

# Effect of Rabbit urine on Larval Behaviour, Egg Hatchability, Pupal Emergence and Oviposition Preference of the Fall Armyworm (*Spodoptera Frugiperda* J.E. Smith)

**Diana Kemunto**

International Centre of Insect Physiology and Ecology

**David Mfuti**

International Centre of Insect Physiology and Ecology

**Evanson R. Omuse**

International Centre of Insect Physiology and Ecology

**Amanuel Tamiru**

International Centre of Insect Physiology and Ecology

**Girma Hailu**

International Centre of Insect Physiology and Ecology

**Ignath Rwiza**

Tanzania Agricultural Research Institute (TARI), Ukiriguru Center

**Yeneneh T. Belayneh**

USAID/BHA/TPQ

**Sevgan Subramanian**

International Centre of Insect Physiology and Ecology

**Saliou Niassy** (✉ [sniassy@icipe.org](mailto:sniassy@icipe.org))

International Centre of Insect Physiology and Ecology

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## Research Article

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# Abstract

The fall armyworm (FAW) (*Spodoptera frugiperda*) is a major cereal pest threatening food security in Africa. African smallholder farmers apply various indigenous pest management practices including the application of rabbit urine; however, there is no scientific evidence on its efficacy and mode of action. We evaluated the effect of rabbit urine on FAW 1st (neonates), 2nd and 3rd instar larvae. Larvae were exposed to rabbit urine treated and untreated maize leaves (control). Larval settlement, larval survival, pupal emergence, adult oviposition preference and egg hatchability were evaluated. More FAW larvae (55.5–73.0%) oriented on the untreated leaves compared to those (25.5–41.5%) that oriented on the rabbit urine-treated leaves. Rabbit urine caused 66, 69 and 72% reduction of damage caused by neonates, 2nd instars and 3rd instars, respectively, after 24 hours of exposure. Rabbit urine significantly reduced survival of FAW and had lethal time (LT<sub>50</sub>) of 5.0, 7.0 and 8.7 days and lethal dose (LD<sub>50</sub>) of 49, 94, and 55% for neonates, 2nd instars and 3rd instars, respectively. Additionally, egg hatchability and pupal emergence were reduced by 55.0% and 13.3%, respectively. When provided a choice, FAW female moth laid more eggs on rabbit urine treated plants (647 ± 153 eggs) than untreated plants (72 ± 64 eggs). This study confirms farmers' assertions that the application of rabbit urine can manage FAW and, therefore, can be integrated into the FAW IPM package in Africa. Additional studies on the chemistry of rabbit urine, behavioural response and cost implication might be required for scaling up.

## Introduction

The fall armyworm (FAW) (*Spodoptera frugiperda* J.E Smith) is a serious pest of cereals in Africa. FAW originated from the Americas and was reported in 2016 in Africa [1–4]. It has now spread in the entire continent and beyond. The impact of FAW on maize productivity is increasingly threatening agricultural productivity and, consequently, the livelihood and escalating food insecurity, especially in Africa [5]. Since its arrival, FAW has caused substantial losses in the cereal value chain. In Africa, maize yield losses due to FAW are estimated at 21–53% [5], accounting for about \$16 billion loss annually [6]. The impact of FAW on the value chain is far-reaching as it also affects the livelihood of smallholder farmers and other actors in the cereal industry, such as seed producers, feed companies and the fodder industry.

Over the past 50 years, crop protection has relied heavily on chemical pesticides [7]. However, chemical insecticides are increasingly becoming unreliable due to health and environmental risks and the development of resistant pest populations [2, 7, 8]. Over-reliance on chemical insecticides due to economic and social constraints and their misuse are not sustainable [10]. Therefore, there is widespread interest in alternative and sustainable control strategies [2]. Agroecological farming practices, including legume intercropping and crop diversification, are considered potential alternatives for pest management worldwide [6, 9]. Though sustainable, many of these agroecological approaches are either not accessible to smallholder farmers or knowledge-intensive. For the longest time, African farmers have relied on indigenous knowledge, including cultural farming practices, to manage the pests. The use of smallholder farmer indigenous knowledge in pest management practices are considered effective and can be scaled to complement other agroecological farming practices [6].

Indigenous knowledge is usually accessible, affordable, and most resource-poor farmers rely on it to solve the emerging pest infestation problem [6]. These practices are usually based on enhancing certain ecosystem services, but the extent of their efficacy is yet to be documented. Unless adequate protocols are established to elucidate the effectiveness of these technologies, they will not be widely known or promoted. Smallholder farmers have used rabbit urine to control FAW in Africa; however, this indigenous pest management practice remains unexplored. Rabbit urine is commonly used as a biopesticide against devastating crop pests and pathogens [12]. Several reports from smallholder farmers indicated that rabbit urine is highly effective against FAW; however, there is no empirical data to confirm such assertions.

Rabbit farming is a low-input livestock activity recognized in many African countries as a tool to promote food security and alleviate poverty among smallholder farmers [11]. Elucidating the mode of action of rabbit urine on FAW becomes necessary to expand the integrated pest management package.

