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Effect of Rabbit Urine on the Larval Behavior, Larval Mortality, Egg Hatchability, Adult Emergence and Oviposition Preference of the Fall Armyworm (*Spodoptera frugiperda* J.E. Smith)

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Abstract: The fall armyworm (FAW) (*Spodoptera frugiperda* J.E. Smith) is a major cereal pest threatening food security in Africa. African smallholder farmers apply various indigenous pest management practices, including rabbit urine; however, there is no scientific evidence for its efficacy. The FAW eggs, first, second and third instar larvae and moths were exposed to rabbit urine-treated maize leaves alongside untreated maize leaves (control). More FAW larvae (46.0–70.0%) remained on the untreated leaves than those (27.0–43.0%) on the rabbit urine-treated leaves. Rabbit urine caused 6.4 and 12.8% damage reduction of the second and third instars, respectively, 24 h post-exposure. Rabbit urine significantly reduced the survival of FAW, had a lethal time (LT_{50}) of 5.0, 7.3 and 8.7 days and a lethal dose (LD_{50}) of 48, 94, and 55% for the first, second and third instars, respectively. Egg hatchability and adult emergence were reduced by 55.0 and 13.3%, respectively. The FAW female moths laid more eggs on the rabbit urine-treated plants (647 $\pm$ 153 eggs) than they did on the untreated plants (72 $\pm$ 64 eggs). This study confirms farmers' assertions about using rabbit urine to manage FAW. For successful integration into the FAW IPM package, additional studies on the chemistry of rabbit urine, the behavioral response and the field might be required.

Keywords: agroecological farming systems; biopesticides; indigenous knowledge; maize; IPM package; oviposition

Citation: Kemunto, D.; Omuse, E.R.; Mfuti, D.K.; Tamiru, A.; Hailu, G.; Rwiza, I.; Belayneh, Y.T.; Subramanian, S.; Niassy, S. Effect of Rabbit Urine on the Larval Behavior, Larval Mortality, Egg Hatchability, Adult Emergence and Oviposition Preference of the Fall Armyworm (*Spodoptera frugiperda* J.E. Smith). *Agriculture* **2022**, *12*, 1282. https://doi.org/10.3390/agriculture12081282

Academic Editors: Susana Pascual, Francisco J. Beitia and Gloria Nombela

Received: 13 July 2022
Accepted: 18 August 2022
Published: 22 August 2022

1. Introduction

The fall armyworm (FAW) (*Spodoptera frugiperda* J.E. Smith) is a devastating pest of cereals in Africa. The FAW originated from the Americas and was reported in 2016 in Africa [1]. It has now spread widely in Africa and beyond [2,3]. The FAW was first reported in western regions of Kenya in 2017, but by early 2018, it was confirmed in more than 42 counties throughout the country [4]. Reportedly, the FAW has been found to attack 186 plant species from 42 families [5], including economically important crops such as maize, rice, sugarcane, sorghum, beet, tomato, potato, cotton and pasture grasses [6,7]. The impact of the FAW on maize productivity is increasingly threatening agricultural productivity and, consequently, livelihood and escalating food insecurity, particularly in Africa [7]. Since its arrival, the FAW has caused substantial losses in the cereal value chain. For instance, smallholder farmers have been heavily impacted [8]. In Africa, maize yield losses due to the FAW are estimated at 21–53% [7], accounting for about $16 billion in losses annually [9]. In Kenya, the yield loss due to FAW infestation is estimated at 47% (1381 kg/ha) [10–12].

Over the past 50 years, crop protection against insect pests has relied heavily on chemical pesticides [13]. However, chemical insecticides are increasingly becoming unreliable due to health and environmental risks [14]. The development of resistant populations of the

FAW to chemical insecticides has also been reported [15]. Therefore, there is widespread interest in alternative and sustainable control strategies [16]. Agroecological farming practices, including legume intercropping and crop diversification, are considered potential alternatives for pest management [9,17]. Though sustainable, many of these agroecological approaches are either not accessible for smallholder farmers or knowledge-intensive. For the longest time, African farmers have relied on indigenous knowledge, including cultural farming practices, to manage pests. The use of smallholder farmers' indigenous knowledge in pest management practices is considered effective and can be scaled to complement other agroecological farming practices.

Indigenous knowledge is usually accessible and affordable, and most resource-poor farmers rely on it to manage the FAW [18]. Some of this documented knowledge includes handpicking, the killing of larvae, placing soil or ash to plant whorls, drenching tobacco extracts, early planting, deep ploughing to kill pupae, burning stubbles after the harvesting of infested crops, intercropping and the rotation of maize with non-host crops [18]. The uses of ash, urea, soils, soaps and botanical extracts such as tobacco, garlic, datura and green pepper are also some of the traditional management options for the FAW in Africa by smallholder farmers [19,20]. 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 the 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 [21]. Apart from being used as a biopesticide, rabbit urine is also an excellent organic fertilizer. For instance, rabbit urine has been used as a biofertilizer to improve the viability of seeds of crops such as cinnamon (*Cinnamomum burmannii* L.) [22] and the growth and yield of pagoda (*Brassica narinosa* L.) [23] and tomato (*Lycopersicon esculentum* Mill.) [24]. Rabbit urine has been used in African countries (e.g., Ethiopia, Kenya, Uganda, Tanzania, Malawi and Nigeria) as a pesticide and fertilizer. In Uganda, for instance, it was observed that the use of rabbit urine collected from the farm could improve soil fertility and kill aphids [25]. This would not only help to reduce the high cost of organic farming but also increase the quantity and quality of the crop produced. Smallholder farmers have asserted that rabbit urine spray helps manage a wide range of insect pests, especially caterpillars, aphids, moths, leaf miners, mites and whiteflies attacking crops such as vegetables, maize, watermelons, coffee, bananas and avocados, among others. Rabbit urine has several merits when it is used as a fertilizer and pesticide—it can be cheaply sourced in sufficient volumes, it contains a high level of nitrates, phosphorus and potassium, which are needed by the plant to grow, and it is environmentally friendly and non-toxic [26]. Several reports from smallholder farmers indicated that rabbit urine is highly effective against the FAW; however, there are no empirical data to confirm such assertions.

