High pesticide tolerance of *S. mansoni*: implications for the risk of schistosomiasis

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Abstract

Background Schistosomiasis is a neglected tropical disease caused by trematodes of the genus Schistosoma. The pathogen is transmitted via freshwater snails. These snails are highly tolerant to agricultural pesticides and indirectly benefit from exposure due to adverse effects on their more sensitive enemy species. Pesticides in surface waters may thus increase the risk of schistosomiasis transmission unless they also affect the pathogen. We tested the tolerance of the free-swimming life stages (miracidia and cercariae) of *Schistosoma mansoni* to the insecticides diazinon and imidacloprid. We also investigated whether these pesticides decrease the ability of miracidia to infect and further develop as sporocysts within the host snail *Biomphalaria pfeifferi*.

Results Exposure to imidacloprid for 6 and 12 h immobilized 50% of miracidia at 150 and 16 µg/L, respectively (nominal EC50); 50% of cercariae were immobilized at 403 and 284 µg/L. Diazinon immobilized 50% of miracidia at 51 and 21 µg/L after 6 and 12 h; 50% of cercariae were immobilized at 25 and 13 µg/L. The observed insecticide tolerance is lower than those of the host snail *B. pfeifferi* and comparable to those of other commonly tested freshwater invertebrates. Exposure for up to 6 h decreased the infectivity of miracidia at high sublethal concentrations (48.8 µg imidacloprid/L and 10.5 µg diazinon/L, i.e. 20–33% of EC50), but not at lower concentrations commonly observed in the field (4.88 µg imidacloprid/L and 1.05 µg diazinon/L). The development of sporocysts within the snail host was not affected at any of these test concentrations.

Conclusions Insecticides did not affect the performance of *S. mansoni* at environmentally relevant concentrations. Particularly within its host snail the pathogen can escape exposure peaks that have been shown to affect other sensitive invertebrates and their biological control of host snails. Our findings suggest that freshwater pollution with agricultural pesticides increases the risk of schistosomiasis; they illustrate the need to integrate an environmental and public health risk assessment and management.

Keywords Schistosomiasis, Neglected tropical diseases, Pesticides, Freshwater pollution, *Schistosoma mansoni*, *Biomphalaria pfeifferi*

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Introduction

Schistosomiasis remains a major public health problem in many parts of the world [1] despite the effort to eliminate this disease. It is caused by the parasitic trematodes of the genus *Schistosoma* that use freshwater snails as intermediate hosts [2]. In western Kenya, which is considered a highly endemic area, two forms of the disease that are relevant to human health are present, intestinal schistosomiasis and urinary schistosomiasis [3]. Here, we focused on intestinal schistosomiasis caused by *S. mansoni* which parasitize planorbid snails from the genus *Biomphalaria* [4]. The trematodes penetrate the snails as free-swimming larvae (miracidia) and undergo asexual reproduction as sporocysts within the snail before maturation into human-infecting free-swimming cercariae. This process takes about four weeks within the snail, depending on temperature [2].

Agricultural activities have been shown to increase the risk of schistosomiasis by creating habitats such as dams and irrigation canals which are suitable for host snails whilst preventing their predators, such as river prawns, from accessing them [5]. Moreover, agricultural pesticides indirectly foster the highly tolerant host snails by affecting their more sensitive competitors [6] and predators [7, 8]. E.g. a recent study from western Kenya has shown that contamination of rural freshwater bodies with agricultural pesticides can increase the likelihood of finding host snails in potential habitats, as well as the density of existing host snail populations [6]. Pesticide—particularly insecticide—residues found in the freshwater samples and within freshwater snails were most toxic to freshwater macroinvertebrates (represented by the standard test species *Daphnia magna*), followed by fish (represented by *Oncorhynchus mykiss*) [9]. Accordingly, the increase in host snails was associated with a decline in potential competitor species from the macroinvertebrate community, such as mayfly larvae and freshwater shrimps; host snails (*Bulinus africanus* and *Biomphalaria pfeifferi*) were more tolerant to insecticides than any of the other 13 macroinvertebrate taxa collected at the study sites and tested [6]. In mesocosms, pesticides increased the abundance of host snails mainly via adverse effects on more sensitive crayfish that preys on host snails [7, 8]. Pesticides may also affect the biological control of host snails by fish, since efficient snail feeders are mainly found within the family of cichlids [10–13] that seems generally not more tolerant to insecticides than *O. mykiss* [14]. Consequently, pesticide pollution may increase the risk of schistosomiasis transmission by supporting increased numbers of intermediate host snails. However, for an overall assessment of this effect it is crucial to also understand how pesticides might affect the pathogen *Schistosoma* itself.

In this study, we investigated effects of agricultural pesticides on the various larval stages of *S. mansoni* and on their interaction with the intermediate host snail *Biomphalaria pfeifferi*. Miracidia of *S. mansoni* hatch from eggs excreted with human faeces and need to find a suitable snail host within 24 h before their energetic reserves have been depleted. During this period, the miracidia can be exposed to pesticides in water while they chemotactically navigate to find their host [15, 16]. We assessed whether miracidia host seeking is affected at high sublethal concentrations serving as positive control (20–33% of the identified acute EC50, i.e. the median effective concentration that immobilizes half of the test organisms) and at environmentally relevant concentrations (2–3% of the EC50). The lower test concentrations represent the upper range of pesticide toxicity that has been observed in rural freshwater bodies in western Kenya [9] and also around the world (i.e. a toxic unit, defined as $log_{10}\left(\frac{\text{Environmental concentration}}{\text{acute EC 50 of standard test organism}}\right)$, of 0 to − 1; [6, 17, 18]. After successful infection, a single miracidium produces a sporocyst within a snail which can self-replicate to thousands of sporocysts. Therefore, maturation and replication of *Schistosoma* relies on nutrient supply from the snail [19]. As a consequence, sporocysts are indirectly susceptible to environmental conditions that affect the energetic reserves but also the immune system of the snails [20]; these conditions potentially include pesticides. Additionally, sporocysts may be directly affected by pesticide residues that enter the body of snails. Finally, the sporocysts produce cercariae that are shed into the water where they can be exposed to pesticides. Cercariae need to chemotactically find and infect humans via dermal contact within 24 h [4].