Therefore, this study aims to evaluate the effect of rabbit urine on the following aspects were assessed: (1) larval behaviour (orientation and settlement); (2) larval feeding (including damage score); (3) larval survival; (4) pupal emergence; (5) egg hatchability; and (6) oviposition preference. Given that indigenous practices are affordable and accepted by the community, the findings of this study will aid in optimizing and standardizing this practice for scaling and wide adoption.

## Materials And Methods

### Study plants and insects

Maize plants (*Zea mays* L.) for the experiments were grown from seeds in a planting pot of 25.5 cm (height) by 30 cm (diameter). The plants were maintained in the laboratory at controlled mean temperatures of  $25.5 \pm 2^\circ\text{C}$  during the day and  $23.5 \pm 2^\circ\text{C}$  at night with  $70 \pm 5\%$  relative humidity (RH) and L12:D12 (light:dark) photophase. Three-week-old plants were used in this study. Three larval stages of FAW: First instar (neonates), 2nd instar and 3rd instar, were obtained from the insect rearing unit at the International Centre of Insect Physiology and Ecology (*icipe*). Rearing was done under laboratory conditions of  $25 \pm 2^\circ\text{C}$ ,  $72 \pm 3\%$  RH and L12:D12 with the larvae feeding on maize leaves. Prior to the exposure assay, the insects were starved for 24 hours before any behavioural feeding experiment for accurate results.

### Rabbit rearing and urine collection

Rabbits used for this experiment were obtained from *icipe*. Prior to this study, the rabbits had been reared following recommended rabbit keeping protocols provided by Animal welfare Victoria [13] and the United States Agency for International Development (USAID) [14]. Following these recommendations, we reared two mid-aged rabbits in a well-ventilated metal cage (50 × 70 × 40 cm) with *ad libitum* provision of fresh grass and legumes supplemented with conventional feed at a rate of 60–80 g/kg body weight/day and clean drinking water. Feed and water were provided in hoppers and crocks, respectively. These rabbits

were maintained at  $25 \pm 2^\circ\text{C}$ , 50–80% RH and L12:D12. General cleanliness of the cages and veterinary care of rabbits were conducted regularly based on USAID [14] and McGlone [15]. A total of 8 one-year-old rabbits were reared for the study. The metal cages had  $50 \times 70$  cm metallic mesh at the bottom to allow passage of the rabbit urine to the collection gutter. The metallic mesh prevented the contamination and mixing of rabbits' faecal pellets and rabbit urine. The rabbit urine was transferred to a 5 mL bottle, and the collection gutter was rinsed prior to collecting the next batch. The rabbit urine collection was done every 24 hours in the morning between 10:00–11:00 am, and 150 mL was collected. The rabbit urine was freshly used for the experiments.

## Determination of larval arrestment and settlement

To determine larval arrestment, maize leaf disks (3-cm) were exposed to undiluted rabbit urine by dipping for 5 minutes. They were then placed individually in Petri dishes lined with filter paper. Ten FAW larvae of the three stages were separately placed on the adaxial sides of the leaf in each Petri dish sealed with Parafilm®. The arrestment behaviour was evaluated by counting the larvae on the leaf disk 6 hours and 24 hours after the release. The experiment was replicated ten times for each larval stage.

The larval settlement was assessed based on a modified protocol described by Scheidegger et al. [23]. FAW larvae were exposed to 3-cm maize leaf disks treated with either undiluted rabbit urine or distilled water (control). The leaf disks were dipped in rabbit urine or distilled water for 5 minutes. The leaf disks were then placed in 15 cm (diameter) Petri dishes lined inside with filter paper (Whatman®). Two maize disks were placed 13 cm apart, with their adaxial sides facing up. Ten neonates, 2nd instar and 3rd instar of FAW larvae, were separately released at the center of a Petri dish. Petri dishes were then sealed with Parafilm® and immediately placed in a wooden cage covered by a black velvet cloth. The experiment was replicated ten times for each FAW larval stage. The number of larvae on or underneath each leaf disk was recorded after 6 hours and 24 hours to assess the larval settlement.

## Assessment of larval feeding and dose-damage response

Maize leaves were cut into 3-cm circular disks and dipped in distilled water (control), and different concentrations of rabbit urine: 10%, 25%, 50%, 75% and 100%; made using distilled water. Treated leaf disks were placed individually in a 15-cm-diameter Petri dish. Ten FAW neonates were released in the Petri dish in 10 replicates. The same procedure was repeated for the 2nd and 3rd instars. Leaf area consumed by FAW larval stages was recorded after 6 hours and 24 hours using a mobile application (Petiole) [16]. The consumed area of the leaf disk was converted into damage score:

$$\text{Damage score (\%)} = \frac{A_1 - A_2}{A_1} \times 100$$

where  $A_1$  is the initial area of the leaf disk, and  $A_2$  is the area of the leaf disk after 6 hours or 24 hours of exposure to FAW larvae.

