Contact toxicity, Electrophysiology, Anti-mating and Repellent effects of Piper guineense against Spodoptera frugiperda

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Research Article

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Abstract

The fall armyworm *Spodoptera frugiperda* (J. E. Smith) is a long-distance migratory pest, which invaded the African continent in 2016, causing enormous losses to agricultural crops, especially maize. Synthetic insecticides are primarily used in the management of *S. frugiperda*, but they leave residues on human food and animal feed and also cause environmental hazards. Assessing the toxicity of plant extracts on *S. frugiperda* may offer a more effective control which can reduce the excessive use of synthetic insecticides. We evaluated the crude ethanolic extract of *Piper guineense* fruits for contact toxicity on *S. frugiperda* larvae and determined the lethal concentration (LC_{50}) of the extract. Additionally, we conducted electrophysiological (EAG) experiment to determine the responses of male and female adult *S. frugiperda* to *P. guineense* extract. We also determined whether the extract influenced mating, oviposition and repellence to adult female *S. frugiperda.* We found that *P. guineense* extract caused significantly higher mortality to *S. frugiperda* larvae than an ethanol control. Electrophysiologically, we observed significantly higher responses to the extracts than control, with some variations in response between the sexes. When checking the repellent effect of *P. guineense* extract on adult *S. frugiperda* females in a wind tunnel, we found that females moved more towards the control than towards the extract. Taken together, our results confirm *P. guineense* extract as a potent extract that could be incorporated in the integrated management of *S. frugiperda.* Future research should explore the responses of *S. frugiperda* to *P. guineense* extract on a field scale.

Key message

We studied the effect of *Piper guineense* extract on *Spodoptera frugiperda larvae and adult*

A positive relationship was found between *P. guineense* extract concentration and *S. frugiperda mortality*

We observed a sex difference in EAG response of adult *S. frugiperda* to *P. guineense* extract

P. guineense elicited repellent effect on adult female *S. frugiperda* at close distance.

Our findings highlight potential application of *P. guineense* extract for control of *S. frugiperda*

Introduction

The fall armyworm (*Spodoptera frugiperda* J.E. Smith) (FAW) is a long-distance migratory pest, with adult moths capable of flying over 100 km in a single night (Johnson, 1987), that invaded the African continent in 2016 (Georgen et al. 2016), causing enormous losses to agricultural crops. It is now found throughout sub-Sahara Africa (Nagoshi et al. 2022), Asia (Guo et al. 2018, Nagoshi et al. 2020), and Australia (Maino et al. 2021), causing significant damages to many important crops like grass species and cereals, especially maize (Early et al. 2018). As maize is a primary staple food crop in Africa, recent invasion of *S. frugiperda* threatens food security of millions of people.

In the management of *S. frugiperda*, synthetic insecticides belonging to the organophosphates, carbamates and pyrethroids are primarily used by farmers in Africa (Kumela et al. 2019; Tambo et al. 2019). To mitigate the impact of *S. frugiperda* in Africa, governments have subsidized the use of synthetic insecticides as emergency control programs (Rwomushana et al. 2018; Makgoba et al. 2021). However, indiscriminate use of these chemicals has led to accumulation of chemical residues in food (Akeme et al. 2021; Van den Berg and Du Plessi. 2022). In addition, *S. frugiperda* is highly adaptable and well known to evolve resistance against synthetic pesticides (Huang et al. 2014; Santos-Amaya et al. 2015), which threatens the sustained use of pesticides.

A sustainable and efficient management of *S. frugiperda* should include an approach that is eco-friendly, without further harm to the environment. Incidentally, the use of plant parts and their extracts been investigated as one of many potential low-cost control options that build on local knowledge and ecological principle (Pavela 2016; Khan et al. 2017). In addition, since crop production is mostly done by many smallholders who lack financial resources to purchase chemical pesticides (Wyckhuys and O'Neil 2010), there is a need to explore more biodegradable, less toxic and more eco-friendly products from tropical plants to control *S. frugiperda.*

Some plant-based insecticides have already been found to cause mortality on *S. frugiperda* (Roel and Vendramim 2006; Silva et al. 2015; Sisay et al. 2019; Rioba and Stevenson 2020; Phambala et al. 2020; Kona et al. 2021) and several have been recommended for field trials and bioassays (Bateman et al. 2018; Kumar et al. 2022). Generally, plants extracts contain specific phytochemicals such as alkaloids, flavonoids, saponins, terpenoids, phenolic compounds and quinones, which have the potential of showing acute toxicity, repellency, antifeedant and growth regulation against many agricultural pests (Isman and Akhtar 2007; Henegamade et al. 2023). *Piper* species are among the plants whose insecticidal properties have been explored as a preventative against many insect pests, including *S. frugiperda* (Soberón-Risco et al. 2012; Alves et al. 2014; Celis et al. 2014; Tanyi et al. 2020; Kona et al. 2021; Lina et al. 2023; Amadi et al. 2024). Application of extracts of *P. el-bancoanum* and *P. arboreum* at different dosages resulted in over 80% mortality rate of *S. frugiperda* larvae similar to the application of *Bacillus thuringiensis* and synthetic chemical, Chlorpyrifos (Celis et al. 2014).

Even though the effects of *Piper* extracts on *S. frugiperda* larvae have been documented, so far it is not clear what the effects are on all life stages, including adult behavior. To develop an integrated pest management approach against *S. fugiperda*, we evaluated the effects of crude extract of *P. guineense* fruits not only on larvae, but also on mating and oviposition behavior, as well as their potential repellent effects on adult female *S. frugiperda.* Additionally, we conducted an electrophysiological experiment to determine physiological responses of male and female *S. frugiperda* adults when exposed to *P. guineense* extracts.

Materials and methods

Insects

Populations of *S. frugiperda* from Kenya (SFK) and Nigeria (SFN) were used for this study. The SFK was collected from the lab population of the International Centre of Insect Physiology and Ecology (icipe), Kenya. The lab population was established with specimens collected from several areas in Kenya at different time points. At icipe, the population was kept at a temperature of 25 ± 2°C, 70% ± 5% relative humidity and a 12:12 h (light: darkness) photoperiod and on an artificial pinto bean diet. Samples of this laboratory population were received at the University of Amsterdam (UvA) in December 2020 and August 2022. The SFN was collected from maize fields in Southern Oyo State and from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, between January 2022 and May 2022 and was received at UvA in October 2022. At UvA, immature stages (eggs and larvae) of both populations were reared on an artificial pinto bean diet in climate chambers set at 25°C and humidity of 60–65%, with reversed light/dark cycle (lights off at 12.00, on at 22.00) and 14:10 light/dark photoperiod. Adults were continuously fed with 10% sugar water, ad libitum.

