Pesticide exposure among organic and conventional smallholder farmers in Costa Rica and Uganda: biomarker evidence on exposure determinants

Anouk Petitpierre, Aggrey Atuhaire, Christian H Lindh, Ana M Mora, Martin Röösli, Philipp Staudacher, Samuel Fuhrimann

Summary

Background Pesticides play a crucial role in pest control; however, exposure remains high, particularly among smallholder farmers in low-income and middle-income countries (LMICs) with little access to pesticide safety training. This study aimed to provide comparative biomarker-based evidence from Latin America and Africa and identify actionable determinants of exposure to inform context-specific interventions and sustainable agricultural policies.

Methods We collected urine samples from 601 conventional and organic smallholder farmers in Zarcero County, Costa Rica, and Wakiso District, Uganda, on two occasions during the primary spraying season. Samples were analysed for seven biomarkers of six commonly used pesticides: mancozeb (ETU); 2,4-D; glyphosate; pyrethroids (3-PBA, DCCA); diazinon (IMPy); and chlorpyrifos (TCPy). Structured questionnaires assessed sociodemographic, farm-related, and pesticide-related exposure determinants. Linear mixed-effects models were used to identify exposure drivers within and between the countries. The outcome variables were the concentrations of the seven biomarkers measured in urine samples from farmers in both Costa Rica and Uganda.

Findings All pesticide biomarkers were detected in nearly all participants, confirming widespread exposure. Statistical evidence was found for the following associations: compared with Uganda, farmers in Costa Rica showed lower concentrations of fungicides and 2,4-D by 77–84%, while glyphosate and insecticide biomarkers were higher by 67–376%. Lower biomarker concentrations were significantly associated with organic and mixed farming practices (24–68%) and with previous training on pesticide safety (17–27%). In contrast, increasing age was consistently linked to higher herbicide and insecticide concentrations, and pesticide use within the last week was consistently linked to higher concentrations of all biomarkers.

Interpretation Smallholder farmers in tropical LMICs are consistently and heavily exposed to pesticides. Evidence from this study highlights the urgent need for tailored interventions—including farmer training and promotion of organic practices—to reduce health risks. Integrating biomarker-based monitoring into planetary health strategies is essential to ensure more sustainable and equitable agricultural transitions in these vulnerable settings.

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Introduction

Pesticides are used to protect crops from pests and secure agricultural yield.[1] Their use is increasing worldwide as populations and economies grow, but trends are uneven, with declines in Europe and continued increases in Africa and Latin America.[2,3] However, occupational human exposure to pesticides has been linked to adverse health effects, such as respiratory symptoms, neurological disorders, and cancer.[4,5] Smallholder farmers in tropical low-income and middle-income countries (LMICs) are especially vulnerable to pesticide-related health risks. Smallholder farmers often use pesticides as their primary pest management strategy, leading to high exposure levels.[6] Tropical settings provide a favourable environment for pests, thereby increasing the need for effective control measures such as pesticides.[7] In LMICs, effective context-specific training programmes to professionalise farmers are often in short supply, and the uptake of farming practices that reduce pesticide use, such as integrated pest management and organic or regenerative agriculture, remains slow.[8,9]

There is compelling evidence that several factors can reduce pesticide exposure, including organic farming practices, higher education level, training on safe pesticide handling, and the use of personal protective equipment (PPE).[10–14] A toxicological comparison of European pesticide active substances found that those approved for organic farming were less hazardous than those approved

Research in context

Evidence before this study

Before the initial data collection, we conducted a non-systematic literature review that was published as part of our study protocol. In addition, we carried out a systematic literature review using observational epidemiological studies in MEDLINE and Embase published 1993 to 2017 on global pesticide exposure and occupational health. For the present manuscript, we updated this evidence base through searches of PubMed covering articles on global pesticide exposure assessments in adult agricultural workers from observational epidemiological studies including articles up to Feb 28, 2025. We also incorporated relevant studies published by our research groups that applied comparable exposure assessment approaches, with a primary focus on occupational pesticide exposure among agricultural populations in low-income and middle-income countries (LMICs). Smallholder farmers in tropical LMICs are widely recognised as being highly exposed to pesticides due to frequent applications driven by high pest pressure and little adoption of safety practices. Survey-based studies consistently report poor use of personal protective equipment (PPE) and unsafe pesticide handling practices. Organic farming has shown lower exposure, but evidence from LMICs is mostly self-reported and there are few high quality large biomonitoring studies. This evidence gap hampers subsequent epidemiological research aiming to identify health risks and develop effective mitigation strategies tailored to local contexts and exposure patterns.

Added value of this study

To the best of our knowledge, this study is the first pooled pesticide exposure assessment comparing organic and conventional smallholder farmers in two socioeconomically and culturally diverse tropical LMICs, using a harmonised study design and objective biomonitoring data. Seven urinary biomarkers for six common pesticides (eg, pyrethroids, chlorpyrifos, glyphosate, and mancozeb) revealed that pesticide application within the previous week increased exposure by up to 314%, while organic farming and safety training reduced it by up to 68% and 27%, respectively. Unlike previous self-reported studies, we found no protective effect of PPE use. Incorrect glove use was even associated with elevated biomarker levels of the insecticide diazinon. These findings strengthen the foundation for future LMIC-specific exposure algorithms, which reveal far greater exposure contrasts during pesticide application. They also underscore the need for practical, evidence-based behaviour-change interventions, beyond PPE, grounded in farmers' knowledge, attitudes, and practices in real-world smallholder contexts in LMICs.