## Assessment of larval survival

To assess the larval survival, leaf sections from a three-week-old maize plant were exposed to rabbit urine at different concentrations of 50% and 100% by foliar application through the use of a spray bottle. Distilled water was used as a control. Treated leaf sections were introduced in a 2000 mL screwed-top transparent plastic jar (one leaf section per jar). The plastic jars were lined at the bottom with moist paper towels. The leaf sections were removed from the jars every two days and replaced with freshly exposed ones. Five FAW neonates were released in each jar and sealed with a paper towel and a lid. Five replications were made for each concentration. The same procedure was adopted for the 2nd and 3rd instars. Larval mortality was recorded every 24 hours for 9 days after exposure.

## **Assessment of egg hatchability and pupal emergence**

FAW eggs were collected from the oviposition cages by cutting sections of leaves containing FAW eggs. FAW eggs on maize sections were sprayed with undiluted rabbit urine or distilled water (control) using a hand spray bottle. The leaf cuts with the eggs were then attached to white papers using an adhesive. The eggs were monitored for 3–4 days, and the number of hatched eggs was scored.

The effect of rabbit urine on the emergence of FAW pupae was assessed using an immersion protocol [17]. Ten FAW pupae were immersed in 15 mL of undiluted rabbit urine or distilled water (control) for 24 hours in four replications. The pupae were removed and recovered on filter papers in open Petri dishes. The pupae were monitored until emergence within 9 days.

## **Assessment of oviposition preference of the adult moth**

One-choice and two-choice behavioural tests were adopted to assess the oviposition preference of gravid FAW moth. The test was conducted in a sleeved Perspex cage (50 × 50 × 77 cm). Using a hand spray pump, one potted three-week-old maize plant sprayed with either undiluted rabbit urine or distilled water was placed in a cage. Five gravid FAW moths were released individually in a cage and repeated twice in the one choice experiment. Two three-week-old maize plants sprayed with either undiluted rabbit urine or distilled water using a hand spray pump were placed 45 cm apart in a cage in the two-choice experiments. Five gravid FAW moths were released individually in a cage, and the experiment was repeated twice. In both one-choice and two-choice experiments, the moths were allowed to oviposit on maize leaves for 24 hours in a dark room. To provide the moth with drinking water, a ball of cotton wool was dipped in distilled water and placed at the centre of the Petri dish. The leaves were examined after 24 hours, and all eggs were recovered and counted under a dissecting microscope.

## **Data analysis**

Analysis was performed in R software [18]. The larval settlement, arrestment, and pupal emergence datasets were analyzed using a logistic regression model. Dataset of larval feeding was subjected to a generalized linear model. In these models, the larval development stages, post-exposure time and treatment were used as fixed variables. The damage score in each treatment dose was corrected using Abbots' formula [19] to eliminate the damage score that naturally occurs in untreated maize:

$$\text{Corrected damage score (\%)} = 100 - \frac{Cd - Td}{Cd} \times 100$$

where  $Cd$  and  $Td$  are the damage scores recorded in the control and treatment, respectively. The same formula was applied for daily mortality data. The lethal dose-damage response and lethal time (days) required to reduce damage by 50% ( $LD_{50}$ ) and to cause 50% mortality ( $LT_{50}$ ) of all populations for each stage and corresponding regression slopes and 95% fiducial limits were computed using the probit regression model implemented in the *ecotox* package [20]. Differences in  $LT_{50}$  estimates across the stages were compared based on the degree of overlaps in the 95% fiducial limits. The survival data were subjected to the Cox regression model and summarized using Kaplan-Meier survival distribution curves. Oviposition preference was analysed using the Poisson regression model and egg hatchability subjected to logistic regression. Mean separation was performed whenever there was a significant difference between the treatments using the *lsmeans* package [21] with the Tukey p-value adjustment method.

## Results

### Larval arrestment and settlement

Larval arrestment was not significantly different across FAW developmental stages ( $\chi^2 = 0.1$ ,  $df = 2$ ,  $p = 0.97$ ) and treatment exposure time ( $\chi^2 = 0.2$ ,  $df = 1$ ,  $p = 0.67$ ); however, there was significant effect of the treatments ( $\chi^2 = 148.86$ ,  $df = 1$ ,  $p < 0.0001$ ) and the interaction of the developmental stage and treatment ( $\chi^2 = 29.55$ ,  $df = 2$ ,  $p < 0.0001$ ). Arrestment of neonates, 2nd and 3rd instars of FAW larval stages were significantly reduced when exposed to maize leaves treated with rabbit urine compared to those exposed to maize leaves treated with distilled water (Table 1).

