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Camel Urine as a Novel Control Agent Against Tomato Leaf Miner (*Tuta absoluta* Meyrick) in Darfur, Sudan

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Abstract: A survey was conducted during the winter season of 2013/14 in the main tomato production areas in Darfur, i.e Kotum, Kebkabiya, Elsyah, Surf Omra and Abu Skein. Five fields in each location were monitored and inspected for the potential hosts of insect and signs of adult insects. The results indicated that population of *Tuta absoluta* was higher in Kotum by 41.5% followed by Surf Omar 27%, then Kebkabiya 26.6% and Abu Skein 25.7% and lastly Elsyah 16.3%. *In vitro* and *in vivo* experiments were conducted to evaluate the efficacy of camel urine against the adults, 4th and 2nd larvae instars of *T. absoluta*. The different stages of insect were kept in separate cages then sprayed with three different concentrations (15%, 25% and 50%) of camel urine and the recommended dose of the insecticide Malathion® 57% as positive control. The evaluation was based on mortality percentage of the treated insects. The lethal effect of urine against the 2ndlarval instar was significantly ($P \leq 0.05$) the highest with mortality level of 100%. The *in vivo* inhibition effect of the three concentrations of camel urine against *T. absoluta* was investigated in a field experiment during the winter season of 2013/14 in comparison with two recommended insecticides; Malathion®57% and Diazinon®60%. The significantly ($P \leq 0.05$) highest mortality percentages were recorded in the first day of the experiment. The urine concentrations of 15%, 25% and 50% were resulted in mortality percentages of 26.5, 38.7 and 57.7, respectively. While Malathion and Diazinon, resulted in 59.0 and 76.3 respectively.

Key words: Survey · *In vitro* · *In vivo* · Camel urine · *Tuta absoluta* · Lethal effect

INTRODUCTION

Tomato leaf miner, *Tuta absoluta* (Meyrick,) is considered the most important tomato pest [1]. It belongs to the family Gelechiidae (Lepidoptera) and also known as South American tomato moth, tomato borer, South American tomato pinworm.

The presence of *T. absoluta* for the first time in Sudan was reported by Mohamed *et al*. [2] during the tomato cultivation season 2010. Reports from Sudan confirmed the identification of *T. absoluta* presence in green houses close to the capital Khartoum. The Agricultural Research Centre who made the initial identification has also carried out pheromone trapping exercise to further confirm the identity of the pest. The expansion of *T. absoluta* to Egypt and Sudan will open the door for further expansion along the east African coast. No official announcement has been made yet although it understood that the Ministry of Agriculture is in the process of drafting a strategy to manage this new challenging pest.

Larvae can damage tomato plants during all growth stages, producing large galleries in their leaves, burrowing stalks, apical buds, green and ripe fruits. It can cause important yield losses in different production regions and under diverse production systems, Baetani *et al*. [3].

The principal method for *T. absoluta* control is blanket spraying with insecticides that are harmful to both humans and the environment, Picanco *et al*. [4] and Silva *et al*. [5]. Moreover, effectiveness of chemical control is

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limited due to the insect's nature of damage as well as its capability of rapidly developing insecticide resistant strains, Siqueira *et al*. [6] and Lietti *et al*. [7].

Different strategies might be applied in an Integrated Pest Management (IPM) program to control *T. absoluta* outbreaks including insecticides and biological control and the association of both. Studies have being done on the use of synthetic sex pheromones in order to monitor population levels and trigger applications of chemicals on the right moment, Michereff-Filho *et al*. [8] and Salas [9]. Chemical control has been the main method of control used against *T. absoluta* and growers normally choose the insecticide in a diversity of options officially registered and recommended. The effectiveness of insecticides alone might be sometimes impaired because of the mine-feeding behaviour of larvae or deficient spraying technology, Lietti *et al*. [7]. Usually, several sprayings are required per growing season and it is noted a decrease of the efficacy of products used against *T. absoluta* since the 1980s in tomato crops. Resistance to some active ingredients has been reported in several countries, for example to abamectin, cartap and permethrin in Brazil, Siqueira *et al*. [6].

Latterly, farmers are successfully transitioning to safer, more environmentally friendly compounds to combat pests and diseases. Recently there is an obvious decline in the use of older, traditional broad based chemistries in favour of more targeted, softer and often organically approved pest and disease control alternatives. The objective of this work is to monitor the distribution and geographic expansion of *T. absoluta* in Darfur scheme in Sudan and to evaluate the prospects of camel urine as new control agent against this leaf miner in order to establish a new environmentally safer management strategy.