In the context of a sustainable food system, agricultural diversification, and intensification, livestock integration becomes critical [27]. Rabbit farming is a low-input livestock activity recognized in many African countries (e.g., Kenya, Rwanda, Algeria, Malawi, South Africa, Tunisia, Botswana, Burundi, Egypt, Ghana, Cameroon and Sierra Leone) as a tool to promote food security and alleviate poverty among smallholder farmers [28]. Rabbits are being farmed for local consumption and as a source of income for farmers through the sale of meat [28]. Provided that rabbit urine can be sourced cheaply by smallholder farmers, elucidating its mode of action on the 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 that were assessed: (1) larval behavior (orientation and settlement); (2) larval feeding (including damage score); (3) larval survival; (4) adult 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 guide the optimization and standardization of this practice for wide adoption.

2. Materials and Methods

2.1. 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 ± 2 °C during the day and 23.5 ± 2 °C at night with 70 ± 5% relative humidity (RH) and a L12:D12 (light:dark) photoperiod. Three-week-old plants were used in this study. Three larval stages of the FAW—first, second and third instars—were obtained from the insect rearing unit at the International Center of Insect Physiology and Ecology (*icipe*). Rearing was done under laboratory conditions of 25 ± 2 °C, 72 ± 3% RH and L12:D12, with the larvae feeding on maize leaves. Prior to the exposure assay, the insects were starved for 24 h before any behavioral feeding experiment for accurate results.

2.2. Rabbit Rearing and Urine Collection

The rabbits used for this experiment were obtained from *icipe*. Prior to this study, the rabbits had been reared following the recommended rabbit-keeping protocols provided by Animal Welfare Victoria [29] and the United States Agency for International Development (USAID) [30]. Following these recommendations, we reared eight mid-aged rabbits in eight well-ventilated metal cages (50 × 70 × 40 cm). The cages were constructed at the *icipe* workshop, Duduville, Nairobi, Kenya. The rabbits were *ad libitum* provided with 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. The rabbits were maintained at 25 ± 2 °C, 50–80% RH and L12:D12. The general cleanliness of the cages was maintained, and the veterinary care for the rabbits was conducted regularly based on the USAID [30] and McGlone [31]. The metal cages had 50 × 70 cm metallic mesh at the bottom to allow for the passage of the rabbit urine to the collection gutter. The metallic mesh prevented the contamination and mixing of the rabbits' fecal pellets and urine. The rabbit urine was transferred to a 5 mL bottle, and the collection gutter was cleaned prior to collecting the next batch. The rabbit urine collection was done every 24 h in the morning between 10:00, and 11:00 am, and a total of 150 mL was collected. The rabbit urine was freshly used for the experiments.

2.3. Determination of Larval Arrestment and Settlement

The arrestment and settlement were considered as the behavior of larvae to orient and settle on the maize leaves. No-choice and two-choice experiments were conducted to determine the larval arrestment and settlement based on a modified protocol described by Cheruiyot et al. [32]. In a no-choice experiment, maize leaf disks (3-cm) were exposed to undiluted rabbit urine or distilled water (control) by dipping for 5 min. They were then placed singly in Petri dishes (Thermo Fisher Scientific, Waltham, MA, USA) lined with filter paper (Figure 1). Ten FAW larvae of the neonate stage (first instars) were placed on the adaxial side of the leaf in a Petri dish. The Petri dish was then sealed with Parafilm® (Heathrow Scientific, Vernon Hills, IL, USA). This was repeated five times with new first instar larvae and the rabbit urine-treated maize leaf disks in the Petri dishes. The same procedure was done for the second instar larval stage. To avoid potential cannibalism, the third instar larvae were singly placed on the adaxial side of the leaf in a Petri dish. The Petri dishes were then sealed with Parafilm® and immediately placed in a wooden cage covered by a black velvet cloth. The arrestment behavior was evaluated by counting the larvae on or underneath the leaf disk 6 and 24 h after the release.