Among agricultural pesticides, insecticides potentially have the strongest effects on *S. mansoni* due to the relationship of insects and trematodes as part of the invertebrates. Therefore, we assessed effects of the neonicotinoid insecticide imidacloprid and of the organophosphorous insecticide diazinon on the survival and infectivity of miracidia, on sporocyst development, and on the survival of cercariae. The test compounds represent two widely used classes of insecticides with different modes of action; they were found to drive the overall pesticide toxicity to macroinvertebrates (*D. magna*) in rural freshwater bodies of western Kenya [9] and have been used to compare the sensitivity of host snails and other macroinvertebrates from these sites [6]. First, we assessed the acute median effective concentration of each compound that immobilizes half of the tested miracidia after constant exposure for six hours (6 h EC50) and for 12 h; immobilized organisms were considered to be ecologically dead. Then we assessed whether miracidia host seeking and maturation into sporocysts and cercariae is

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affected at high sublethal concentrations (20–33% of the identified 6 h EC50, serving as positive control) and at lower, environmentally relevant concentrations (2–3% of the 6 h EC50). The lower test concentrations represent the upper range of the agricultural pesticide toxicity that has been observed in rural freshwater bodies in western Kenya and also around the world (see materials and methods for details).

Materials and methods

Study location

All experiments were conducted at the International Centre for Insect Physiology and Ecology (*icipe*) Thomas Odhiambo Campus (TOC), Mbita, western Kenya.

Snail collection and rearing

Biomphalaria snails were collected from the shores of Lake Victoria with a snail catcher and a pool net. Species were identified with a field identification key [21]. Collected snails were placed in open plastic containers along with some vegetation from the collection site for shade and cooling during transport. No water was provided during transport to avoid excess mortality due to warming. In the laboratory, snails were placed in large plastic tubs (45×35×28 cm) with 5 L of lake water and reared with boiled kale (*Brassica oleracea L*) and tropical fish food. These tubs were kept at ambient conditions in a greenhouse with netting screened walls at *icipe* TOC. The day after collection, snails were screened for infection with a *Schistosoma* parasite by placing them individually in 24-well plates and exposing them to indirect sunlight for 2 h to cause shedding of cercariae., similar to what was done by Opisa et al*.* [22]. After 2 h, the well plates were observed under a dissecting microscope (Zeiss AxioCam5 100–400×) for *Schistosoma* cercariae which would indicate which snails were infected. Infected snails were separated—to more easily access cercariae for experiments—but were otherwise reared similarly to uninfected snails in aerated, dechlorinated water and fed with boiled kales. Uninfected snails were reared for an additional 5 weeks at ambient temperature before rechecking for cercarial shedding, after which uninfected snails were considered fit for experiments that required infection such as the miracidia host seeking and sporocyst development assays.

Schistosoma cercariae collection

Cercariae were obtained from *Schistosoma* positive snails. On the days of experiments, the snails were placed in 24-well plates under artificial light at 9 am to allow for cercariae shedding for two hours before setting up assays. The well plates were then observed under a microscope (Zeiss AxioCam5 100–400×) for cercariae showing the visible 'curling' forked tail prongs. When found, the snail from the appropriate well was removed, and cercariae were pipetted into the test containers for experiments as described in the acute toxicity tests section below. After the experiment, the snails used to shed the cercariae were placed in a freezer to kill them.

Schistosoma miracidia collection

Miracidia were obtained from *Schistosoma* eggs extracted from stool samples obtained from primary school children with due consent from both parent and child, and ethical approval from the relevant national authorizing body. Over the course of the experiment, 145 children were recruited for screening from Kombe (− 0.440028, 34.220040) and Wasulwa A (-0.435084, 34.211620) villages in Homa Bay County and Katito (− 0.314557, 35.006869), Kisumu County. Stool samples of standard size − 41 mg were obtained from the children and tested for infection through the Kato–Katz method [1, 23]. Briefly, the stool was placed on a template that approximates the sample to about 43 mg per slide. The samples were then pressed with a cellophane strip coated with Malachite green dye, and slides were observed under a compound microscope (Axiocam ERc5s at 400× magnification) for *S. mansoni* eggs. The number of eggs per gram (epg) for each slide was counted. Forty-five children were found to be positive, of which those with 200 epg or more were recruited to provide additional stool samples to supply eggs as a source of eggs for miracidia while those with low egg burdens were immediately treated with praziquantel, according to the Kenya Government Ministry of Health guidelines using a Ugandan-model dose pole [24]. The children who provided samples were treated afterwards with a single dose of praziquantel (40 mg/kg). All treatments were done under the supervision of a qualified and competent clinician. The stool was collected in plastic containers with lids sealed with cling film. *Schistosoma* eggs were isolated from the stool sample by passing it through a series of sieves of different pore sizes (212, 180, 150, 45 µms) using 8.5% saline solution to harvest eggs and ensure they do not hatch. The isolated eggs were stored overnight in falcon tubes with saline, and experiments were conducted the day after egg collection to ensure their viability was not affected by storage time or overexposure to cold temperatures. When miracidia were needed for experiments, the falcon tubes with the eggs were poured into 5 L of bottled water in a large conical flask. The flask was left on a bench near a window for 2 h to allow the ova to hatch into miracidia. Afterwards, the flask was covered with a piece of aluminium foil so that the phototropic miracidia swam up to the water surface,

where they were collected and transferred into a petri dish and utilized in the experiment.

