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ENTOMOLOGY | RESEARCH ARTICLE

Control of cocoa mirids using aqueous extracts of *Thevetia peruviana* and *Azadirachta indica*

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Abstract: Mirids, *Sahlbergella singularis,* are the most harmful cacao pest in Africa. Chemical management, although controversial, is currently the only effective option for controlling these pests. Based on *in vitro* and field experiments, this study evaluated and compared the effectiveness of aqueous extracts obtained from the seeds of *Thevetia peruviana* (TP) and *Azadirachta indica* (AI) in controlling cocoa mirids, *S. singularis*. *Thevetia peruviana* extracts proved to be more effective than *A. indica* extracts and were as effective as Actara 25 WG, the benchmark insecticide, in reducing mirids in the field. All the aqueous extracts tested *in vitro* displayed insecticidal activities within maximum eight days and some repellent effects at both 10 and 25% w/v. Treatment of mirid food with the extracts also led to high mortality rates and to significant avoidance of pods treated with the TP25, TP10 and AI25%. The use of plant aqueous extracts to control mirids in the field thus offers promising prospects for the control of this pest, given the availability of the plants, their simple preparation, their effectiveness and their eco-friendly features.

Subjects: Agriculture & Environmental Sciences; Entomology; Ecology - Environment Studies

Keywords: *Sahlbergella singularis*; agroforestry system; biopesticide; Cameroon; neem; yellow oleander; integrated pest management

1. Introduction

Worldwide cocoa production is currently four million tons per year, of which 72% comes from Africa (Ivory Coast, Ghana, Nigeria and Cameroon) (ICCO, 2016). The majority is produced on small family farms. In Cameroon, 85% of cocoa farms are owned by smallholders who farm plots of less than

ABOUT THE AUTHORS

The work presented here is part of the research program of the Department of Biology and Vegetal Physiology of the Faculty of Science of the University of Yaoundé1, Cameroon. This research program conducted by Pr Zachée Ambang, aims at developing biopesticide to control pest and disease of agricultural crops mainly cocoa. In this lab, crude extracts derivated from local plants (*Azadirachta indica, Thevetia peruviana*) are tested against many insects' pests and pathogens (*nematodes: Meloidogyne sp, Glomus sp, fungus: Cercospora sp*, stramenopile: *Phytophthora sp*) responsible for main losses in crop production. The study was conducted by Dr Serge Mboussi who did his PhD in this Department of University of Yaoundé1.

PUBLIC INTEREST STATEMENT

Our work is of major interest for the protection of African cocoa as it represents a real alternative solution to the use of chemical insecticide for the control of the most important pest, by smallholder farmers. We investigated the potential of aqueous extracts derived from local plants (neem and yellow oleander) for the control of mirid bugs. Yellow oleander extracts were more effective than neem extracts and as effective as the benchmark insecticide, in reducing mirid bugs in the field. These biopesticides are effective, easy to produce and environmentally friendly and could be highly beneficial for cocoa growers. They could be part of integrated pest management programmes in the cacao based agroforestry system in Cameroon.

three ha (Alemagi et al., 2014). Cocoa is an important source of income for approximately 1.4 million people in the country (Alemagi et al., 2014). National production is seriously affected by black pod rot (up to 80–90% losses), a disease caused by *Phytophthora megakarya* Brasier and Griffin (Guest, 2007) and by the damage caused by mirids, *Sahlbergella singularis* Haglund, and *Distantiella theobroma* Distant (Heteroptera: Miridae) (Babin et al., 2010). These species, which originate from the forests of central Africa, are by far the most frequent and serious pest of West African cacao (*Theobroma cacao* L., Malvaceae) (Ayenor, Huis, Obeng-Ofori, Padi, & Röling, 2007; Babin et al., 2012). They have very similar life histories and regularly live together in cacao-based agroforestry systems (Babin, Bisseleua, Dibog, & Lumaret, 2008).

The immature (five instars) and adult stages of these sucking insects feed on the sap of young semi-lignified branches, on plant tissues by injecting a digestive saliva, on buds and on fruits (Lavabre, 1977). The damage inflicted on the growing vegetative parts causes the branches to dry out. Losses due to mirids are difficult to estimate, but can reach 30–40% of potential production (Adu-Acheampong et al., 2014; Awudzi et al., 2016). In plantations, mirids gather in open canopy zones, known as mirid pockets (Babin et al., 2010). Population densities, although fairly low overall, reach maximum during the pod growth period (Anikwe, Okelana, & Omoloye, 2010).

