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Full Length Research

Evaluation of the repellent activity of the oil extract of garlic (*Allium sativum,* L.) against *Glossina palpalis gambiensis,* (V.)

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ABSTRACT: Transmission of Human African Trypanosomiasis is caused mainly by the bites of tsetse fly (*Glossina* spp) infected with the species of *Trypanosoma brucei* in sub-Saharan Africa. The tsetse fly constitutes a major threat, and the disease, trypanosomiasis, poses a big socio-economic burden in sub-Saharan African countries. This study aimed to evaluate the repellent activity of the oil extract of fresh garlic bulbs against *Glossina palpalis gambiensis.* The oil was extracted using cold maceration with n-hexane. The repellent activity of garlic oil extract against laboratory-reared teneral *Glossina palpalis gambiensis* was evaluated on the fore-arm skin of five human subjects under laboratory conditions. The data generated were subjected to statistical analysis using One-way analysis of variance (ANOVA). All graded concentrations of garlic oil extract (50, 75, 87.5 and 100%) showed a significant difference ($p<0.01$) in their mean values in preventing tsetse flies from landing and probing on human skin. The high repellent effect of 100% concentration of garlic oil extract against *Glossina palpalis gambiensis* makes it a possible tool in the control of tsetse flies, as it prevents human skin contact with the tsetse flies, therefore eliciting its potential to reduce fly-human contact and thus reduce trypanosomiasis transmission and disease burden. Garlic oil extract applied at a concentration of 100% on skin may protect humans and livestock from the bites of tsetse flies, and so curb trypanosomiasis disease transmission and prevalence.

Keywords: Garlic oil; *Glossina palpalis gambiensis*, repellent activity, Trypanosomiasis.

INTRODUCTION

Human African Trypanosomiasis (HAT), also known as sleeping sickness, is a neglected tropical disease, caused by *Trypanosoma brucei* parasites transmitted by tsetse flies (Genus: *Glossina* spp), in sub-Saharan Africa (Leak, 1998). Two different subspecies of pathogens cause two forms of HAT: *Trypanosoma brucei gambiense* causes a slowly progressing disease in western and central Africa, and *Trypanosoma brucei rhodesiense* causes a more acute form of the disease in eastern and southern Africa (Büscher *et al.,* 2017). Control efforts have reduced the number of annual cases. In 2009, under 10,000 cases were reported, and the number of cases continues to drop. In 2020, fewer than 700 combined cases were reported by WHO (Franco *et al.,* 2022).

Glossina palpalis gambiensis (*G. p. gambiensis*) is one of the vectors of sleeping sickness, a tsetse fly subspecies which transmits *Trypanosoma brucei gambiense,* which causes gambiense HAT (gHAT) throughout western and central Africa. The disease poses a socio-economic burden in sub-Saharan African countries (Swallow, 2000).

Biting of humans and livestock by infected tsetse flies is crucial in the transmission of trypanosomiasis. In view of its economic importance, research strategies aimed at breaking the cycle of transmission had been centred on interrupting the tsetse fly-human contact. Vector control strategies are therefore crucial in reducing the trypanosomiasis challenge, and the most effective control is to avoid contact with tsetse flies. Among the methods in use to achieve this is the use of synthetic insecticides such as DDT (dichloro-diphenyl-trichloroethane) and Malathion. The use of synthetic insecticides in the suppression of tsetse flies is not ecologically friendly, as most synthetic insecticides are highly toxic to humans and non-targeted organisms, being harmful to aquatic life and terrestrial animals (Uilenberg, 1998). Phytochemicals are used by plants for self-defence against insects, pests, pathogens and environmental hazards, and this is capitalised on to produce medicinal, industrial, agricultural and commercial applications (Mazid *et al.,* 2011). Phytochemicals are responsible for the organoleptic properties of plants such as the smell of garlic (*Allium sativum* Linnaeus), and are important in the development of environmentally friendly insecticides, pesticides and herbicides (Mazid *et al.,* 2011). This study was therefore initiated to evaluate the effect of *A. sativum* oil extract as a repellent to protect humans and livestock from tsetse fly bites and disease transmission, at the *Insectarium de Bobo-Dioulasso – Campagne d'Eradication de la Mouche Tsee-tsee et de la Trypanosomose (IBD-CETT), Bobo-Dioulasso, Burkina Faso.*

