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Research

Effects of Aqueous Extracts from Amazon Plants on *Plutella xylostella* (Lepidoptera: Plutellidae) and *Brevicoryne brassicae* (Homoptera: Aphididae) in Laboratory, Semifield, and field trials

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Abstract

We evaluated the insecticide activities of aqueous extracts of five species of plants from the Ecuadorian Amazon (*Deguelia utilis* (ACSm.) AMGAZEVEDO (Leguminosae: Papilionoideae), *Xanthosoma purpuratum* K. Krause (Alismatales: Araceae), *Clibadium* sp. (Asteracea: Asterales), *Witheringia solanacea* L'Hér (Solanales: Solanaceae), and *Dieffenbachia costata* H. Karst. ex Schott (Alismatales: Araceae)) plus *Cymbopogon citratus* Stapf. (Poales: Poaceae) under laboratory, open-field conditions in *Plutella xylostella* L. (diamondback moth), and semifield conditions in *Brevicoryne brassicae* L. Tap water was used as a negative control, and synthetic insecticides were used as positive controls. In a laboratory bioassay, aqueous extracts of *D. utilis* resulted in *P. xylostella* larval mortality. In contrast to chlorpyrifos, all botanicals were oviposition deterrents. All extracts except *Clibadium* sp. decreased leaf consumption by *P. xylostella* larvae. In semifield experiments, *D. utilis*, *Clibadium* sp., *D. costata*, and *X. purpuratum* initially controlled the population of *B. brassicae*, but 7 d after application, all botanicals except the *D. utilis* lost their ability to control the pest. In field experiments on broccoli crops in both dry and rainy seasons, the extracts did not control the abundance of *P. xylostella*, where as a mixture of two insecticides (chlorpyrifos + lambda cyhalothrin) did. These results show some incongruences from laboratory to semifield and field conditions, indicating that more studies, including the identification of the chemicals responsible for the biological activity, its stability, and the effects of chemotypes on insecticidal activity, are needed to understand the potential of these plant species as botanical insecticides.

Key words: Ecuador Amazon, oviposition deterrence, botanical insecticide, food preference

One of the major problems in agriculture is the excessive use of synthetic insecticides, particularly in regard to their negative effects on biodiversity (European Commission 2013, Carvalho 2017). Another serious side effect of this type of compounds is the risk to lead to the evolution of resistance in insect pests, making entire groups of these plant protection products ineffective (IRAC 2017).

Novel natural insecticides originating from plants (hereafter referred to as 'botanical insecticides') can help to avoid such problems because instead of being lethal to a range of pests and nontarget arthropods alike, they act specifically as oviposition and feeding deterrents (Miresmailli and Isman 2014). Unfortunately, many of such substances of natural origin are subject to rapid environmental degradation but,

unlike their synthetic counterparts, they contain numerous chemicals with different and complex mechanisms of action that should delay the evolution of insecticide resistance (Miresmailli and Isman 2014).

According to Miresmailli and Isman (2014) and Isman (2017), the number of scientific articles on botanical insecticides has grown immensely. Unfortunately, progress toward their commercialization and use in agriculture is limited, and to date, there are only four categories of effective commercial botanical products: pyrethrins, rotenones, azadirachtin, and essential oils. However, some scientists (Isman and Grieneisen 2014, Velasques et al. 2017) have argued that most of these articles were based on laboratory observations, only one third includes chemical data, one quarter includes positive controls, and only a few report field tests.

The tropics have the highest plant biodiversity on Earth and contain a plethora of chemical plant–insect interactions (Miller and Dyer 2009, Richter et al. 2009). In this sense, in recent years, sub-Saharan Africa has been the focus of multinational efforts to study botanical pesticides in the field, and hundreds of species with pesticidal properties have been reported (Anjarwalla et al. 2016, Stevenson et al. 2017). Thus, in the Ecuadorian Amazon, which has one of the highest levels of plant species richness in the world (Myers et al. 2000), we would expect to find a wide range of potential botanical insecticides with unknown compounds, completing our knowledge on rather the effectiveness of neotropical plant extracts as substitutes for synthetic insecticides.

The diamondback moth, *Plutella xylostella* L., and the aphid *Brevicoryne brassicae* L. are among the most important pests of brassicas grown around the world (Ahuja et al. 2010, Furlong et al. 2013). They are also among the pests most difficult to control, and they have developed resistance to a range of synthetic insecticides (Mazhawidza and Mvumi 2017).[AU: As per journal style, insect name or common name of any species should not be abbreviated. Hence, 'DBM' across the article has been spelled out as 'diamondback moth'. Please check and confirm.]