MATERIALS AND METHOD

Survey: Geographic expansion of *Tuta absoluta* in Darfur States was investigated through survey conducted in five locations (Latitude 4 - 16°N and longitude 25 - 28°E) in Kotum, Kebkabiay, Elsyah, Surf Omra and Abu Sekein. Five fields in each location were chosen, an area of about one hectare was taken from four sides and one from the middle of the field. The data were collected from the four edges and the middle area of every field as a total number of tomato plants and the number of infested plants. Tomato leaves, fruits, young tender shoots, sepals of unripe fruits, flowers and stems from the fields and the nurseries were investigated for larvae penetrating and egg depositing which expect to be found. Also areas around demonstrated tomato fields were monitored and inspected for the potential hosts of insect and signs of adult insects. Farmers and village's leader were interviewed.

Collecting and rearing of *Tuta absoluta*: Adults and eggs of *Tuta absoluta* were collected at the end of February, 2014 from infested tomato fields in Darfur States and kept in metal frame cages covered with muslin clothes, after eggs were hatching the emerging larvae were reared in these cages. Tomato leaves and fruits were added for the feeding of insects.

Camel Urine Collection and Preparation: Fresh female camel urine was collected at the end of February 2014 from Al fasher and Kotum in Darfur. Three different concentrations of camel urine 15%, 25% and 50% were prepared by adding 85ml, 75ml and 50ml distilled sterilized water; respectively; to the concentrated urine to get 100ml of each concentration.

***In vitro* Inhibition Effect of Camel Urine:** Five metal frame cages covered with muslin clothes were used to assess the *in vitro* inhibitory effect of camel urine. Twenty adult insects were kept in each cage as one treatment. Insects in each cage were sprayed with one of the three concentrations (15%, 25% and 50%) of the urine and with the insecticide Malathion® 57% by hand knapsack sprayer. Twenty other insects were kept in a separate cage and were sprayed with distilled sterilized water as a control treatment. Three replications of each treatment were adopted. Mortality was recorded daily for 3days.

Thirty of the 4th larval instars were kept in separate cages each as one treatment. Larvae in the cages were sprayed with one of the three concentrations of the urine, Malathion and with distilled sterilized water as a control treatment. Three replications of each treatment were adopted. The mortality data were recorded daily for 3days.

Ten of the 2nd larval instars were kept in separate cages each cage as one treatment. Larvae in the cages were sprayed by one of the three concentrations of the urine, Malathion and distilled sterilized water as a control treatment. Three replications of each treatment were adopted. The mortality data were recorded daily for. 3days.

***In vivo* Inhibition Effect of Camel Urine:** The *in vivo* inhibition effect of urine was evaluated under field conditions. The experiment conducted in the winter growing season of 2013/14. The recommended dose of the insecticides Diazinon® 60% and Malathion® 57% were

used for comparison. Tomato seeds were sown in 10 cm diameter plastic bags 7seeds/bag in the nursery. Three weeks later in mid February, seedlings were transplanted to the field. Plants were irrigated weekly. A stand count was conducted two weeks after transplanting. Six weeks later, tomato plants were sprayed separately with the concentrations 15%, 25% and 50% of the urine, Diazinon, Malathion and with distilled sterilized water as a control treatment, the experiment repeated three times and average mean calculated.

The experimental design adopted was Complete Randomize Design (CRD). Each treatment was replicated three times. Mortality percentages of the insects were recorded daily.

The percentage data were converted to square root and arcsine values for analysis, McDonald [10]. Then statistical analysis was accomplished in SPSS and the Duncan`s multiple range test (DMRT) was adopted to compare means. Least significant difference values at $P \leq 0.05$ were used to separate treatment means when ANOVA indicated a significant F value. Standard error and coefficient of variation were calculated for each treatment according to Gomez and Gomez [11].

RESULTS

Survey: The insect has been observed in nearly all fields surveyed, except fields with early harvested tomato, they were varied considerably with harvest period and between the places which were surveyed. Results in Table 1 show that the highest distribution level of *T. absoluta* was in Kotum location, the number of infested tomato plants was 395.2 plants out of 951.2 plants/ha which displayed 41.5% infestation. Followed by Surf Omra which recorded 27% infestation by 222.8 infested plants out of 825.4 plants/ha. Then Kebkabiya location with 26.6% infestation, the number of infested plants was 281.8 plants out of 1060.4 plants/ha. The number of infested plants/ha in Abu Skein location was 227 plants out of 881.6 plants which displayed 25.7% infestation. While the least infestation percentage was recorded from Elsyah location which was16.3 by 110.4 infested plants out of 676.2 plants/ha.