Table 1

Mean number ( $\pm$  SE) of fall armyworm larvae that remained on the maize leaves treated with rabbit urine and distilled water (control) within 24 hours of exposure

Stage	Control	Rabbit urine
Neonates	73.00 $\pm$ 3.33a	25.50 $\pm$ 3.52b
2nd Instars	55.50 $\pm$ 3.20a	41.50 $\pm$ 3.19b
3rd Instars	70.00 $\pm$ 2.51a	27.00 $\pm$ 2.19b

Different small letters adjacent to figures indicate significant differences between control and exposed maize leaves on FAW settlement at  $p = 0.05$  (Tukey test). \*SE: Standard errors

Likewise, larvae settlement did not differ significantly across FAW developmental stages ( $\chi^2 = 1.32$ ,  $df = 2$ ,  $p = 0.51$ ) or treatment exposure time ( $\chi^2 = 0.36$ ,  $df = 1$ ,  $p = 0.55$ ) but differed significantly between the treatments ( $\chi^2 = 55.18$ ,  $df = 1$ ,  $p < 0.006$ ) and interaction of treatment\*time\*treatment ( $\chi^2 = 14.50$ ,  $df = 2$ ,  $p = 0.0007$ ). The FAW larvae released to maize leaves treated with distilled water had a higher settlement compared to those treated with rabbit urine (Table 2). Most of the neonates settled on untreated leaves

compared to rabbit urine treated leaves after 6 hours and 24 hours of exposure. For the 2nd instars, a significantly high proportion of larvae settled on untreated leaves after 24 hours of exposure. On the other hand, the 3rd instars preferred to settle on untreated maize after 6 hours of exposure.

Table 2

Mean number ( $\pm$  SE) of fall armyworm larvae that settled on the maize leaves treated with rabbit urine and distilled water (control) within 6 and 24 hours of exposure

Stages of <i>Spodoptera frugiperda</i>	6 hours post exposure		24 hours post exposure	
	Control	Rabbit urine	Control	Rabbit urine
Neonates	66.00 $\pm$ 4.27b	34.00 $\pm$ 4.27a	70.00 $\pm$ 4.22b	27.00 $\pm$ 4.96a
2nd Instars	50.00 $\pm$ 6.32a	50.00 $\pm$ 6.32a	57.00 $\pm$ 6.33b	43.00 $\pm$ 6.33a
3rd Instars	66.00 $\pm$ 7.18b	30.00 $\pm$ 7.30a	46.00 $\pm$ 6.00a	43.00 $\pm$ 5.39a

Different small letters adjacent to figures indicate significant differences between control and exposed maize leaves on FAW settlement at  $p = 0.05$  (Tukey test). \*SE: Standard errors

## Larval feeding and dose-damage response

Larval feeding differed significantly across the FAW larval stages ( $\chi^2 = 306.18$ ,  $df = 2$ ,  $p < 0.0001$ ), between treatment ( $\chi^2 = 9.71$ ,  $df = 1$ ,  $p = 0.002$ ), between the post-exposure time ( $\chi^2 = 80.47$ ,  $df = 1$ ,  $p < 0.001$ ) and there were significant interactions between FAW larval stages and post-exposure time ( $\chi^2 = 25.37$ ,  $df = 2$ ,  $p < 0.001$ ) and between treatment and post-exposure time ( $\chi^2 = 9.97$ ,  $df = 2$ ,  $p = 0.002$ ). Notable effect of rabbit urine on larval feeding was detected after 24 hours of treatment exposure on stages of fall armyworm: 2nd instars ( $\chi^2 = 8.94$ ,  $df = 1$ ,  $p = 0.003$ ) and 3rd instars ( $\chi^2 = 7.45$ ,  $df = 1$ ,  $p = 0.006$ ) (Fig. 1). In relation to untreated maize leaves, maize leaves treated with rabbit urine reduced damage due to feeding of neonates, 2nd instars and 3rd instars by 66, 69 and 72%, respectively, after 24 hours of exposure.

The probit regression shows that within 24 hours of exposure, the damage caused by FAW was reduced by 50% when the neonates, 2nd instars and 3rd instars larvae were exposed to 49, 94 and 55% of rabbit urine (Table 3). The lower doses of rabbit urine are required to reduce the damage caused by neonates and 3rd instars compared to doses required to reduce damages caused by 2nd instars.

Table 3

Dose-damage response (LD) and regression slope and fiducial limits (FL) of the fall armyworm (*Spodoptera frugiperda*) developmental stages feeding on maize treated with rabbit urine

Stages of <i>Spodoptera frugiperda</i>	<sup>a</sup> Slope ( $\pm$ <sup>b</sup> SE)	LD <sub>25</sub>	LD <sub>50</sub>
Neonates	1.51 $\pm$ 0.01	17.4 (13.1, 21.4) a	48 (42, 57) a
2nd Instar	4.61 $\pm$ 0.02	67.1 (64.0, 69.9) b	94 (90, 99) b
3rd Instar	2.24 $\pm$ 0.01	27.5 (24.3, 30.5) a	55 (50, 60) a

Figures enclosed in brackets are 95% lower and upper FL for LD<sub>25</sub> or LD<sub>50</sub> (%). Different small letters adjacent to figures indicate significant differences among the FAW developmental stages at p = 0.05 according to the degree of overlap in the FL values. <sup>a</sup>Slope is the regression slope, <sup>b</sup>SE: Standard error

## Larval lethal time-mortality response and survival

Rabbit urine showed insecticidal activity against the neonates, 2nd instars and 3rd instars of FAW. Rabbit urine caused 50% mortality within 5.0, 7.0 and 8.6 days to neonates, 2nd instars and 3rd instars, respectively (Table 4).