In this context, the objectives of this study were 1) to evaluate the effect of aqueous extracts from five readily available Ecuadorian Amazon plants *Deguelia utilis* (local name Barbasco), *Xanthosoma purpuratum* (local name Shungu panga), *Clibadium* sp. (local name Kakllambi), *Witheringia solanacea* (local name Tsimbio), and *Dieffenbachia costata* (local name Lalu) on *P. xylostella* leaf consumption, larvicidal, and oviposition deterrence under laboratory conditions and 2) to determine whether these five plant extracts and aqueous extracts from *Cymbopogon citratus* (local name Hierba Luisa) are effective in reducing the abundance of *P. xylostella* in an open-field test and the abundance of *B. brassicae* in semifield trials.

The plants in this study are common in the wild and are used in traditional medicine or as natural products by the native people of the Amazon (Bennett 1992, De la Torre and Macia 2008). *Deguelia utilis* is a shrubby plant of the legume family Fabaceae and is found throughout the Amazon; the active ingredient is the insecticide rotenone type II (World Health Organization 2010). In South America, *Deguelia urucu* and *D. utilis* are the two principal native species used for insecticide production, but for commercial purposes, the names *Derris urucu* and *Derris nicou* have been retained (Dos Santos et al. 2000). In Ecuador, this plant is known only from cultivation by indigenous farmers and rarely produces flowers or fruits; in previous ethnobotanical and phytochemical literature, it has incorrectly been referred to as *Lonchocarpus nicou* (Aubl.) DC (Camargo and Goulart de Azevedo 2014). The root of *D. utilis* is traditionally used to control pest species in the Amazon region (Dos Santos et al. 2000, Lawson et al. 2010, Torres et al. 2013, Zubairi et al. 2016). However, the use of the roots destroys the entire plant, and therefore, we investigated whether fresh leaves can also act as a botanical insecticide. *Cymbopogon citratus* is a tropical grass species native to Southeast Asia (Nakahara et al. 2003), introduced and acclimated as an unknown variety to the Ecuadorian Amazon. Bioactive essential oils can be extracted from the grass through steam distillation and are commonly used as an ingredient of plant-based insect repellents (Setiawati et al. 2011, Solomon et al. 2012).

Materials and Methods

Laboratory Bioassay to Evaluate the Leaf Consumption, Larvicidal, and Oviposition Deterrence Effect of the Aqueous Extracts on *P. xylostella*

Plant Sample Collection

Leaves from *D. utilis* (A.C.Sm.) A.M.G. Azevedo (local name Barbasco), *X. purpuratum* K. Krause (local name Shungu panga), *Clibadium* sp. (local name Kakllambi), *W. solanacea* L'Hér. (Solanaceae) (local name Tsimbio), *C. citratus* (DC.) Stapf (local name Hierba Luisa), and *D. costata* H. Karst. ex Schott (local name Lalu) were collected within 3 km of the Centro de Investigación Posgrado y Conservación Amazónica (CIPCA) of the Universidad Estatal Amazónica (S 1°14′18.85″, W 77°53′4.30″) Santa Clara in Pastaza, Ecuador, from June to September 2014 and from June to December 2015. The collection site is within the Amazon primary forest and is characterized by moist tropical rain forest (Consejo de la Provincia de Pastaza 2012). Mean minimum and maximum ambient temperatures were 22 and 31°C, respectively, with a mean relative humidity ranging from 65 to 80%. Voucher specimens of all plant species were deposited at the Herbarium Amazónica del Ecuador (ECUAMZ) at the Universidad Estatal Amazonica (UEA) Santa Clara, Ecuador, and the Herbarium of the Escuela Superior Politécnica de Chimborazo (ESPOCH), Riobamba, Ecuador.

Botanical Extracts

The vegetative materials from at least three specimens of each plant species were thoroughly mixed, mainly because the concentrations of secondary products in the tissues can vary among individual specimens; this method therefore is an accepted approach to control against the confounding factors of natural chemical variation in active compounds across the material tested (Amoabeng et al. 2013). The mixed vegetative material was oven-dried at 60°C for 48 h and milled using a Thomas mill into a fine powder. The dry leaf powder from each plant was suspended in tap water for 48 h to produce an aqueous plant extract at concentrations (weight by volume) ranging from 0.04 to 0.55 g/ml: *D. utilis*, 0.13 g/ml; *Clibadium* sp., 0.08 g/ml; *D. costata*, 0.04 g/ml; *X. purpuratum*, 0.55 g/ml; and *W. solanacea*, 0.06 g/ml. The observed differences in the concentrations of each extract were probably due to the different proportions of fiber present in each plant. The extracts were filtered with a vacuum pump and stored at 4°C in airtight glass vials in the dark until subsequent use.