MATERIALS AND METHODS

The study was carried out at the Insectarium de Bobo-Dioulasso, Burkina Faso, located in the village of Darsalamy, 15 km from the town of Bobo-Dioulasso (IBD), Burkina Faso (Pagabeleguem *et al.,* 2021). The IBD is a facility that specialises in the mass-rearing of tsetse flies. It was built under the auspices of the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) initiative to satisfy the needs of sterile male tsetse flies for the PATTEC program in Burkina Faso and other countries infested by tsetse flies (Percoma *et al.,* 2018).

One thousand (1000) tsetse flies (*G. p. gambiensis*), from the IBD colony were used for this research study in the experimental insectary arm of the mass rearing complex. The colony from where the experimental tsetse flies were obtained was fed *in vitro* on defibrinated and irradiated blood collected from slaughtered cows at the Bobo-Dioulasso abattoir, and maintained under the climatic conditions of 25±1°C and 75±5% relative humidity. Each experimental batch of tsetse flies consisted of 30 starved laboratory-reared teneral *G. p. gambiensis* held in a Roubaud cage (13.5 × 8 × 4.5 cm) and introduced into each experimental cage. The tsetse fly experimental cages used had some modifications to the WHO (2009) recommended experimental cage, with frames of size 45 cm per side with a clear transparent netting on all four sides for viewing, and a fabric sleeve for access on the front.

Fresh garlic bulbs

Fresh garlic bulbs were bought at Yankura market in Kano, Kano State, Nigeria. The fresh garlic bulbs were cultivated at Kofa town, Bebeji Local Government Area of Kano State. Identification and authentication of the fresh garlic bulbs were done at the Herbarium of the Department of Biological Sciences, Faculty of Science, Nigerian Defence Academy, Kaduna, with Herbarium voucher reference number NDA/BIOH/2022/12. The fresh garlic bulbs were taken to the laboratory of the Department of Pharmacognosy and Herbal Medicine, Faculty of Pharmaceutical Sciences, College of Natural and Pharmaceutical Sciences, Bayero University, Kano, for garlic oil extraction, using cold maceration (Zhang *et al.,* 2007). The extracted garlic oil was highly volatile and was preserved in a dark bottle with tight-fitting lid and kept in the refrigerator until used.

Volunteers and inclusion criteria

Testing of repellents on human subjects yielded results that were relevant to the actual conditions of use (Harrington *et al.,* 2020). Five (5) apparently healthy adult male human test volunteers were used as human subjects in this study. Test volunteers selected were people who did not smoke cigarettes or use tobacco, and did not have wounds or irritated skin. They were instructed to avoid the use of odorous substances such as fragranced soaps, fragranced creams, perfumes and repellent products for 12 hours before and during testing because such factors may alter a person's attractiveness to tsetse flies.

Ethical clearance and signed informed consent

Ethical approval to conduct this study was obtained from the Institutional Ethics Committee for Health Sciences Research, Ministry of Health, Bobo-Dioulasso, Burkina Faso, with reference number N/Ref. A042-2022/CEIRES/IRSS. Written informed consents were obtained from the five human subjects before commencing tests; volunteers were informed of potential risks and benefits.

Experimental procedures

Allergy screening for test volunteers

Allergy screening was carried out for test volunteers using a 0.25 ml test dose of 100% concentration of garlic oil applied to an area of 4 cm^2 of each test volunteer's forearm skin. Test volunteers with allergy reactions such as inflammation or skin irritation were excluded from participating in the study.

