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SCIENTIFIC NOTE

LABORATORY EVALUATION OF EFFICACY OF THE LARVICIDE SPINOSAD AGAINST *ANOPHELES STEPHENSI* IN JIGJIGA, ETHIOPIA

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ABSTRACT. We report the efficacy of a commercial formulation of the insecticide spinosad against larvae of *Anopheles stephensi* populations found in the city of Jigjiga, Somali Region, eastern Ethiopia. Batches of 25 larvae (late III to early IV instars) collected from large water storage reservoirs associated with construction sites (the primary *An. stephensi* larval site in the dry season) were tested under laboratory conditions against each insecticide at a dose recommended by the manufacturer (Natular® G30, 0.02 g/5 liter), following World Health Organization guidelines. Mortality at 24–48 h postexposure was 100%. Results show that spinosad is effective against *An. stephensi* larvae and suggest that it may be a useful tool as part of larval source management plans aimed at controlling this invasive malaria vector in Ethiopia.

KEY WORDS *Anopheles stephensi*, larval source management, larvicide, urban malaria

The introduction and rapid geographic range expansion of the Asian urban malaria vector *Anopheles stephensi* (Liston) in African cities has created concern among international health organizations and in the countries impacted (WHO 2022). Since it was first detected in Djibouti in 2012, *An. stephensi* has spread to Ethiopia, Somalia, Nigeria, Kenya, and Ghana (WHO 2022), with modeling predicting suitable environmental conditions throughout tropical African cities (Sinka et al. 2020, Whittaker et al. 2023). The World Health Organization (WHO) considers the spread of *An. stephensi*, an efficient and competent vector for both *Plasmodium falciparum (*Welch) and *P. vivax* (Grassi and Feletti), to be a major potential threat to malaria control and elimination in Africa (WHO 2022).

The WHO has launched a global initiative to stop the spread and to support an effective response to *An. stephensi* in Africa (WHO 2022). Since the alert, WHO has urged countries, especially those in and around the Horn of Africa, to take immediate action enhancing and expanding surveillance and control activities with the aim of eliminating this species from the invaded areas (WHO 2022). Larval source management to control *An. stephensi* is one of the key recommended activities and includes the treatment with WHO prequalified chemical or biological larvicides of those breeding sites that cannot be removed or modified immediately (WHO 2005). Evidence from Ethiopia indicates that *An. stephensi*, while resistant to adulticide insecticides such as pyrethroids, carbamates, organophosphates, and organochlorines (Balkew et al. 2020, 2021; Yared et al. 2020), are susceptible to the organophosphate temephos and microbial larvicide *(Bacillus thuringiensis var. israelensis*, *de Barjac)* (Teshome et al. 2023).

On March 6–14, 2023, we collected larvae of *An. stephensi* from Jigjiga city (9°21′0.5″N; 42°48′01.8″E) during the peak of the dry season to conduct larvicide efficacy studies. Jigjiga (population 763,509) is the capital of the Somali Region of Ethiopia and a rapidly growing urban center (Fig. 1). Although not endemic for malaria, Jigjiga has reported the presence of *An. stephensi* since 2018 (Balkew et al. 2021). For this study we identified sites with high potential to contain water with *An. stephensi* larvae. From all habitats surveyed (metal/plastic cisterns, 200 liter drums, buckets, truck tires) temporary water storage reservoirs located at houses under construction and/or reserved for production of cement blocks were found positive for *Anopheles* sp. larvae by regular dipping, supplemented with fish net collections (Fig. 1). Such construction pits consisted of a hole in the dirt, covered with orange plastic tarp (Fig 1) with a capacity of 8–40 m^3 and used to store water for construction purposes. Prior experience of our team indicates that these sites are also positive for *An. stephensi* in the rainy season in other Ethiopian cities (Balkew et al. 2020, 2021).