We selected the aqueous extraction based on locally available plants that grow wild around farming areas, so that the insecticides can be made available with little effort and at no or minimal cost by the small-scale Ecuador Amazon farmers.

In all tests, leaves dipped in tap water were used as negative control. Leaves dipped in the insecticide chlorpyrifos (LORSBAN 480TM, Dow AgroSciences, EEUU; Cortes et al. 2012, Edifarm 2016) were used as positive control.

Insects

We collected 400 *P. xylostella* pupae from a cabbage field in Izamba parish, north of Ambato, Ecuador (El Pisque Sector, Tungurahua Province, W 78°35′25.71″, S 1°12′25.14″), and reared them in cages containing potted white heading cabbage, *Brassica oleracea* convar.

capitata L. var. Alba OMBRIOS RS 91020 (Agripac C.A., Ecuador), using the method described by Cerda et al. (2003). Briefly, *P. xylostella* pupae collected from the field were placed into containers (14 cm diameter × 20 cm height) until the adults emerged. The containers included cabbage leaf discs (4 cm diameter; *B. oleracea* above) for oviposition. At the top of each container, there was a 4.0-cm opening where a piece of cotton soaked in a 10% honey solution was placed to feed *P. xylostella* adults. Each container had two lateral openings (5.5 × 5.5 cm) covered with plastic wrap through which the cabbage leaf discs containing eggs were removed. These discs were then transferred to plastic boxes (6.5 × 25 × 15 cm) containing additional cabbage leaves, which were replenished as necessary until pupation was reached by all larvae. The pupae were then reintroduced to the rearing containers. The complete cycle was repeated once the adults had emerged.

The populations were maintained at room temperature (20–25°C) in 60–80% relative humidity under a natural photoperiod.

Bioassay to Evaluate the Effect of the Botanical Extract on the Percentage of Leaf Consumption by *P. xylostella* larvae

The leaf consumption effect of the studied extracts on *P. xylostella* larvae was based on the leaf dip method described by Tabashnik et al. (1990). Leaf discs (4 cm in diameter) were cut from the middle leaves of 6-wk-old cabbage plants using a sharp metal hole punch. Each disc was immersed with gentle agitation into the test solution of the aqueous plant extract for 10 s. The discs were then drained for 10 s to allow the run-off of surplus solution and then dried with the ad axial surface up on corrugated sheets of aluminum foil for 1 h at room temperature. Dried leaf discs were placed in an individual Petri dish (5 cm in diameter) containing a single layer of moistened filter paper (Whatman No. 1, 4.5 cm in diameter) to prevent wilting. Five third-instar larvae were placed on each leaf using a clean camel hairbrush. Each Petri dish was sealed with parafilm (Pechiney Plastic Packaging, EEUU) to prevent the larvae from escaping and was kept for 5 d under laboratory conditions. Each treatment, including the control, was replicated 5–10 times; a total of 25–50 larvae were tested for each treatment. The treatments were completely randomized. The leaf discs were scanned at the beginning (day 1) and end of the 5-d experimental period (day 5) with a Hewlett Packard Deskjet Ink Advantage 1515 scanner, and the area of each disk was quantified using the Scion Image software (Scion Corporation, EEUU). The normality of the data was tested using the Darling test when the data set did not meet the assumption of normality, even after transformation; a Mann–Whitney test was used to compare treated against control samples, and the difference was considered statistically significant at $P < 0.05$. The percentage leave consumption between treatments was compared with a Kruskal–Wallis test with a level of significance of $P < 0.05$.

Bioassay to Evaluate the Botanical Extract Larvicidal Effect on *P. xylostella*

At the end of the 5-d experiment, the number of dead larvae on the treated and control discs was determined. The normality of the data was tested using the Darling test when the dataset did not meet the assumption of normality, even after transformation. The treatments were compared with a Kruskal–Wallis test at $P < 0.05$.

Bioassay to Evaluate the Botanical Extract Oviposition Deterrence Effect on *P. xylostella*

For each extract, two cabbage leaf discs (4.0 cm diameter) were prepared. One disc was dipped in the aqueous plant extract solution and the other in tap water only. The discs were drained and dried as described for the feeding deterrence bioassay. One hour after treatment, the control and extract-treated leaf discs were placed in a plastic container (14 cm diameter × 20 cm height) containing 20 couples of *P. xylostella* adults, 5–7 d old, and the females were allowed to choose to oviposit on either the extract-treated disc or the control disc. After 2 d, the two leaf discs were removed from the container and the numbers of eggs along the main vein of both leaf discs were counted. Each treatment, including the control, was replicated 5–10 times. The results are presented as the mean ± SEM of eggs after treatment. The number of eggs from each treatment was compared with the number of eggs in the tap water control. The normality of the data was tested using the Darling test when the dataset did not meet the assumption of normality, even after transformation, a Mann–Whitney test at a significance level of $P < 0.05$. The number of eggs between treatments was compared with a Kruskal–Wallis test with a level of significance of $P < 0.05$.