Chemical pesticides are still the main input used in Cameroon's cacao-based agroforestry systems (Matthews, Wiles, & Baleguel, 2003). Management of these pests is based on the use of synthetic insecticides of the neonicotinoid family, such as imidacloprid and thiametoxam (Ayenor et al., 2007). However, small-scale producers cannot procure these products regularly, and their price is extremely high (Sonwa, Coulibaly, Weise, Akinwumi Adesina, & Janssens, 2008), resulting in an insufficient overall impact of the treatments used to control the pest at an economic level. Synthetic insecticides are often mishandled and misapplied, especially by inexperienced farmers, resulting in poor effectiveness of the treatments (Coulibaly, Cherry, Nouhoheflin, Aitchedji, & Al-Hassan, 2007; Ntow, Gijzen, Kelderman, & Drechsel, 2006). Recurrent use of chemical insecticides is also widely questioned due to the adverse effects they have on ecosystems (Fosu-Mensah, Okoffo, Darko, & Gordon, 2016; Pimentel et al., 1992), as well as their toxic effects on human health (Tijani, 2006). What is more, massive pesticide use can irreversibly upset the ecological equilibrium of agrosystems. This is extremely important in Cameroon's agroforestry systems, which contain high levels of plant and animal diversity, and high biocontrol potential.

Alternative cocoa pest control methods have been developed to overcome these problems. They include cultural management (Babin et al., 2010), varietal management (Dibog et al., 2008), as well as semiochemical management with the use of synthetic sexual pheromone traps (Mahob et al., 2011). Another option currently under development is the use of plant extracts as pesticides (Ayenor et al., 2007; N'Guessan, Kouassi, & Atindehou, 2006; Padi, Adu-Acheampong, Asamoah, & Aneani, 2003).

Plant-based biopesticides have the advantage of being environmentally friendly and are often not dangerous for human health, as well as being biodegradable (Isman, 2006). Although they have the reputation of being more sensitive to variations in temperature, humidity or light radiation and less effective than chemical insecticides, they are a subject of growing interest for crop protection, especially as part of integrated pest management strategies (Campos, de Oliveira, Pascoli, de Lima, & Fraceto, 2016; Isman, 2015). Some plant-derived products including pyrethrum, neem (Schmutterer, 1990) and essential oils (Isman, 2006) are well known and have already been used for many years against different agricultural pests (Gopalakrishnan et al., 2014).

Neem oil is an insecticide extracted from the seeds of *Azadirachta indica* A. Juss. (Meliacea), a tree originating from India and South-East Asia (Schmutterer, 1990). Neem seeds contain more than a dozen insecticide analogues, of which azadirachtin, a mixture of seven isomers of tetranortriterpenoid, is the major active ingredient (Isman, 2006; Schmutterer, 1990). Neem disrupts morphogenesis and the development of the insect embryo (Correia et al., 2013). Neem also significantly affects insect fitness (Ahmad, Ansari, & Muslim, 2015) via its anti-feeding, repellent and growth retardant actions and its restrictive effects on egg laying (Isman, 2006; Schmutterer, 1990).

The insecticidal properties of the seeds of *Thevetia peruviana* (Pers.) K. Schum., which belongs to the Apocynaceae family and is commonly known as yellow oleander, have also been studied in Africa and Asia (India, Bangladesh) (Emeasor, Ogbuji, & Emosairue, 2005; Mollah & Islam, 2007; Reed, Freedman, & Ladd, 1982; Yadav, Singh, & Mittal, 2013). However, less information is available on the insecticidal properties of *T. peruviana* in comparison to neem. *Thevetia peruviana* is a decorative bush originating from Central America and is currently cultivated in different parts of Africa (Ghana, Uganda, Cameroon, Mali, Ivory Coast, Benin) and Asia (India, Sri Lanka). Solutions prepared from ground *T. peruviana* seeds have proven to be highly effective in destroying the chitinous cuticle of insects (Reed et al., 1982). *Thevetia peruviana* seeds contain a variety of compounds including terpenes, glycosides and sterols, which are known to have antifungal (Gata-Gonçalves, Nogueira, Matos, & Bruno de Sousa, 2003; Naz, Bano, Wilson, Guest, & Roberts, 2014), insecticidal (McLaughlin, Freedman, Powell, & Smith, 1980) and bacterial effects (Bandara, Weinstein, White, & Eddleston, 2010). Several botanically-derived pesticides offer effective control of insect pests, but few have been systematically tested on heteropterans (Jaastad, Trandem, Hovland, & Mogan, 2009). A few studies conducted in Ivory Coast, Ghana and Cameroon have revealed the toxic effects of aqueous extracts of neem on cocoa mirids in the laboratory (Ambang et al., 2010; N'Guessan et al., 2006; Padi et al., 2003).

The aim of the present work was to assess and compare the effects on cocoa mirids (*S. singularis*) of aqueous extracts of *T. peruviana* and *A. indica* harvested in Cameroon. Laboratory studies were conducted to investigate the toxic, repellent and anti-feeding effects of plant extracts on mirids via direct application and treatment of mirid food. Field studies were also conducted in cacao-based agroforestry systems to confirm the effectiveness of the plant extracts in controlling mirid populations over long periods.