For our study, immature mosquito specimens (larvae and pupae) were collected with standard dippers and entomological aquatic nets (WHO 2011) and

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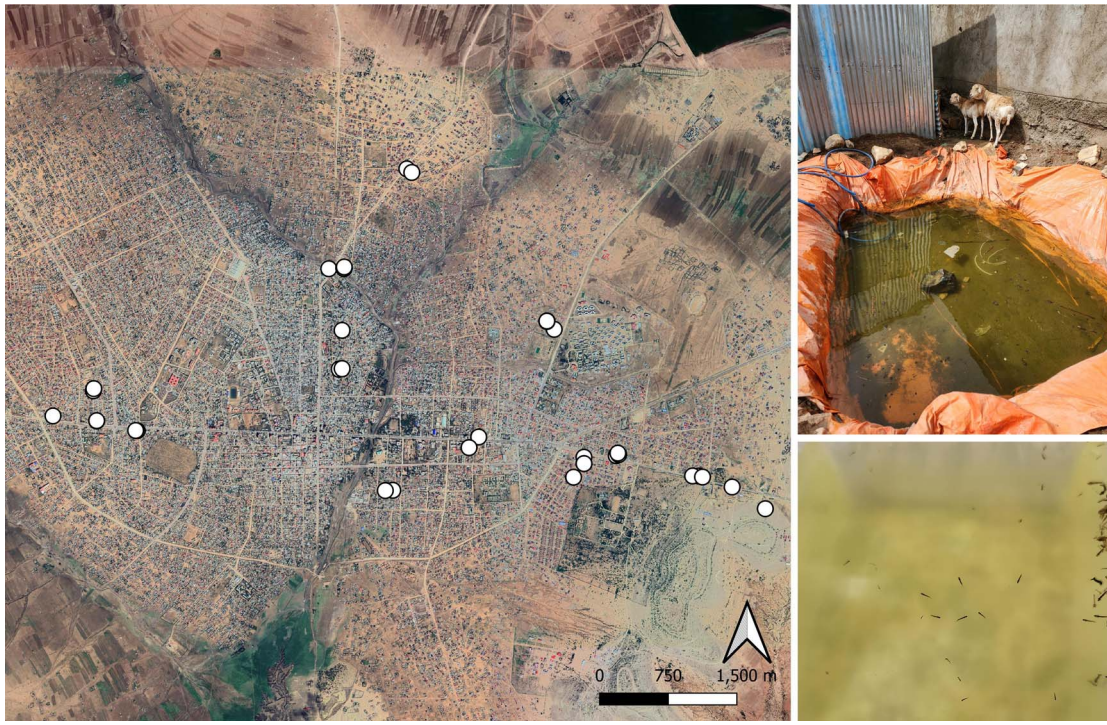

Fig. 1. Map of Jigjiga (left) marking the locations where *Anopheles stephensi* larvae and pupae were obtained for larvicide testing (white circles) and images showing the predominant site (construction pits, top right) and sample of *Anopheles* sp. larvae collected from such site (bottom right).

transported alive in plastic jars with water from their breeding sites to the insectary facility at the University of Jigjiga. All *Anopheles* larvae were separated, and a sample was reared to adults in cages (BugDorm-1Insect Rearing Cages, 30 × 30 × 30 cm) at the Entomology Laboratory of the University of Jigjiga. Although larvae were used in the bioassays, all *Anopheles sp.* pupae collected in the field were reared to adults in the laboratory inside 20 × 20 × 20 plastic cages (Bugdorm) to rule out the presence of other anopheline species cohabiting with *An. stephensi*. All adult female *Anopheles* mosquitoes were aspirated from cages and confirmed to be *An. stephensi* using the updated key to the females of Afrotropical *Anopheles* mosquitoes (Coetzee 2020). All emerged *Anopheles* adults aspirated from the cage were *An. stephensi*. All the adults visually identified as *An. stephensi* were molecularly confirmed to be this species using an allele-specific polymerase chain reaction and the sequencing of internal transcribed spacer 2 (ITS2) and cytochrome *c* oxidase subunit I (COI) loci (Carter et al. 2018).

For the bioassays, WHO guidelines for laboratory testing of mosquito larvicides were followed (WHO 2005). In 1 liter plastic containers, pooled batches of 25 larvae (late III to early IV instars) collected from the field were tested against spinosad (Natular® 30G), formulated as an extended-release granule and applied at a rate of 0.004 g per 1 liter of tap water. A total of 100 larvae were exposed to spinosad in 4 replicates with 25 larvae each. Four replicated containers water containing 25 larva each were used as controls. Larval mortality was recorded after 24 and 48 h. Dead larvae were those that could not be induced to move when they were probed with a needle in the cervical region, and moribund larvae were those incapable of rising to the surface or not showing the characteristic diving reaction when the water was disturbed or sank to the bottom (WHO 2005). The mortality of the test sample was calculated by summing the number of dead larvae across all exposure replicates expressed as a percentage of the total number of exposed larvae. The residual efficacy of the larvicide was not included in this study. All larvae were found dead at 24 h postexposure to spinosad at a concentration of 0.004 g/liter of water and remained dead at 48 h, indicating strong efficacy of spinosad against *An. stephensi*. No larval mortality was observed in the control.