Repellent activity

A 600 cm^2 area of skin on the left fore-arm (between the hand and elbow) of each test volunteer was washed with unscented soap, rinsed with clean water, and allowed to dry prior to testing. One thousand (1000) laboratory-reared teneral *G. p. gambiensis* starved for 48 hours were used to evaluate the repellent activities of graded concentrations of 50%, 75%, 87.5% garlic oil extract reconstituted in 100% BETIS® Extra Virgin olive oil, and 100% concentration of garlic oil extract. The different concentrations of the garlic oil were applied separately on the fore-arm skin of test volunteers for a duration of 1 hour prior to exposure to these tsetse flies in different experimental cages, each cage containing thirty (30) starved laboratory-reared teneral *G. p. gambiensis*, in the laboratory, under conditions of normal room temperature and daylight. At each hour, each volunteer introduced his hand into the experimental cage containing 30 starved tsetse flies for a period of 5 minutes. The number of tsetse flies that landed and/or commenced to probe the skin was counted each time by the test volunteer and recorded. This process was repeated two more times in each hour, at an interval of 20 minutes. The test duration was 5 hours. A control group with untreated skin was included to baseline tsetse behaviour.

Determination of protection provided by garlic oil extract against G. p. gambiensis landing and/or probing on human skin

The protection provided by the graded concentrations of garlic oil extract against *G. p. gambiensis* landing and/or probing on human skin was determined by the number of tsetse flies landing and/or probing on the skin, counted each time by test volunteers. The protection provided by garlic oil against *G. p. gambiensis* landing and/or probing on human skin was calculated as the number of hours that elapsed between the time when the fore-arms treated with the various concentrations of garlic oil extract were inserted into different experimental cages, each containing 30 starved tsetse flies, and the first tsetse fly that commenced to probe the skin. Data generated were expressed as mean ± standard error of the mean (mean ± SEM). The data were subjected to statistical analysis using One-way Analysis of Variance (ANOVA). Graphpad Prism version 8.2 for Windows (Graphpad Software, San Diego, California, USA) was used to analyse all the data. Values of $p<0.01$ were considered significant.

RESULTS

Mean number of *Glossina palpalis gambiensis* landing, and probing on skin post-application of 50% and 75% concentrations of garlic oil extract

At the 3rd hour post-application of 50% concentration of garlic oil extract on skin exposed to starved laboratory-reared teneral *G. p. gambiensis*, garlic oil ($p<0.01$) had 8.0±0.00 mean number of *G. p. gambiensis* landing on skin (Figure 1a).

At the 3rd hour post-application of 50% concentration of garlic oil on skin exposed to starved laboratory-reared teneral *G. p. gambiensis*, garlic oil ($p< 0.01$) had a 5.0±1.2 mean number of *G. p. gambiensis* probing on skin (Figure 1b).

At the 3rd hour post-application of 75% concentration of garlic oil extract on skin exposed to starved laboratory-reared teneral *G. p. gambiensis*, garlic oil ($p<0.01$) had 7.0±0.58 mean number of *G. p. gambiensis* landing on skin (Figure 2a).

At the 3rd hour post-application of 75% concentration of garlic oil on skin exposed to starved laboratory-reared teneral *G. p. gambiensis*, garlic oil ($p<0.01$) had a 2.0±0.0 mean number of *G. p. gambiensis* probing on skin (Figure 2b).

The repellent efficacies of 50% and 75% concentrations of garlic oil against the landing and probing of *G. p. gambiensis* were time-dependent. At 50% and 75% concentrations post-application on skin, garlic oil provided 0 hours protection each against the landing and probing of *G. p. gambiensis* (Figure 5a and b).

Mean number of *Glossina palpalis gambiensis* landing, and probing on skin post-application of 87.5% and 100% concentrations of garlic oil extract

At the 3rd hour post-application of 87.5% concentration of garlic oil extract on skin exposed to starved laboratory-reared teneral *G. p. gambiensis*, garlic oil ($p<0.01$) had a 4.0±0.00 mean number of *G. p. gambiensis* landing on skin (Figure 3a).