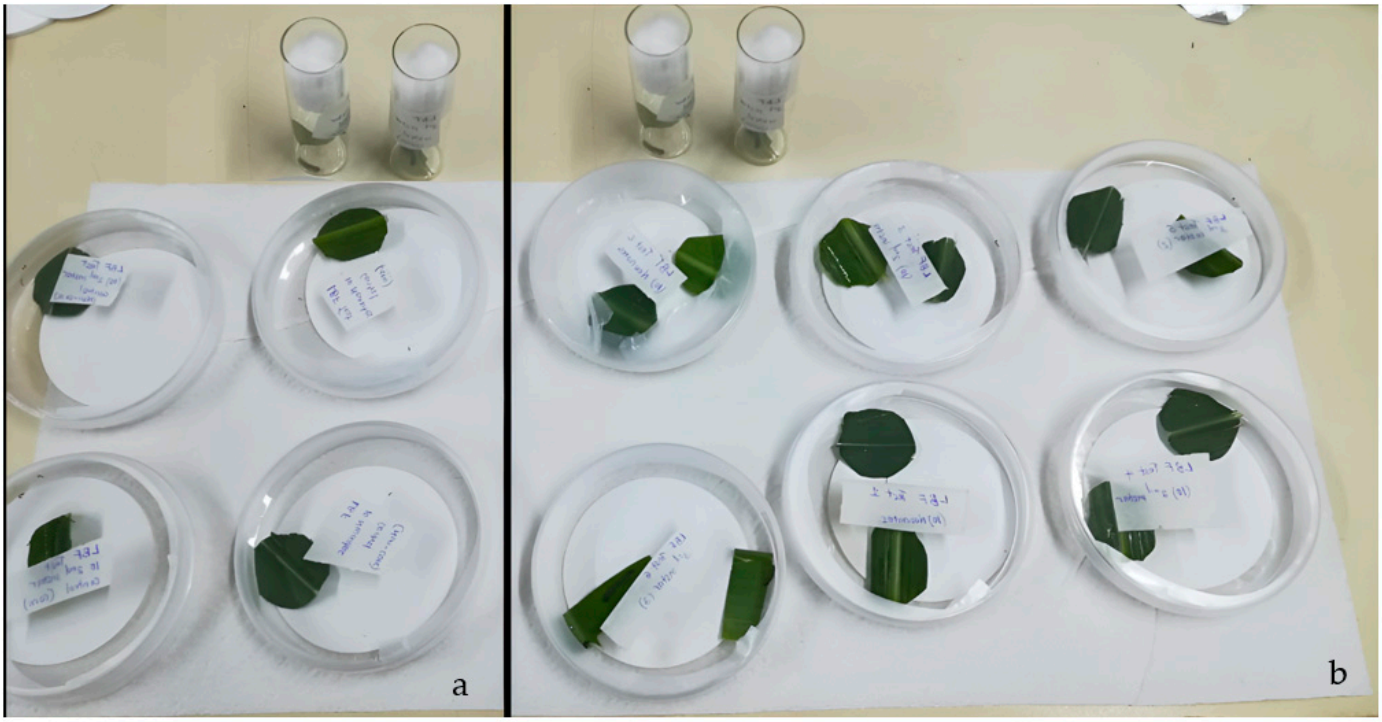

Figure 1. Experimental set-up for (**a**) no-choice experiment and (**b**) two-choice experiment for larval arrestment and settlement assessment.

In the two-choice experiment, the 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 min. The leaf disks were then placed in 15 cm (diameter) Petri dishes lined on the inside with filter paper (Whatman®, Vancouver, Canada). Two maize disks (one rabbit urine-treated and one untreated) were placed 13 cm apart, with their adaxial sides facing up (Figure 1). Ten first, second and third instars of the FAW larvae were individually and separately released at the center of a Petri dish (one larva per Petri dish). The 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 and 24 h to assess the larval orientation and settlement.

2.4. Assessment of Larval Feeding and Dose-Damage Response

Maize leaves were cut into 3 cm circular disks and dipped in distilled water (control) or different doses of rabbit urine—10, 25, 50, 75 and 100%—that were prepared using distilled water (Figure 2). Serial dilutions were used to establish the effect of the rabbit urine through a dose-response relationship. The treated leaf disks were placed singly in a 15 cm-diameter Petri dish. A FAW first instar larva was released in the Petri dish and was allowed to consume the leaf disk. This was replicated 10 times with the new first instars. The same procedure was repeated for the second and third instars. The leaf area (mm^2) consumed by the FAW larval stages was recorded after 6 h and 24 h using a mobile application (Petiole) [33]. With the application downloaded alongside its calibration pad and installed, the android mobile phone (Tecno Camon 17, Shenzhen, China) was fixed at the optimal height (11 cm) above the calibration pad using a measuring stand. The mobile phone camera was calibrated using the calibration pad printed on a paper at the optimal height. The leaf samples were placed in front of the mobile camera on a measuring stand, and the estimated leaf area was recorded. The surface area consumed indicated the tissue of

leaves that was damaged by the larvae. The consumed area of the leaf disk was converted into a damage score:

$$Damage\ score\ (\%) = \frac{A^1 - A^2}{A^1} \times 100$$

where A^1 is the initial area of the leaf disk, and A^2 is the area of the leaf disk after 6 h or 24 h of exposure to FAW larvae.

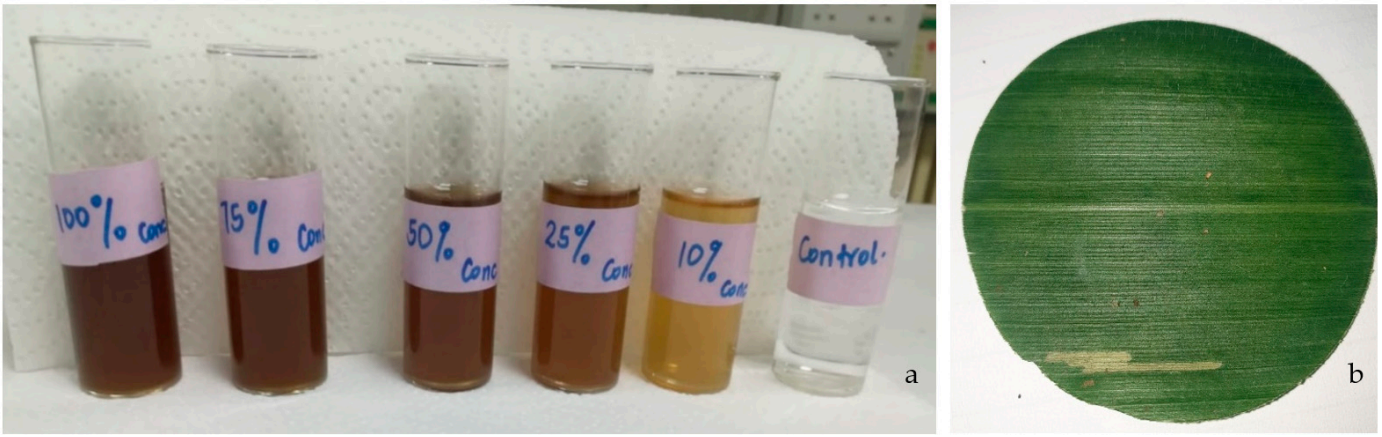

Figure 2. (**a**) Preparation of five rabbit urine doses and the control, and (**b**) a section of a leaf disk with damaged tissue as a result of feeding by fall armyworm larva.

2.5. Assessment of Larval Lethal Time-Mortality Response and Survival

To assess the larval survival, leaf sections from a three-week-old maize plant were exposed to rabbit urine at different doses of 50 and 100% by a foliar application using a hand spray bottle (KAPI Ltd., Nakuru, Kenya). Distilled water was used as a control. The treated leaf sections were introduced in a 2000 mL screwed-top transparent plastic jar (Kenpoly Manufacturers Ltd., Nairobi, Kenya) (one leaf section per jar). The plastic jars were lined at the bottom with moist paper towels to reduce desiccation. Five FAW first instars were released in each jar and sealed with a paper towel and a lid. Five replications were made for each dose. The same procedure was used for the second instars. However, for the third instar, an individual larva was introduced in a glass jar containing treated or untreated sections of maize leaves. The experiment with the third instars was replicated 25 times. The leaf sections were removed from the jars every two days and replaced with sections freshly exposed to the same dose as the previously introduced leaves. Larval mortality was recorded every 24 h for 9 days after exposure.

