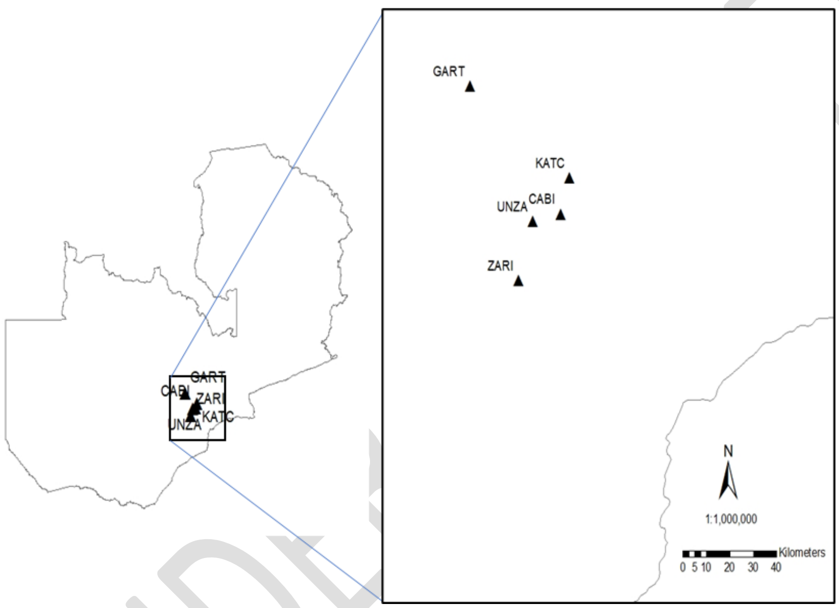

Figure 1. Five sites where *T. absoluta* and potential natural enemies were surveyed and sampled. i.e., GART (Chisamba), Kasisi Agricultural Training Centre, CABI (Chalimbana), University of Zambia field station and Mount Makulu.

2.2 Tomato plant establishment

Tomato nurseries of free *T. absoluta* Tengeru select variety, obtained from Chisamba were established in the entire sites understudy (**Figure 1**). The plants were planted in uniform plot sizes of 10m x 20m prepared to fine tilth. Nursery establishment was done both in the rainy and dry seasons with supplementary irrigation especially during dry spells in the rainy season.

Tomato seedlings were transplanted at 4 weeks at the recommended spacing of 50 cm x 75 cm with the total plant population of 534 seedlings per site. Transplanting was done late in the afternoon to prevent seedlings from wilting especially in the dry season. Prior to planting, a basal fertilizer application rate of 200 kg/ha compound D was applied to the soil. 100 Kg/ha Muriate of potash (MOP) and 150 Kg/ha Ammonium nitrate (AN). Ammonium nitrate was applied fortnightly to tomato upon fruiting except for Kasisi Agricultural Training centre where compost manure was used at the rate of 200 Kg/ ha this was because the institution is strictly organic and therefore no artificial chemicals were allowed. Other standard cultural practices in tomato production such as weeding were followed.

2.3 Sampling for *Tuta absoluta* and its natural enemies

Bi weekly check-up and sampling were conducted in all the five locations for three months. *Tuta absoluta* samples and its suspected natural enemy samples collected were later sent to CABI Plantwise Laboratory (CABI's science centre at Egham) in United Kingdom for identification. The procedure for identification of potential natural enemies was conducted as by [10]. Details of surveying for predators and parasitoids are explained in the sub-sections below.

2.3.1 Surveying for Predators

Firstly, the mined leaves of tomato by *T. absoluta* were identified in each plot early morning and close observation of any preying activities on either *T. absoluta* eggs or different stages of larvae were done. The inverted bright coloured umbrella pink in colour was placed under the *T. absoluta* infested plants and thereafter the plants were shaken in order to collect the insects that were found either preying or resting on the plants and in particular the leaves. Insects that fell on the umbrella were sucked using the improvised aspirator and transferred to 5mL small insect collection bottles and 30mm diameter Petri dishes. The collected insects could be predators of *T. absoluta* eggs, larvae and pupae. Particular attention was paid to predatory hemipteran bugs, ladybirds, and lacewings with an exception of termites which have proved to be difficult to study [11].

Insect samples were stored in 70% alcohol labelled vials at University of Zambia, Department of Plant Science Insectarium Laboratory, indicating the site and date of collection. The insect samples were then sent to CABI Plantwise Laboratory in the United Kingdom for identification.