2. Materials and methods

2.1. Mirid source

The mirids used in the bioassays were reared in the entomology laboratory at IRAD (Agricultural Research Institute for Development) in Nkolbisson, Cameroon, according to the method described by Babin et al. (2008). Mating, egg laying and embryo development took place *in situ* on an isolated pod on a cacao tree located next to the laboratory. Nymph development and emergence of the adults took place in the rearing room under controlled conditions (temperature: 24.7 ± 0.9°C; relative humidity: 84.5 ± 6.8%; photoperiod: 12: 12). Only F2 individuals were used in the experiments. F0 nymphs of *S. singularis* were collected in the field. The emerged adults were transferred to a cacao branch outdoors (next to the laboratory) for egg laying. The emerged nymphs were transferred to the laboratory until emergence of the adults. After mating, these adults were transferred to a cacao branch outdoors. The emerging nymphs were used for the experiment when they were at the 4th and 5th instar stages. All the mirid nymphs used in the laboratory experiments were left unfed for 12 h before the beginning of the experiment.

2.2. Preparation of aqueous extracts

The aqueous extracts were prepared from seeds obtained from ripe fruits of *T. peruviana* collected around Yaoundé (Central Region of Cameroon) and of *A. indica* collected around Maroua (Far North Region). The ripe fruits were picked directly from the tree. Fruit seeds were dried at ambient temperature for three weeks and crushed using a manual grinding mill (Corona® Hand Mill). Two hundred and fifty grams of *T. peruviana* and *A. indica* seed powder, wrapped in muslin cloth, was left to soak in two litres of water for 12 h. The aqueous extracts were obtained by mixing the filtrate from soaking with 15 L of water to which 10 g of powdered household pure soap was added as a wetting agent (Stoll, 2000). The concentration of crude aqueous extracts (primary suspension) obtained for both plants was 14.7 g/L.

2.3. Laboratory assays

The bioassays were conducted in the IRAD entomology and phytopathology laboratories in Nkolbisson. The two aqueous extracts of *A. indica* and *T. peruviana* were used at 10% and 25% dilution. The 10% dilution (AI10%, TP10%) consisted of 100 mL of primary suspension in 1 L of water (resulting concentration 1.47 g/L). The 25% dilution (AI25%, TP25%) consisted of 250 mL of primary suspension in 1 L of water (resulting concentration 3.67 g/L). The positive control was the benchmark chemical insecticide, Actara®25 WG, Syngenta (25% Thiomexam, WG granules, 4 g/15 L sprayer) (Actara) used following the manufacturer's recommendations (0.26 g/L). This insecticide is used in alternation with imidaclorpide to treat mirids in the field. The negative control was treatment with water only. Two experiments were conducted in the laboratory to evaluate the effectiveness of the aqueous extracts: one to test direct contact toxicity and another to test residue contact toxicity (McDonald, Guy, & Speirs, 1970).

2.3.1. Direct contact toxicity

In this experiment, aqueous extracts were applied directly onto groups of five mirids (4th and 5th instars). The effect of the four aqueous extracts (AI10, AI25, TP10, TP25%) was tested in separate batches. The aqueous extract was sprayed onto the mirids placed in a Petri dish (diameter: 9 cm) lined with absorbent paper using a 15 mL plastic hand pump trigger sprayer. Five minutes after being sprayed, a brush was used to transfer the mirids to a mature pod in an aerated bucket (25 × 12 × 20 cm) lined with absorbent paper. The pod was the only source of food throughout the bioassay, which lasted 15 days (corresponding to the time needed for the pod to deteriorate). Every day at a set time, the number of moults indicating changes in the development stage were counted, along with the corpses of dead individuals. Necrotic feeding lesions (usually appearing as a black plug of dead tissue) caused by mirids on the pod were also counted for each treatment. Each aqueous extract and positive and negative controls were the subject of five replications.

2.3.2. Residue toxicity

Two treated pods were placed in an aerated plastic bowl (50 × 29 × 16 cm) placed 45 cm apart. One pod was immersed for one minute in one of the aqueous extracts or in Actara, and the other one was immersed in water only. After immersion, the pods were dried in the open air for 10 min. Five mirid instars (4th and 5th) were placed in the centre of the plastic bowls at an equal distance from the two pods (five replications were carried out). The mirids could move freely and independently to either of the pods to feed. To assess feeding preference, the number of feeding lesions were counted on each pod each day for a period of 15 days. The mean number of lesions per living individual per day on the treated and untreated pods were compared in the same bowl. The numbers of lesions on the pods treated with the aqueous extracts and with Actara were also compared with each other, since their effect was always evaluated against a pod treated with water.