Bioassay Data Analysis

The statistical analysis was performed using the MINITAB 16 statistical program for PCs (Minitab 2010).

Plutella xylostella Open-Field Test

Study Site and Agronomic Broccoli Cultivation

The study site was located in the Andes Sierra, which has a near constant year-round temperature averaging between 23 and 14°C. There are two distinct seasons: dry (June to August) and wet (September to December). The elevation (2,754 m) and proximity to Chimborazo result in solar radiation that is among the most intense in the world (CONELEC 2008). Temperature and precipitation during the experiment were monitored at a field weather station (INAMHI 2010), located 500 m from the field plot. Standard agronomic practices for broccoli cultivation such as ploughing, weed control, watering, and organic soil amendments were implemented at the experimental fields of the Department of Horticulture of the Polytechnic School of Chimborazo (ESPOCH; S 1°41′05″, W 78°40′20″) Riobamba, Chimborazo Province, Ecuador. As insect abundance usually varies at dry and rainy seasons, we test the treatments in both seasons to make a better assessment of their performance against *P. xylostella* abundance: dry season (June–August 2015) and wet season (September–December 2015). The plants were sown on June 20 for cultivation in the dry season and September 3 for cultivation in the wet season.

Botanical Extracts

The samples from the six plant species were dried outdoors and ground using a Thomas mill to obtain a fine powder. A 45.4-g subsample of each processed plant was mixed with 1 liter tap water containing a 0.2% detergent solution (TIPS, Calbaq S.A., Ecuador) to obtain a concentration of 0.0454 g/ml; the solution was filtered through fine linen for immediate application. A separate 5-liter knapsack sprayer (Petrul FUT-5LP; Truper S.A., Jilotepec, México) was used to apply each solution to the point of run-off, including the underside of the leaves.

Experimental Design

Brassica oleracea var. Avenger was grown from certified seeds sown on a raised bed in the field. The young seedlings were protected from pests with insect-proof netting and were grown in a closed greenhouse. The seedlings were transplanted at the four true leaf stage (30 d after sowing). The spacing was 0.3 × 0.6 m between plants, and the plots measured 3 m × 1.8 m, resulting in 30 plants per plot. Two-meter-wide unplanted alleys were left between the plots to avoid spray drift between adjacent plots. In total, 1,200 plants were used in the trial.

The design of the trial was a completely randomized block with six treatments and two controls with five replications, with a total of 40 experimental units. The treatments were the aqueous extracts

of *W. solanacea*, *D. costata*, *X. purpuratum*, *Clibadium* sp., *D. utilis*, and *C. nardus*. The negative control was a 0.2% tap water detergent solution (TIPS, Calbaq S.A., Ecuador). The positive insecticide control was a mix of 48% chlorpyrifos (LORSBAN 480TM, Dow AgroSciences, EEUU) and 25% lambda cyhalothrin (ZERO 5EC, ANASAC, Chile) at established dosages of 600 and 40 ml/ha, respectively (Cortes et al. 2012, Edifarm 2016).

The applications began 30 d after the plants were transplanted into the field and were re-applied for 7 wk at 8-d intervals. Eight days after each spraying, the number of *P. xylostella* live larvae was determined in situ on 10 plants from the innermost row of each plot. The larvae were counted leaf by leaf on each of these plants, and the total number was recorded. The results are presented as the weekly mean (± SEM) of live *P. xylostella* larvae after treatment in the two seasons.

Open-Field Data Analysis

The normality of the data was tested using the Darling test. When the data set did not meet the assumption of normality, even after transformation, a Friedman nonparametric analysis of variance was applied. When a significant ($P < 0.05$) effect was found in an experiment, the Conover Iman test of nonparametric multiple comparisons was used (Conover 1999). All statistical analyses were performed with the software packages Infostat (Di Rienzo et al. 2011) and Minitab 16 (Minitab 2010).

Brevicoryne brassicae Semifield Test

Botanical Extracts

The plant extracts were prepared as in the field experiments, and hand sprayers were used for immediate application.