Insecticides

We tested the effects of two insecticides with different modes of action, the neonicotinoid imidacloprid acting on the nicotinic acetylcholine receptors and the organophosphate diazinon inhibiting the enzyme acetylcholinesterase (AChE). Both compounds are among those that typically drive the overall risk of agricultural pesticides to freshwater invertebrates in the study area [9]. Imidacloprid was provided with the formulated plant protection product Loyalty® 700 WDG (distributed by Greenlife Crop Protection Africa, Nairobi; manufactured by Shandong United Pesticide Industry China) containing 700 g imidacloprid per kg as the active ingredient. Diazinon was provided as the product Diazol® 60 EC (emulsified concentration, repacked and distributed by Laibuta Chemicals Ltd, Nairobi, an insecticide of the chloronicotinyl class; and containing an insecticide that contains diazinon 600 g per kg, an organophosphate, as an active ingredient. Both formulated products are commonly sold in the study area. Fresh stock solutions based on the required active ingredient concentration were prepared the night before the experiments. The stock solutions were then left to stir overnight in amber glass bottles covered with foil and used to produce the remaining concentrations through dilutions on the morning of experimentation.

Miracidia and cercariae acute toxicity assay

Miracidia and cercariae were constantly exposed to imidacloprid and diazinon for 24 h. Immobilization was recorded after 1.5, 3, 6, 12 and 24 h under a dissecting microscope (Zeiss AxioCam5 100-400×). As the tested miracidia showed little immobilization after up to 3 h and high immobilization of 100% even in the controls after 24 h, later analysis was limited to observations after 6 and 12 h (see below). Test concentrations were chosen based on preliminary experiments such that they covered the range of 5–95% immobilization to estimate the median effective concentration required to immobilize 50% of the individuals (EC50) in 12 h. Miracidia were tested at ambient temperate conditions (approx. 25 °C) with the following nominal test concentrations of both imidacloprid and diazinon: control (no compounds), 1, 4, 14, 55 and 209 µg/L). Each petri dish contained ten miracidia in 2 ml test concentration. Cercariae were tested with the following concentrations of both pesticides: control (no compounds), 1, 4, 14, 55, 209 and 792 µg/L. Each petri dish contained ten cercariae in 2 ml test concentration. Cercariae in the first petri

dishes were tested at approx. 25 °C like the miracidia. However, we observed high immobilization in the controls so that subsequent tests with cercariae were done in a temperature-controlled room at 18 °C. Data from a recent field survey in the area found temperatures as low as 16.9 °C [6]. Tests were done in triplicate on three separate days, except for cercariae exposed to imidacloprid, to which a fourth day with three replicates was also done (Additional file 2: Table S1). To account for potential host-mediated variability, the tested cercariae were collected and mixed from different snails, and miracidia were collected and mixed from egg batches from different children.

Miracidia host-seeking assay

We exposed miracidia to different concentrations of pesticides before allowing them access to a snail host. Using a pipette under a dissecting microscope, we distributed 1080 miracidia to six petri dishes (60×20 mm, PYREX 1480102D). Two petri dishes served as control, while each of the other dishes contained one of the following nominal test concentrations: 4.88 µg/L imidacloprid (~3% of the 6 h EC50 for miracidia), 48.8 µg/L imidacloprid (~33% of the 6 h EC50), 1.05 µg/L diazinon (~2% of the 6 h EC50 for miracidia), and 10.5 µg/L diazinon (20% of the 6 h EC50). The lower test concentrations are environmentally relevant: their toxicity to aquatic invertebrates reaches -1 (for imidacloprid) to 0 (for diazinon) toxic units, defined as $log_{10}\left(\frac{\text{Concentration}}{\text{acute EC 50 of standard test organism}}\right)$, when considering a 96h EC50 of 55 µg/L imidacloprid for the sensitive standard test species *Chironomus riparius* and a 48 h EC50 of 1 µg/L diazinon for *Daphnia magna* [29]. Toxic units of various pesticides calculated in the same way typically range up to ca. − 1.5 in grab samples of agricultural freshwater bodies in western Kenya [6, 9] and also in Europe and Australia [17, 18]. However, effects on the macroinvertebrate community are typically driven by short-term exposure peaks due to run-off from agricultural fields after rainfall; such peaks last for few hours and have been shown to exceed background concentrations from grab samples by an average factor of 54 and to reach toxic units up to 0 [17], resembling the toxicity of our test concentrations.

We tested 180 miracidia per compound and concentration. After pesticide exposure for two, four and six hours, respectively, 60 miracidia were collected from each petri dish and distributed into twelve 100-ml borosilicate crystallizing glasses (70×40 mm, PYREX). Each cup contained 70 ml bottled water and a single non-contaminated *Biomphalaria pfeifferi* snail, such that each of the 12 snails per pesticide, concentration and exposure time was exposed to five miracidia. The

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miracidia were given 6 h to infect their host snail. The snails were then removed from the oviposition cup and washed by shaking vigorously in distilled water followed by spraying with distilled water from a wash bottle. They were reared in round plastic tubs (48 cm diameter) with 2.5 L of lake water in the screenhouse as above, for three days to ensure successful infection has taken hold within the snail. After the three days, the snails were frozen and stored for molecular analysis to confirm successful penetration by the miracidia as described below.