Spinosad as an insecticide is composed of a mixture of spinosyn A and D as the active ingredient (AI) and derived from the soil actinomycete *Saccharopolyspora spinosa* (Mertz and Yao) under aerobic fermentation condition (Prabhu et al. 2011). Within 24 h, spinosad functions as both a contact and stomach poison for mosquito larvae, altering the function of the GABA-gated nicotinic channel and the nicotinic receptor, producing mainly involuntary muscle contractions, tremors, paralysis, and death (Prabhu et al. 2011). Natular G30 has

previously been studied for its efficacy against *An. arabiensis* (Patton), *An. gambiae* (Giles), and *An. funestus* (Giles) in both permanent and semipermanent habitats in sub-Saharan Africa (Gimnig et al. 2020). In that study, 2 formulations of pinosad (emulsifiable concentrate and extended-release granules) were tested in semifield habitats and in a field trial in 2 villages, showing significant reductions in *Anopheles* sp. that resulted in significant reductions in larval occupancy, larval densities, and adult densities (Gimnig et al. 2020). Spinosad has not exhibited cross-resistance with other insecticides and can be alternated with all other classes of mosquito larvicides currently in use (WHO 2013).

Our findings are the first reporting of the efficacy of spinosad under laboratory conditions against larvae of the invasive *An. stephensi* in Africa. Our results also validate the results with different spinosad formulations against *An. stephensi* larvae in Asian countries (Kumar et al. 2011, Prabhu et al. 2011). The WHO guidelines for laboratory and field testing of mosquito larvicides indicate larval mortality should be recorded after 24 h exposure and 48 h for slow-acting larvicides (WHO 2005). In this study, the effects of spinosad were observed 24 h after exposure as an erratic diving or lack of mobility, and after 48 h all larvae were immobile at the bottom of the containers.

The preference of *An. stephensi* larvae for developing in construction pits and other artificial containers, particularly during the dry season, suggests possible control through the application of larvicides to these fixed habitats. In addition to treatment applied to water-holding containers during the dry season, the preventive use of granules to dry containers known to fill during the rainy season could also be considered (Aldridge et al. 2018, Britch et al. 2018, Golden et al. 2018). Further laboratory and field-based studies are necessary to determine the efficacy of spinosad larvicide against *An. stephensi* and other malaria vectors at different localities and under field settings. Exhaustive assessment of larval habitats and the identification of the cohabitants of this vector can also help in identifying effective tool(s) to control these vectors in an integrated approach.

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REFERENCES CITED

Aldridge RL, Golden FV, Britch SC, Blersch J, Linthicum KJ. 2018. Truck-mounted Natular 2EC (spinosad) ULV residual treatment in a simulated urban environment to control *Aedes Aegypti* and *Aedes albopictus* in North Florida. *J Am Mosq Control Assoc* 34:53–57.

Balkew M, Mumba P, Dengela D, Yohannes G, Getachew D, Yared S, Chibsa S, Murphy M, George K, Lopez K, Janies D, Choi SH, Spear J, Irish SR, Carter TE. 2020. Geographical distribution of *Anopheles stephensi* in eastern Ethiopia. *Parasit Vectors* 13:35.

Balkew M, Mumba P, Yohannes G, Abiy E, Getachew D, Yared S, Worku A, Gebresilassie A, Tadesse FG, Gadisa E, Esayas E, Ashine T, Ejeta D, Dugassa S, Yohannes M, Lemma W, Yewhalaw D, Chibsa S, Teka H, Murphy M, Yoshimizu M, Dengela D, Zohdy S, Irish S. 2021. An update on the distribution, bionomics, and insecticide susceptibility of *Anopheles stephensi* in Ethiopia, 2018–2020. *Malar J* 20:263.

Britch SC, Linthicum KJ, Aldridge RL, Golden FV, Pongsiri A, Khongtak P, Ponlawat A. 2018. Ultra-low volume application of spinosad (Natular 2EC) larvicide as a residual in a tropical environment against *Aedes* and *Anopheles* species. *J Am Mosq Control Assoc* 34:58–62.