Insecticidal material and preparation of extract concentrations

Fruit extract of *Piper guineense* Schum and Thonn (family Piperaceae) was used in this study. Fresh fruits of the plant were purchased from a local market in Kajola, Osun State, Nigeria (latitude 7° 31' 0.01" and longitude 4° 30' 0"; terrain elevation of 257 m above the sea level) in August 2021. Five hundred grams (500 g) of the fruits were air-dried and grounded. The sample was extracted with ethanol (95%) in a Soxhlet apparatus and two hundred grams (200 g) of the crude extract was concentrated *in vacuo* on a rotary evaporator at 40°C, under reduced pressure and stored. Sample was transfered to UvA in October 2022 and was further stored in -20˚C until the time of bioassays. To produce varying concentrations of the extract, 30 mL of the crude extract was dissolved in 70 mL of ethanol to a concentration of 30% (vol/vol) stock solution. Stock solution was further diluted into concentrations of 15%, 3%, 1.5%, 0.3% (v/v). These concentrations, including the stock, were used for bioassays against *S. frugiperda*, while ethanol only, i.e., 0% (v/v) was used as a negative control.

Effects of extracts on larvae and larval development

To assess the toxicity of plant extracts on larvae, third instar larvae were individually placed in a small cup (4 cm ×3 cm; 40 mL) and fed on artificial diet as above. Contact toxicity was performed by pipetting aliquots (10 µL) of each concentration of *P. guineense* extract and applying the extracts to the bodies of the larvae. The cups were then covered with a lid. Each concentration was used to treat 20 *S. frugiperda* larvae of each population (SFN and SFK). Larval mortality was recorded one day, two days and seven days post treatments by counting the number of dead larvae. For each day, the median lethal concentration (LC_{50}) of the extract concentrations were determined. The larvae were considered dead if they displayed no observable response to a mechanical stimulus, i.e., short-term pressure applied with a camel hairbrush. Surviving larvae in each vial were followed through pupation. Emerging pupae were checked for survival and weighed daily.

Electrophysiogical responses in adults

To measure antennal responses to *P. guineense* extract at concentrations of 0.3%, 1.5%, 3.0% and 15% v/v, electroantennogram (EAG) experiments of 0–4 day old virgin males and females were performed with both populations (SFN and SFK). The procedures described in Akinbuluma et al (2024) were slightly modified and used for the EAG set up. Briefly, live male and female insects were individually placed in a plastic pipette tip and one antenna was immobilized with a small strip of parafilm pressing the antennal base against the head. Silver wires nserted in glass microelectrodes (GC150TF-10; Warner Instruments, Hamzen, CT, USA) with insect Ringer's solution were used to make electrical contact. The reference electrode made contact with the cut antennal tip, while the recording electrode was inserted at the base of the antenna. The amplitude of the EAG was measured using an IDAC-4 amplifier equipped with a high impedance (> 10^9 Ohm) head stage and recorded with GC-EAD/2014 software (Syntech, Kirchzarten, Germany).

Ten µl of each concentration were pipetted onto a small piece of filter paper (1 cm^2) and placed into a glass Pasteur pipette. Each trial included two control treatments, with the pipettes either containing filter paper with 10 µL ethanol alone or completely empty. Additionally, we used the multicomponent blends (MCBs) of *S. frugiperda* sex pheromone containing five identified sex compounds, as a positive control. A stimulus controller (CS-55, Syntech) produced a flow of 1 l/min charcoal-filtered air over the antennae. Antennae were stimulated with 0.5 s odor puffs, using a randomized sequence for each recording. The control stimuli were presented in between runs of the different concentrations. The inter-stimulus interval was 60 s. Signals were recorded with Syntech software (Autospike Version 3.9).

Mating and oviposition behaviour in presence of extract

To determine whether *P. guineense* extract influenced mating in adult *S. frugiperda*, single pair matings of SFK and SFN populations were set up. Virgin adults (1–4 day-old) were set up inn clear plastic cups (17oz.) containing a small a sauce cup (4 cm ×3 cm; 40 mL) filled with cotton wool soaked in a 10% sugar solution. Matings were set up in a climate chamber (26°C, 70% RH, L:D 14:10) at least two hours before scotophase, period of darkness in a 24-hour light-dark cycle, see Yuan et al. (2023). Within each mating cup, 10 µL of either 0.3%, 1.5%, 3.0% or 15% *P. guineense* concentrations were added to a small strip of filter paper (2 cm), which was covered with muslin cloth. For the controls, two mating cups received 10 µL of ethanol only, which we refer to as 0.0% (v/v) *P. guineense* concentration, and two mating cups did not receive any paper strip (empty control). Each SFK and SFN population thus comprised six treatments, which were replicated four times and arranged in a completely randomized design. Couples were observed throughout the ten hours of scotophase, with 30 min intervals, for three consecutive nights after setup. Furthermore, oviposition by *S. frugiperda* females was recorded a day during the observation period by counting the number of egg masses in each cup.

Female behavioral responses to extracts

To determine behavioral responses of adult *S. frugiperda* females to *P. guineense* extract, we conducted a wind tunnel assay, using the procedures described in Verschut et al. (2019), with some modifications. Ten µL of the extract (test solution) at concentration of 1.5% (v/v) and ethanol (control) were individually applied to a piece of filter paper, placed inside lure holders and hung 40 cm apart on a retort stand placed at 50 cm from the upwind end of a wind tunnel (200 cm long × 100 cm wide × 100 cm high plexiglass flight section). The wind tunnel was lit from above by red LED strips and the experiment was conducted under controlled environmental conditions (27°C, 22% RH) and wind speed of 0.24 m/s (using an anemometer). Insects were allowed to acclimatize to the wind tunnel room conditions for 1 h before testing.

Twenty 2-day old adult *S. frugiperda* females of each population were individually introduced through a side-panel at the downwind end of the wind tunnel (130 cm from the odour release point). For each insect taking flight and first landing within a 12 min period, the distance from the source (control or test) was recorded in distance classes: 0–5 cm, 5–10 cm, 10–15 cm, 15–20 cm and 50 cm (for any choice farther away than 50cm). Odor sources were swapped after testing 4 insects and the bioassay was conducted between 15.00 and 23.00.