2.3.1 Surveying for Parasitoids

2.3.1.1 Raising of *Tuta absoluta* eggs

Tuta absoluta colony was established by collecting infested tomato leaves in Lusaka and Central provinces of Zambia. Sampling for *T. absoluta* was done in the field using Zig Zag or "Z" sampling procedure as conducted by [12]. Thirty leaves were collected per sampling day from each site and at least two leaves per plant were collected and kept in a ventilated lunch box with a moistened filter paper at the bottom.

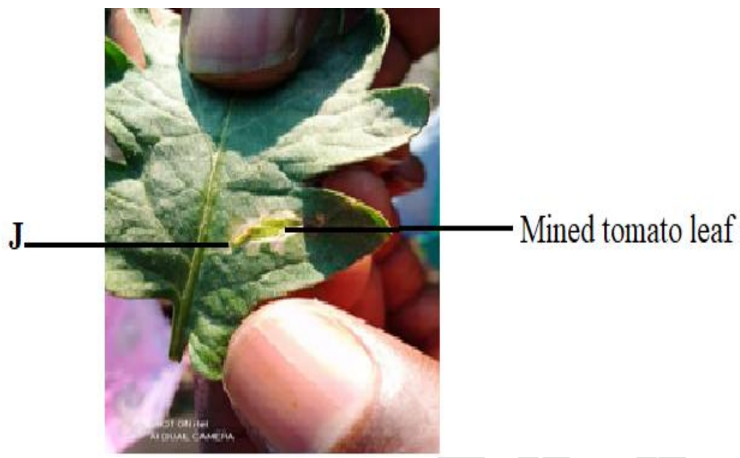

Figure 2. Tomato leaf infected with *Tuta absoluta*. **J-***Tuta absoluta* larvae

Upon collection, the tomato leaf miner infested leaves placed on a Petri dish with moist filter paper at the bottom. The Petri dishes were labelled with site and date of collection. The larvae were left to develop into an adult stage and later on transferred into a rearing cage (45cm x 45cm x 45cm) having honey water solution (10%) which was used to fed the adult *Tuta absoluta* moth. In each rearing cage, six tomato plants at three leaf stages were placed and changed every after three days in order to allow mating of adult *Tuta absoluta* thereby enabling the females to oviposit onto the tomato leaves (inoculating tomato leaves with *T. absoluta* eggs). The infested (inoculated) tomato plants were removed after 72 hours and eggs were observed under stereoscopic binocular microscope. With the aid of a brush, eggs were removed from the tomato plants and placed on leaflets which were kept inside a Petri dish (9 cm diameter) until the adult *T. absoluta* emerged for colony maintenance. The rearing of *T. absoluta* was done continuously so as to provide a constant supply of eggs which were used later on to perform experiments on in order to determine the efficacy of the predators and parasitoids if found.

2.3.1.2 Surveying for parasitoids in the field

Tomato infested leaves were collected from the fields by direct inspection of cultivated tomato leaves in open fields in all the sites (UNZA, Kasisi, Chalimbana, Chilanga and Chisamba). The samples were collected in all the fields initially was from February 2019 to April 2019 and when no parasitoids emerged other sampling were conducted from September 2019 to November 2019 on a bi-weekly basis. The tomato infested leaves were collected in the all cultivated period (s), each sampling consisted of 30 infested leaves, and care was taken to ensure that only a maximum of two leaves were collected per plant.

To avoid collecting empty old mines (without *T. absoluta* larvae), since *T. absoluta* attack moves towards the apical leaves in a plant as the crop advances in season, higher (top most) leaves bearing active infestation were progressively selected. Leaves were then observed under a stereo microscope to register the number of *T. absoluta* larvae and in some cases if available *T. absoluta* eggs.

Each foliage (leaf) with apparently healthy *T. absoluta* larvae were stored in aerated box to isolate possible emerging adult parasitoids. In addition, tomato apical with shoots mines were collected to search for *T. absoluta* eggs and assess the egg parasitoid activity. The parasitoid activities were observed by mainly cutting open a mined portion of the stem and searching for cocoons for wasps using an electronic microscope in the insectarium at UNZA.