Implications of all the available evidence

The findings offer a crucial evidence base to inform and improve educational programmes on the health risks of conventional pesticide use, the benefits of organic farming, and safe pesticide handling practices. Such programmes, especially those addressing the correct use of PPE, should be tailored to the needs of vulnerable groups, particularly smallholder farmers in tropical LMICs. To facilitate transitioning to organic practices without risking crop loss and financial ruin, and to guarantee access to PPE for smallholder farmers, both financial and systemic support will be essential. Future research should expand study cohorts of smallholder farmers in tropical LMICs or other high-exposure populations and explore locally relevant behaviour-change intervention strategies to reduce their pesticide exposure.

for conventional farming,[10] and analyses from the Pesticide Use in Tropical Settings (PESTROP) study in Costa Rica similarly found lower estimated weekly exposure intensity scores among applicators working on organic farms compared with conventional farming.[12,14] In the 2024 IMPRESS study, urinary glyphosate concentration increased with illiteracy among Ugandan farmers and with lower education among farmers in the UK,[13] whereas PESTROP data indicated reduced exposure scores among Costa Rican applicators who had received training on safe pesticide handling.[12,14] A systematic review of studies published between 1999 and 2005 on the determinants of pesticide exposure in occupational and non-occupational settings found that PPE reduced exposure,[11] whereas more recent analyses from the IMPRESS study observed lower urinary glyphosate and 3-PBA concentrations among UK farmers who used either gloves or a facemask during pesticide handling than those who did not.[13] Further, exposure levels increased with the duration and frequency of application, as shown by higher urinary glyphosate and 3-PBA concentrations with longer spraying durations in Malaysia[13] and by elevated urinary biomarkers among recent (within the previous 12 months) or current applicators in Costa Rica.[14] Within the same Costa Rican study population, increased biomarker concentrations were observed in male workers, workers of Nicaraguan origin, and ever-smokers.[12,14]

However, a systematic review of methods used to assess exposure to pesticides in occupational epidemiology studies found little research in LMICs, and a lack of studies relying on direct exposure assessment methods, such as biomonitoring or personal sampling to measure exposure levels in the target population. Conversely, indirect methods estimate exposure using self-reported information, expert assessment, or exposure models.[15] Although direct exposure assessment methods of non-persistent pesticides might not always provide a comprehensive picture due to their short half-lives, they offer a more objective basis for evaluating exposure determinants than estimates derived from indirect exposure assessment methods, which often incorporate some of the same determinants under investigation.

In our study, we aimed to characterise exposure to frequently applied pesticides, ie, one fungicide (mancozeb), two herbicides (2,4-D and glyphosate), and three insecticides (pyrethroids, diazinon, and chlorpyrifos), and identify determinants of exposure to these pesticides, such as farming practice and country, in tropical LMICs.

Methods

Study design

Our study was part of the PESTROP project, an interdisciplinary and transdisciplinary research project studying environmental, health, and institutional dimensions of pesticide use in tropical settings. We conducted a harmonised study design among conventional and organic smallholder farmers in a tropical region in Costa Rica and a tropical region in Uganda, with minor site-specific adaptations to the questionnaire. We conducted exposure measurements at two timepoints, spaced two to four weeks apart during the primary spraying season, to assess the temporal variability of pesticide exposure among smallholder farmers.[16,17]

The Costa Rican participants were from commercial small-scale horticultural farms within the Tapezco River catchment area in Zarcero County. The main crops grown in this study area include potatoes, carrots, and coriander for conventional farmers, and tomatoes, bell peppers, and lettuce for organic farmers. The Ugandan participants were from subsistence-based mixed horticultural and livestock production farms within the Mayanja River catchment area in Wakiso District. The main crops grown include beans, maize, (sweet) potato, and bananas, across all farming practices. In Costa Rica, we collected data from June to September, 2016, and in Uganda, we collected data from September to November, 2017. Both time frames lie within the countries' primary spraying season.[17] From here onward, we use Costa Rica and Uganda to refer to Zarcero County and Wakiso District; however, this does not mean that statements are generalisable to these countries.

All study materials were approved by the human subjects committee of the Universidad Nacional in Costa Rica (UNA-CECUNA-ACUE-04-2016), the Higher Degrees, Research and Ethics Committee (HDREC) of Makerere University in Uganda (HDREC 522), and the Ethical Board of the Ethikkommission Nordwest- und Zentralschweiz in Switzerland (EKNZ-UBE 2016-00771).

Participants

To be eligible for study participation, participants had to be aged 18 years or older, and they had to own, live, or work on a smallholder farm located within the study area. Farmers were identified through local farmer lists, community leaders, and field visits, and a random subset of those expressing interest were enrolled, aiming for an equal distribution of conventional and organic farmers to ensure a contrast of related characteristics in our study population. We enrolled 300 farmers in Costa Rica, and 302 farmers in Uganda. At enrolment, each participant gave written informed consent (if literate) or was read the study details and provided a fingerprint as consent. More detailed information on participant recruitment is found in published study protocols.[16,17]

Procedures

Trained research assistants collected spot urine samples and anthropometric data, and conducted structured interviews to gather sociodemographic, farm-related, and pesticide-related information. Urine samples were analysed for pesticide biomarkers using liquid chromatography-triple quadrupole mass spectrometry at Lund University, Sweden.[18,19] The laboratory participates in G-EQUAS for analysis of glyphosate, TCPy, and 3-PBA in urine (appendix p 3).

See Online for appendix

Data sources and time of data collection for all collected data used in this study are summarised in figure 1. More detailed information on procedures is found in the appendix (p 2) and the published study protocols.[16,17]