Statistical analyses

All analyses were performed in R (version 4.3.2, R Development Core Team, 2020). Larval mortality was analysed using a generalized linear mixed model (GLMM), whereby insect mortality was set as the response variable, while population, concentration, day and their interactions were set as fixed variables. Replicate was added as a random effect. LC_{50} values were calculated for each day with dose response model (DRM) from the dose response curve (DRC) package (Ritz et al. 2015) and the differences were compared using the compParm and confint functions from the same package. Differences in the number of pupae and pupal weight between control and treatments were analysed with GLMs. For pupation (yes or no) a binomial distribution was used and a Gaussian distribution for pupal weight. In both models, concentration and population were the explanatory variables. Posthoc testing was done with emmeans (Lenth 2024) and Tukey correction for multiple comparisons.

The electrophysiology data was analysed with a linear mixed model (LME), the response variable was amplitude which was square root transformed to meet the assumptions of homoscedasticity and normality. The fixed variables were population, exposure, sex and their interactions and replicate was included as a random effect. To analyse the impact on mating probability, we used a GLMM with a binomial distribution. Population and exposure were fixed variables and replicate set as a random effect.

In the case of length of mating time, we used a LME and set mating length as the response variable, population, treatment and time period as the fixed variables and again replicate was the random effect. However, the variance and standard deviation for the fixed effect in the model were both zero. Therefore, the random effect was removed and a linear model was used instead. The response variable and fixed variables remained the same.

To assess the impacts of extracts on oviposition, a GLMM with a poisson distribution was used. Cumulative oviposition was used as the response variable and population, exposure and their interactions as the fixed variables. Replicate was included as a random effect.

For the behavioral responses of females in the wind tunnel experiment, the final dataset contained in total 32 insects from 2 populations (SFK and SFN), as 8 females did not respond within the time frame of 12 min, collected over two measuring days (day 1or day 2). To determine if population, day, time in scotophase and distance from source influenced female choices, a binomial GLM was constructed, with the chosen first landing side as response. We tested the zero hypothesis of equal probability of choice for treatment and control side with the binomial test and the effect of distance to source on side.

In all cases, a model simplification procedure was followed. The full model was fitted, after which the least significant term (from the three-way interaction downwards) was removed if the removal resulted in an insignificant increase in deviance. Significance of simplified models was assessed by performing a likelihood ratio test. The model simplification process was repeated until the model contained only significant terms (level $p < 0.05$). To check for overdispersion and nonuniformity of the residuals in the fitted models we used the Residual Diagnostics for HierARchical Models (DHARMa) package (version 0.4.6; Hartig 2022). Our final models had no significant overdispersion or nonuniformity of the residuals.

Results

Larval responses to extract

Topical application of *P. guinense* to *S. frugiperda* larvae showed a strong concentration- response for all the extract concentrations across days during the trials (Fig. 1). We found a significant effect of the extract concentrations ($df = 338$, $p = 0.000$) and day ($df = 338$, $p = 0.001$), but no significant effect of population or any interaction effect. The highest mortality (100%) of *S. frugiperda* larvae was observed with 30% (v/v) of *P. guinense* extract. Except with 0.3% (v/v) concentration of *P. guineense*, mortality of *S. frugiperda* larvae in other treatments was significantly different ($p < 0.05$) from the mortality in the control. The LC_{50} values of *P. guineense* (ranging between 0.86% and 3.46%) were not significantly different between day 1, 2 and 7 (Table S1a and S1b). Percentage pupation was significantly higher in the ethanol control than in the *P. guineense* treatments and none of the larvae made it to pupation at concentration ≥ 15% (v/v). Also, the pupae in the control treatment weighed significantly more than pupae from the *P. guineense* extract-treated larvae ($p < 0.05$).

Electrophysiology

In assessing the electrophysiological responses, the multicomponent (MCB) control only had a few samples for females of both populations ($n = 2$ for SFK and $n = 3$ for SFN). Given this low sample size, responses to MCB were excluded from the analysis but included in the figure for visual comparison with other concentrations used. We found that the response of *S. frugiperda* from both populations and sexes

differed when exposed to *P. guineense* extract concentrations, an increase in extract concentration of *P. guineense* led to an increase in EAG response in both populations and both sexes. We found a significant interaction effect between population, sex and the extract concentration (LME Population:Exposure:Sex; estimate = 0.51 ± 0.17se, d.f. = 163.78, t-value = 2.98, p = 0.003) (Fig. 2). Males of the two populations differed in their response to an exposure of 0.3% (v/v) (pairs contrast: estimate = 0.34 ± 0.08, d.f. = 169, t-ratio = 4.27, p < 0.0001) and 1.5% (v/v) concentration (pairs contrast: estimate = 0.22 ± 0.08, d.f. = 169, t-ratio = 2.74, p = 0.007) (Fig. 2, horizontal red lines underneath the bars). When comparing the response between sexes within each population, in the SFK population females and males differed in their EAG response at the 0% (v/v) extract concentration (pairs contrast: estimate = 0.22 ± 0.08, d.f. = 169, t-ratio = 2.80, p = 0.006), with males having a higher response than females. In the SFN population, the sexes differed in their EAG response at the 0.3% (v/v) extract concentration (pairs contrast: estimate = 0.26 ± 0.09, d.f. = 165, t-ratio = 2.77, p = 0.006), with males having a higher response than females, and the 15% (v/v) extract concentration (pairs contrast: estimate = -0.20 ± 0.09, d.f. = 165, t-ratio = -2.20, p = 0.029), with females having a higher response than males (Fig. 2, vertical red lines).

Effects of extracts on mating and oviposition

Mating probability was similar in all treatments (Fig. S1). Model results indicated a significant declining trend in mating probability when comparing an exposure concentration of 15% (v/v) with the empty control, where an exposure concentration of 15% (v/v) had a significantly lower probability of mating than the empty control (Estimate = 3.21 ± 1.40se, z-value = -2.29, p-value = 0.02). However, after Tukey correction for multiple comparisons, this difference was no longer significant (estimate = 3.21 ± 1.40 se, z-ratio = 2.29, p-value = 0.199). Mating time (duration) varied across exposure concentrations and time periods (Fig. 3), but was not significantly affected by population (anova: d.f. = 1, F = 0.002, p = 0.968), exposure concentration (anova: d.f. = 5, F = 1.42, p = 0.26) or time period (anova: d.f. = 3, F = 1.20, p = 0.33). There was a significant interaction between population and extract concentration on oviposition (GLMM: estimate = -2.43 ± 0.83se, z-value. = -2.93, p = 0.003). The SFK population did not show any difference in oviposition with increasing extract concentration (Fig. 4, Table S2). However, in the SFN population we found reduced oviposition at the 0.3% (estimate = 3.43 ± 1.02se, z-ratio = 3.38, p-value = 0.03), 1.5% (estimate = 2.74 ± 0.73se, z-ratio = 3.76, p-value = 0.009) and 15% (estimate = 2.34 ± 0.60se, z-ratio = 3.87, p-value = 0.006) extract concentrations when compared to the empty control (Fig. 4, Table S2).