At the 1st, 2nd, and 3rd hours post-application of 87.5% concentration of garlic oil on skin exposed to starved laboratory-reared teneral *G. p. gambiensis*, 0.0 ±0.00 mean numbers of *G. p. gambiensis* probed on skin ($p<0.01$).

At the 4th hour post-application of 87.5% concentration of garlic oil on skin exposed to starved laboratory-reared

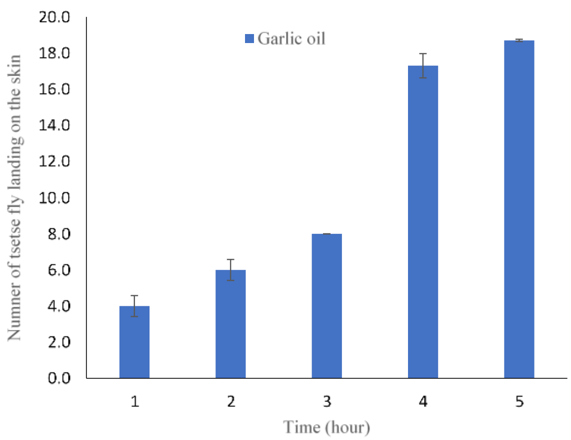

Figure 1a. Showing the mean number of *Glossina palpalis gambiensis* landing on skin after application of 50% garlic oil (p<0.01).

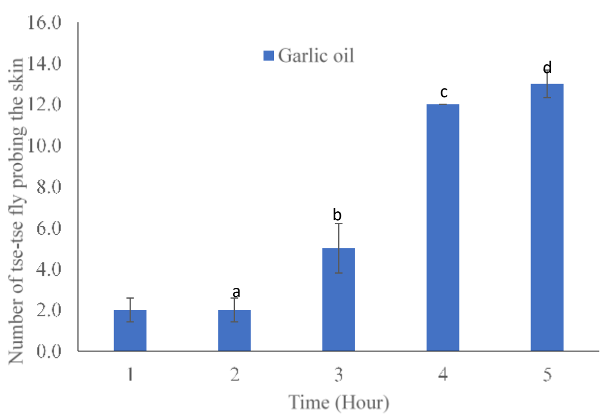

Figure 1b. Showing the mean number of *Glossina palpalis gambiensis* probing on skin after application of 50% garlic oil. Means with different superscript letters ($^{a-d}$) in different bars differ significantly (p<0.01).

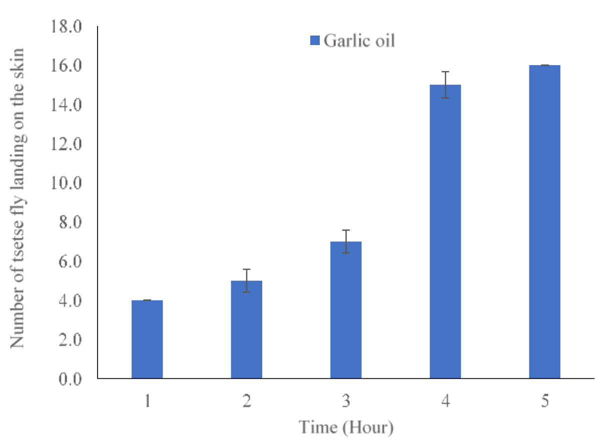

Figure 2a. Showing the mean number of *Glossina palpalis gambiensis* landing on skin after application of 75% garlic oil (p<0.01).