Insects

Brevicoryne brassicae were collected from broccoli (*B. oleracea*. var. Avenger) in the experimental fields of the Department of Horticulture of the Escuela Politecnica de Chimborazo (ESPOCH; S 1°41′05″, W 78°40′20″) Riobamba, Chimborazo Province, Ecuador, during September 2015. The aphids were reared on seven independently potted broccoli plants covered with insect-proof netting with 0.87-mm mesh, which prevented the aphids from escaping and any other insects from entering the potted broccoli plants.

Experimental Design

Semifield Outdoor Enclosures

The semifield trials were carried out using a partially covered garden shed located at the Department of Horticulture of the Escuela Superior Politecnica de Chimborazo. The shed had a transparent roof and walls that were half-open; the wind and solar radiation were largely blocked; humidity and temperature were not controlled.

Plants were arranged 50 cm apart inside the garden shed. For each experiment, 30-d-old potted broccoli plants (*B. oleracea* var. Avenger) with six true leaves were covered with an insect-proof net fitted with an elastic band at the base and a zipper at the side to enable access. The potted plants were covered with the netting immediately after potting. Seedlings for potting were raised on a seed bed that was completely covered with an insect-proof net from the day of sowing until the seedlings were potted. The plants were artificially infested with 20 adult cabbage aphids each, which were allowed to establish colonies for 7 d before treatment.

The treatments consisted of two positive controls, two negative controls, and six botanical treatments and were set up in a completely randomized block design with five replications. The plant extracts were prepared as in the field experiments, and precompression manual 2-liter sprayer (Tramontina, Rio Grande do Sul, Brazil) was used for immediate application. The positive controls were two synthetic insecticides, imidacloprid and chlorpyrifos, and the negative controls consisted of 0.2% tap water detergent solution (TIPS) and tap water. Imidacloprid and chlorpyrifos were applied at the recommended concentrations of 0.0025 and 0.6 ml/liter of water, respectively (Cortes et al. 2012, Edifarm 2016).

All solutions were applied to the point of run-off to infested potted plants through a zipper at the side of the cage. A single application was made in each experiment. To measure the effectiveness of the treatments, the number of live individuals was counted at 3, 7, and 15 d after application.

Data Analysis

Because the data could not be normalized, a nonparametric Kruskal–Wallis test was used. When a significant ($P < 0.05$) effect was found in an experiment, nonparametric multiple comparisons were applied (Conover 1999) using Infostat (Di Rienzo et al. 2011). The results are presented as the mean ± SEM.

Results

Effect of Plant Extracts on *P. xylostella* Percent Leaf Consumption, Larvicidal, and Oviposition Deterrence

Plutella xylostella Percent Leaf Consumption

Application of the aqueous extracts from *D. utilis*, *X. purpuratum*, *D. costata*, *W. solanacea*, and the insecticide chlorpyrifos resulted in a lower percentage of leaf consumption by *P. xylostella* larvae compared with the negative water controls (Table 1). On the contrary, *Clibadium* sp. did not show any significant decrease in the percentage of leaf consumption compared with the negative water controls (Table 1). The application of botanical extracts resulted

Table 1. Percent leaf consumption (median) by *P. xylostella* larvae after exposure to aqueous extracts of five Ecuadorian Amazon plants and chlorpyrifos versus their respective water controls

Plant extract	Treatment	Control	Whitney test statistic (*W* value)	P^a	n^b
Chlorpyrifos	0.0	7.7	40	0.01**	5
X. purpuratum	0.4	5.5	145.0	0.003**	10
D. utilis	0.5	5.7	61.0	0.001***	10
D. costata	0.6	5.5	143.0	0.005**	10
W. solanacea	1.6	5.5	134.5	0.028*	10
Clibadium sp.	3.9	5.5	93.0	0.385ns	10

[a]Mann–Whitney test: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
[b]n = number of replicates.

in a lower percentage of leaf consumption than the application of chlorpyrifos (H =14.96, df = 4, P = 0.0048).

Larvicidal Effect of plant extracts on *P. xylostella*

Only the *D. utilis* extract exhibited a significant negative effect on larval survival compare with water controls (Table 2). Chlorpyrifos was three times more lethal to the larvae than *D. utilis* (W = 17.5, P = 0.0472). The effects of the other botanical extracts were not significantly different from those observed in the water controls.

Oviposition Deterrence Effect of Plant Extracts on *P. xylostella*

The oviposition by female *P. xylostella* was significantly lower ($P \leq 0.001$) on the leaf discs treated with botanical extracts than on the discs dipped in water (Table 3). Particularly, the extract from *D. costata* showed a strong negative effect on diamondback moth oviposition, with a mean of 1.1 ± 0.5 eggs laid per leaf disc in 5 d versus 44.2 ± 6.3 eggs in the water control (W = 155.0, P = 0.0002). Interestingly, chlorpyrifos did not deter oviposition (W = 26.0, P = 0.8345) compared with the control discs dipped in water (Table 3). The oviposition deterrence of the extracts from *D. utilis*, *Clibadium* sp., *X. purpuratum*, and *W. solanacea* were similar (5.1–6.4 eggs in 5 d; H = 0.7, df = 3, P = 0.8720), and all botanical extracts had a significantly higher oviposition deterrence effect than chlorpyrifos (42.0 ± 16.5 eggs; H = 10.69, df = 4, P = 0.0289).