Behavioral response of females to extracts

In the wind tunnel experiment, we found no difference in response between SFK and SFN females (d.f = 28, p = 0.127), or between the time of measurements within the scotophase (df = 28, p = 0.923). However, significantly lower number of *S. frugiperda* females chose the *P. guineense* odour source over the control odour source at the measured distances (p = 0.019) (Fig. 5). Females that landed closer towards the odour sources (0–5 or 5–10 cm) landed more often towards the control odour than towards the plant

odour source (GLM, p = 0.008, df = 30), while females that landed at further distance from the source were less repelled.

Discussion

Many tropical plants produce secondary compounds to protect themselves against herbivores and pathogens and some have been used for pest management (Pino et al. 2013; Pavela, 2016; Khan et al. 2017; Bhosle et al. 2025). Apart from causing death, botanical insecticides can disrupt the biological activity of insect pests, including suppression of feeding activity, reduction of pupal weights, prolonging developmental time and influencing mating and oviposition, and their odours can have repellent effects (Isman 2006; Widayani et al. 2023; Oliveira et al. 2024; Shai et al. 2024). In this study, we tested the effect of *P. guineense* extracts on different life stages of *S. frugiperda*, because this is a promising pesticidal plant species to manage this pest.

Contact toxicity, pupation and pupal weight

The response of *S. frugiperda* larvae to the topical applications of *P. guineense* extracts showed a positive relationship between concentration, time of exposure and mortality. This agrees with the results of Lina et al. (2023) who reported that an increase in the larval mortality of *S. frugiperda* was proportional to the increase in the testing concentration. As *Piper guineense* extract caused up to 100% mortality at the 30% (v/v) of the extract after 2 days exposure, the high larvicidal effect of this plant is obvious. In general, botanical pesticides cause 80% mortality under laboratory conditions (Tavares et al. 2010). In Ethiopia, *Nicotiana tabacum* caused only 50% mortality after 72 h exposure to third instar *S. frugiperda* larvae (Sisay et al. 2019), a value which is considerably lower than the mortality observed in our bioassay. Neem extracts (Silva et al. 2015; Babendreier et al. 2020) and extracts from *Eucalyptus urograndis* (Hruska et al. 2019) were also very effective in causing mortality of *S. frugiperda.* It is likely that the mortality caused by the extract of *P. guineense* is related to the secondary metabolites present in the extract. Earlier phytochemical and chromatographic analyses revealed the presence of a range of secondary metabolites in *P. guineense* solvent fractions, including phenolic compounds, alkaloids and terpenoids (Paula et al. 2000; Akinbuluma et al. 2017).

The fact that we found high levels of mortality already within one day of exposure to the plant extract (not significantly different from other days of observation) suggest a rapid toxic action on the insects, which is in line with other reports (Batista-Pereira et al. 2006; Soberón-Risco et al. 2012; Lina et al. 2023; Widayani et al. 2023).

The plant extracts also resulted in significantly lower percentages of pupation and lighter pupae in the insects that survived, which can thus add to its effectivity. When testing the potential of three botanicals, including *Piper aduncum* on *S. frugiperda, P. aduncum* did not only prolong larval development but also caused lower pupal weight than those in the control (Widayani et al. 2023). Thus, *P. guineense* extract can disrupt the physiological processes of *S. frugiperda* larvae.

Electrophysiological responses to plant extract

Overall, an increase in extract concentration of *P. guineense* led to an increase in EAG response in both Kenyan and Nigerian populations of *S. frugiperda*, revealing that antennal responses to the extract was concentration-dependent. That adult male and female *S. frugiperda* responded differently to the highest concentration of *P. guineense* signifies a sex difference in electrophysiological response of the insect. To our knowledge, this is the first report on the EAG responses of male and female *S. frugiperda* to *P. guineense* extract.

Although included in the MCB of synthetic sex pheromone blends for only visual comparison, we observed that, relative to the extract concentrations, the MCB produced very high EAGs on male *S. frugiperda* from both SFK and SFN populations.

Several authors have reported that sex pheromone compounds released by female *S. frugiperda* elicited responses to conspecific males and have been used to capture the latter (Howse et al. 1998; Malo et al. 2001, 2018; Koffi et al. 2021). However, only little EAG responses were observed with the female populations, agreeing with earlier evidence that females of certain Lepidoptera species (Grant 1970; Yang et al. 2009) including *S. frugiperda* (Malo et al. 2004; Cruz-Díaz et al. 2022) can autodetect their sex pheromone. More studies involving this autodection of *S. frugiperda* to their sex pheromones are needed.

Effects of plant extracts on mating and oviposition

As we found no significant differences in the mating duration between mating couples with *P. guineense* extract and untreated controls showed that the extract did not disrupt matings at the concentrations tested. This finding is in contrast to Sammani et al (2020), who reported a greater percentage of successful mating in *Cadra cautella* (Lepidoptera: Pyralidae) when exposed to camphor oil, citronella oil and neem oil. Plant extracts may thus boost mating success among moths or have no effect on mating.