Carter TE, Yared S, Gebresilassie A, Bonnell V, Damodaran L, Lopez K, Ibrahim M, Mohammed S, Janies D. 2018. First detection of *Anopheles stephensi* Liston, 1901 (Diptera: culicidae) in Ethiopia using molecular and morphological approaches. *Acta Trop* 188:180–186.

Coetzee M. 2020. Key to the females of Afrotropical *Anopheles* mosquitoes (Diptera: Culicidae). *Malar J* 19:70.

Gimnig JE, Ombok M, Bayoh N, Mathias D, Ochomo E, Jany W, Walker ED. 2020. Efficacy of extended release formulations of Natular (spinosad) against larvae and adults of *Anopheles* mosquitoes in western Kenya. *Malar J* 19:436.

Golden FV, Britch SC, Aldridge RL, Wittie J, Gutierrez A, Snelling M, Henke JA, Linthicum KJ. 2018. Ultra-low volume application of spinosad (Natular 2EC) as a residual in a hot-arid environment against *Aedes aegypti. J Am Mosq Control Assoc* 34:63–66.

Kumar AN, Murugan K, Madhiyazhagan P, Prabhu K. 2011. Spinosad and neem seed kernel extract as bio-controlling agents for malarial vector, *Anopheles stephensi* and non-biting midge, *Chironomus circumdatus*. *Asian Pac J Trop Med* 4:614–618.

Prabhu K, Murugan K, Nareshkumar A, Bragadeeswaran S. 2011. Larvicidal and pupicidal activity of spinosad against the malarial vector *Anopheles stephensi*. *Asian Pac J Trop Med* 4:610–613.

Sinka ME, Pironon S, Massey NC, Longbottom J, Hemingway J, Moyes CL, Willis KJ. 2020. A new malaria vector in Africa: predicting the expansion range of *Anopheles stephensi* and identifying the urban populations at risk. *Proc Natl Acad Sci USA* 117:24900–24908.

Teshome A, Erko B, Golassa L, Yohannes G, Irish SR, Zohdy S, Dugassa S. 2023. Laboratory-based efficacy evaluation of *Bacillus thuringiensis var. israelensis* and *temephos* larvicides against larvae of *Anopheles stephensi* in Ethiopia. *Malar J* 22:48.

Whittaker C, Hamlet A, Sherrard-Smith E, Winskill P, Cuomo-Dannenburg G, Walker PGT, Sinka M, Pironon S, Kumar A, Ghani A, Bhatt S, Churcher TS. 2023. Seasonal dynamics of *Anopheles stephensi* and its implications for mosquito detection and emergent malaria control in the Horn of Africa. *Proc Natl Acad Sci USA* 120:e2216142120.

WHO [World Health Organization]. 2005. *Guidelines for laboratory and field testing of mosquito larvicides* [Internet]. WHO/CDS/WHOPES/GCDPP/2005.13. Geneva, Switzerland: World Health Organization. [accessed July 8, 2023]. Available from: https://www.who.int/publications/i/item/WHO-CDS-WHOPES-GCDPP-2005.13.

WHO [World Health Organization]. 2011. *Operational guide for assessing the productivity of Aedes aegypti breeding sites* [Internet]. Geneva, Switzerland: World Health Organization. [accessed July 8, 2023]. Available from: https://tdr.who.int/publications/m/item/2011-10-31-operational-guide-for-assessing-the-productivity-of-aedes-aegypti-breeding-sites.

WHO [World Health Organization]. 2013. *Report of the sixteenth WHOPES working group meeting: WHO/HQ, Geneva, 22–30 July 2013: review of Pirimiphos-methyl 300 CS, Chlorfenapyr 240 SC, Deltamethrin 62.5 SC-PE, Duranet LN, Netprotect LN, Yahe LN, Spinosad 83.3 Monolayer DT, Spinosad 25 Extended release GR* [Internet]. Geneva, Switzerland: World Health Organization. [accessed July 8, 2023]. Available from: https://iris.who.int/handle/10665/90976.

WHO [World Health Organization]. 2022. *WHO initiative to stop the spread of Anopheles stephensi in Africa* [Internet]. Geneva, Switzerland: World Health Organization. [accessed July 8, 2023]. Available from: https://www.who.int/publications/i/item/WHO-UCN-GMP-2022.06.

Yared S, Gebressielasie A, Damodaran L, Bonnell V, Lopez K, Janies D, Carter TE. 2020. Insecticide resistance in *Anopheles stephensi* in Somali Region, eastern Ethiopia. *Malar J* 19:180.