2.6. Assessment of Egg Hatchability and Adult Emergence

The FAW eggs were collected from the oviposition cages by cutting sections of leaves containing FAW eggs. The cages were constructed at the *icipe* workshop, Duduville, Nairobi, Kenya. The FAW eggs on the maize sections were sprayed with undiluted rabbit urine or distilled water (control) using a hand spray bottle until the leaf was soaking wet (Figure 3). The leaf cuts with the eggs were then air-dried for 1 h and attached to white paper using an adhesive. The eggs were monitored for 3–4 days, and the number of hatched eggs was recorded.

The effect of rabbit urine on the emergence of FAW pupae was assessed using an immersion protocol [34]. Ten FAW pupae were immersed in 15 mL of undiluted rabbit urine or distilled water (control) for 24 h in four replications (Figure 3). The pupae were removed and recovered on filter papers in open Petri dishes. The pupae were monitored until adult emergence within 9 days.

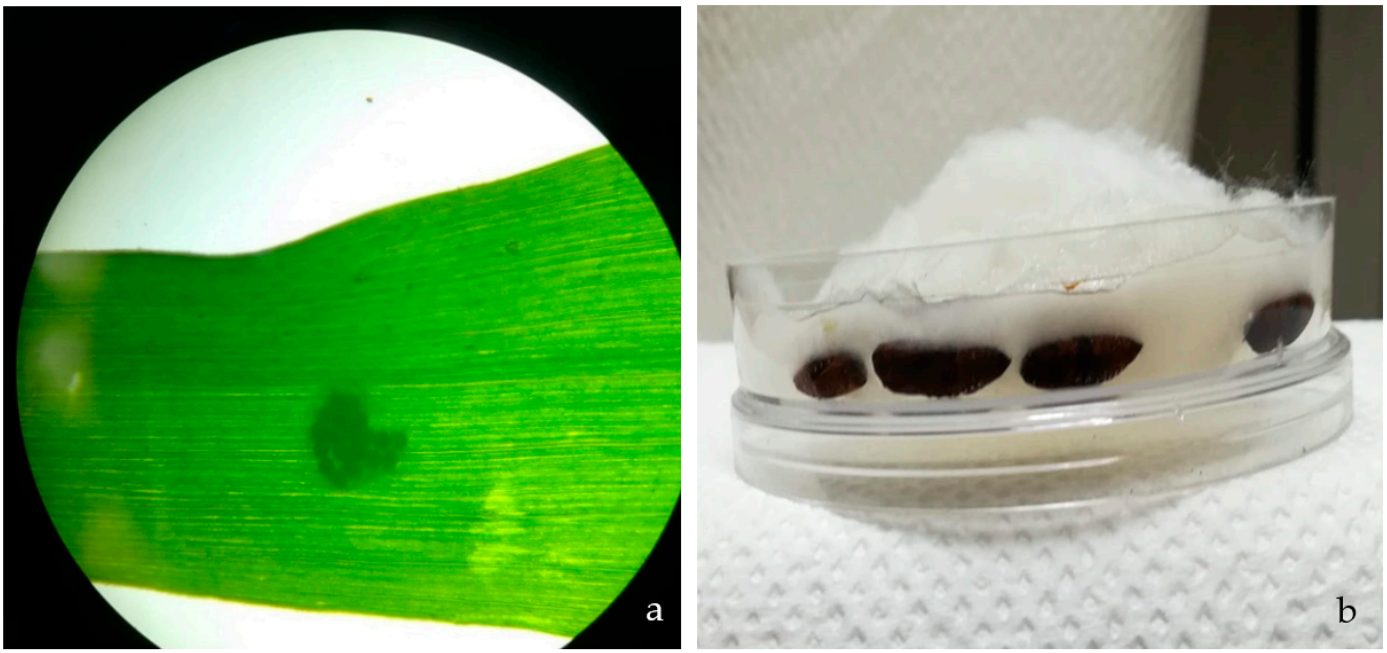

Figure 3. (**a**) Section of maize leaf containing fall armyworm eggs that have been sprayed with rabbit urine and (**b**) pupal immersed in rabbit urine before incubation for adult emergence assessment.

2.7. Assessment of Oviposition Preference of the Adult Female Moth

No-choice and two-choice behavioral tests were adopted to assess the oviposition preference of gravid female FAW moths. The test was conducted in a sleeved Perspex cage (50 × 50 × 77 cm). The cages were constructed at the *icipe* workshop, Duduville, Nairobi, Kenya. Using a hand spray pump, ten three-week-old maize plants in a 2 L plastic pot (Kenpoly Manufacturers Ltd., Nairobi, Kenya) were sprayed with either undiluted rabbit urine or distilled water (control) and placed in a cage. Five gravid FAW moths were released individually in a cage and repeated twice in the no-choice experiment. Two pots, each containing ten 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 (Figure 4). Five gravid FAW moths were released individually in a cage, and the experiment was repeated twice. In both the no-choice and two-choice experiments, the moths were allowed to oviposit on the maize leaves for 24 h 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 center of the Petri dish. The leaves were examined after 24 h, and all eggs were recovered and counted under a dissecting microscope (New York Microscope Co., New York, NY, USA).

2.8. Data Analysis

The analysis was performed in the R software (Version 4.0.3, R Foundation for Statistical Computing, Vienna, Austria) [35]. The larval arrestment and settlement and the pupal emergence datasets were analyzed using a logistic regression model. The dataset of the damage score due to 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. Prior to the analysis, the damage score in each treatment dose was corrected using Abbots' formula [36] to eliminate the damage score that occurred in the untreated maize:

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

where *Cd* and *Td* are the damage scores recorded in the control and treatment, respectively. The same formula was applied for the daily mortality data. The lethal dose-damage response that is required to reduce damage by 25% (LD_{25}) and 50% (LD_{50}), the lethal time (days) required to cause 25% (LT_{25}) and 50% mortality (LT_{50}), the corresponding regression slopes and 95% fiducial limits were computed using the Probit regression model implemented in the *ecotox* package [37]. Differences in LT_{50} estimates across the stages were compared based on the degree of overlap 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 analyzed using the Poisson regression model, and egg hatchability was subjected to the logistic regression model. Mean separation was performed whenever there was a significant difference between the treatments using the *lsmeans* package [38] on the Tukey-adjusted *p*-values.