We found that oviposition of *S. frugiperda* Nigerian population was influenced by *P. guineense* extract, as fewer egg batches were laid at higher concentrations. Previous studies also found that *Piper hispidinervum* C. affected spermatogenesis and egg laying in *S. frugiperda* (Alves et al. 2012; Rioba and Stevenson 2020). However, we did not observe any differences in egg batches in the Kenya population. In the Indian meal moth *Plodia interpunctella* (Hübner), botanical extracts from olive, sunflower and corn did not reduce oviposition but enhanced it on several food hosts (Nansen and Phillips 2003), suggesting that different populations may behave differently to different plant extracts. More research is needed to test the effect of extracts of different plants on different populations of *S. frugiperda.*

Behavioral responses of females to plant extract

In the wind tunnel bioassay, we found that *S. frugiperda* females landed more in the vicinity of the ethanol control than the *P. guineense* odor source. Previously, a repellent effect of *Piper* extracts was also found on *S. frugiperda* larvae (Celis et al. 2014; Amadi et al. 2024). In addition, *Piper* extract repelled

coleopterous insect pests in storage, such as the banana weevil, *Cosmopolites sordidus* (Germar) (Inyang and Emosairue 2005) and the maize weevil, *Sitophilus zeamais* (Akinbuluma et al. 2017). However, our observation that at further distances from the odour source the repellent effect of *P. guineense* disappeared, suggests that repellence only works at short distances. Many activities of extracts from *Piper* species, including its repellent effect, are related to the secondary metabolites in the extract, including alkaloids, triterpenoids, tannins, coumarins, and terpene lactones (Gutierrez et al. 2013; Salehi et al. 2019). Among the several secondary metabolites in plants, alkaloids have been reported to largely affect the nervous system, regulate growth, and act as a repellent against *S. frugiperda* larvae (Celis et al. 2008; Gutierrez et al. 2013; Celis et al. 2014). Therefore, repellent effect of *P. guineense* in this study may largely be due to the numerous alkaloids (Akinbuluma et al. 2021).

Conclusion

Our study revealed that the extract of *Piper guineense* was toxic to *Spodoptera frugiperda* in a concentration-dependent way. Specifically, the extract showed larvicidal activity against *S. frugiperda*, produced high electrophysiological responses in both adult males and females, and was repellent to females at close distance. Future research should determine the exact mode of action(s) of the extract against the insect pest as well as explore the responses of *S. frugiperda* to *P. guineense* extract on a field scale.

Abbreviations

EAG Electroantennogram

LC_{50} Lethal concentration

SFK *Spodoptera frugiperda* from Kenya

SFN *Spodoptera frugiperda* from Nigeria

ICIPE International Centre of Insect Physiology and Ecology

IITA International Institute of Tropical Agriculture

UvA University of Amsterdam

DHARMa Residual Diagnostics for HierARchical Models

MCB Multicomponent blend

RH Relative Humidity

L:D Light: Dark

DRM Dose response model

DRC Dose response curve

LME Linear mixed model

GLMM Generalized linear mixed model

mV millivolt

µL microlitre

mL millilitre

Declarations

Ethical Approval and consent to participate: Not applicable

Consent for publication: Not applicable

Availability of data and material: The authors confirm that data supporting the findings of this study are available from the corresponding author on request.

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Authors' contributions: MDA and ATG planned, conceived and prepared the study. MDA, JAD and PR analyzed data. All authors wrote, edited and approved the final version of the manuscript.

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Figures

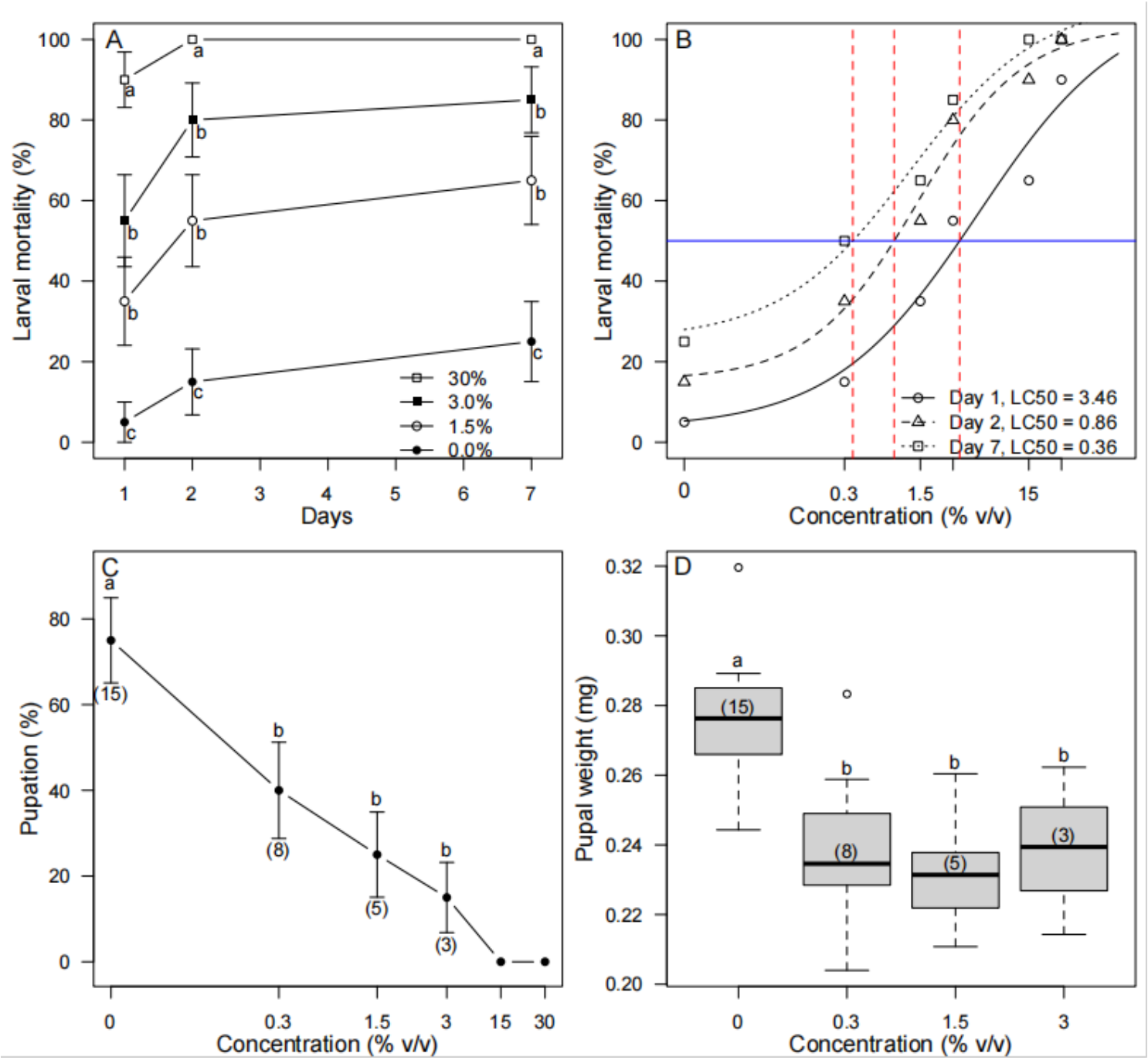

Figure 1

Effect of contact toxicity of *Piper guineense* on *Spodoptera frugiperda* larvae and pupae. *A. Effects of different concentrations of P. guineense* extract on *S. frugiperda* larvae mortality after one, two and seven days of exposure (0.0% – solid circle; 1.5% – open circle; 3.0% – solid square; 30% – open square). For visual clarity, concentrations 0.3% v/v and 15% v/v were omitted from the graph, because the concentrations were not significantly different from the control (0.0%) or the highest concentration