Table 4

Lethal time-response mortality (LT) of the fall armyworm (*Spodoptera frugiperda*) developmental stages treated with rabbit urine

Stages of FAW	Slope ( $\pm$ SE*)	LT <sub>25</sub>	LT <sub>50</sub>	LT <sub>75</sub>	LT <sub>99</sub>
Neonates	2.95 $\pm$ 0.04	2.9 (1.8, 3.8) a	5.0 (3.9, 6.4) a	8.4 (6.5, 14.0) a	30.5 (16.9, 131.0) a
2nd Instar	3.13 $\pm$ 0.04	4.5 (3.7, 5.1) ab	7.3 (6.5, 8.6) b	12.0 (10.0, 16.2) a	40.6 (26.5, 85.0) a
3rd Instar	2.84 $\pm$ 0.04	5.0 (4.6, 5.3) b	8.6 (8.0, 9.5) bc	15.0 (13.2, 17.6) a	57.2 (43.0, 83.5) a

Figures enclosed in brackets are 95% lower and upper fiducial limits (FL) for LT<sub>25</sub>, LT<sub>50</sub>, LT<sub>75</sub> and LT<sub>99</sub> (days). Different small letters adjacent to figures indicate significant differences among the FAW developmental stages at p = 0.05 according to the degree of overlap in the FL values. \*SE: Standard error

Survival of FAW differed significantly among tested developmental stages ( $\chi^2 = 6.10$ , df = 2, p = 0.047) and between treatments ( $\chi^2 = 42.14$ , df = 1, p < 0.001) but not across different treatment concentrations ( $\chi^2 = 1.05$ , df = 1, p = 0.31) and stage\*treatment\*concentration interaction ( $\chi^2 = 3.06$ , df = 1, p = 0.22). Compared to controls, neonates (Z = 4.15, p < 0.001) were more susceptible to treatments followed by 2nd instars (Z = 2.59, p = 0.010) and 3rd instars (Z = 2.44, p = 0.015) (Fig. 2).

## Egg hatchability and pupal emergence

The hatchability of the FAW egg was significantly affected by the treatments ( $\chi^2 = 1361.60$ ,  $df = 1$ ,  $p < 0.001$ , rabbit urine;  $156.0 \pm 69.0$ , control;  $723.0 \pm 66.0$ ). Likewise, pupal emergence was significantly affected by treatment ( $\chi^2 = 13.75$ ,  $df = 1$ ,  $p = 0.0002$ ). The proportion of pupae that emerged as adults was significantly higher after their larval stages were fed on untreated maize leaves ( $80.0 \pm 0.0\%$ ) than when they were fed on rabbit urine treated leaves ( $66.7 \pm 13.3\%$ ).

## Oviposition preference of the adult female moth

The ability of female FAW moth to lay eggs varied significantly between oviposition substrates in one-choice ( $\chi^2 = 174.53$ ,  $df = 1$ ,  $p < 0.001$ ) and two-choice experiment ( $\chi^2 = 5272.90$ ,  $df = 1$ ,  $p < 0.001$ ). The female FAW moth laid more eggs on treated maize leaves (Fig. 3).

## Discussion

Indigenous knowledge is a set of practices and skills acquired by local people through the accumulation of experience, informal experiments, and understanding of their environment [22]. This study investigated the veracity of indigenous knowledge used by African smallholder farmers to control FAW using rabbit urine. The study showed that rabbit urine influences the survival, behaviour (host selection, settlement, feeding and egg-laying), egg hatchability and pupal emergence of the FAW.

FAW feeds on over 350 host plants, with cereals, especially maize being the most preferred host [23]. FAW larvae damage host crops during their active growth stage and can entirely or partly destroy the plant [24]. Our results show that treating maize plants with rabbit urine repels the larvae, reducing their feeding and consequently damage to maize plants. Rabbit urine causes antixenosis, a mechanism that renders the treated maize plants undesirable to FAW larvae for food, shelter, or reproduction [25].

All larval stages of the FAW are destructive [24]. Nevertheless, our results on the leaf-feeding showed some variance in the different FAW larval stages. This can be attributed to the proportion of food required to support the growth and development of different FAW larval stages. The 2nd and 3rd instars of FAW had high feeding rates than the neonates. Our results corroborate Coy et al. [26, 27], who reported that the destruction of host plants by FAW increases concomitantly with larval stages. The susceptibility of FAW larvae to the insecticidal compounds may differ significantly with the larval stages. For instance, Ghidiu and Andaloro [28] found that the susceptibility of FAW larvae to the toxicity of insecticides (methomyl and fenvalerate) decreased as the age increased. The rabbit urine may contain specific compounds that account for FAW avoidance. An investigation conducted by Mutai [29] indicated that the chemical composition of rabbit urine contained 1.05% nitrogen, 0.01% phosphorus, 0.85% potassium and 0.12% calcium. Rabbit urine also contains other chemical compounds which may have a deleterious effect on FAW larvae. The robenidine hydrochloride [30] and ammonium excretion [31] are some of the most reported compounds.