Figure 4. Two-choice experimental set-up with rabbit urine-treated and untreated potted maize plants for gravid female moths of the fall armyworm to lay eggs during the oviposition assay.

3. Results

3.1. Larval Arrestment and Settlement

In the no-choice experiment, the number of larvae that oriented and settled on the maize leaves was not significantly different across FAW developmental stages ($\chi^2 = 0.1$, df = 2, $p = 0.97$) and treatment exposure times ($\chi^2 = 0.2$, df = 1, $p = 0.67$); however, there was a significant effect of the treatments ($\chi^2 = 148.86$, df = 1, $p < 0.0001$) and the interaction of the developmental stage and the treatments ($\chi^2 = 29.55$, df = 2, $p < 0.0001$). The arrestment and settlement of the first, second and third instars of the FAW larval stages were significantly reduced by 25.5, 41.5 and 27.0% when exposed to the maize, leaves treated with rabbit urine compared to those exposed to the maize leaves treated with distilled water (73.0, 55.5 and 70.0%, respectively) (Table 1).

Table 1. Mean number (±SE) of fall armyworm larvae that remained on the maize leaves treated with rabbit urine and distilled water (control) within 24 h of exposure in the no-choice experiment.

Stages of *Spodoptera frugiperda*	Control	Rabbit Urine
Neonates	73.00 ± 3.33 [a]	25.50 ± 3.52 [b]
Second Instars	55.50 ± 3.20 [a]	41.50 ± 3.19 [b]
Third Instars	70.00 ± 2.51 [a]	27.00 ± 2.19 [b]

Different small letters adjacent to the figures indicate significant differences between the control and exposed maize leaves on the *S. frugiperda* settlement at $p = 0.05$ (Tukey test).

Likewise, in the two-choice experiment, the number of larvae that oriented and settled on the maize leaves did not differ significantly across FAW developmental stages ($\chi^2 = 1.32$, df = 2, $p = 0.51$) or treatment exposure times ($\chi^2 = 0.36$, df = 1, $p = 0.55$) but differed significantly between the treatments ($\chi^2 = 55.18$, df = 1, $p < 0.006$) and the interaction of the treatment, time and treatment ($\chi^2 = 14.50$, df = 2, $p = 0.0007$). Generally, the FAW larvae released to the maize leaves treated with distilled water had a higher settlement compared to those released to the leaves treated with rabbit urine (Table 2). Most of the first instars (66 and 70%) settled on the untreated leaves rather than (34 and 27%) the rabbit urine-treated leaves after 6 h and 24 h of exposure, respectively. For the second instars, a significantly high proportion (57%) of larvae settled on the untreated leaves compared to those that settled (43%) on the rabbit urine-treated leaves after 24 h of exposure. On the other hand, the third instars preferred to settle on the untreated maize (66%) rather than the rabbit urine-treated leaves (30%) after 6 h of exposure.

Table 2. Mean number (±SE) of fall armyworm larvae that oriented and settled on the maize leaves treated with rabbit urine and distilled water (control) within 6 and 24 h of exposure in the two-choice experiment.

Stages of *Spodoptera frugiperda*	6 h Post-Exposure		24 h Post-Exposure	
	Control	Rabbit Urine	Control	Rabbit Urine
Neonates	66.00 ± 4.27 [b]	34.00 ± 4.27 [a]	70.00 ± 4.22 [b]	27.00 ± 4.96 [a]
Second Instars	50.00 ± 6.32 [a]	50.00 ± 6.32 [a]	57.00 ± 6.33 [b]	43.00 ± 6.33 [a]
Third Instars	66.00 ± 7.18 [b]	30.00 ± 7.30 [a]	46.00 ± 6.00 [a]	43.00 ± 5.39 [a]

Different small letters adjacent to the figures indicate significant differences between the control and exposed maize leaves on the *S. frugiperda* settlement at $p = 0.05$ (Tukey test).

3.2. 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 treatments ($\chi^2 = 9.71$, df = 1, $p = 0.002$) and between post-exposure times ($\chi^2 = 80.47$, df = 1, $p < 0.001$). A notable effect of rabbit urine on larval feeding was detected after 24 h of treatment exposure on the following stages of fall armyworm: the second instars ($\chi^2 = 8.94$, df = 1, $p = 0.003$) and third instars ($\chi^2 = 7.45$, df = 1, $p = 0.006$) (Figure 5). Compared to the untreated maize leaves, the maize leaves treated with rabbit urine exhibited estimated reduced damage by 6.4 and 12.8% due to the feeding of the second and third instars, respectively, after 24 h of exposure.

The Probit regression shows that, within 24 h of exposure, the damage caused by the FAW can be reduced by 50% when the first, second and third instar larvae are exposed to 48, 94 and 55% of rabbit urine, respectively (Table 3). Lower doses of rabbit urine are required to reduce the damage caused by the first and third instars compared to the doses required to reduce the damage caused by the second instars.

Table 3. Lethal dose (LD), regression slope and fiducial limits (FL) of the rabbit urine needed to reduce the damage of the fall armyworm (*Spodoptera frugiperda*) larval stages feeding on maize treated by 25 and 50%.

Stages of *Spodoptera frugiperda*	[a] Slope (± [b] SE)	LD_{25}	LD_{50}
Neonates	1.51 ± 0.01	17.4 (13.1, 21.4) [a]	48 (42, 57) [a]
Second Instar	4.61 ± 0.02	67.1 (64.0, 69.9) [b]	94 (90, 99) [b]
Third Instar	2.24 ± 0.01	27.5 (24.3, 30.5) [a]	55 (50, 60) [a]

The figures enclosed in brackets are the 95% lower and upper FL for LD_{25} or LD_{50} (%). Different small letters adjacent to the figures indicate significant differences among the *S. frugiperda* developmental stages at $p = 0.05$, according to the degree of overlap in the FL values. [a] Slope is the regression slope. [b] SE: Standard error.