Sporocyst development assay

To test the effect of pesticide exposure on the growth and maturation of sporocysts, we first exposed 240 non-infected snails to fresh miracidia, with the aim to infect them. This was done by placing each snail with five miracidia in a glass dish with 100 ml of bottled water for six hours indoors at ambient temperature. We used bottled water to provide optimum conditions for the miracidia to penetrate and infect the snails. After infection, the snails were distributed into six 48 cm circular plastic tubs containing 40 snails each and reared in an outdoor greenhouse at ambient conditions. The tubs were then randomly assigned to two non-contaminated controls and two test concentrations per pesticide similar to those in the miracidia host-seeking assay (see above). All test concentrations were below 0.01% of the 24 h acute median lethal concentration (LC50) for *B. pfeifferi* [6]. To mimic pulse exposure in the field, the infected snails were exposed to the described nominal pesticide concentrations at ambient temperature in glass bowls (48 cm diameter) for 24 h once a week, beginning three days post-infection. After pesticide exposure the snails were washed with lake water before being placed in their original tubs with lake water. The snails were otherwise reared in lake water, as above, that was changed weekly, and were fed with boiled kale. Beginning four weeks after the first exposure, the snails were checked every two days for cercariae shedding by exposing them to artificial light and observing them under a compound microscope. All positive snails were immediately removed for storage in 70% ethanol. Dead snails were also collected in 70% ethanol. Molecular screening was done on all stored samples for *Schistosoma* DNA to confirm the infection status as well as to detect prepatent infections that did not lead to cercarial shedding. The experiment concluded when all snails had died or been collected.

Molecular analysis

To ensure penetration of snails by miracidia had occurred, snails were tested for *Schistosoma* infection using polymerase chain reaction (PCR) assays to amplify any *Schistosoma* DNA within the snail using primers from Sady et al., [26]. At point of testing, snails were removed from the freezer and the soft body extracted from the shell using forceps, and cut into small pieces. The bodies were then transferred to an Eppendorf tube and homogenized using a motorized homogeniser, and DNA was extracted using standard protocols [25]. The DNA obtained was then amplified using conventional PCR [26] using the following primers: ShbmF (5′-TTTTTTGGTCATCCTGAGGTGTAT-3′), ShR (5′-TGATAATCAATGACCCTGCAATAA-3′) and SmR 5′-TGCAGATAAAGCCACCCCTGTG-3′). Briefly, a total volume of 50 µl containing 10 mM Tris–HCl (pH 9.0), 50 mM KCl, 0.1% Triton® X-100, 200 µM dNTP (Promega, Madison, WI, USA), 2.5 mM MgCl₂, 0.2 µM of each primer, 1 unit of *Taq* polymerase (Promega, Madison, WI, USA), and approximately 75 ng of schistosome genomic DNA. The thermal cycling profile included an initial denaturation step at 94 °C for 5 min, followed by 35 cycles of 45 s at 94 °C, 45 s at 58 °C, 30 s at 72 °C and a final step of 7 min at 72 °C using a GeneAmp 2400 (Applied Biosystems, Foster City, CA, USA) thermal cycler. Amplicons were electrophoresed in a 10% agarose gel, stained with ethidium bromide and visualized in a UV chamber (ngenius syngene bio imaging. A 100bp DNA ladder was used to determine the product sizes. Presence of *Schistosoma* DNA was confirmed by a band on the agarose gel at 250 bps, indicating miracidia penetration of a snail (Additional file 1: Fig. S1).

Data analysis

Datasets were analysed using RStudio for Windows (version 4.1,1, 2021-08-10) and R for Windows (software R 3.6.2) [27]. When analysing the acute toxicity tests with miracidia and cercariae, only replicate tests in which ≤ 60% of the control organisms in showed immobilization were considered. Moreover, when two out of three replicates from the same day showed higher immobilization in the controls, all three replicates were discarded. Ultimately, this resulted in two to five petri dishes per life stage, compound and concentration being analysed after constant exposure for 12 h, and in six to ten petri dishes per life stage, compound and concentration analysed after constant exposure for 6 h (see Additional file 2: Table S1 for details). Each dish contained ten miracidia or cercariae, respectively.

First, we tested whether the different test temperatures had a significant effect on the observed concentration vs. immobilization relationship for cercariae. Therefore, we fitted a quasi-binomial generalized linear model (GLM) to the data from tests with both pesticides imidacloprid and diazinon together. We specified the following effects (explanatory variables) incl. all their interaction terms: pesticide

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identity, pesticide concentration, exposure time (6 and 12 h) and temperature. To improve model diagnostics, pesticide concentration was log-transformed and 0.5 (half of the lowest test concentration) was added to avoid negative infinite values for the control. We used a probit link function which provided the best fit. Then we applied backward selection by successively removing all effect terms that were considered non-significant ($p > 0.05$) based on χ^2-tests for the increase in residual deviance due to effect term removal [28]. The resulting minimal adequate model contained only pesticide identity, pesticide concentration, exposure time, and the two-way interaction of pesticide identity and pesticide concentration, but not temperature (see Results). Therefore, temperature was not considered relevant in the following analysis.

We estimated the acute median effective concentration which increased immobilization of miracidia and cercariae by 50% (EC50) after 6 h and 12 h, respectively, from non-linear regression using the drc package 3.0–1. While it is more difficult with these models to test complex effect interactions, model fitting is more flexible as the upper limit of the fitted dose–response curve is variable and can be estimated from survival in the controls. This way, the EC50 may be estimated with higher precision as compared to GLMs. We used separate three-parameter binomial log-logistic models for each pesticide, exposure time and life stage (miracidia and cercariae). Only in case a three-parameter model provided a bad fit (assessed visually), we used a five-parameter model instead; this was the case for the analyses of the effects of diazinon on miracidia after 6 h and on cercariae after 12 h. The five-parameter models included an additional parameter for the lower boundary of the fitted log(dose)–response curve (which was pre-set to zero) and a shape parameter to enable the fitting of asymmetric log(dose)–response curves. Because the different test temperatures for cercariae showed no significant effect on the log(dose)–response relationship in the GLM (see above), we did not differentiate according to temperature in the non-linear models.