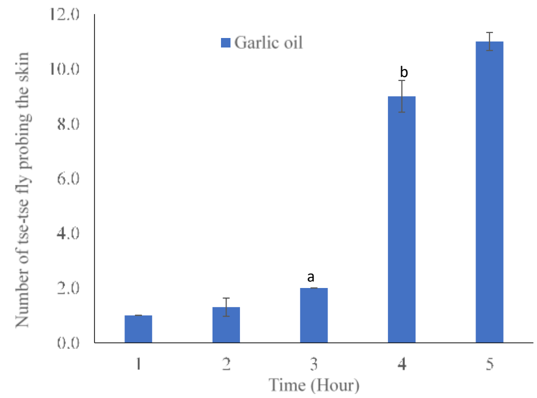

Figure 2b. Showing the mean number of *Glossina palpalis gambiensis* probing on skin after application of 75% garlic oil. Means with different superscript letters ($^{a-b}$) in different bars differ significantly (p<0.01).

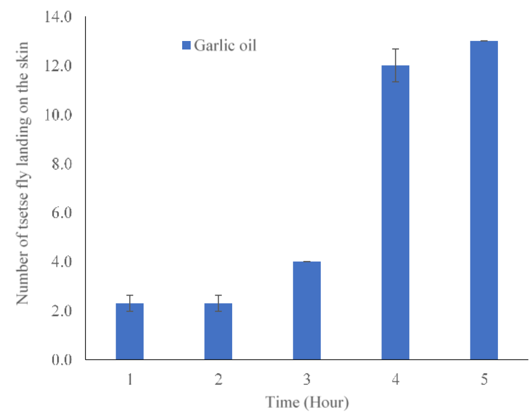

Figure 3a. Showing the mean number of *Glossina palpalis gambiensis* landing on skin after application of 87.5% garlic oil (p<0.01).

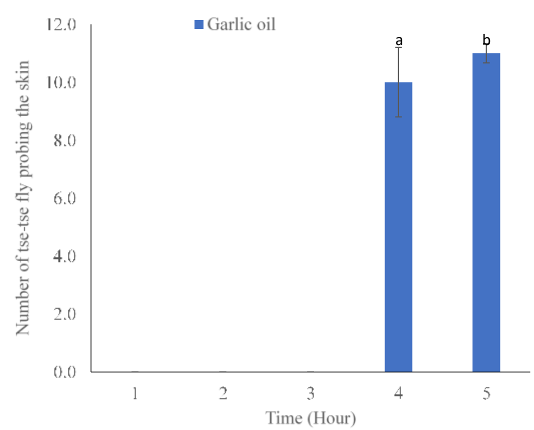

Figure 3b. Showing the mean number of *Glossina palpalis gambiensis* probing on skin after application of 87.5% garlic oil. Means with different superscript letters ($^{a-b}$) in different bars differ significantly (p<0.01).

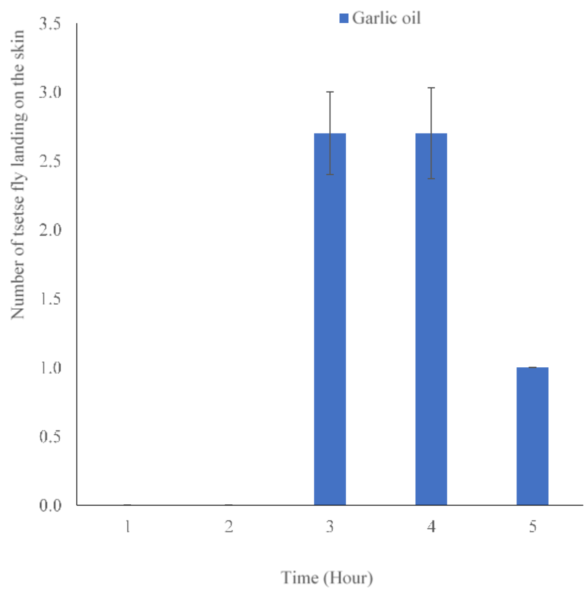

Figure 4a. Showing the mean number of *Glossina palpalis gambiensis* landing on skin after application of 100% garlic oil (p<0.01).