Our study established that rabbit urine reduces the survivorship of the three tested larval stages of FAW. However, the neonates are more susceptible, followed by the 2nd instar and 3rd instar, which support the

dose-response relationship. Apart from controlling FAW directly, rabbit urine improves crop health as it is utilized as a biofertilizer and an organic pesticide against black bean aphids (*Aphis fabae* APHIFA) on common beans (*Phaseolus vulgaris* L.) [32]. Therefore, the application of rabbit urine could have a double effect on the plant: (1) reducing pest load and (2) boosting crop health, but this needs to be demonstrated further.

Interestingly, treating maize plants with rabbit urine significantly increased oviposition preference by gravid FAW moth. This can be attributed to the olfactory response of the FAW female moth, given the strong smell of rabbit urine. The chemical response of FAW female moth to the rabbit urine requires further investigations. Nevertheless, this finding can reinforce some agroecological systems such as the 'Push-pull' technology by spraying the rabbit urine on the trap plant to make it more attractive to the FAW female moth oviposition. Eggs would not suitably survive or pupae will not emerge in high numbers since rabbit urine drastically reduced hatchability and pupal emergence. Push-pull was initially designed to control *Striga* and stemborers. Although it has also been demonstrated to control FAW, the attractiveness of *Brachiaria* grass to gravid FAW moth is being investigated.

## Conclusions

The use of rabbit urine by African smallholder farmers is an affordable strategy to manage FAW. Rabbit urine acts as a repellent by reducing larval feeding leading to mortality. FAW neonates are the most susceptible to the toxic effect of rabbit urine. Rabbit urine also has a detrimental effect on FAW egg hatchability, suggesting its application as a preventive measure once eggs are detected and/or damages on leaves are identified. Results of this study could aid in developing optimized formulations after proper field scouting. Female FAW oviposition preference could be used to optimize deterrent tactics such as push-pull technology. Our study suggests further chemical analysis and additional behavioural responses.

## Abbreviations

ANOVA

Analysis of variance

FAW

Fall armyworm

L12

D12

12 hours of light and 12 hours of dark

LD

Lethal dose

LT

Lethal Time

USAID

United States Agency for International Development.

## **Declarations**

### **Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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### **Author contributions**

SN conceived the idea, DK prepared the manuscript and was the main data collector, ERO analysed the data, DMK, ERO, AT, GH, IG, SS, SN, and YB reviewed and edited the manuscript. Lastly, the final manuscript was read and confirmed by all authors.

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### **Ethics approval**

Ethical approval for the study was provided by the IACUC of the Kenya Agricultural and Livestock Research Organization—Veterinary Science Research Institute; approval code: KALRO-VSRI/IACUC019/30082019.

### **Consent for publication**

Not applicable.

### **Competing interests**

The authors declare that they have no competing interests.

## Author details

<sup>1</sup>International Centre of Insect Physiology and Ecology (*icipe*), P.O. Box 30772–00100, Nairobi, Kenya

<sup>2</sup>Tanzania Agricultural Research Institute (TARI), Ukiriguru Center, Shinyanga Road, P. O. Box 1433, Mwanza, Tanzania