(30%), respectively. Comparisons were made between concentrations for each day (n=20). B. Log-linear concentration response curves for the three days of observation (Day 1 – open circle and solid line; Day 2 - open triangle and dashed line; Day 3 – open square and dotted line). The LC_{50} is indicated by the red line. C. Percentage pupation (i.e number of surviving pupae) of *S. frugiperda* larvae at different concentrations of the extract (number of surviving insects in each concentration is presented in parenthesis under the error bars). D. Weights of *S. frugiperda pupae after exposure of larvae to concentrations of P. guineense* extract. The number of surviving insects in each concentration is given in parenthesis within each box. In A, C and D, different letters indicate significant differences across treatments ($p < 0.05$).

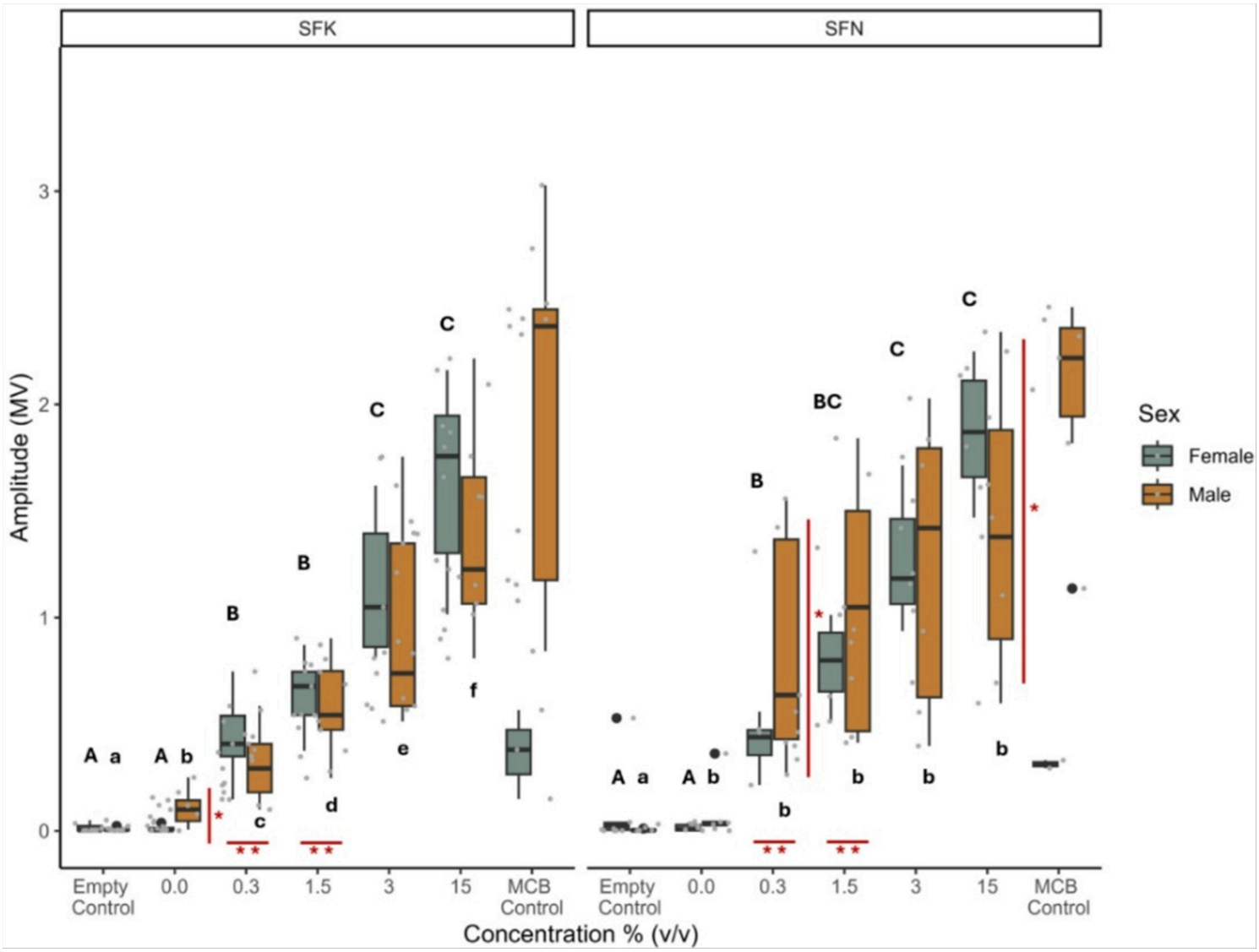

Figure 2

Electrophysiological responses of *Spodoptera frugiperda* females and males from Kenya (SFK) and Nigeria (SFN) to *Piper guineense* extracts. Box and whisker plots of electroantennography (EAG) responses (amplitude in millivolt (mV)) of the SFK and SFN population and both sexes. Left panel represents the SFK population and the right panel the SFN population with light grey indicating responses of females and orange the male responses. Raw data are indicated by grey points. Extract

concentrations of *P. guineense* are in percentage volume to volume (% (v/v)). The empty control represents a negative control. The synthetic multicomponent blend (MCB) containing five female sex pheromone compounds of *S. frugiperda* was included as a visual comparison only and not included in the analysis due to low sample size. Differences in capital letters denote significant differences between exposure treatments for females; differences in small letters denote significant differences between treatments for males. Red vertical line with a single star, to the right of a box plot, indicates significant differences between males and females at the given exposure treatment. Red horizontal line with two stars, below box plots, indicates significant differences between populations. Significant level was set at $p < 0.05$. Sample sizes varied between populations and sexes: SFK – Female: empty control and all concentrations (0.0% (v/v) - 15%(v/v)): n = 6, MCB control n = 2; Male: empty control, all concentrations and MCB control: n = 11. SFN – Female: empty control and all concentrations: n = 7, MCB control, n= 3; Male: empty control, all concentrations and MCB control: n = 7).