The Effects of Botanical Extracts on the Population Abundance of *P. xylostella* Larvae Infield Experiments

Field experiments showed that in both seasons, the abundance of larvae per plant was controlled only by the chemical insecticide (Table 4).

In most treatments, the abundance of *P. xylostella* larvae in both seasons did not differ between the extract treatments and the negative water + detergent controls. During the dry season, in all weeks of observation, none of the extracts produced a statistically significant effects in the abundance of *P. xylostella* larvae compared with the negative water + detergent control (Table 4). During the wet season, significant botanical insecticide effects were observed only at week 1 for *W. solanacea* and *Clibadium* sp. and at week 5 for *W. solanacea*, *C. citratus*, *Clibadium* sp., and *X. purpuratum* (Table 4).

Effects of Botanical Extracts on *B. brassicae* Populations Abundance in Semifield Experiments

On the third day after the application, the extracts of *D. utilis*, *Clibadium* sp., *D. costata*, *X. purpuratum*, and the chemical insecticides significantly reduced the population of *B. brassicae* compared with the water controls ($P < 0.05$; Kruskal–Wallis test; Table 5). On the 7th and 15th days after the application, none of the botanical treatments, except *D. utilis*, differed significantly from the water control in the abundance of *B. brassicae* present ($P < 0.05$; Kruskal–Wallis test; Table 5).

Discussion

Laboratory Studies

When tested against pests, hundreds of plant species have been reported to have activity such as feeding and oviposition deterrence, repellency, acute and chronic toxicity, developmental disruption, or growth inhibition under laboratory conditions (Regnault-Roger et al. 2012, Isman 2017). We contribute with the following new results: the species *D. utilis* caused larval mortality in *P. xylostella*, all of the botanical extracts studied significantly deterred *P. xylostella* oviposition, and with the exception of *Clibadium* sp., all extracts decreased *P. xylostella* leaf consumption.

***Plutella xylostella* Larval Mortality**

None of the extracts tested shown an effective *P. xylostella* larvicidal effect compared with chlorpyrifos (effective 64%), with

Table 2. Percent mortality of *P. xylostella* larvae (mean ± SEM) in response to aqueous extracts of five Ecuadorian Amazon plants and chlorpyrifos versus their respective water controls

Treatment	Mean ± SEM Percent larval mortality	Mean ± SEM Control	Kruskal–Wallis test statistic (Z value)	P^a	n^b
D. costata	4.0 ± 2.7	0.0 ± 0.0	0.76	0.146	50
X. purpuratum	6.0 ± 3.1	0.0 ± 0.0	1.13	0.067	50
Clibadium sp.	6.0 ± 3.1	0.0 ± 0.0	1.13	0.067	50
W. solanacea	8.0 ± 4.4	0.0 ± 0.0	1.13	0.068	50
D. utilis	20.0 ± 6.3	0.0 ± 0.0	2.09	0.017*	25
Chlorpyrifos	64.0 ± 11.7	0.0 ± 0.0	2.61	0.005**	25

[a]Kruskal–Wallis test: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
[b]n = number of larvae used in bioassay.

Table 3. Number of eggs laid by *Plutella xylostella* adults (mean ± SEM) in response to aqueous extracts of five different Ecuadorian Amazon plants and chlorpyrifos versus their respective water controls

Plant extract	Treatment	Control	Mann–Whitney test statistic (W value)	P^a	n^b
D. costata	1.1 ± 0.5	44.2 ± 6.3	155.0	0.0002***	10
D. utilis	5.1 ± 1.9	120.9 ± 15.3	55.0	0.0002***	10
Clibadium sp.	4.5 ± 1.0	43.3 ± 8.2	155.0	0.0002***	10
X. purpuratum	5.7 ± 1.4	65.5 ± 11.8	155.0	0.0002***	10
W. solanacea	6.4 ± 1.9	87.0 ± 17.1	55.0	0.0002***	10
Chlorpyrifos	42.0 ± 16.5	38.8 ± 7.8	26.0	0.8345	5

[a]Mann–Whitney test: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
[b]n = number of replicates.