The mean percentage repellency (McDonald et al., 1970) was used to evaluate repellent activity. Each day at a set time, the number of individuals present on the treated pods (Nt) and untreated pods (Nc) in each plastic bowl were counted over a period of five minutes. The mean percentage repellency ($Rp = (Nc - Nt)/(Nc + Nt) \times 100$) was used to attribute the extracts (McDonald et al., 1970), to one of the repellent effect classes varying from 0 (no repellent effect) to V (highly repellent effect): class 0 ($Rp < 0.1\%$), class I ($Rp = 0.1–20\%$), class II ($Rp = 20.1–40\%$), class III ($Rp = 40.1–60\%$), class IV ($Rp = 60.1–80\%$) and class V ($Rp = 80.1–100\%$).

2.4. Field trials

For the field trials, undiluted aqueous solutions of *A. indica* or *T. peruviana* (at 14.7 g/L) were compared to the positive control (Actara) used following the manufacturer's recommendations (0.27 g/L) and to the negative controls (treatment with water). Field trials were set up in the localities of Biakoa (32 N 0773823; UTM 0426500) and Bindambogo (32 N 0773757; UTM 0501316) in the Department of Mbam and Kim, Central Region, Cameroon. The plantations were 50 and 10 years old, respectively. Spacing between individual trees was 2.5 m × 2.5 m. The trial was monitored over two consecutive cacao seasons, from July to November 2013 and from June to November 2014. These periods correspond to the pod growth and ripening period, and mirid population peaks. Three blocks composed of 200–250 contiguous cacao trees were chosen in each plantation for their similarity in terms of shade and trees density. Each block was divided into four sub-blocks, each of which was treated differently. A minimum of four rows and 10 rows of untreated cacao trees separated the sub-blocks and blocks, respectively, to prevent neighbouring effects. Within a sub-block, 25 cacao trees (trunks and branches) were sprayed using a Screw Pump Matabi Manual Sprayer (Pandora, China), to a height of approx. 4 m from the ground. Each aqueous extract was applied at 15 day intervals and Actara and water were applied once a month during the monitoring period. For each application period, a total of 4.9 L of *A. indica* solution (at 14.7 g/L), 4.9 L of *T. peruviana* solution (at 14.7 g/L), and 4.9 L of water were used to spray the 150 trees (25 trees per sub-block * 3 blocks * 2 sites). In parallel, 1.5 L of Actara solution (at 0.27 g/L) was used for the two sites.

To evaluate the mirid populations in the sub-blocks, 15 trees out of the 25 treated trees were selected per sub-block based on the presence of young fruits and of damage caused by previous mirid attacks (cankers and feeding lesions on the branches and the chupons). Each week, the number of living mirids (nymphs or adults) present on the trees was counted. Observations were made early in the morning (7 am) on the cacao tree trunks and branches between 0 and 2 m from ground level. To evaluate the effects of the treatments on mirid populations, the mean numbers of mirids per site before and after treatment were calculated. Pre-treatment populations (Table 1) were evaluated two weeks before the first treatment. Post-treatment populations (Table 1) corresponded to the mean number of mirids counted each week over the whole monitoring period. The number of mirids per site per treatment per week was evaluated as the sum of the numbers of mirids in the treated sub-blocks/3 (number of blocks).

2.5. Statistical analysis

The data collected in the laboratory and in the field were analysed using R3.1.2 software (R Core Team, 2015). Since the homogeneity between the replications was verified, the various replications were grouped in the same treatment for analyses. Normality could not be achieved with any of the transformations performed on the mirid nymph mortality data. Non-linear regression models (GLMs with binomial error and logit link function) were used to adjust changes in mortality data over time as a function of the different extracts. Using these models, the lethal times 50 and 90 (LT50 and LT90: time after which 50 and 90% of the population dies, respectively) were determined. To assess the anti-feeding effects of the aqueous extracts on mirids, a GLM was built with the type of extract tested and the period as the fixed effect, and the number of lesions per larva per day as the dependent variable. Two periods were defined: a period of up to three days' exposure, and a period of more than three days' exposure to consider the behaviour of surviving individuals in the less toxic treatments. A variance analysis, followed by a Wilcoxon multiple mean comparison test with Bonferroni correction, were used to reveal the significant factors and the differences between the treatments. To assess the effect of the aqueous extracts on mirid feeding preferences, the numbers of lesions on treated and untreated pods in the bowls were compared using Wilcoxon multiple mean comparison tests with Bonferroni correction.

To assess the effects of the application on mirid populations in the field, an analysis of variance (with the drop1 function in the lme4 package) was used on a linear mixed model (with the lmer function in the lme4 package) with the post-treatment population as the dependent variable, the treatment as the fixed effect, and the year, the population before treatment and the interaction between the sites and the blocks as random effects. Post hoc tests with a Tukey HSD test for multiple comparison (with the glht function from the multcomp package) were used to compare the effects of the different treatments.