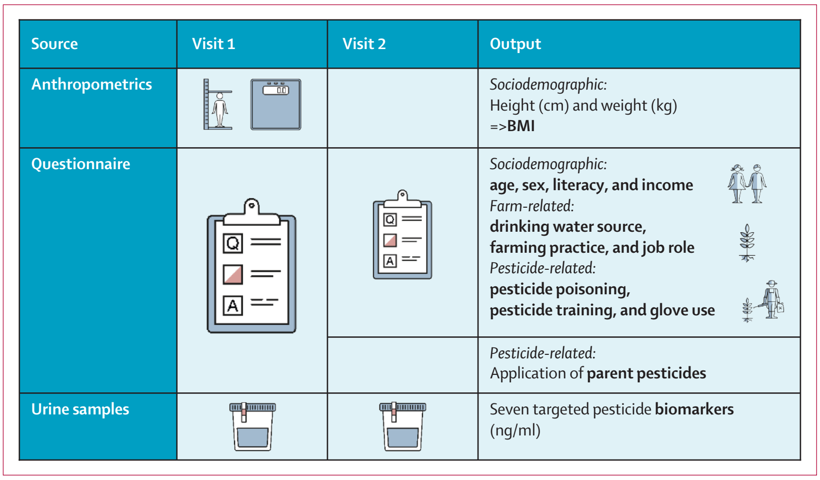
Outcomes

The outcome variables were the concentrations (ng/ml) of the seven biomarkers measured in urine samples from farmers in both Costa Rica and Uganda: a biomarker of the fungicide mancozeb, ethylene thiourea (ETU); the herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and glyphosate; 3-phenoxybenzoic acid (3-PBA), a non-specific pyrethroid insecticide biomarker, 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid (DCCA), a biomarker of the pyrethroid insecticide cypermethrin; 2-isopropyl-4-methyl-6-hydrocypyrimidine (IMPy), a biomarker of the organophosphate insecticide diazinon; and a biomarker of the organophosphate insecticide chlorpyrifos, 3,5,6-trichloro-2-pyridinol (TCPy). An overview of each biomarker's parent pesticide, pesticide type, chemical class, and limit of detection (ng/ml) is found in the appendix (p 6). Biomarker concentrations below the limit of detection were retained as machine-read values for statistical analyses.[20,21] Biomarkers were adjusted for urinary dilution using specific gravity and $\log_{10}$-transformed to remove right-skewness.[14]

Predictors

Exposure predictors included sex assigned at birth (male or female), age in years, BMI (underweight [$<18·5$ kg/m^2], normal weight [18·5–24·9 kg/m^2], overweight [$\geq 25·0$ kg/m^2], or unknown), the ability to read and write (no or yes), monthly household income, converted to purchasing power parity (PPP), per capita below the global poverty line (no or yes), drinking water source (pipes, open water bodies, pipes and open water bodies, or unknown), history of pesticide poisoning (no, yes, or unknown), farming practice (conventional, mixed, organic, or non-applicator), job role (in Costa Rica farm owner or farm worker; in Uganda crop farmer or other), training on safe pesticide handling (no or yes), glove use during pesticide application (no, yes, or unknown), and time since last application of a pesticide from the biomarker's parent pesticide type (never, last year, last week, or unknown).

The global poverty line was set at PPP 5·50 per day per capita for Costa Rica, according to the defined global poverty line for upper-middle-income countries from 2011 to



Source	Visit 1	Visit 2	Output
Anthropometrics			*Sociodemographic:* Height (cm) and weight (kg) **=>BMI**
Questionnaire			*Sociodemographic:* **age, sex, literacy, and income** *Farm-related:* **drinking water source, farming practice, and job role** *Pesticide-related:* **pesticide poisoning, pesticide training, and glove use**
			Pesticide-related: Application of **parent pesticides**
Urine samples			Seven targeted pesticide **biomarkers** (ng/ml)

Figure 1: **Overview of all variables' data sources and time of data collection**

2016, and at PPP 2·15 per day per capita for Uganda, according to the defined global poverty line for low-income countries from 2017 to 2020.[22] Farming practices were reclassified based on self-reported use of synthetic pesticides, as definitions of conventional and organic farming varied across sites in Costa Rica and Uganda. Some farms were certified as organic for specific crops but used conventional practices for others. Additionally, some workers reported employment on organic farms but also worked on farms using synthetic pesticides. We defined conventional farming as the exclusive use of synthetic pesticides, organic farming as the exclusive use of non-synthetic pesticides, mixed farming as the use of both, and non-applicator as the absence of any pesticide application.[17] More detailed information on variable modification can be found in the appendix (p 5).

Statistical analysis

The study size calculation was based on a subsequent health risk assessment, as described in the study protocol,[16] which recommended a minimum sample size of 300 farmers per country. For variable selection, we created a directed acyclic graph based on expert opinion and evidence from previous literature.[12–14,17] When variables were correlated (eg, height and BMI), the variable of primary interest was retained. The process of variable selection is described in more detail in the appendix (p 4).

We created boxplots of specific gravity-adjusted biomarker concentrations in both Costa Rica and Uganda. We computed between-biomarker correlations and intraclass correlation coefficients to investigate between-to-within-subject correlations of measured urinary biomarkers.

For each biomarker, we used a linear mixed-effects model with all determinants as predictors and participant identification as a random effect to adjust for repeated measurements. Additionally, we conducted a pooled analysis of all biomarkers belonging to the same pesticide type, eg, a pooled analysis of 2,4-D and urinary glyphosate to estimate the common effect of exposure determinants on herbicides. To this end, we Z standardised all biomarker concentrations and used a linear mixed-effects model with all biomarkers belonging to the respective types as outcomes, all determinants as predictors, and biomarker as a fixed effect. All models were run both stratified by country and pooled. In the pooled models, only the exposure determinants with the most consistent associations in the stratified models were included, namely age, sex, drinking water source, farming practice, training on safe pesticide handling, use of gloves, and time of last pesticide application. Moreover, country was added as a fixed effect to the determinants.

The statistical significance level was set at 5%. Regarding multiple testing, we followed the *Guidelines for Multiple Testing in Impact Evaluations of Education Interventions*, which state that adjustment for multiple testing is not required for exploratory analyses such as ours, but that authors might wish to adjust for multiple testing to avoid spurious correlations if the data can be structured appropriately and statistical power levels are deemed to be tolerable.[23] We thus used Holm's method to adjust for multiple testing within countries and predictors, and reported both unadjusted and adjusted p values.

Additionally, we visualised between-biomarker Spearman correlation coefficients (r_S), the resulting Akaike Information Criteria of models with and without plausible interaction effects (eg, pesticide training and glove use), and the distributions of biomarker concentrations and intraclass correlation coefficients by farming practice and applicator status as shown in the appendix (pp 10, 17–19). Missing observations in categorical determinants (BMI, drinking water source, and time of last application of parent pesticide) were replaced by an additional category labelled unknown. Missing observations in continuous determinants (monthly household income) were imputed using the R package missForest: a non-parametric single imputation tool using random forest.[24,25] Missing outcome observations (biomarker concentrations) were excluded from data analysis (figure 2). All statistical analyses were performed in RStudio (R version 4.3.2 [2023-10-31 ucrt]).