***In vitro* Inhibition Effect of Camel Urine Against *T. absoluta*:** All concentrations of camel urine used resulted in significantly higher mortality percentages at ($P \leq 0.05$) among adult stage compared to the control treatment at the 1st, 2nd and 3rd day of the commencement of the experiment. Mortality of adult stage was 85% when 15% urine was applied and was 100% when the concentrations 25% and 50% of camel urine were used. The two highest concentrations 25% and 50% of urine resulted in mortality percentages equal to the recommended insecticide Malathion after 24 hrs of treatment. There was no significant difference ($P \leq 0.05$) in mortality percentages resulted from the recommended dose of Malathion and the highest concentration of camel urine 50% after 48 hrs of application (Table 2).

Table 1: Infestation of tomato plants by *Tuta absoluta*

					Galleries in infested plants%	
Location	Total No. of plants/ha	No. of infested plants/ha	Infested plants/ha%	Infested fruits%	Fruits	Leaves
Kotum	951.2	395.2	41.5	37.2	20.2	29.2
Surf Omra	825.4	222.8	27	23.8	16.6	12.8
Kebkabiya	1060.4	281.8	26.6	29	26	17.6
Abu Skein	881.6	227	25.7	21.4	6.6	16.4
Elsyah	676.2	110.4	16.3	17.6	9.2	15.6

Table 2: *In vitro* mortality% of *Tuta absoluta* adult stage treated with Malathion 57% and different concentrations of camel urine

	Mortality (%)		
	Days after treatment		
Treatments Camel urine%	1st	2nd	3rd
15%	17.2^{c}	7.9^{b}	14.1^{a}
25%	21.6^{b}	11.5^{a}	7.3^{b}
50%	22.7^{b}	11.3^{ab}	4.3^{c}
Malathion 57%	25.6^{a}	5.1^{c}	1.9^{c}
Control	0.7^{d}	5.7^{bc}	0.0^{d}
SE±	1.4	1.8	0.7
CV%	13.8	37.6	51.2

Percentage data were transformed to Arc sine and root square.

Means followed by the same letter(s) are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test (DMRT).

Table 3: *In vitro* mortality % of *Tuta absoluta* 2nd and 4th instars treated with Malathion 57% and different concentrations of camel urine

	Mortality (%) of the 2nd instars			Mortality (%) of the 4th instars		
	Days after treatment			Days after treatment		
Treatments Camel urine%	1st	2nd	3rd	1st	2nd	3rd
15%	13.2^{c}	16.5^{c}	4.4^{a}	11.4^{c}	16.5^{c}	5.4^{c}
25%	15.3^{b}	19.6^{b}	1.8^{c}	14.1^{bc}	19.6^{b}	12.3^{a}
50%	18.1^{a}	23.6^{a}	3,1^{b}	16.2^{b}	23.6^{a}	11.3^{ab}
Malathion 57%	18 4^{a}	16.4^{c}	1.8^{c}	22^{a}	16.4^{c}	14.1^{a}
Control	0.7^{d}	0.7^{d}	0.0^{d}	0.7^{d}	8.1^{c}	0.0^{d}
SE±	0.6	0.6	0.8	0.94	0.63	1.2
CV%	7.7	14.6	50.1	12.4	6.5	20.3

Percentage data were transformed to Arc sine and root square.

Means followed by the same letter(s) are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test (DMRT).

Table 4: *In vivo* mortality% of *Tuta absoluta* treated with Malathion 57%, Diazinon 60% and different concentrations of camel urine

	Mortality (%)		
	Days after treatment		
Treatments Camel urine%	1st	2nd	3rd
15%	26.5^{d}	19.9^{d}	5.3^{e}
25%	38.7^{c}	27.4^{c}	11.3^{c}
50%	57.7^{b}	46.2^{b}	28.1^{b}
Malathion 57%	59.0^{b}	47.7^{b}	30.2^{b}
Diazinon60%	76.3^{a}	58.7^{a}	40.2^{a}
Control	0.7^{e}	2.0^{e}	0.0^{d}
SE±	2.0	1.7	2.2
CV%	8.1	8.6	17.6

Percentage data were transformed to Arc sine and root square.

Means followed by the same letter(s) are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test (DMRT).

Camel urine proved to have highly inhibition effect against 2nd larval instar (Table 3). All concentrations (15%, 25% and 50%) resulted in significantly ($P \leq 0.05$) higher mortality percentages of 50, 70 and 100; respectively; after 24hrs of application. The concentrations 15% and 25% resulted in mortality of 40% and 30% after 48hrs of application and 10% after 72hrs of application; respectively; comparing to the control treatment. Malathion resulted in the highest mortality percentages of 100% after 24hrs of application. Results appeared that there was a significant difference among urine treatments, the concentration 50% resulted in significant mortality percentage of 100% which was equal to Malathion after 24hrs. However, there was no significant difference at $P \leq 0.05$ level in mortality percentages between urine concentrations 15%, 25% and 50% and Malathion after 72hrs of application; all treatments resulted in 100% mortality.