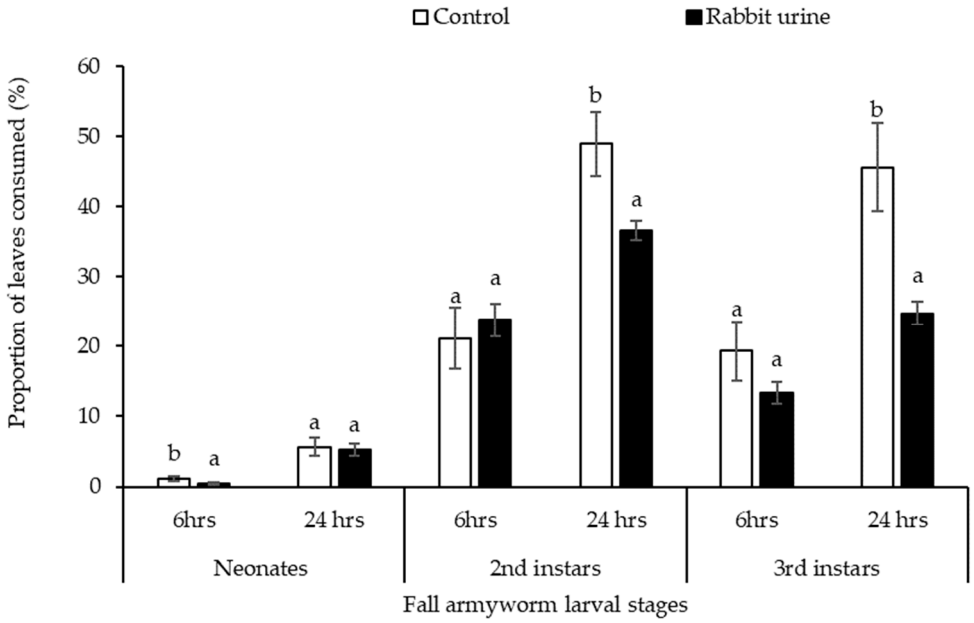

Figure 5. Proportion of leaf area consumed by fall armyworm (FAW) larval stages. The maize leaves were treated with rabbit urine (undiluted form) and distilled water (control) and were exposed to FAW larvae for 6 and 24 h. Error bars indicate standard errors. Different small letters in the columns indicate significant differences at $p = 0.05$ (Tukey test). Neonates are the first instar larval stage.

3.3. Larval Lethal Time-Response Mortality and Survival

Rabbit urine showed insecticidal activity against the first, second and third instars of the FAW. Rabbit urine caused 50% mortality within 5.0, 7.3 and 8.6 days to the first, second and third 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 *Spodoptera frugiperda*	Slope (±SE *)	LT_{25}	LT_{50}
Neonates	2.95 ± 0.04	2.9 (1.8, 3.8) ^{a}	5.0 (3.9, 6.4) ^{a}
Second Instar	3.13 ± 0.04	4.5 (3.7, 5.1) ^{ab}	7.3 (6.5, 8.6) ^{b}
Third Instar	2.84 ± 0.04	5.0 (4.6, 5.3) ^{b}	8.6 (8.0, 9.5) ^{bc}

The figures enclosed in brackets are the 95% lower and upper fiducial limits (FL) for LT_{25} and LT_{50} (days). Different small letters adjacent to the figures indicate significant differences among the *S. frugiperda* developmental stages at $p = 0.05$, according to the degree of overlap in the FL values. * SE: Standard error.

The survival of the FAW larvae was significantly different when they were exposed to 50 and 100% doses of rabbit urine ($\chi^2 = 1.05$, df = 1, $p = 0.31$); therefore, the survival datasets from these two doses were pooled for the subsequent analysis. The survival of the FAW differed significantly among the 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 the stage*treatment interaction ($\chi^2 = 3.13$, df = 2, $p = 0.21$). Compared to the controls, the first instars ($Z = 4.15$, $p < 0.001$) were more susceptible to the treatments, followed by the second instars ($Z = 2.59$, $p = 0.010$) and the third instars ($Z = 2.44$, $p = 0.015$) (Figure 6).

3.4. Egg Hatchability and Adult 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 ± 69.0, control; 723.0 ± 66.0). Likewise, adult emergence was significantly affected by the treatment ($\chi^2 = 13.75$, df = 1, $p = 0.0002$). The proportion of adults that emerged from pupae was significantly higher

after their larval stages were fed on untreated maize leaves (80.0 ± 0.0%) than it was when they were fed on rabbit urine-treated leaves (66.7 ± 13.3%).