Role of the funding source

The funders had no role in study design, data collection, data analysis, data interpretation, writing of the manuscript, or the decision to submit for publication.

Results

The number of participants who were eligible for study participation and included in the regression models after exclusion of participants with missing biomarker measurements at both study visits is shown in figure 2.

We observed differences in age, BMI, poverty rate, and drinking water sources between countries. In both countries, most participants were male, with a more pronounced sex imbalance in Costa Rica, where only 27 (9%) of 299 participants were female (table 1). In Costa Rica,

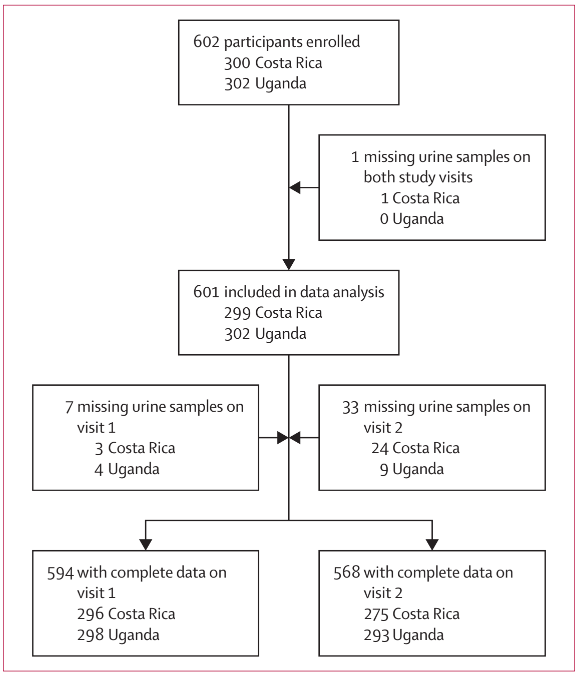


Figure 2: **Flowchart of study participants**

30 (11%) of 279 lived below the global poverty line, whereas in Uganda, 233 (78%) of 297 participants lived below the poverty line. Although 85% of participants in both countries (n=254 in Costa Rica and n=256 in Uganda) reported no history of pesticide poisoning, the distribution of the remaining responses differed. In Costa Rica, most of the remaining participants reported a history of previous pesticide poisoning, while in Uganda, ten (3%) reported poisoning, whereas the rest were uncertain. A larger proportion of participants in Costa Rica reported having received training on safe pesticide handling (123 [41%]) compared with Uganda (69 [23%]). Similarly, the use of gloves during pesticide applications was more frequently reported in Costa Rica (86 [29%]) than in Uganda (37 [12%]).

There were approximately twice as many fungicide and herbicide applicators in Costa Rica as in Uganda, and a higher percentage of Costa Rican farmers reported applications within the last week, indicating a higher application frequency (table 2). The percentage of insecticide applicators was similar in both countries; however, the percentage of applications within the last week appeared to be almost twice as high in Costa Rica as in Uganda.

Limits of detection are shown as dashed horizontal lines, and intraclass correlation coefficients are provided by biomarker and country (figure 3). Detection frequencies were close to 100% for almost all biomarkers, regardless of study visit and country, except for urinary glyphosate in Uganda (detection frequency 60–70%). Intraclass correlation coefficients between first and second visits were overall low, except for TCPy, which had intraclass correlation coefficients of 0·72 in Costa Rica and 0·87 for Uganda. The highest concentrations were observed for TCPy in both countries. The limit of detection, detection frequency, minimum, maximum, median, and IQR of each biomarker by country and study visit are summarised in the appendix (p 8).

A simplified visualisation of the regression models' effect estimates is shown in figure 4. An effect estimate of value x is to be interpreted as the average x-fold change in the concentration of the respective individual biomarker, or of the respective pesticide type. Alternatively, an x-fold change corresponds to an $(x-1)*100\%$ difference. A complete table of all effect estimates, corresponding 95% CIs, and p values is found in the appendix (p 11). Here, we only summarise statistically significant effect estimates between exposure determinants and individual biomarkers.

Fungicide (ETU) concentrations were lower in Costa Rica than in Uganda by 77% and decreased with age by 10% per ten years in Uganda. Concentrations were also lower in women compared with men in Uganda by 33% and were reduced by 27% in mixed farms and by 41% in organic farms compared with conventional farms in Costa Rica. In contrast, farmers who were overweight in Uganda had higher concentrations than those who were a normal weight by 101%. Recent fungicide application was associated with increased concentrations, with applications within the past year resulting in 40% to 43% higher concentrations, and applications within the past week resulting in 182% to 248% higher concentrations (figure 4).

Overall, herbicide concentrations were lower in Costa Rica than in Uganda by 44%, with different patterns across biomarkers: while 2,4-D was reduced by 84%, urinary glyphosate was increased by 74%. Concentrations increased with age, ranging from 7% to 19% per ten years depending on the biomarker and country. Females had lower herbicide concentrations than males, with reductions ranging from 31% to 52% across pooled and country-specific biomarkers. Mixed farming was associated with lower herbicide levels compared with conventional farming, with decreases ranging from 27% to 47%. Recent herbicide applications were associated with higher concentrations: applications within the past year increased levels by 23% overall, whereas applications within the past week showed stronger increases, ranging from 64% to 314% (figure 4).