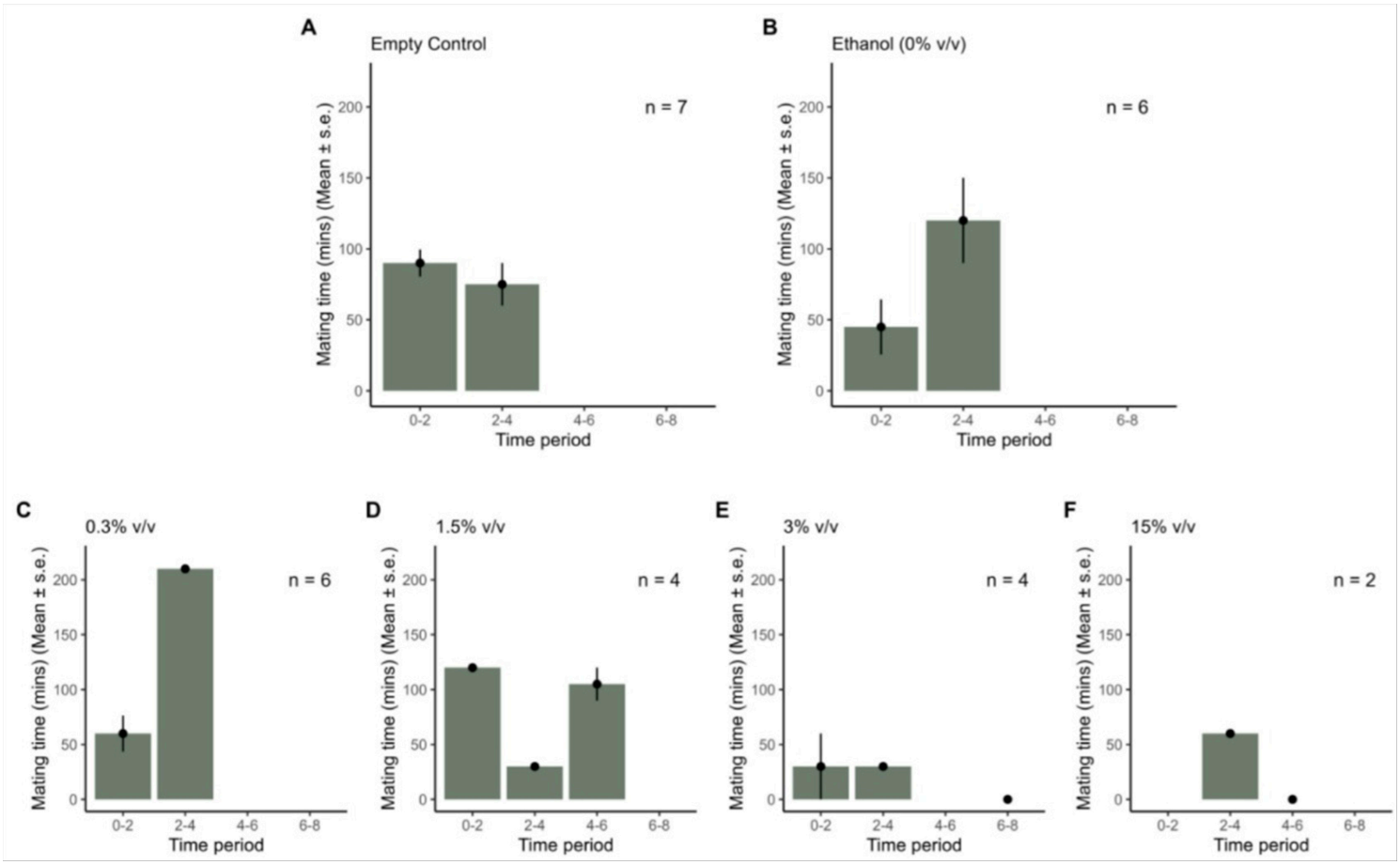

Figure 3

Duration of mating of *Spodoptera frugiperda* as influenced by exposure from *Piper guineense* extract within ten hours. (A) Empty control containing paper strip only; (B) paper strip exposed to ethanol (equivalent to 0% (v/v)); (C-F) paper strips treated with *P. guineense* extract at concentrations of 0.3%, 1.5%, 3% and 15% (v/v). There was no significant difference between treatments or time periods