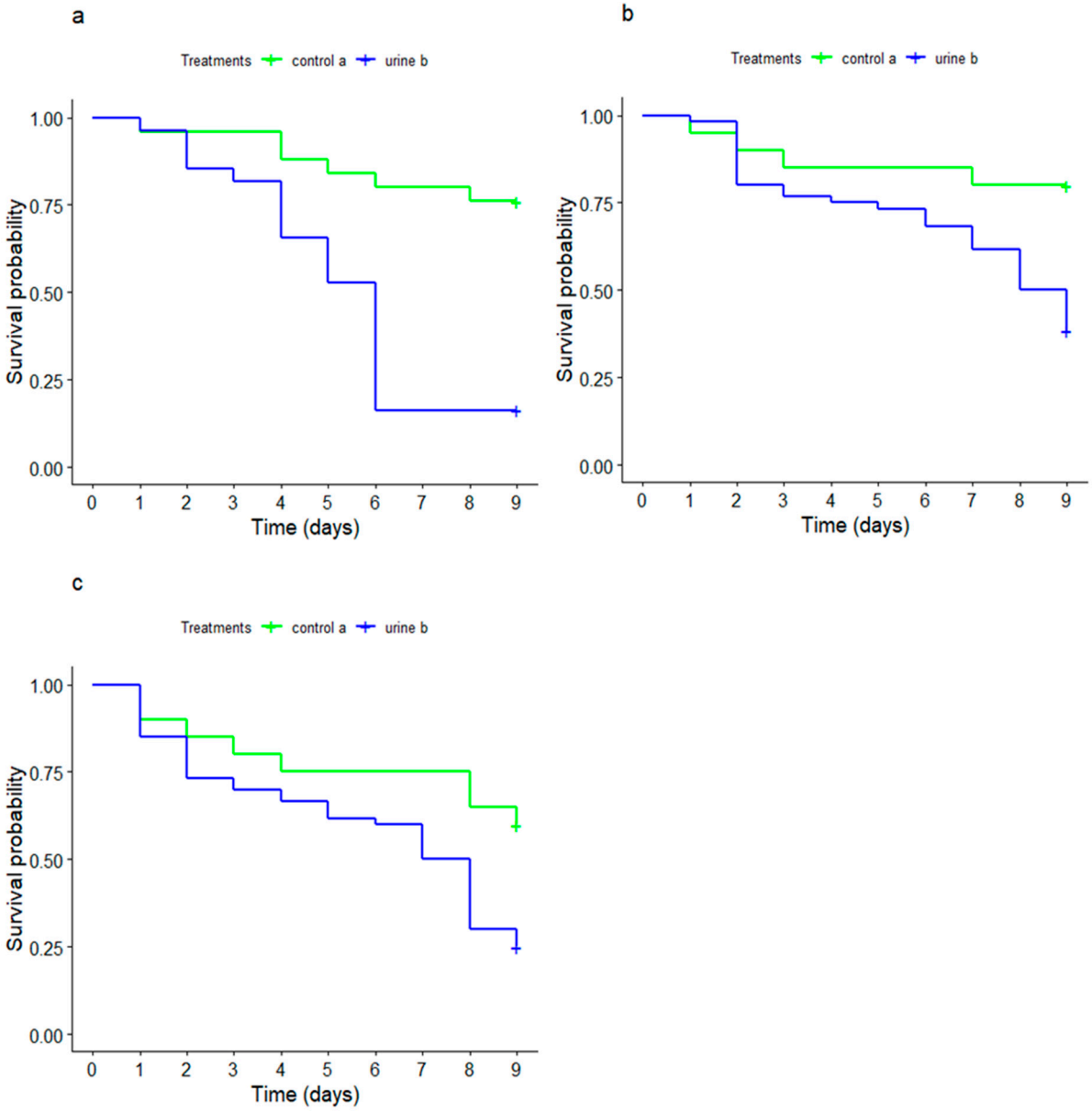

Figure 6. Kaplan–Meier survival distribution curves of the fall armyworm first instar larvae (**a**), second instar larvae (**b**) and third instar larvae (**c**) exposed to rabbit urine or distilled water (control). "+" indicates right censorship. Small letters after the legends indicate a significant difference between the control and rabbit urine treatments.

3.5. Oviposition Preference of the Adult Female Moth

The ability of the female FAW moth to lay eggs varied significantly between oviposition substrates in the no-choice experiment (χ^2 = 174.53, df = 1, $p < 0.001$) and two-choice experiment (χ^2 = 5272.90, df = 1, $p < 0.001$). The female FAW moth laid more eggs on the treated maize leaves in both behavioral experiments (Figure 7).

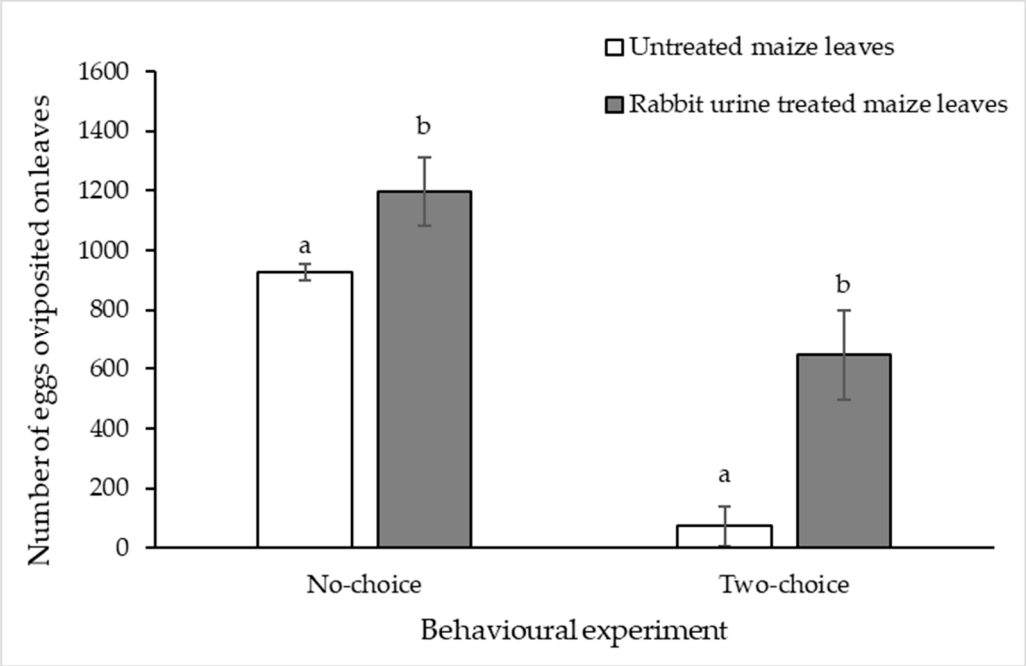

Figure 7. Mean number of eggs oviposited by the gravid female FAW moth on maize leaves treated with rabbit urine and distilled water in no-choice and two-choice behavioral experiments. Error bars represent standard errors. Different small letters above the error bars represent a significant difference between treatments at $p = 0.05$, according to the Tukey test.

4. Discussion

Indigenous knowledge is a set of practices and skills acquired by local people through the accumulation of experience, informal experiments and an understanding of their environment [39]. Since the arrival of the FAW in Africa [1], its control has often been done through chemical control methods [8]. However, due to the unaffordability of chemical products and their adverse environmental effects, indigenous management options are also used by farmers. Among the traditional options used, various particles such as ash, limes, oils, soaps, soils and botanical extracts have been applied to control the arthropod pests [19,40]. Furthermore, cultural and mechanical methods are also used through intercropping or the removal of infested plants from the fields [18]. Attempts to use rabbit urine as a biopesticide to control the FAW have been tried in Eastern Africa (e.g., Uganda) [25]; however, elucidating the mechanism of action is needed for further scientific testing and documentation. Therefore, this study investigated the veracity of using rabbit urine as an indigenous pest management option to control the FAW, which is done by the smallholder farmers in Africa. The study showed that rabbit urine influences survival, behavior (host selection, settlement, feeding and egg-laying), egg hatchability and pupal emergence of the FAW.