<sup>3</sup>USAID/BHA/TPQ, Washington, D.C., United States of America

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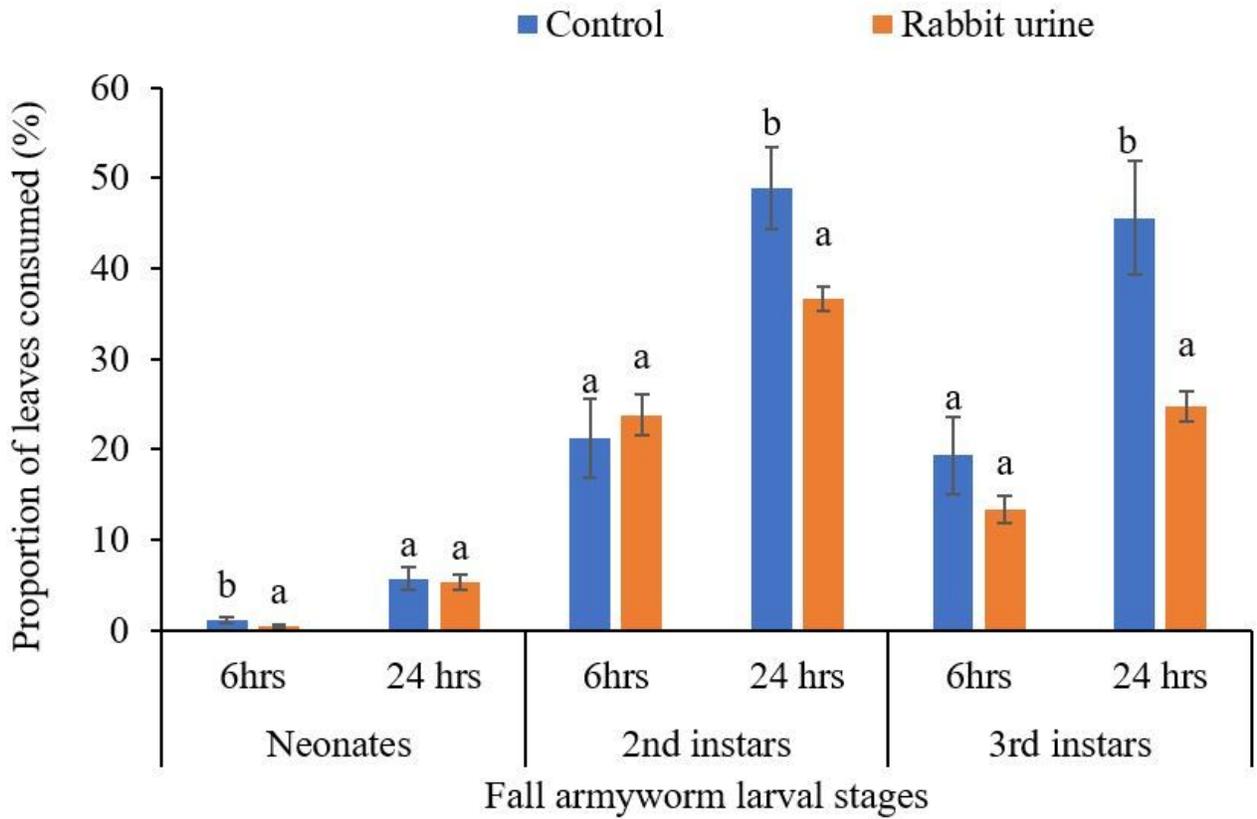
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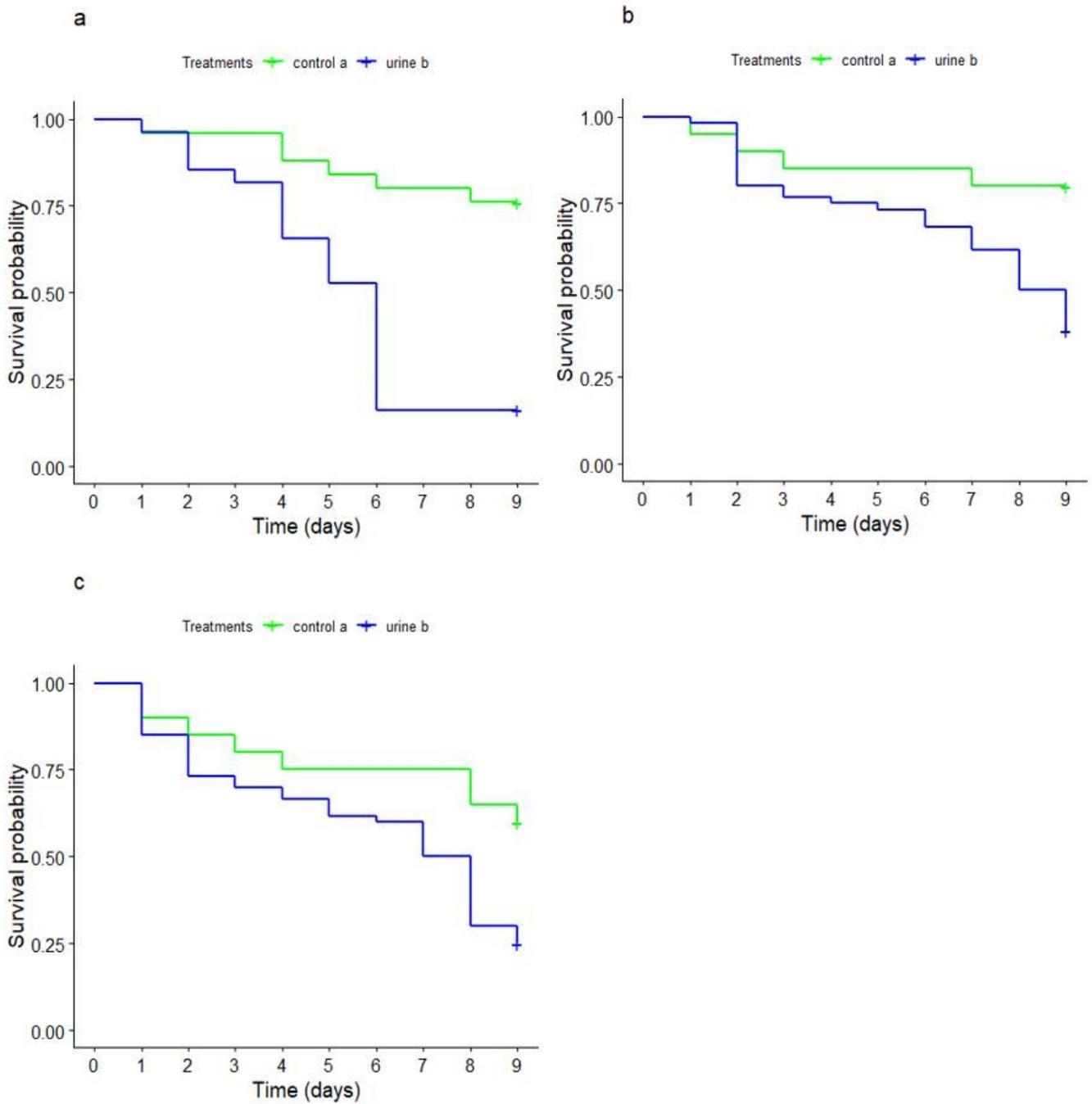
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## Figures