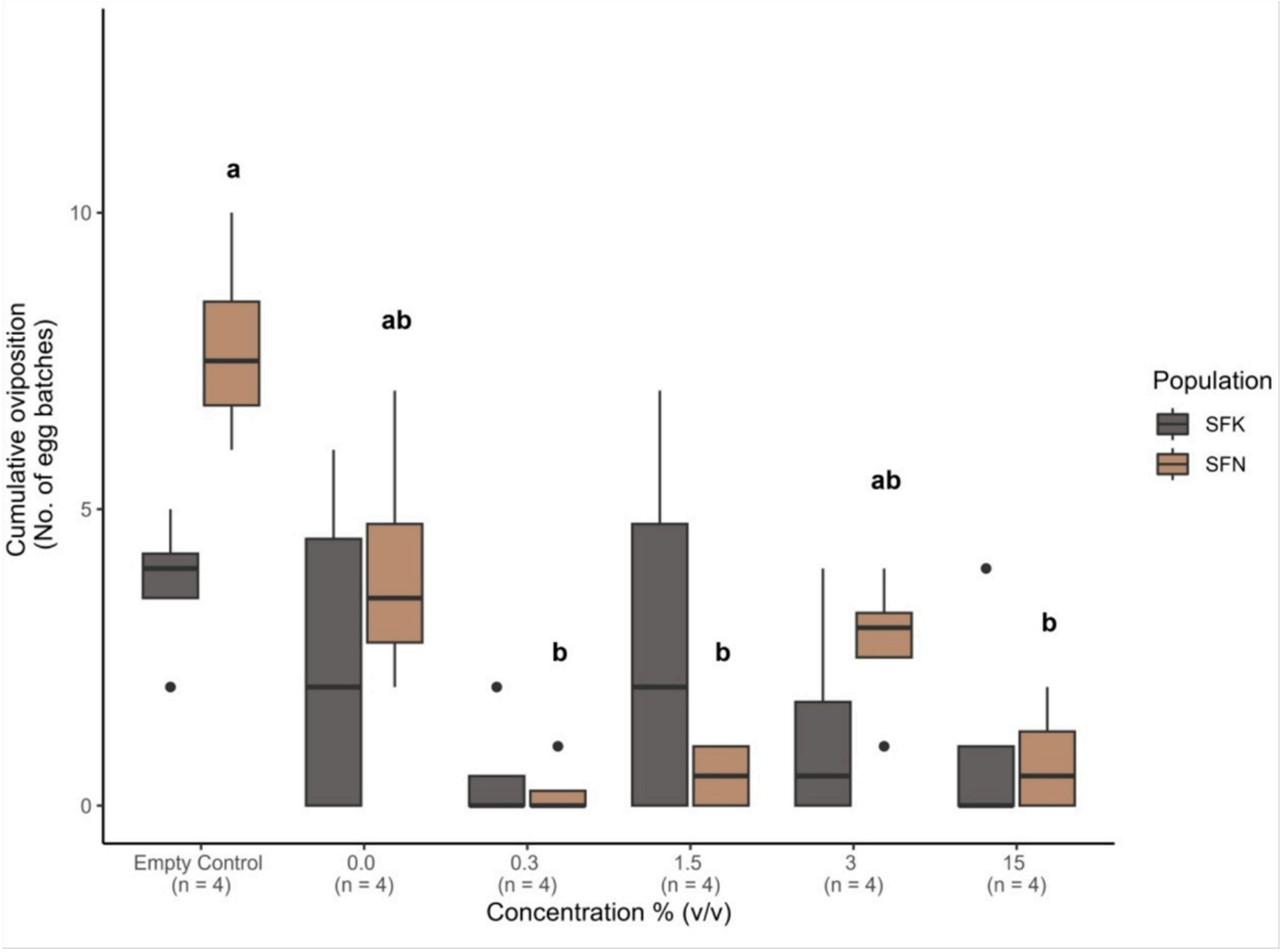

Figure 4

Effect of *Piper guineense* extract concentrations on *Spodoptera frugiperda* oviposition. Box and whisker plots of the number of egg batches of females exposed to *P. guineense* extract at concentrations of 0.3%, 1.5%, 3% and 15% v/v. Dark grey boxes represent females from the Kenya population (SFK) and dark cream boxes represent females from the Nigeria population (SFN). Different small letters within the graph represent significant differences ($p < 0.05$) between treatments in the SFN population. The SFK population showed no significant difference between exposure treatments.

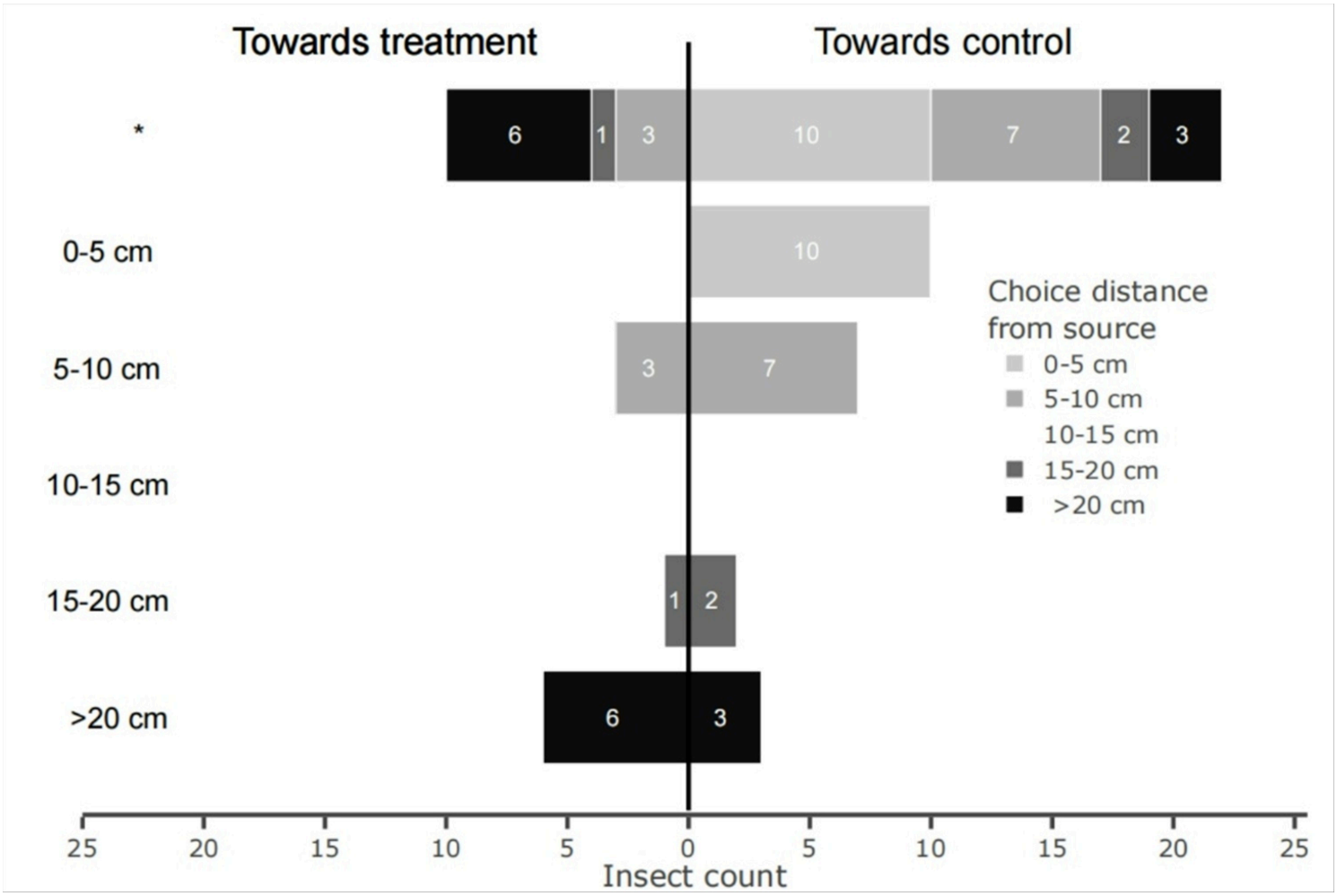

Figure 5

Repellent effect of *Piper guineense* extract on adult female *Spodoptera frugiperda*. First landing responses (towards treatment or control) of adult *S. frugiperda* females (n=32) by the distance to the source measured within a time frame of 12 min are represented by the first line of bars. Landing responses are split by the distances of the choices (indicated by the direction of the bars) for treatment or control by each insect. Numbers inside the bars represent the number of responses.

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