2.3.1.3 Surveying for parasitoids in inoculated tomato plants in the laboratory

Tomato potted plants were collected at four leaf stage and exposed to adult *T. absoluta* (15 males and 15 females per cage) in cages. Four rearing cages were used for inoculation purposes of tomato plants with *T. absoluta* eggs. In each cage, six tomato potted plants were put and later on 15 males and also 15 females adult *T. absoluta* were introduced in each rearing cage and allowed to mate for 2 days thereafter, the tomato potted plants were removed and kept at the field station. Two hundred and fifty tomato potted plants were inoculated with *T. absoluta* using the above mentioned method. Then the inoculated tomato plants with *T. absoluta* were distributed equally in all the 5 sites i.e. 50 *T. absoluta* eggs inoculated plants per site. The infested plants were placed in all the sites and on a bi-weekly basis, two leaves (with *T. absoluta* larvae) were collected per plant and transferred to the Petri dishes with moistened filter paper at the bottom in the laboratory. The Petri dishes were observed on a daily basis to see any emerged insect and seemingly inactive or paralyzed instars were isolated to observe whether adult wasp could emerge at the expense of *T. absoluta* adult.

Each petri dish was labelled showing the site where the leaf samples were collected and the date of collection.

For both (field sampled and *T. absoluta* inoculated plants), dead larvae of *T. absoluta* were recorded as well as the kind of insect emerging was noted and kept in the vials in order to assess the parasitism rate in an event parasitoid emerged as was demonstrated by [13].

2.4 Data Collection and Analysis

During surveys, data on the site, location, altitude, position coordinates, dates of survey, number of potential natural enemies collected during the survey were recorded. Specimens (potential natural enemies) were put in vial containing 70% and labeled (date and site) which were later sent for identification using molecular methods at CABI Plantwise laboratory.

Seventeen samples labelled S1, S2 to S17 representing 4, 3, 4, 3 and 3 samples obtained from CABI, GART, KATC, UNZA and ZARI respectively. The samples were then sent for identification using molecular methods at CABI Plantwise laboratory in the United Kingdom where Molecular assays were carried out on each sample using nucleic acid as a template.

3.0 RESULTS

Results from CABI Plantwise laboratory shows that out of the 17 samples sent, only three samples that were labeled S1, S2 and S3 representing samples collected from KATC, CABI and UNZA respectively as shown in **Table 2**, 100% matched *Nesidiocoris tenuis* (**Figure 3**) which is a predator for *T. absoluta* in the rainy season. This predator (*N. tenuis*) was found in all the sites during the dry season when a similar survey conducted in the rainy season was replicated.

Table 2. Summary of results from CABI Plantwise Laboratory

Senders Identification	Processing number	Summary of key results	% Match	Insect Type	Site
S1	E513001	*Nesidiocoris tenuis* (tomato bug)	100	Predator	KATC
S2	E513002	*Nesidiocoris tenuis* (tomato bug)	100	Predator	CABI
S3	E513003	*Nesidiocoris tenuis* (tomato bug)	100	Predator	UNZA
S4	E513004	Yeast	_	_	UNZA
S5	E513005	No ID	_	_	GART
S6	E513006	Rhyparochromidae (Seed bug)	100	Herbivore	ZARI
S7	E513007	Oxycarenidae	>97	Herbivore	KATC
S8	E513008	Miridae	>99	Herbivore	CABI
S9	E513009	No ID	_	_	ZARI
S10	E5130010	*Solenopsis* sp.	>99	Herbivore	GART
S11	E5130011	No ID	_	_	CABI
S12	E5130012	Miridae	>99	Herbivore	KATC
S13	E5130013	Platygastridae	100	Parasitoid of true bug	KATC
S14	E5130014	*Gryon* sp.	>99	Parasitoid of true bug	CABI
S15	E5130015	Lygaeidae	>99	Herbivore	ZARI
S16	E5130016	Coreidae	>98	Herbivore	UNZA
S17	E5130017	Rhyparochromidae (Seed bug)	>99	Herbivore	GART