The FAW feeds on over 186 host plant species, with cereals, especially maize, being the most preferred host [5]. FAW larvae damage host crops during their active growth stages and can entirely or partly destroy plants [41]. Our results show that treating maize plants with rabbit urine repels the larvae, reducing their feeding ability and, consequently, the damage they can inflict to maize plants.

All larval stages of the FAW are destructive [41]. 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 second and third instars of the FAW had high feeding rates compared to the first instars. However, spraying the maize leaves with rabbit urine variably reduced the damage caused by the different larval stages through feeding. Our results corroborate Coy et al. [42], who reported that the destruction of host plants by the FAW increases

concomitantly with larval stages. Slightly diluted rabbit urine (94%) was required to reduce the damage of the second instars by 50%, while diluted rabbit urine (48 and 55%) was required to reduce the damage of the first and third instars by 50%, respectively. The authors understand that the farmers rarely dilute rabbit urine before applying it to the crops to control FAW. Therefore, the diagnosis of feeding symptoms of the FAW can guide the rate of application of rabbit urine.

Compared to the control, the survival of larval instars feeding on rabbit urine-treated maize reduced significantly. The rabbit urine may contain specific compounds that account for FAW avoidance. Rabbit urine also contains other chemical compounds which may have a repellent or deleterious effect on FAW larvae. The robenidine hydrochloride [43] and ammonium excretion [44] are some of the most reported compounds.

Our study established that rabbit urine reduces the survivorship of the three tested larval stages of the FAW. However, the first instars are more susceptible, followed by the second instar and the third instar, which support the dose-response relationship. Rabbit urine has a pungent smell which may repel pests, rendering the treated plants unpalatable. The FAW larvae may have died because of reduced feeding. Previous studies have indicated that the susceptibility of FAW larvae to the insecticidal compounds may differ significantly with the larval stages. For instance, Ghidiu and Andaloro [45] found that the susceptibility of FAW larvae to the toxicity of insecticides (methomyl and fenvalerate) decreased as the age increased.

Interestingly, treating maize plants with rabbit urine significantly increased oviposition preference by the 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 the FAW female moth to the rabbit urine requires further investigation. Nevertheless, this finding can reinforce some agroecological systems, such as the 'push-pull' technology, by which the rabbit urine is sprayed on the trap plant to make it more attractive to the FAW female moth oviposition. Eggs would not suitably survive, or pupae would 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 the FAW, the attractiveness of *Brachiaria* grass to the gravid FAW moth is being further investigated.

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.) [46]. An investigation conducted by Mutai [47] indicated that the chemical composition of rabbit urine contained 1.05% nitrogen, 0.01% phosphorus, 0.85% potassium and 0.12% calcium. These chemical elements make the rabbit urine considered as an excellent fertilizer [23,24]. 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. We understand that, despite little research having been conducted on the effect of rabbit urine on plant growth and pest control, farmers in Eastern Africa are promoting its use as a foliar biofertilizer and as a biopesticide in organic farming to reduce their expenditure in using synthetic products.

5. Conclusions

The use of rabbit urine by the smallholder farmers in Africa is an affordable strategy to manage the FAW. Rabbit urine acts as a repellent by reducing larval feeding, leading to mortality. The FAW first instars 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 damage on leaves is identified. The results of this study could aid in developing optimized formulations after proper field scouting and the identification of signs and symptoms of the FAW before preparing an appropriate formulation of the rabbit urine. Female FAW oviposition preference could be used to optimize deterrent tactics such as push-pull technology. Using rabbit urine as an organic pesticide is beneficial since it also acts as a biofertilizer, and it only repels insects

that aid in pollination and does not kill them. Therefore, application before active blooming seasons is recommended. Our study warrants further chemical analysis and additional behavioral responses, and field experiments using rabbit urine in order for it to be adopted as an IPM package.

Author Contributions: Conceptualization, D.K.M., A.T., S.S., I.R., Y.T.B. and S.N.; Methodology, D.K., D.K.M. and S.N.; Validation, D.K.M., D.K. and S.N.; Formal Analysis, E.R.O.; Investigation, D.K.; Resources, S.N.; Data Curation, E.R.O. and S.N.; Writing—Original Draft Preparation, D.K. and E.R.O.; Writing—Review & Editing, D.K.M., D.K., E.R.O., A.T., G.H., I.R., Y.T.B., S.S. and S.N.; Visualization, E.R.O.; Supervision, S.N.; Project Administration, S.N.; Funding Acquisition, S.N. All authors have read and agreed to the published version of the manuscript.

Funding: The authors gratefully acknowledge the financial support for this research by USAID/OFDA and USAID/BHA through the project titled "Reinforcing and Expanding the Community-Based Fall Armyworm *Spodoptera frugiperda* (Smith) Monitoring, Forecasting for Early Warning and Timely Management to Protect Food Security and Improve Livelihoods of Vulnerable Communities—CBFAMFEW II" grant number 720FDA20IO00133 and the European Union (EU) funded project Integrated pest management strategy to counter the threat of invasive fall armyworm to food security and Eastern Africa (FAW-IPM) GRANT NUMBER FOOD/2018402-634. We also acknowledge support from the Swedish International Development Cooperation Agency (Sida); the Swiss Agency for Development and Cooperation (SDC); the Federal Democratic Republic of Ethiopia; and the Government of the Republic of Kenya. The views expressed herein do not necessarily reflect the official opinion of the donors.

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

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets generated during and/or analyzed during the current study and the R software custom codes for the data analysis are available from the corresponding author on reasonable request.

Acknowledgments: The authors are grateful for the technical support of the staff at the *icipe* Animal Rearing and Containment Unit (ARCU).

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses or interpretation of the data; in the writing of the manuscript; or in the decision to publish the results.

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