As shown in Table 3 the number of dead 4th larval stage was increased with the increment of camel urine concentration, the highest mortality percentages of 20%, 33.3% and 43.3% were recorded respectively after 24 hrs of application, all camel urine concentrations proved significantly ($P \leq 0.05$) higher mortality percentages after 24, 48 and 72hrs of application comparing to the control treatment. The concentrations 25% and 50% of urine resulted in significant higher mortality percentages at $P \leq 0.05$ levels than Malathion treatment after 48hrs of application. However, after 72hrs of application the mortality of 30.7%, 56.6%, 83.4% and 93.4% were recorded for urine concentration 15%, 25%, 50% and the recommended dose of Malathion, respectively.

***In vivo* Inhibition Effect of Camel Urine Against *T. absoluta*:** When urine applied under field conditions, the number of dead insects increased with the increment of the concentration. All urine concentrations resulted in the highest mortality percentages in comparison with the control treatment after the 1st, 2nd and 3rd days of application. The highest mortality percentages of 57.7 and 46.2 were resulted from urine concentration 50% in the 1st and 2nd day of application, respectively. The concentrations 15% and 25% of urine resulted in significant lower mortality percentages of 5.3% and 11.3%; respectively; when compared to Malathion treatment which resulted in mortality of 30.2% after three

days of application. The highest urine concentration 50% resulted in similar mortality percentages to that recorded from Malathion treatment after two days of application. However, results showed that all camel urine concentrations gave significantly lower mortality percentages than the insecticide Diazinon which recorded 76.3%, 58.7% and 40.2% at the 1st, 2nd and 3rd days of application, respectively (Table 4).

DISCUSSION

In the last four decades many organic formulations have proven to be as potent as many conventional synthetic pesticides, even at low concentrations. Organic insecticides have gained great attention over synthetic pesticides as a major control agent in organic farming. This study was aimed to evaluate the lethal effect of camel urine against *Tuta absoluta*.

Studies of *T. absoluta* distribution and its impact in Darfur are still in the initial phase. Primary surveys strongly indicated that *T. absoluta* was established well in Darfur area. The massive outbreak recorded from Kotum (Table 1), is of particular concern because of the potential spread of this pest via trade and exporting to other regions of Darfur States and to the neighbour States. Significant loss of tomato production was observed in several tomato producing areas, which can be attributable to the high percentages recorded of infested tomato plants by this insect. This ongoing spread of *T. absoluta* throughout Darfur and its localities as a severe pest on tomato combined by the lack of fully satisfaction effective management strategies will lead to outbreak of this insect, which are in agreement with Desneux *et al*. [12] who mentioned the importance of integrated pest management strategies and plant resistance to manage infestation of *T. absoluta*.

The *in vitro* evaluation of the efficacy of camel urine against *T. absoluta* cleared that the mortality percentages of 4th and 2nd larval instars caused by urine concentrations 25% and 50% were equal to that caused by the recommended dose of the insecticide Malathion after 48hr of application. The highest mortality percentages of these larvae stages were obtained 24hrs after application, which can be agreed with the observations of Al-Bashan [13] who observed that the antimicrobial activity of camel urine was very slightly and determinedly evident after 48 hours of application.

In vivo experiment showed that all camel urine concentrations resulted in significantly ($P \leq 0.05$) higher mortality percentages comparing to the control treatment. The urine at 50% and the recommended dose of Malathion resulted in almost similar mortality percentages of 57.7%, 59.0%, 46.2% for urine and 47.7%, 28.1%, 30.2% for Malathion after 1st, 2nd and 3rd days of application; respectively; which is a good indication of the effectiveness of this concentration against *T. absoluta*. However, results revealed the superiority of the insecticide Diazinon after 1st, 2nd and 3rd days of experiment over both Malathion and camel urine.

Although information of camel urine characteristics and its medicinal benefits also its mode of action is limited, Al-Bashan [13]. This study proved the ability of camel urine to affect the morphology and behaviour of *T. absoluta*; this effect appeared as deformation of treated larvae instars, also as failure of development and repellent or anti feeding effect observed. In addition urine reduced the mean number of eggs with increasing of the concentration. The results also showed that camel urine has the ability of prolongation of larvae period and shortening in lifespan of adult insect. Generally, the higher doses resulted in significantly higher and quick mortality rate compared to low mortality rate caused by lower doses. No fecundity was observed in the control treatment. The fertility and development were reduced significantly ($P \leq 0.05$), therefore no larvae developed in all urine concentrations compared to control treatment which showed a new hatching and larvae development.

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