The infection success of miracidia on snails was analysed using a binomial generalized linear model with a probit link function. The model initially contained pesticide identity, pesticide treatment (control, low and high concentration), exposure time, and all possible interactions. Pesticide treatment was specified as a categorical factor and not as a numeric variable to test for significant effects at the limited number of test concentrations rather than fitting a full dose response curve. The model was reduced using backward selection as described above; only the main effect of

pesticide treatment remained in the final model. The model was analysed using the R packages MASS 7.3–51.5 and effects 4.1–4. The different pesticide treatment levels in the model were compared using likelihood ratio χ^2 tests with the phia package 0.2–1.

Pesticide effects on the sporocyst development and on the mortality of snails were analysed with a similar generalized linear modelling approach. The initial models contained pesticide identity, pesticide treatment and their interaction as fixed effects and a logit link function. After backward selection, no effect terms remained in the minimum adequate models for sporocyst development and for snail mortality (see results).

Ethical clearance
Ethical Clearance was granted from the Kenya Medical Research Institute's (KEMRI) Scientific and Ethical Review Unit (SERU) to collect stool samples from schoolchildren to obtain miracidia for experiments (KEMRI/SERU/CBRD/194/3836).

Results
Miracidia and cercariae acute toxicity tests
The life span of miracidia and cercariae is limited to approximately 24 h, such that we observed a drastic reduction in control survival of miracidia after 12 h. Thus, we limited the analyses of EC50s to effects after exposure for 6 and 12 h, rather than for 24 h which is more common in ecotoxicological testing of other species. Based on non-linear regression analysis, the exposure to imidacloprid immobilized 50% of miracidia at 149.7 (12.8–1746) µg/L after 6 h (mean ± 95% confidence interval) and at 15.6 (7.8–31.3) µg/L after 12 h (Fig. 1). Exposure to diazinon immobilized 50% of miracidia at 51.3 (37.9–69.4) µg/L after 6 h and at 20.9 (14.7–29.8) µg/L after 12 h.