Insecticide concentrations were generally higher in Costa Rica than in Uganda, ranging from 67% to 476% increases depending on the biomarker. Concentrations increased with age, with a 7% to 22% increase per ten years. Females had 52% to 69% lower concentrations than males in Costa Rica, whereas in Uganda DCCA concentrations were 31% higher in females than males. Mixed farming was associated with 25% lower insecticide concentrations, while organic farming showed stronger reductions, ranging from 24% to 68%. Farmers who had received training on safe pesticide handling had 17% to 27% lower

	Costa Rica (n=299)	Uganda (n=302)	Missing (%)
Age, years	33 (25–49)	49 (38–57)	0
Sex			0
Female	27 (9%)	125 (41%)	
Male	272 (91%)	177 (59%)	
BMI			3 (1%)
Healthy	120/296 (41%)	201 (67%)	..
Underweight	0/296	16 (5%)	..
Overweight	176/296 (59%)	85 (28%)	..
Literate	283 (95%)	271 (90%)	0
Monthly household income per capita below global poverty level*	30/279 (11%)	233/297 (78%)	25 (4%)
Drinking water source			98 (16%)
Pipes	192/203 (95%)	82/300 (27%)	..
Open water	11/203 (5%)	93/300 (31%)	..
Pipes and open water	0/203	125/300 (42%)	..
History of pesticide poisoning			0
No	254 (85%)	256 (85%)	
Yes	43 (14%)	10 (3%)	
Unknown	2 (1%)	36 (12%)	
Farming practices			0
Conventional	148 (49%)	150 (50%)	
Mixed	71 (24%)	84 (28%)	
Organic	34 (11%)	40 (13%)	
Non-applicator	46 (15%)	28 (9%)	
Job role†			0
Crop farmer	0	227 (75%)	
Other	0	75 (25%)	
Producer	113 (38%)	0	
Farm worker	186 (62%)	0	
Had training	123 (41%)	69 (23%)	0
Glove use during pesticide applications			0
No‡	213 (71%)	263 (87%)	
Yes	86 (29%)	37 (12%)	
Unknown	0	2 (1%)	

Data are median (IQR), n (%), or n/n with available data (%). Summary statistics are stratified by country. The percentage of missing observations corresponds to raw observations before data manipulation. *Defined in purchasing power parity (PPP) according to the World Bank (2025). The Costa Rica poverty line for upper-middle-income countries as defined in 2011 (PPP 5·50 per capita per day). The Uganda poverty line for low-income countries as defined in 2017 (PPP 2·15 per capita per day). †Job role was defined differently between countries to reflect local practices. In Uganda, we asked for the main profession (either crop farmer or other). In Costa Rica, we asked whether farmers were farm owners (producers) or farm workers. ‡Non-applicators were automatically categorised as non-users.

Table 1: **Overview of constant (unchanged between study visits) determinants**

concentrations. Recent insecticide application increased concentrations: applications within the past year increased levels by 23% to 41%, and applications within the past week increased concentrations by 37% to 113%. Other factors showed less consistent associations, with some biomarker-specific effects: farmers who were overweight had 39% lower IMPy concentrations, farmers who were literate had 71% lower IMPy concentrations, using both pipes and open water decreased 3-PBA and DCCA by 27% to 32%, farm workers in Costa Rica had 54% higher IMPy and TCPy than farm owners, and glove use increased IMPy concentrations by 43% to 110% (figure 4).

Discussion

All seven biomarkers were detected in nearly all participants in both countries, confirming widespread pesticide exposure of smallholder farmers in tropical LMICs. Urinary insecticide and urinary glyphosate concentrations were higher in Costa Rica, whereas 2,4-D and fungicide concentrations were higher in Uganda. The highest increase in biomarker concentrations was observed among applicators, particularly among those who had sprayed within the past week. Lower biomarker concentrations were found in organic farmers, and farmers with pesticide safety training.

Differences between countries likely reflect variations in pest pressure, climate, crops, and farming practices. For example, on the one hand higher ETU concentrations in Uganda are consistent with its higher elevation and temperature, which support fungal growth.[26] Higher 2,4-D concentrations in Uganda are consistent with its use on maize, a key crop in Wakiso District.[17] On the other hand, higher urinary glyphosate, IMPy, and TCPy concentrations in Costa Rica could result from the region's emphasis on commercial farming, which requires higher yields and more intense spraying.[2]

Although Costa Rica had a higher number of applicators overall and more participants reporting pesticide application within the past week—suggesting a potentially higher application frequency—biomarker concentrations were not consistently elevated, probably due to lower pesticide quantities used per application, increased training, and greater PPE use. The distribution of biomarker concentrations remained stable across study visits, despite considerable variability within individuals. This observation indicates that broader, population-level factors mainly influence exposure, and although a cross-sectional study design is adequate for assessing exposure at the population level, a longitudinal design is necessary to examine exposure at the individual level.

Higher biomarker concentrations with increasing age could be due to slower metabolism and differences in fat distribution and body water compared with younger patients, as these factors alter the distribution and elimination of lipophilic compounds, such as pyrethroids.[27] Lower exposure levels in females could result from stricter adherence to safety guidelines compared with males.[28] An unexpected finding was the positive relationship between IMPy concentrations and glove use. During data collection, we observed that many farmers used gloves inconsistently and did not wash their gloves or hands after handling pesticides. Inconsistent use and neglecting handwashing could have negated the protective effects of glove use. Previous research indicates that gloves only reduce exposure when combined with proper hygiene practices.[29] Using inappropriate gloves or failing to wash gloves after

	Costa Rica visit 1 (n=296)	Costa Rica visit 2 (n=275)	Uganda visit 1 (n=298)	Uganda visit 2 (n=293)	Missing (%)
Any fungicide last applied					100 (9%)
Never	66/244 (27%)	69/227 (30%)	184 (62%)	178 (61%)	..
Last year*	97/244 (40%)	103/227 (45%)	72 (24%)	77 (26%)	..
Last week	57/244 (23%)	51/227 (22%)	42 (14%)	38 (13%)	..
Unknown	24/244 (10%)	4/227 (2%)	0	0	..
Any herbicide last applied					100 (9%)
Never	59/244 (24%)	56/227 (25%)	128 (43%)	122 (42%)	..
Last year*	132/244 (54%)	150/227 (66%)	149 (50%)	144 (49%)	..
Last week	41/244 (17%)	20/227 (9%)	21 (7%)	27 (9%)	..
Unknown	12/244 (5%)	1/227 (0%)	0	0	..
Any insecticide last applied					1 (0%)
Never	115/295 (39%)	97 (35%)	130 (44%)	121 (41%)	..
Last year*	83/295 (28%)	91 (33%)	120 (40%)	118 (40%)	..
Last week	98/295 (33%)	86 (31%)	48 (16%)	54 (18%)	..
Unknown	0/295	0	0	0	..

Data are n (%) or n/N (%). The percentage of missing information corresponds to the raw observations before data manipulation. Application recency by use type has been pooled from the originally reported application recency by individual pesticide due to incomplete reporting. An overview of application recency by individual pesticides is found in the appendix (p 7). *Does not include participants who applied within the last week.