Table 1. Average number of mirids per treatment and per site before and after application of the aqueous extracts during the 2013 and 2014 cacao seasons

Sites	Year	Treatments	Pre treatment population* mean±se	Post treatment population ** mean±se
Biakoa	2013	TP	5.33 ± 0.33	1.21 ± 0.11
		AI	5.67 ± 0.99	1.98 ± 0.14
		Actara	4.83 ± 0.70	1.67 ± 0.13
		Water	6.33 ± 1.14	8.95 ± 1.18
	2014	TP	2.00 ± 0.26	0.78 ± 0.09
		AI	2.50 ± 0.34	1.1.35 ± 0.18
		Actara	1.50 ± 0.43	0.65 ± 0.07
		Water	3.00 ± 0.45	4.38 ± 0.23
Bidambogo	2013	TP	0.33 ± 0.21	1.19 ± 0.12
		AI	0.33 ± 0.21	2.02 ± 0.06
		Actara	1.67 ± 0.67	0.83 ± 0.17
		Water	2.00 ± 0.73	7.31 ± 1.67
	2014	TP	2.00 ± 0.26	0.26 ± 0.32
		AI	2.17 ± 0.17	0.90 ±0.10
		Actara	1.67 ± 0.21	0.21 ± 0.04
		Water	2.50 ± 0.34	3.15 ± 0.03

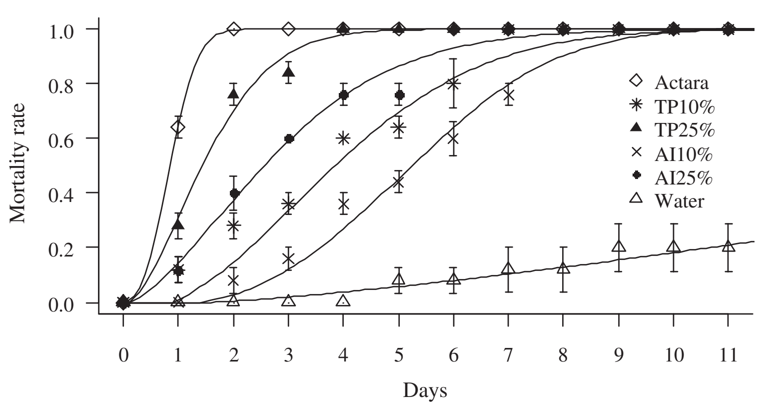
Note: Mean values were obtained from 45 trees per plantation (15 trees * 3 blocks).

*Mean values of mirids two weeks before the treatment.

**Mean values of mirids over the whole monitoring period after treatment.

Figure 1. Effect on the mirid mortality rate of aqueous extracts of *T. peruviana* and *A. indica* applied directly on mirid bugs.

Notes: TP 10%: 10% aqueous extract of *T. peruviana*, TP 25%: 25% aqueous extract of *T. peruviana*; AI 10% and AI 25%: 10% and 25% aqueous extract of *A. indica*.



3. Results

3.1. Direct contact toxicity

The most concentrated aqueous extracts, in particular TP25%, proved to have strong insecticidal effects. The extracts significantly influenced the temporal evolution of mortality of these insects ($P_{df=5', \ dev=304} < 0.001$) compared to the control treatment with only water. Significant differences were observed between TP and AI and between the concentrations applied (Figure 1). Fifty percent of the mortality rate was achieved after 1.44 days with TP25%, 3.85 days with TP10%, 2.56 days with AI25% and 5.24 days with AI10% (Figure 1). In comparison, the positive control Actara resulted in 100% mortality after 2 days, whereas with the control treatment with only water, no mortality was observed before five days. Eight days after exposure, no mirids had survived in the presence of the extracts, regardless of the concentration. With TP10% and AI10%, three and four mirids respectively