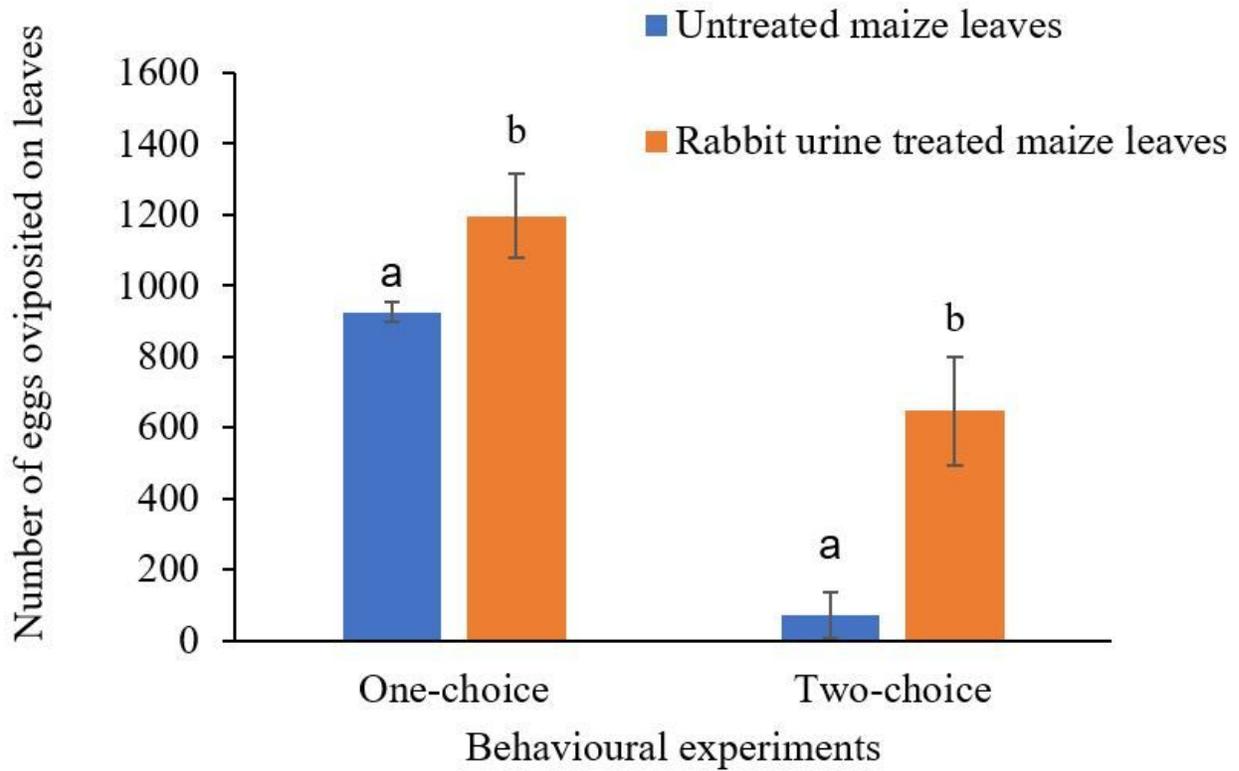
**Figure 1**

Proportion of leaf area consumed by fall armyworm (FAW) larval stages. The maize leaves were treated with rabbit urine (undiluted form) and distilled water (control) and exposed to FAW larvae for 6 and 24 hours. Error bars indicate standard errors. Different small letters in columns indicate significant differences at  $p = 0.05$  (Tukey test)



**Figure 2**

Kaplan-Meier survival distribution curves of fall armyworm neonates (a), 2<sup>nd</sup> instar larvae (b) and 3<sup>rd</sup> instar larvae (c) exposed to rabbit urine or distilled waters (control). "+" indicates right censorship. Small letters after the legends indicate a significant difference between control and rabbit urine treatments



**Figure 3**

Mean number of eggs oviposited by gravid female FAW moth on maize leaves treated with rabbit urine and distilled water in one-choice and two-choice behavioural experiments. Error bars represent standard errors. Different small letters above error bars represent a significant difference between treatments at  $p = 0.05$  according to the Tukey test