Table 4. Mean (± SEM) of *Plutella xylostella* larvae per plant in field experiments and the effects of six different aqueous plant extracts, synthetic insecticides and water + detergent, performed in Riobamba, Ecuador, during the dry (June to Aug.) and wet (Oct. to Dec.) seasons in 2015

Treatments	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
(A) Dry season							
W. solanacea	2.2 ± 0.5B	4.0 ± 1.1AB	2.0 ± 0.7AB	11.4 ± 1.0BC	9.0 ± 3.1B	6.8 ± 1.1B	3.8 ± 0.6A
D. costata	0.8 ± 0.8AB	2.0 ± 0.6AB	5.8 ± 2.0AB	8.4 ± 2.1BC	6.6 ± 1.9B	6.2 ± 0.9B	2.0 ± 0.6A
D. utilis	1.2 ± 0.8AB	2.2 ± 1.5AB	5.6 ± 1.6B	11.8 ± 3.4BC	10.2 ± 3.0B	7.2 ± 1.9B	2.8 ± 1.1A
Clibadium sp.	1.4 ± 0.7AB	3.6 ± 1.1B	6.8 ± 2.2B	10.0 ± 2.0BC	8.6 ± 2.9B	4.4 ± 0.7B	2.8 ± 0.7A
C. citratus	1.4 ± 0.7AB	2.2 ± 0.7AB	2.8 ± 0.8AB	11.2 ± 2.3BC	10.8 ± 2.9B	8.0 ± 1.5B	3.4 ± 0.7A
X. purpuratum	2.4 ± 1.5AB	3.4 ± 0.8B	6.8 ± 2.4B	17.8 ± 2.1C	9.0 ± 1.4B	9.8 ± 2.2B	3.6 ± 0.7A
Water + detergent	2.6 ± 0.6B	2.8 ± 0.7AB	2.6 ± 0.8AB	6.4 ± 1.4B	5.2 ± 1.5B	8.2 ± 1.2B	1.4 ± 0.5A
Insecticide (chlorpyrifos and lambda cyhalothrin)	0.0 ± 0.0A	0.2 ± 0.2A	0.4 ± 0.2A	0.6 ± 0.4A	0.0 ± 0.0A	0.4 ± 0.2A	0.2 ± 0.2A
(B) Wet season							
W. solanacea	0.8 ± 0.6AB	1.4 ± 0.4A	1.6 ± 0.2BC	0.8 ± 0.4AB	2.6 ± 0.5B	6.4 ± 1.4BC	17.8 ± 1.5BC
D. costata	3.0 ± 0.9C	4.4 ± 0.5B	1.4 ± 0.2ABC	1.0 ± 0.3AB	12.8 ± 2.5D	11.8 ± 1.6C	20.2 ± 1.8BCD
D. utilis	2.4 ± 0.7C	3.6 ± 0.7AB	1.0 ± 0.3B	4.8 ± 0.4C	12.6 ± 1.1D	7.4 ± 0.9BC	21.8 ± 2.0BCD
Clibadium sp.	0.8 ± 0.6AB	1.4 ± 0.2A	1.0 ± 0.5ABC	2.4 ± 0.7B	3.4 ± 0.5BC	4.6 ± 1.0B	21.8 ± 6.0CD
C. citratus	2.0 ± 0.8BC	4.8 ± 0.9BC	2.2 ± 0.4C	0.8 ± 0.2AB	6.4 ± 1.3C	8.6 ± 2.0BC	34.2 ± 3.1E
X. purpuratum	2.4 ± 1.4BC	8.4 ± 0.7C	2.0 ± 0.3C	2.4 ± 0.5B	6.4 ± 0.7C	6.2 ± 0.9BC	26.4 ± 4.0DE
Water + detergent	1.6 ± 0.7C	3.4 ± 0.9AB	1.6 ± 0.2AB	1.2 ± 0.4A	0.6 ± 0.4D	3.0 ± 0.3BC	4.2 ± 0.7AB
Insecticide (chlorpyrifos and lambda cyhalothrin)	0.0 ± 0.0A	1.6 ± 0.7A	0.0 ± 0.0A	0.2 ± 0.2A	0.2 ± 0.2A	0.4 ± 0.2A	0.8 ± 0.4A

Means in a column with the same letter are not significantly different ($P \leq 0.05$) by Friedman nonparametric analysis of variance.