Table 2: **Overview of time-varying exposure determinants, stratified by country and study visit**

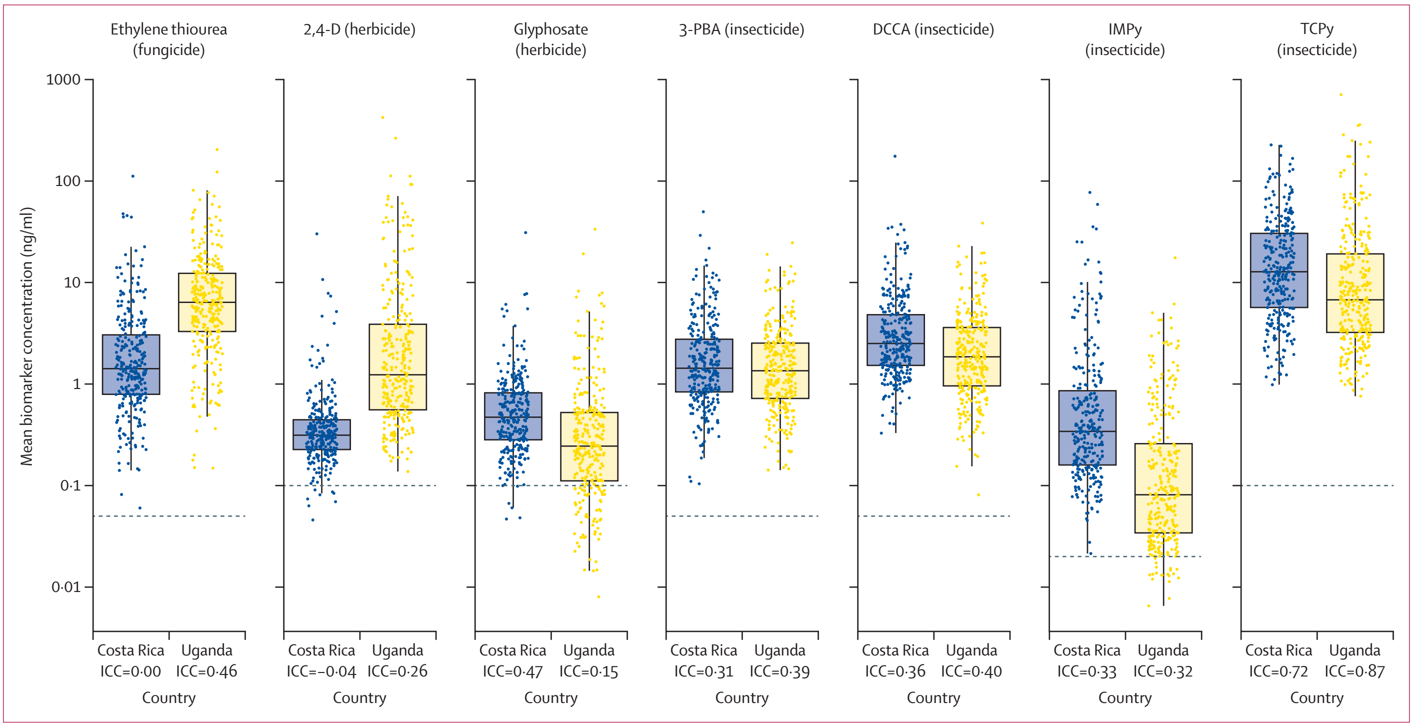
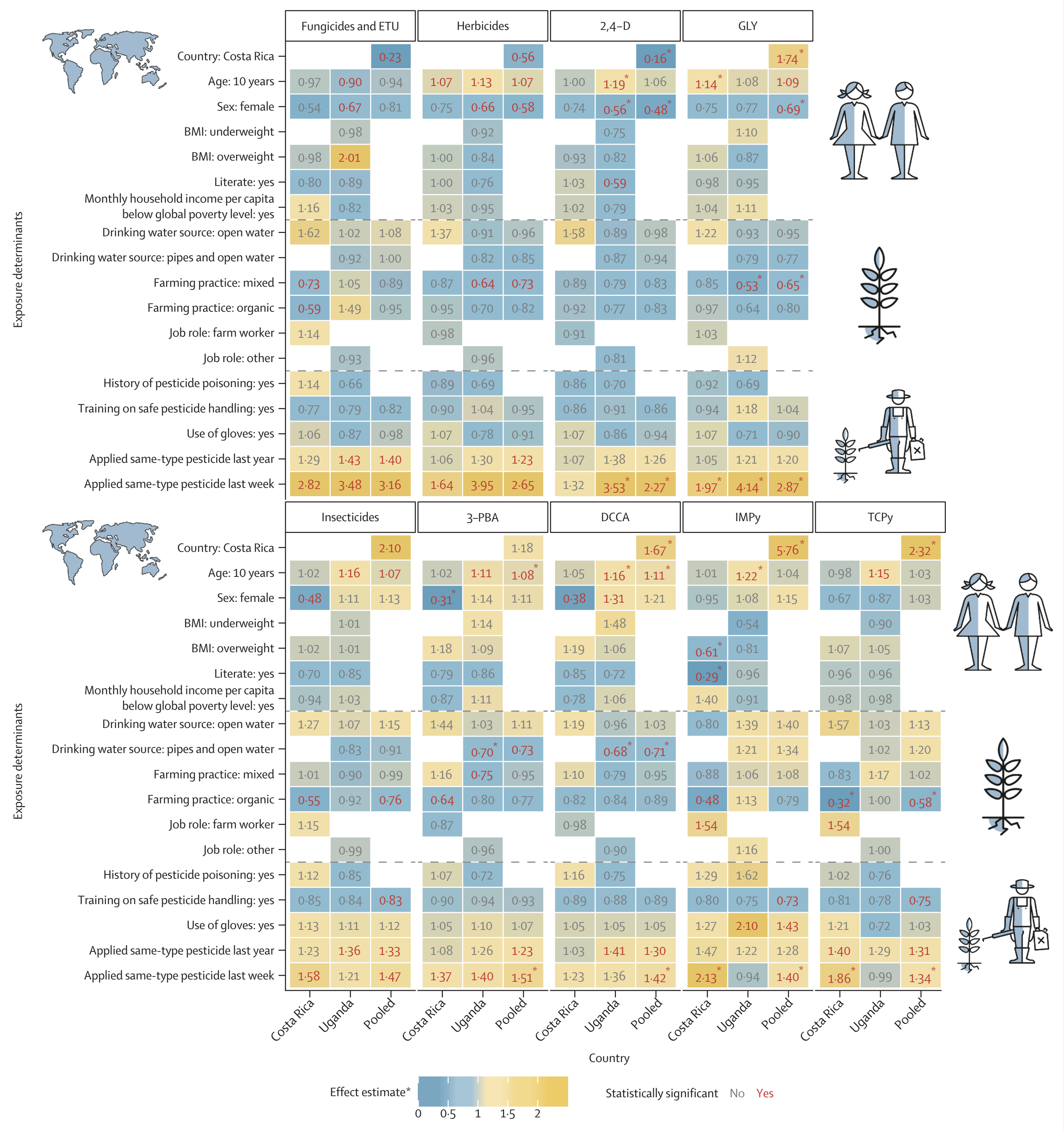