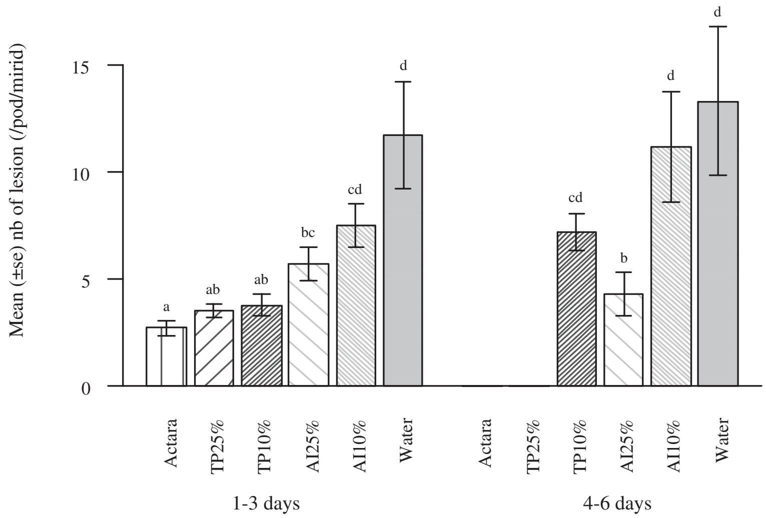


Figure 2. Effect on the number of pod lesions of aqueous extracts of *T. peruviana* and *A. indica* applied directly on mirid bugs for two periods (one to three days and four to six days post direct application). Different letters refer to significant differences (Wilcoxon test, $p < 0.05$).

reached the adult stage before dying on the fourth day after exposure in all five replications. In comparison, after 15 days, a total of 15 adults were present with the control treatment with only water.

The different aqueous extracts also significantly reduced the feeding activities of the mirids ($p_{5'97} < 0.001$). Over the first three days after exposure, the mean numbers of lesions per pod caused by surviving individuals were similar in TP25% (3.4), TP10% (3.7), AI25% (5.6) and in the positive control Actara (2.7) (Figure 2). The mean number of feeding lesions was significantly higher with AI10% (7.5) and with the control treatment with only water (11.5) (Wilcoxon test, $p < 0.05$). Over the period ranging from four to six days after exposure, the individuals which had survived the TP10% treatment caused a mean of 7.2 feeding lesions per pod, which was comparable to the number caused by the individuals treated with AI10% (11.2) and the control treatment (13.3) (Wilcoxon, $p > 0.005$) (Figure 2). However, the mean number of feeding lesions per pod for AI25% (4.3) remained the same as over the previous period (Wilcoxon, $p > 0.005$).

3.2. Residue toxicity

The choice experiment showed that the mirids fed significantly less on pods treated with the various extracts or with Actara than on water-treated pods ($p < 0.05$, Wilcoxon test) (Figure 3). The pods treated with TP25% had more lesions (3.9 lesions per day per mirid) than those treated with Actara (2 lesions per day per mirid). These pods were significantly less attacked than those treated with TP10% (12 lesions per day per mirid), AI25% (7.9 lesions per day per mirid) and AI10% (23.6 lesions per day per mirid) (Figure 3). High and rapid mirid mortality occurred in the plastic bowls containing the pods treated with TP25% and Actara. One hundred percent of individuals died on the 6th and 3rd day, respectively, in both cases (data not shown). In the presence of the pods treated with AI25% and TP10%, 6 and 11 mirids out of 25, respectively, became adults during the experiment, all of which died between the 7th and 8th day. With AI10%, 14 mirids out of 25 became adults, all of which died on the 12th day.

During the daily five-minute observations, the mirids were most often found on untreated pods. No mirids were observed on pods treated with TP25%, or on the pods treated with Actara. The presence of some lesions (Figure 3), however, proves that certain individuals fed on the treated pods. According to the classification of McDonald et al. (1970), TP25% (Rp = 100%) and AI25% (Rp = 92%) belong to repellent class V, TP 10% (51%) belongs to class III and AI10% (24%) belongs to class II. AI10% ceased to have any repellent effects after five days, with individuals found on both the treated and untreated pods.

Figure 3. Effect on pod lesions of aqueous extracts of *T. peruviana* and *A. indica* applied to pods (residue toxicity).

Notes: *Refers to a significant difference in the mean number of bites between pods treated with extracts and pods treated with water (Wilcoxon test, *$p < 0.05$; **$p < 0.01$, ***$p < 0.001$). Different letters refer to significant differences between the pods treated with the extracts.

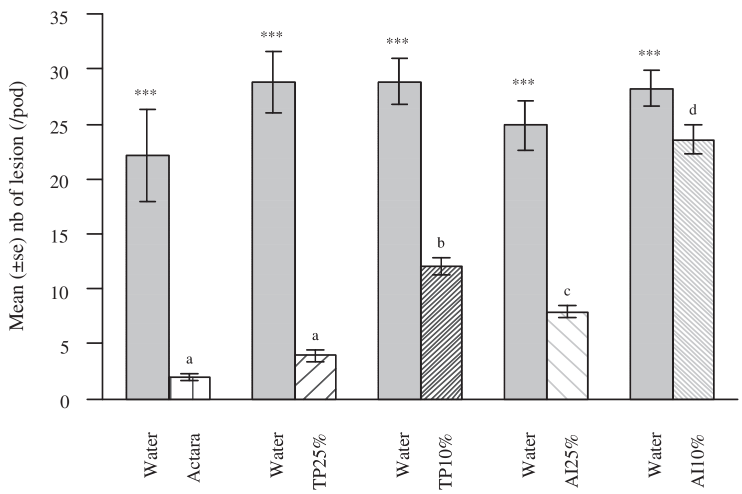


Table 2. Results of the summary of the linear mixed model used to explain the significant effect of treatment on the mean population of mirids over the monitoring period