Cercariae were partly tested under ambient temperature conditions (ca. 25 °C) and under controlled conditions (18 °C). Across both temperature regimes, diazinon showed a significantly steeper concentration–immobilization relationship than imidacloprid (concentration:pesticide interaction in the final model: $\chi^2 = 35.94$; df = 1; $p < 0.001$). However, temperature had no significant effect on the concentration–immobilization relationship ($p = 0.213$, deviance = 1.75, d.f. = 1, residual d.f. = 126 for the comparison of quasi-binomial GLMs with and without the temperature:pesticide concentration interaction term during backward selection). Therefore, tests with cercariae from both temperature regimes were merged in the following analysis using non-linear regression. Overall, cercariae showed greater tolerance than miracidia to imidacloprid, with an average median effective concentration (EC50) to imidacloprid of
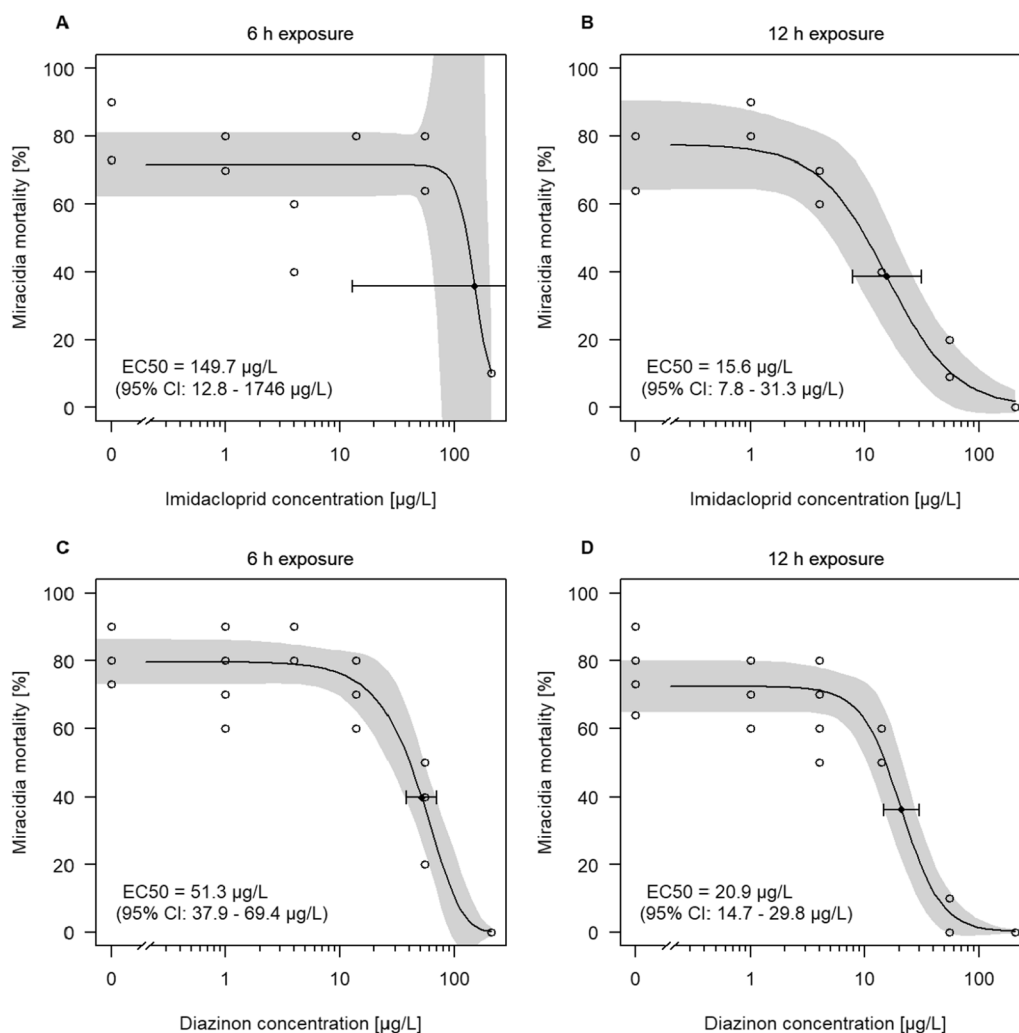
Fig. 1 Immobilization of *S. mansoni* miracidia after exposure to imidacloprid (**A**, **B**) and diazinon (**C**, **D**) for 6 and 12 h, respectively. Data points represent survival from different replicate tests, solid lines show the fitted concentration—response relationships, and the shaded areas correspond to the 95% confidence intervals. The EC50 (black dot) is shown together with the upper and lower limit of its associated 95% confidence interval
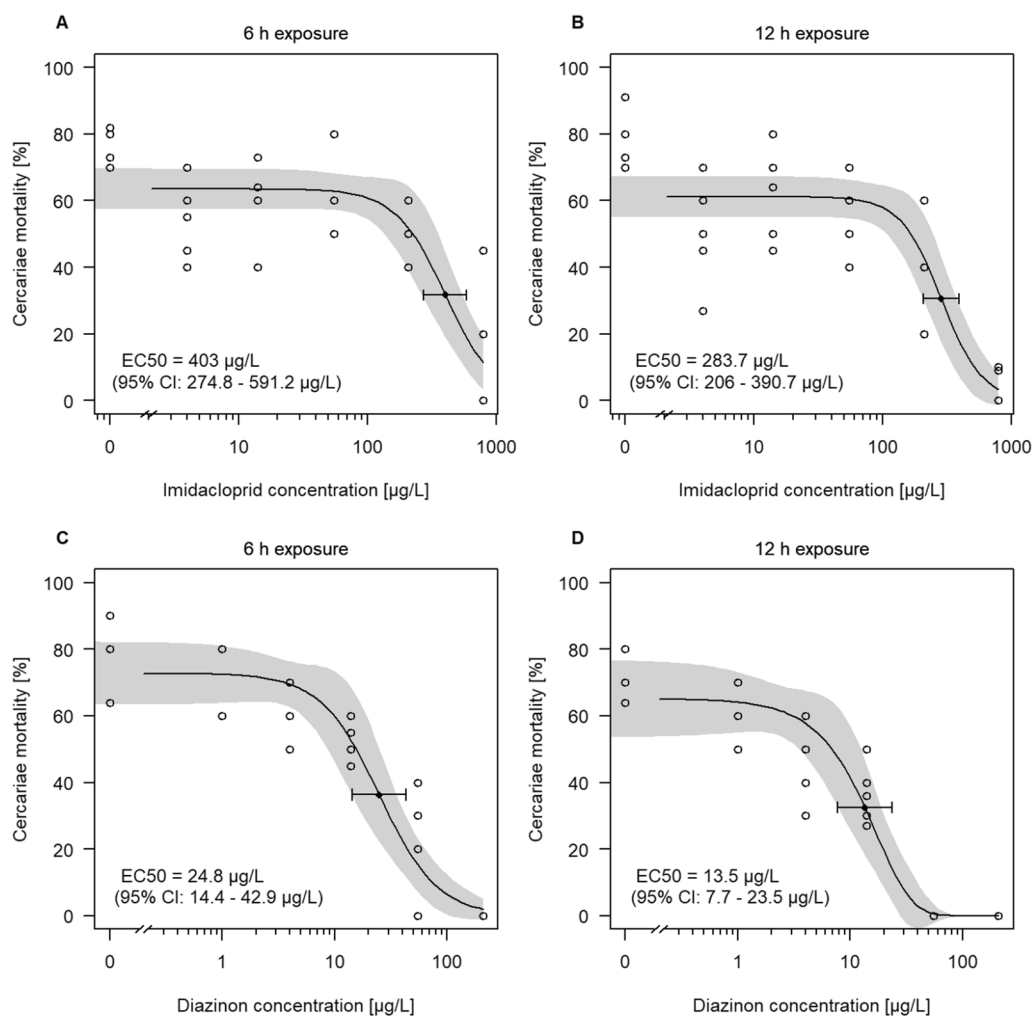
403.0 (274.8–591.2) μg/L after 6 h (Fig. 2A) and of 283.7 (206.0–390.7) μg/L after 12 h (Fig. 2B). The tolerance of cercariae to diazinon was lower, with an EC50 of 24.8 (14.4–42.9) μg/L after 6 h (Fig. 2C) and of 13.5 (7.7–23.5) μg/L after 12 h (Fig. 2D).

Miracidia host-seeking assay

To test the effect of pesticide exposure on the infectivity of miracidia, we exposed miracidia to bottled water (control) and to low or high concentrations of imidacloprid or diazinon for two, four or six hours before they had access to a snail. Infection success was identified based on the number of snails that were PCR positive for *Schistosoma* DNA (Additional file 1: Table S2). Based on the observed acute toxicity (Fig. 1), the low test

concentrations were considered to immobilize less than 1%, and the high test concentrations were considered to immobilize less than 5% of the miracidia after exposure for six hours($<$6 h EC5).