Figure 3: **Distribution of outcomes (biomarker concentrations)**

The boxplots show the distribution of individual mean concentrations (specific-gravity-adjusted and $\log_{10}$-transformed) as well as the ICC across the two study visits, stratified by biomarker and country. Dashed lines represent each biomarker's limit of detection. DCCA=3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid (pyrethroid insecticide). ICC=intraclass correlation coefficient. IMPy=2-isopropyl-4-methyl-6-hydrocypyrimidine (organophosphate insecticide). TCPy=3,5,6-trichloro-2-pyridinol (organophosphate insecticide). 2,4-D=2,4-dichlorophenoxyacetic acid. 3–PBA=3-phenoxybenzoic acid (pyrethroid insecticide).

application could lead to pesticide buildup on the gloves, and repeated use could increase rather than decrease personal exposure. Additionally, our study's observational nature prevents ruling out reverse causality; farmers who do not use gloves might be less likely to handle hazardous pesticides such as diazinon.



Exposure determinants	Fungicides and ETU			Herbicides			2,4–D			GLY		
	Costa Rica	Uganda	Pooled	Costa Rica	Uganda	Pooled	Costa Rica	Uganda	Pooled	Costa Rica	Uganda	Pooled
Country: Costa Rica			0·23			0·56			0·16*			1·74*
Age: 10 years	0·97	0·90	0·94	1·07	1·13	1·07	1·00	1·19*	1·06	1·14*	1·08	1·09
Sex: female	0·54	0·67	0·81	0·75	0·66	0·58	0·74	0·56*	0·48*	0·75	0·77	0·69*
BMI: underweight		0·98			0·92			0·75			1·10	
BMI: overweight	0·98	2·01		1·00	0·84		0·93	0·82		1·06	0·87	
Literate: yes	0·80	0·89		1·00	0·76		1·03	0·59		0·98	0·95	
Monthly household income per capita below global poverty level: yes	1·16	0·82		1·03	0·95		1·02	0·79		1·04	1·11	
Drinking water source: open water	1·62	1·02	1·08	1·37	0·91	0·96	1·58	0·89	0·98	1·22	0·93	0·95
Drinking water source: pipes and open water		0·92	1·00		0·82	0·85		0·87	0·94		0·79	0·77
Farming practice: mixed	0·73	1·05	0·89	0·87	0·64	0·73	0·89	0·79	0·83	0·85	0·53*	0·65*
Farming practice: organic	0·59	1·49	0·95	0·95	0·70	0·82	0·92	0·77	0·83	0·97	0·64	0·80
Job role: farm worker	1·14			0·98			0·91			1·03		
Job role: other		0·93			0·96			0·81			1·12	
History of pesticide poisoning: yes	1·14	0·66		0·89	0·69		0·86	0·70		0·92	0·69	
Training on safe pesticide handling: yes	0·77	0·79	0·82	0·90	1·04	0·95	0·86	0·91	0·86	0·94	1·18	1·04
Use of gloves: yes	1·06	0·87	0·98	1·07	0·78	0·91	1·07	0·86	0·94	1·07	0·71	0·90
Applied same-type pesticide last year	1·29	1·43	1·40	1·06	1·30	1·23	1·07	1·38	1·26	1·05	1·21	1·20
Applied same-type pesticide last week	2·82	3·48	3·16	1·64	3·95	2·65	1·32	3·53*	2·27*	1·97*	4·14*	2·87*

Exposure determinants	Insecticides			3–PBA			DCCA			IMPy			TCPy		
	Costa Rica	Uganda	Pooled	Costa Rica	Uganda	Pooled	Costa Rica	Uganda	Pooled	Costa Rica	Uganda	Pooled	Costa Rica	Uganda	Pooled
Country: Costa Rica			2·10			1·18			1·67*			5·76*			2·32*
Age: 10 years	1·02	1·16	1·07	1·02	1·11	1·08*	1·05	1·16*	1·11*	1·01	1·22*	1·04	0·98	1·15	1·03
Sex: female	0·48	1·11	1·13	0·31*	1·14	1·11	0·38	1·31	1·21	0·95	1·08	1·15	0·67	0·87	1·03
BMI: underweight		1·01			1·14			1·48			0·54			0·90	
BMI: overweight	1·02	1·01		1·18	1·09		1·19	1·06		0·61*	0·81		1·07	1·05	
Literate: yes	0·70	0·85		0·79	0·86		0·85	0·72		0·29*	0·96		0·96	0·96	
Monthly household income per capita below global poverty level: yes	0·94	1·03		0·87	1·11		0·78	1·06		1·40	0·91		0·98	0·98	
Drinking water source: open water	1·27	1·07	1·15	1·44	1·03	1·11	1·19	0·96	1·03	0·80	1·39	1·40	1·57	1·03	1·13
Drinking water source: pipes and open water		0·83	0·91		0·70*	0·73		0·68*	0·71*		1·21	1·34		1·02	1·20
Farming practice: mixed	1·01	0·90	0·99	1·16	0·75	0·95	1·10	0·79	0·95	0·88	1·06	1·08	0·83	1·17	1·02
Farming practice: organic	0·55	0·92	0·76	0·64	0·80	0·77	0·82	0·84	0·89	0·48	1·13	0·79	0·32*	1·00	0·58*
Job role: farm worker	1·15			0·87			0·98			1·54			1·54		
Job role: other		0·99			0·96			0·90			1·16			1·00	
History of pesticide poisoning: yes	1·12	0·85		1·07	0·72		1·16	0·75		1·29	1·62		1·02	0·76	
Training on safe pesticide handling: yes	0·85	0·84	0·83	0·90	0·94	0·93	0·89	0·88	0·89	0·80	0·75	0·73	0·81	0·78	0·75
Use of gloves: yes	1·13	1·11	1·12	1·05	1·10	1·07	1·05	1·05	1·05	1·27	2·10	1·43	1·21	0·72	1·03
Applied same-type pesticide last year	1·23	1·36	1·33	1·08	1·26	1·23	1·03	1·41	1·30	1·47	1·22	1·28	1·40	1·29	1·31
Applied same-type pesticide last week	1·58	1·21	1·47	1·37	1·40	1·51*	1·23	1·36	1·42*	2·13*	0·94	1·40*	1·86*	0·99	1·34*