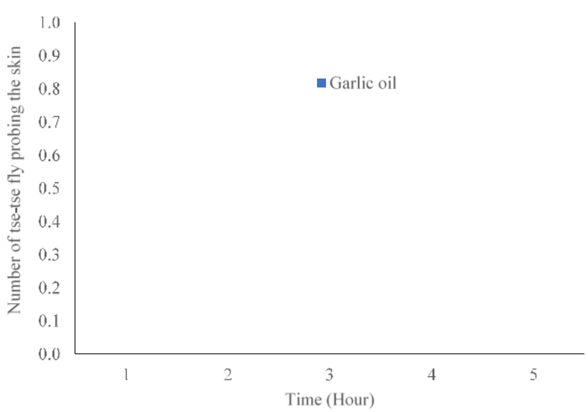

Figure 4b. Showing the mean number of *Glossina palpalis gambiensis* probing on skin after application of 100% garlic oil (p<0.01).

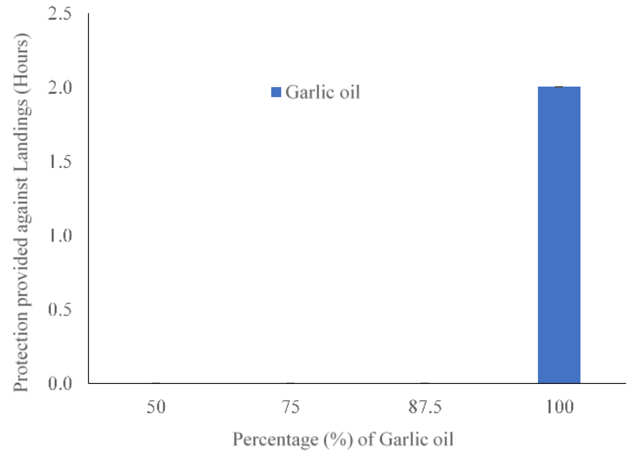

Figure 5a. Showing the mean protection provided by garlic oil after application on skin, against the landings of *Glossina palpalis gambiensis* (p<0.01).

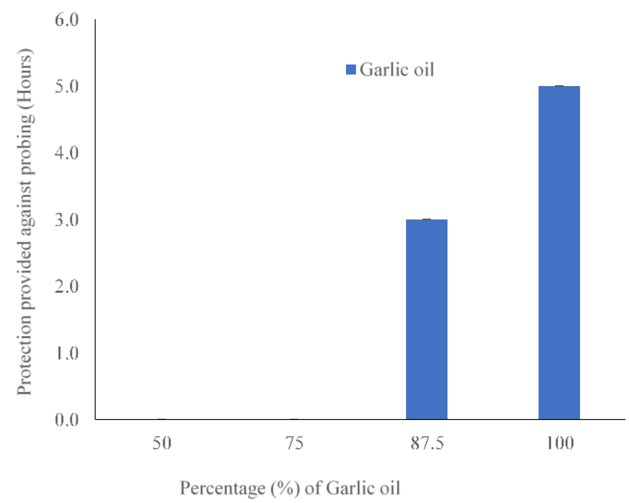

Figure 5b. Showing the Mean protection provided by garlic oil after application on skin, against the probing of *Glossina palpalis gambiensis* (p<0.01).

teneral *G. p. gambiensis*, the efficacy of garlic oil (p< 0.01) had waned with 10.0±1.2 mean number of *G. p. gambiensis* probing on skin (Figure 3b).

At 87.5% concentration post-application on skin, garlic oil provided 0 hours protection against the landing of *G. p. gambiensis,* and 3 hours protection against the probing of *G. p. gambiensis* (Figure 5a and b).

At the 1st and 2nd hour post-application of 100% concentration of garlic oil extract on skin exposed to starved laboratory-reared teneral *G. p. gambiensis*, the mean number of *G. p. gambiensis* landing on skin was 0.0±0.00, and 0.0±0.00, but at the 3rd, 4th, and 5th hour, the efficacy of 100% garlic oil waned in repelling *G. p. gambiensis* from landing on skin (Figure 4a).