Model:			
Post-treatment population ~ Treatment + (1|Site:Block) + (1|Year) + (1|Before treatment population)			
Fixed effects	**Estimate**	**Std. error**	***t* value**
Intercept	5.9464	0.8988	6.616
Treatment-AI	−4.3829	0.4894	−8.956
Treatment-TP	−5.0833	0.4894	−10.387
Treatment-Actara	−5.1071	0.4894	−10.435

3.3. Field trials

Before the treatments, there was no significant difference in the mean number of mirids between the sites ($p < 0.05$, Tukey's test) (Table 1). The Bindambogo site displayed very low infestation levels before treatment in 2013 compared to the Biakoa site. Yet in 2014, the levels were comparable at the two sites. Post-treatment populations were lower than pre-treatment populations (except for Bidambogo in 2013) (Table 1). The application treatment had a significant effect on the mean population of mirids over the monitoring period ($P_{df = 3, F = 50.20} < 0.05$). During this period there were significantly fewer mirids on the trees treated with the aqueous extracts (AI, TP) and with Actara in comparison with the control treatment with only water ($p < 0.0001$, Tukey's test, Table 2). Based on the estimates obtained in the explicative model (Table 2), AI and TP reduced the mean number of mirids by 1.7 and 1.9 (similar to Actara), respectively, in comparison with water. There was no significant difference between the mean number of mirids in the plots treated with the aqueous extracts and Actara ($p < 0.0001$, Tukey test).

4. Discussion

In this study, *Thevetia peruviana* and *A. indica* aqueous extracts were as effective as Actara®25 WG, the benchmark insecticide, in reducing mirid populations in the field. The effectiveness of these aqueous extracts shows them to be a viable option for smallholders who are in need of an easily accessible, less expensive and more environmentally friendly control method. The aqueous extract showed clear insecticidal, anti-feeding and, to some extent, repellent effects at both concentrations (10 and 25% w/v) under laboratory conditions.

Our study showed that, under controlled conditions, *T. peruviana* extracts were more effective than *A. indica* extracts in reducing mirid populations at comparable concentrations. The greater effectiveness of *T. peruviana* over *A. indica* was also confirmed in a comparative study on the effect of different plant extracts in controlling *Callosobrichus masculatus* (Fab.) (Coleoptera: Bruchidae), a pest of stored cowpeas (Emeasor et al., 2005). The insecticidal effect of neem is well known (Ahmad, Ansari, & Hasan, 2012; Ahmad et al., 2015; Asogwa, Ndubuaku, Ugwu, & Awe, 2010; Ayenor et al., 2007; Campos et al., 2016; Schmutterer, 1990) but few studies have been conducted on the effect of neem aqueous extracts (N'Guessan et al., 2006; Oroumchi & Lorra, 1993). Studies on the effect of *T. peruviana* extracts against insect pests (Emeasor et al., 2005; Kareru, Keriko, Kenji, & Gachanja, 2010; Mishra, Gupta, & Kumar, 2015; Mollah & Islam, 2007; Ray, Dutta, Srivastava, Kumar, & Saha, 2012) are even rarer, although all these studies confirmed its high toxicity to insects.

Aqueous extracts of *T. peruviana* and *A. indica* were as effective as the commercial insecticide Actara®25 WG in controlling mirid populations in the field. The use of these biopesticides is thus a very promising alternative to chemical pesticides for the control of cocoa pests. Ayenor et al. (2007) proposed an integrated approach for the management of mirid populations in the field based on mass trapping, the use of sex pheromones, and applications of crude aqueous neem extracts. Many studies have confirmed that aqueous plant extracts can be effective in controlling field populations of sucking bugs (Adesina, 2014; Jaastad et al., 2009), aphids and moths (Amoabeng et al., 2013), and in managing tea pests in India (Roy et al., 2016). Botanical insecticides are increasingly attracting the attention of researchers as they offer novel modes of action that may provide effective control of pests that have already developed resistance to conventional insecticides. What is more, some botanical pesticides, notably neem, are less harmful to beneficial insects than chemical insecticides, as there is no persistent residual effect on the environment (Raguraman & Kannan, 2014). Botanical pesticides also appear to be safer for human health than chemical insecticides, although their effect depends on the plant species and the type of extract used, as evidenced by *Thevetia peruviana,* which contains cardiac glycoside (thevetine, neriifolin), which can cause poisoning that can culminate in death in the case of ingestion by mammals and humans (Bandara et al., 2010). Care should thus be taken when using this plant. Provided they are applied with care and recommendations for their application are respected, plant aqueous extract pesticides appear to be a good way to help reduce the health and environmental risks involved in the use of synthetic chemical pesticides.