Pesticide identity (imidacloprid or diazinon) and exposure time did not significantly affect the percentage of infected snails. However, pesticide treatment (control, low or high test concentration) significantly affected the infectivity of miracidia across both pesticides ($x^2 = 23.1$, d.f. = 2, $p < 0.001$): While the low concentrations showed no significant effect ($x^2 = 0.08$, d.f. = 1, $p = 0.774$), the high concentrations decreased the percentage of infected snails from 25.7% to 2.7% ($x^2 = 10.62$, d.f. = 1, $p = 0.002$) (see Fig. 3).

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Fig. 2 Immobilization of *S. mansoni* cercariae after exposure to imidacloprid (**A**, **B**) and diazinon (**C**, **D**) for 6 and 12 h, respectively. Data points represent survival from different replicate tests, solid lines show the fitted concentration—response relationships, and the shaded areas correspond to the 95% confidence intervals. The EC50 (black dot) is shown together with the upper and lower limit of its associated 95% confidence interval

Sporocyst development assay

We tested the effect of weekly pesticide pulse exposure for 24 h to the same nominal test concentrations as used in the miracidia host-seeking assay on the development of sporocysts within snails. Effects were identified based on the number of snails that had shed cercariae after 21 days (when the first snails started shedding) and after five weeks (Additional file 1: Table S3). No statistically significant effects could be observed. Overall, only 13 out of 118 snails (11%) shed cercariae after five weeks; the proportion of shedding snails did not differ between the controls (9%), low pesticide treatments (13%) and high treatments (12%; $\chi^2 = 0.25$, d.f. = 2, $p = 0.882$ for the elimination of pesticide treatment as the last step in backward model selection).

The pesticide treatments did also not affect the survival of snails. Overall, 124 out of 280 snails (44%) survived until the end of the test; survival did not significantly differ between the controls (48%) and low pesticide treatments (44%) or high treatments (40%; $\chi^2 = 1.18$, d.f. = 2, $p = 0.555$ for the elimination of pesticide treatment as the last step in backward model selection).

Discussion

Our investigation shows that the maximum imidacloprid and diazinon concentrations observed in earlier grab samples from natural water systems of the study area in Western Kenya [9] may not affect the aquatic life-stages of *S. mansoni* directly. The median effective concentrations that immobilized 50% of miracidia and of cercariae after constant exposure for
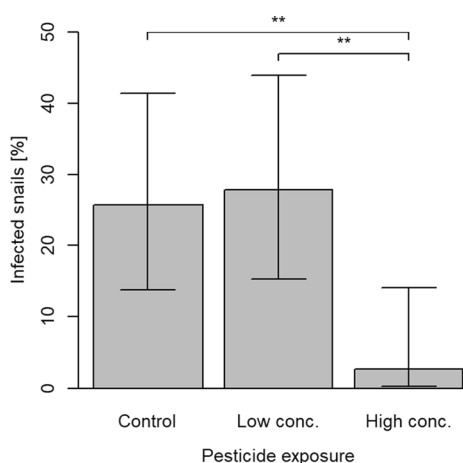
Fig. 3 Percentage (mean and 95% confidence intervals) of infected snails after contact with five miracidia per snail for six hours. The miracidia had been previously exposed to either imidacloprid or diazinon for 2, 4 or 6 h at low concentrations (imidacloprid: 4.88 μg/L; diazinon: 1.05 μg/L; equal to 2–3.3 percent of the 6 h EC50 for miracidia) or at high concentrations (imidacloprid: 48.8 μg/L; diazinon: 10.5 μg/L; equal to 20–33 percent of the 6 h EC50). Asterisks indicate significant changes in infection success as compared to the non-contaminated control (** $p < 0.01$)

6 h (6 h EC50) were three to four orders of magnitude higher than the maximum concentrations observed in the field (imidacloprid: 6 h EC50 = 149.7 and 403.0 μg/L, environment = 0.032 μg/L; diazinon: 6 h EC50 = 51.3 and 24.8 μg/L, environment = 0.020 μg/L) [9]. Sublethal high concentrations of 48.8 μg/L imidacloprid and 10.5 μg/L diazinon reduced the infectivity of miracidia but not the development of sporocysts within host snails. However, these were still 525–1525 times higher than the maximum concentrations observed in the aquatic environment [9]. Lower, environmentally relevant concentrations of 4.8 μg/L imidacloprid and 1.05 μg/L diazinon showed no effects on the development of *S. mansoni*.

The EC50s for the free-swimming life stages of *S. mansoni* are within the same range as those for standard test organisms from the aquatic invertebrate community that are typically used in ecotoxicology: e.g. the 6 h EC50s of imidacloprid for miracidia and cercariae were 2.7 and 7.3 times as high as the acute 96 h EC50 for *Chironomus riparius* (55 μg/L) [27]. The 6 h EC50s of diazinon for miracidia and cercariae were 51 and 25 times as high as the 48 h EC50 for *Daphnia magna* (1 μg/L) [29]. The EC50 values are not directly comparable as the exposure times in the tests differ, such that a simple ranking may overestimate the relative tolerance of *Schistosoma*. Nevertheless, our results suggest that *S. mansoni* is likely to benefit indirectly from pesticide exposure because it can survive together with its host snails in waters where

competitors and predators of the snails cannot. This conclusion is based on three considerations:

First, in contrast to other invertebrates, *S. mansoni* may be rarely exposed to harmful concentrations because its free-swimming life stages are only present in the water column for short periods of time. Results from the sporocysts development assay indicate that after successful infection, *S. mansoni* is well protected from pesticide exposure within the tissue of its host snail. In the field, accumulation of pesticides and thus exposure of sporocysts within the snails might be higher than in the experiment due to additional exposure pathways such as contaminated snail food and because snails may accumulate pesticides already before infection. Nevertheless, in the form of sporocysts, *S. mansoni* can escape high short-term pesticide exposure peaks to the water that follow run-off events and drive the overall risk of pesticides to invertebrates in agricultural streams [17].