The mean numbers of *G. p. gambiensis* probing at 1st, 2nd, 3rd, 4th, and 5th hour post-application of 100% concentration of garlic oil on skin exposed to starved laboratory-reared teneral *G. p. gambiensis*, were 0.0±0.00, 0.0±0.00, 0.0±0.00, 0.0±0.00 and 0.0±0.00 (Figure 4b).

Garlic oil at 100% concentration exhibited efficacy (p<0.01) at completely repelling *G. p. gambiensis* from probing the skin for the 5 hours test duration.

Garlic oil at 100% concentration post-application on skin exposed to starved laboratory-reared teneral *G. p. gambiensis*, provided 2 hours protection against the landing of *G. p. gambiensis*, and 5 hours protection against the probing of *G. p. gambiensis* (Figure 5a and b).

DISCUSSION

Organic chemical compounds such as thiosulfinates (allicin), vinyldithiins (2-vinyl-(4H)-1,3-dithiin, 3-vinyl-(4H)-1,2-dithiin), sulfides (diallyl disulfide) are reported to be largely responsible for the repellent properties of garlic oil (Prowse *et al.,* 2006; Al-Snafi, 2013; Subroto *et al.,* 2021; Tudu *et al.*, 2022). The oil of garlic released a characteristic odour, probably due to the sulfurous compounds identified in it. This might have had powerful properties which have caused the garlic oil to actively repel the tsetse flies.

The repellent efficacies of graded concentrations of garlic oil post-application on skin exposed to starved laboratory-reared teneral *G. p. gambiensis* were evaluated on the landing and the probing behaviour of *G. p. gambiensis*. The repellent efficacy of garlic oil, and the protection in hours that the garlic oil provided against the landing, and the probing of *G. p. gambiensis* were concentration-dependent, and time-dependent. The number of *G. p. gambiensis* repelled from landing, and from probing, and the hours of protection from landing, and from probing given, increased with increasing concentration of garlic oil, and with post-application time. Garlic oil at 87.5 and 100% concentrations provided 3 hours and 5 hours protection, respectively, against *G. p. gambiensis* probing on skin, while garlic oil at 50 and 75% concentrations provided 0 hours protection against *G. p. gambiensis* probing on skin.

The repellent efficacy exhibited by garlic oil against *G. p. gambiensis* landing and probing on human skin could be due to the bioactive potentials, such as insect enzyme-inhibition properties in the garlic oil. Wills (1956) reported that crude garlic extract completely inhibited succinic oxidase, thereby reducing oxygen uptake. Reduction in oxygen uptake could contribute to destabilising the tsetse flies from having enough vitality to feed, causing them to be lethargic. Rattan (2010), stated that the mechanism of action of essential oils on the body of insects is due to the inhibition of acetylcholinesterase activity, which is a key enzyme responsible for nerve impulse transmission. Inhibition of acetylcholinesterase activity could weaken the flight of the tsetse flies, making them not able to actively seek their host. The garlic oil, applied to human fore-arm skin in this study, caused no adverse effect on the human subjects who used it. Strickman *et al.* (2009) had reported that most of the plants used as repellents are also used for food flavouring or in the perfume industry, and this may explain why essential oils from plants are safer natural alternatives to synthetic insecticides.

Conclusion

In this study, 100% garlic oil extract was observed to be effective at repelling *G. p. gambiensis* from probing on skin. Garlic oil extract could be applied at a concentration of 100% at an interval of 5 hours on skin, in a carefully planned husbandry practice, to protect humans and livestock from the bites of tsetse flies, and so curb trypanosomiasis disease transmission and prevalence. Garlic oil should be incorporated into the environmentally friendly methods of integrated control of tsetse flies, being advocated by the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) initiative to curb the transmission of trypanosomiasis. More research should be conducted on the oil of garlic to elicit more novel insights into its underlying mechanisms of action.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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