Figure 4: **Heatmap of effect estimates**

This heatmap visualises the magnitude, direction, and statistical significance of effect estimates for all investigated associations between exposure determinants and biomarker concentrations. Blue fields indicate negative associations (<1), whereas yellow fields indicate positive associations (>1). Associations in red are statistically significant at a 5% level. The study population used in the model is indicated on the x-axis, the exposure determinant is indicated on the y-axis. The heatmap is stratified by outcome. *Effect estimates are defined as 10^{β} for individual biomarkers, and as $10^{\beta/\sigma}$ for pesticide types due to the outcomes' Z standardisation, where σ is the mean (SD) of the $\log_{10}$-transformed biomarkers belonging to the respective use type. Individual biomarkers (not pesticide groups) were adjusted for multiple testing within countries and predictors. Red stars indicate statistical significance after adjustment. DCCA=3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid. ETU=ethylene thiourea. GLY=glyphosate. IMPy=2-isopropyl-4-methyl-6-hydrocypyrimidine. TCPy=3,5,6-trichloro-2-pyridinol. 2,4-D=2,4-dichlorophenoxyacetic acid. 3–PBA=3-phenoxybenzoic acid.

Overall, our results align with previous studies: recent (especially within the previous week) pesticide application increases exposure, while organic farming and pesticide safety training reduce it. Literacy, which is highly correlated with education, was also associated with lower exposure, consistent with previous findings on the protective effect of higher education.[11–13] Beyond known behavioural and occupational factors, we also identified sociodemographic variables such as age, sex, BMI, and household income as key determinants of exposure. To the best of our knowledge, this study is the first pooled pesticide exposure assessment across two socioeconomically and culturally diverse tropical LMICs using a harmonised study design. Given the consistent associations across countries, our findings are likely generalisable to similar populations; however, they might not be applicable in environmental or non-tropical settings with different exposure routes and pest pressures, and they should not be interpreted as representative of the entire countries, as our use of Costa Rica and Uganda refer only to the respective study areas.

A limitation of our study is how we classified farming practices. Although we redefined the classification based on farmers' self-reported use of synthetic pesticides, the risk of misclassification is high and could lead to biased effect estimates. Additionally, self-reported data always carry the risk of recall bias, and cross-sectional analyses cannot rule out potential reverse causality, as discussed previously for PPE. Moreover, as only two urine samples per participant were available, exposure estimates could be affected by random variation and the short biological half-lives of the assessed biomarkers. Notably, all of the investigated biomarkers have half-lives of one to two days, so using application within the past week might not optimally capture recent exposures. Future studies should aim to collect more nuanced information on application recency. Finally, our analyses by use type (ie, fungicide, herbicide, and insecticide) might not capture the full variability within each type, as application practices can differ by active ingredient. Thus, the pooled effect estimates should be interpreted as reflecting only the biomarkers investigated in this study rather than representing the entire use type. Future research should expand study cohorts of smallholder farmers in tropical LMICs or other high-exposure populations to investigate pesticide exposure determinants and explore intervention strategies to reduce exposure and prevent adverse health effects.

Our findings highlight the potential of organic practices to reduce personal exposure, as well as the need for educational programmes for smallholder farmers in tropical LMICs that raise awareness of the health impacts of conventional pesticide use and address context-specific knowledge, attitudes, and practices to promote protective behaviour change.[30] To achieve this, financial and systemic support will be essential to facilitate a transition to organic practices without facing crop loss and economic hardship, and to ensure access to educational programmes and PPE for smallholder farmers. Potential solutions include transition grants, subsidies for PPE and organic certification costs, tax breaks for organic producers, investment in agronomic research focused on organic pest control methods, support for producers to get access to international or national organic markets, or community-supported agricultural systems.[31]

Contributors

AP contributed to the conceptualisation, data curation, formal analysis, methodology, visualisation, and drafting of the original manuscript. AA contributed to funding acquisition, project administration, and data curation. CHL contributed to funding acquisition, methodology, and resources. AMM contributed to funding acquisition, project administration, and data curation. MR contributed to the methodology and provided supervision. PS contributed to data curation. SF oversaw conceptualisation, data curation, funding acquisition, investigation, methodology, project administration, and supervision. SF and PS accessed and verified the data. All authors had access to the study data and reviewed and edited the manuscript's contents. All authors read and approved of the final manuscript. AP had final responsibility for submitting the manuscript for publication.

Declaration of interests

We declare no competing interests.

Data sharing

De-identified individual participant data underlying the results reported in this Article, together with the data dictionary, study protocol, and analytic code, will be made available to researchers who submit a methodologically sound proposal for any purpose to the corresponding author. Data will be available immediately following publication with no end date and can be obtained from the corresponding author under a data access agreement.

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