Second, the standard test organisms in ecotoxicology do not always represent the most sensitive macroinvertebrate species that live in freshwaters. In acute tests, some potential competitor or predator species of host snails such as ephemeropteran species appeared about 50% less tolerant to the tested insecticides [6]. Moreover, our tests covered all relevant aquatic life stages of *S. mansoni*, whereas acute toxicity tests are limited to a single life stage that may not be the most sensitive one. Particularly for insects it has been shown that larvae can survive concentrations in acute tests that result in considerably higher delayed mortality during moulting [30, 31]. Therefore, particularly insects can be more sensitive than they appear from the available acute toxicity tests, while this seems unlikely for *S. mansoni*.

Third, the very high pesticide tolerance of host snails renders indirect effects on sporocyst development due to disturbed nutrient supply within snails unlikely, even under field conditions. The observed 6 h EC50s of imidacloprid for the miracidia and cercariae of *S. mansoni* for imidacloprid fall more than 2,400 times lower than the 24 h EC50 for the highly tolerant host snail *Biomphalaria pfeifferi* (> 1 g/L) [6]. The 6 h EC50s of diazinon for miracidia and cercariae fall more than 390 times lower than the 24 h EC50 for *B. pfeifferi* (~ 20 mg/L) [6]. Given this high tolerance of *B. pfeifferi*, it is not surprising that we also did not observe an indirect positive effect of pesticides on the infectivity of miracidia that could arise from adverse effects on the host snail immune system.

It should be noted that in the field, macroinvertebrates can respond negatively to pesticides at considerably lower concentrations than in the laboratory [6, 32]. This can be related to factors such as additional stressors

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[33, 34] and mixture toxicity [35]. Nevertheless, the observed no-effect concentrations (NOEC) of 4.88 µg/L imidacloprid and 1.05 µg/L diazinon for the overall performance of *S. mansoni* throughout its aquatic life stages were still around 60 times higher than the maximum environmental concentrations observed in western Kenya [9]. For comparison, NOECs in the aquatic environmental risk assessment of pesticides are often divided by an empirically obtained factor of 10–100 in order to account for variability in sensitivity across both species and environmental conditions [35]. However, with information on inter-species variability available, this assessment factor can be lowered, e.g. in Europe to 3 – 6 to account only for variability across environmental conditions [36]. Though the protectiveness of assessment factors has been questioned [37], the lowered factors are an order of magnitude lower than the ratio of our observed NOEC and the environmental concentrations in western Kenya. Therefore, we expect no considerable effects of pesticide exposure on the performance of *S. mansoni* to occur also under natural conditions.

We used field-collected snails that were reared for several weeks prior to the experiments to ensure they were free of schistosome infections. Comparably high mortality during breeding and in the controls indicated suboptimal breeding and test conditions, and limited the number of available replicates. This should be considered when comparing the obtained EC50 values with those from standard tests under optimum conditions. Nevertheless, suboptimum conditions represent additional stress that is likely to increase rather than decrease effects of pesticides (see Discussion above). Therefore, we consider the risk of having missed relevant effects due to suboptimum test conditions low.

Taken together, our results suggest that exposure of surface waters to agricultural pesticides may indirectly increase the risk of schistosomiasis transmission by sparing the pathogen *S. mansoni*, while the natural control of its host snail *B. pfeifferi* is affected [6]. Thus, pesticide mitigation measures should be taken in at-risk areas to prevent further exacerbation of the disease.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12302-024-00856-2.

Additional file 1: Figure S1. Gel image of the PCR results of the miracidia host-seeking assay for imidacloprid. The image shows the results for controls (well 1–12 = controls in substrate for 2 h, 13–24 = controls in substrate for 4 h and 24–36 = controls in substrate for 6 h) versus those exposed to imidacloprid at 2% the average EC50 for miracidia at 6 h (37–72, arranged for time as controls) and those exposed to imidacloprid at 40% the average EC50 for miracidia at 6 h (73–108). Each row starts with a DNA ladder and a positive control (250 bps) except the bottom row which does not have the positive control. **Table S2.** Results of the

miracidia host-seeking assay in raw format. **Table S3.** Results of the sporocyst development assay in raw format.

Additional file 2: Table S1. All acute toxicity tests raw data of miracidia and cercariae exposed to diazinon and imidacloprid and the reasons for exclusion from analysis if any.

Author contributions
ML conceived the approach; AG, UF, ML, UF, FM and EA designed the research; AG and SK conducted the research; JB, NS and AG analysed and interpreted the data; AG drafted the initial version; AG, JB, NS, SK, HH, ML, EA, FM, UF contributed to the final version—all approved the final version of the publication.

Availability of data and materials
All data generated or analysed during this study are included in this published article (and its Additional information files).

Declarations

Ethics approval and consent to participate
Ethical Clearance was granted from the Kenya Medical Research Institute's (KEMRI) Scientific and Ethical Review Unit (SERU) to collect stool samples from schoolchildren to obtain miracidia for experiments (KEMRI/SERU/CBRD/194/3836).

Consent for publication
Not applicable.

Competing interests
The author(s) declare no competing interests.

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