In addition to the effectiveness of these crude plant extracts, they are easy to make and are cost effective. The extracts presented here did not require any solvents as they are cold aqueous extracts with the addition of soap. The soap might play a role in the efficiency of the aqueous extracts tested. More experiments are required to separate aqueous extract effect from adjuvant effect. Amoabeng et al. (2013) stated that, to be a viable technology for most of the world's poor farmers, botanical insecticides must be based on plant materials that are cheap, readily available and easy to prepare. The use of crude plant aqueous extracts to control pests in the field is a realistic method which is already used for pest management in tea crops in India (Roy et al., 2016). In fact, in Indian tea production, the majority of the plant products used are in the form of crude extracts prepared locally. *Thevetia peruviana* is widely distributed in Cameroon's cacao growing zones and very frequently grows near the farmers' homestead. It also has the potential to control *P. megakaya* (Ambang et al., 2010). Using local materials for pest management enables farmers to reduce production costs, as the materials can be obtained with little effort and at minimum cost (Amoabeng et al., 2013). Biopesticides have been described as an inexpensive plant protection strategy that is ideal for smallholders in West Africa (Mkenda et al., 2015).

Under laboratory conditions, both *T. peruviana* and *A. indica* aqueous extracts displayed toxic, anti-feeding and, to some extent, repellent effects on mirids. Both treatment methods (direct contact and residue toxicity) proved to be effective in killing mirids and in preventing them from feeding. The anti-feeding effect is an important mode of action of neem extract in managing herbivore populations (Ahmad et al., 2012). It makes it possible to extend the toxic effect of the application to surviving individuals.

In the field, the mirid population was drastically reduced after the treatments with *A. indica* and *T. peruviana*-based extracts in comparison with the control treatment consisting in only applying water. Based on the protocol presented here, the application of *T. peruviana* and *A. indica*-based extracts needs to be twice as frequent as the application of Actara for comparable effectiveness. Biopesticides are known to persist for a shorter time, which is generally viewed as an advantage in terms of environmental risk management. However, a higher spraying frequency to maintain an acceptable level of control can lead to a larger quantity of product being released into the environment (Siegwart et al., 2015). Some biopesticides can also lead to the development of resistance in pest populations, although the risk is higher with purified extracts of plant components such as azadirachtine (Siegwart et al., 2015). Neem oil biopesticide appears to be better in terms of durability than other biopesticides. For the moment, there are no reports of resistance occurring due to the use of plant aqueous extracts, but it is important to keep these possibilities in mind for the future.

The adoption of botanical plant extracts as biopesticides should be easy in Cameroon as they were already used by farmers many years ago. Following the devaluation of the CFA franc in 1994, farmers in Cameroon developed their own alternative pest management strategies based on traditional knowledge (Coulibaly, Mbila, Sonwa, Adesina, & Bakala, 2002; Sonwa, Coulibaly, Adesina, Weise, & Tchatat, 2002). Plant extracts based on *Cannabis sativa* L. (Cannabaceae) (although strictly forbidden), tobacco, *Nicotiana tabacum* L. (Solanaceae), as well as extracts from wild plants commonly found in cacao plantations such as *Guibourtia tessmannii* (Harms) J. Léonard (Fabaceae), *Erythrophleum ivorense* L. (Fabaceae) or the bark of *Ceiba pentandra* L. Gaertn. (Bombaceae) and *Pachyelasma tessmannii* Harms (Caesalpiniaceae) are used for mirid control (Coulibaly et al., 2002). Research has not shown much interest in this indigenous knowledge to date, nor have extension services. Yet, Coulibaly et al. (2002) and Sonwa et al. (2002) both advocated that research should tap into this knowledge to aid in the development of control practices which make use of natural enemies of cocoa pests, to identify combinations of crops which minimize the expression of agents responsible for cocoa pests and disseminate cropping practices which minimize the use of synthetic pesticides.

In conclusion, *Thevetia peruviana* and *Azadirachta indica*–based aqueous extracts could reduce the impact of mirids on cacao in agroforestry systems, thanks to their insecticidal effects and their anti-feeding potential. It would be useful to set up training programmes targeting extraction and application processes to help farmers produce their own biopesticide as part of an integrated pest management approach. These botanical extracts are effective, less risky for human health and environmentally friendly, and could reduce the financial and environmental costs incurred in phytosanitary management.

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Authors' contributions
SBM, ZA and LBB designed the research. SBM and SK conducted the experiments. SBM and LBB analysed the data and conducted statistical analyses. SBM and LBB wrote the manuscript. All the authors read and approved the manuscrip.

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