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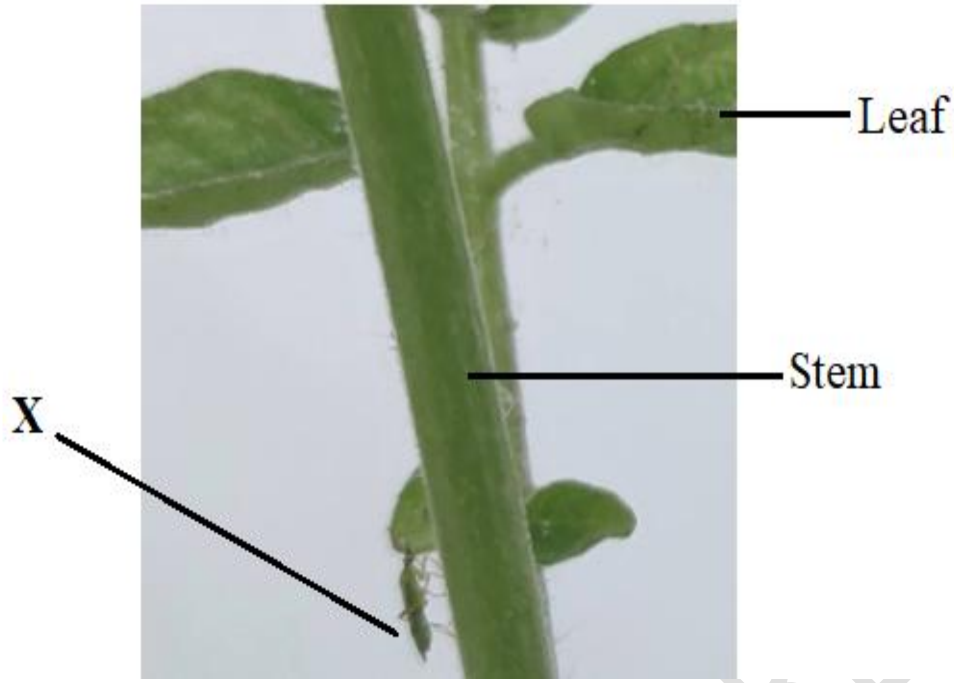

Figure 3. Adult predator on a tomato plant. X- *Nesidiocoris tenuis*.

No parasitoids were found both from our sampling in the rainy season (Field sampling) and dry season (Field and *T. absoluta* inoculated plants sampling).

4.0 DISCUSSSION

Predators attacking *T. absoluta* and other pests on vegetables became more prevalent during the past decade. This is a positive development as these generalist predators can be employed as important agents in biological control against *T. absoluta* or involved in an IPM programme [14].

One species belonging to the Miridae family was found in association with *T. absoluta* and identified as *Nesidiocoris tenuis* (Hemiptera: Miridae) (**Figure 3**). Previous authors identified *N. tenuis* as a predator agreeing with what was obtained in our study [15, 16]. Other reports also suggested that *N. tenuis* plays a role in managing various pests such as lepidoptera, thrips, white flies as well as many other pests in the greenhouse [17, 18]. This predator has been seen to cause necrotic rings on tomatoes though the damage is low, this induces a double defence system by producing Herbivore induced plant volatiles (HIPVs) [19]. The HIPVs non preference of tomato leaves by *T. absoluta* as a result of increased abscise acid and on the other hand they promote production of Jasmonate acid which attract parasitoid white fly Formosa (Gahan) [20].

Elsewhere other predators of *T. absoluta* such as *Dicyphus bolivari* Lindberg, *Dicyphus errans* (Wolf) (Hemiptera: Miridae) and *Nabis pseudoferus ibericus* (Hemptera: Nabidae) were identified [21, 22] respectively. Failure to find other natural enemies (parasitoids, lacewings and predatory ants) during surveys could mean that the environment where surveys were conducted are not conducive for survival and reproduction of these natural enemies or they should be introduced as exotic natural enemies. This however, could be an independent study on effect of introducing such exotic natural enemies in controlling *T. absoluta* in the studied environment and in Zambia as a whole. In addition, the variations in the prevalence of *N. tenuis* where it was more in Kasisi agricultural training centre (KATC) than other sites could imply that environment plays a role on population increase of these predators.

The Kasisi farm utilises an ecological centred farming or organic agricultural practice that leads to greater evenness among predatory insects including those that attack *T. absoluta* on tomato plants [23]. Species evenness has been found to lead to a stronger biocontrol or genesis of greater natural enemy species although little is known about the evenness in response to specific farming practices [24].

CONCLUSION

The study identified the predator *N. tenuis* in both rainy and dry seasons in KATC, UNZA and CABI. On the other hand, the predator was only found in the dry season in GART and ZARI. However, parasitoids were not found in all sites and seasons.

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