Table 5. Effects of six aqueous plant extracts, synthetic insecticides, and detergent solution on the survival of *Brevicoryne brassicae* nymphs and adults (mean ± SEM) in semifield experiments at 3, 7, and 15 d after application (DAA)

	3 d	7 d	15 d
Treatments	DAA	DAA	DAA
Chlorpyrifos	0.0 ± 0.0A	0.0 ± 0.0A	0.0 ± 0.0A
Imidacloprid	2.2 ± 0.7AB	0.0 ± 0.0A	0.0 ± 0.0A
D. utilis	30.2 ± 5.9ABC	21.2 ± 5.8AB	16.4 ± 8.8AB
Clibadium sp.	37.8 ± 6.9BCD	37.8 ± 7.1BC	22.8 ± 8.2BC
D. costata	38.2 ± 5.7BCD	47.2 ± 11.7BC	24.0 ± 7.6BC
X. purpuratum	46.0 ± 7.4CDE	44.4 ± 9.9 BC	32.6 ± 9.3BC
W. solanacea	55.4 ± 9.8CDE	58.0 ± 18.0 BC	27.8 ± 12.4BC
C. citratus	62.0 ± 4.1DE	39.6 ± 6.0 BC	28.2 ± 6.7BC
Water + detergent	63.0 ± 6.7DE	53.8 ± 10.2 BC	38.6 ± 9.0BC
Water	76.6 ± 9.6E	78.0 ± 13.5C	57.4 ± 17.1C

Each experiment was replicated five times. Means within a column with different letters differ significantly ($P < 0.05$; Kruskal–Wallis test).

the exception of *D. utilis*. This is the first time that *D. utilis* has been tested as larvicide against *P. xylostella*. Its lethality, around 20%, is most likely due to the presence of rotenone (Dos Santos et al. 2000, Lawson et al. 2010, Zubairi et al. 2016), although other rotenoids, flavonoids, and saponins are also present in *Deguelia* (Dos Santos et al. 2000) and may contribute to its effectiveness. With larvicidal effect around 20%, the extract of *D. utilis* was, however, significantly less effective than chlorpyrifos which resulted in 64.0% mortality.

Despite the apparent superiority of synthetic compounds, we must remember that one of the major problems of these insecticides is that they can also lead to the evolution of resistance in insect pests (IRAC 2017). Further studies are needed to understand whether an increase in solution concentration or in the number of applications can increase the effectiveness of these extracts and to ensure their rapid environmental degradation. It is also important to evaluate the rate of their environmental degradation.

***Plutella xylostella* Oviposition Deterrence**

All botanical extracts studied here were strong deterrents to oviposition by *P. xylostella*, whereas the chemical insecticide was not. The selection of oviposition sites by the female insect is based on a complex behavioral detection of environmental signals to determine the suitability of the host plant for the completion of the life cycle. Oviposition deterrence could act through behavioral effects or sublethal toxicity (Isman 2002). In previous laboratory studies, extracts of *Enterolobium contortisiliquum* (Vell.) Morong, *Sapindus saponaria*, *Trichilia pallida* (Sw.), *Melia azedarach* (L.), and *Azadirachta indica* significantly reduced oviposition by *P. xylostella* (Chen et al. 1996, Medeiros et al. 2005). In our study, it is possible that the deterrence of oviposition in diamondback moths can be explained by the presence of both chemical compounds and/or physical particles in the extracts. All botanical extracts used in this study were cloudy, indicating the presence of particulates, and stained the broccoli leaves with a greenish-brown color. In contrast, the synthetic insecticides were

crystalline and transparent. The presence of particulate matter can alter the oviposition behavior of insects (Unruh et al. 2000, Puterka et al. 2005, Lapointe et al. 2006, Peng et al. 2011). Scanning electron microscopy analysis could be used to confirm the hypothesis that deterrence may exploit the physical properties of a botanical extract rather than its chemical properties. On the other hand, the oviposition deterrent may be caused by the chemical compounds contained in the extracts, although in this study, a full chemical characterization was not carried out. A further study on this subject could be performed by comparing pure rotenone—and/or *Cymbopogon* essential oil—to our extracts to establish, e.g., whether these compounds could cause the oviposition deterrence observed.

Decrease in Leaf Consumption by *P. xylostella* Larvae

We measured leaf consumption by *P. xylostella* larvae by quantitating the percentage removal of tissue from leaf discs over 5 d. In the same experiment, we determined diamondback moth larval mortality from exposure to residues of extracts. The observed decrease in leaf consumption could have been the result of feeding deterrent or sublethal poisoning, impeding feeding. Both effects can explain our leaf consumption results. Here, the percentage of leaf consumption is understood as an estimate of the antiherbivory efficiency of the botanical extracts studied.

Over the 5-d period, diamondback moth larvae could become sublethally poisoned and not eat the leaves for this reason. For example, throughout the experiment, almost 2/3 of larvae in the positive chemical insecticide control died, as did 20% of those in the *D. utilis* treatment and between 4 and 8% of those subjected to the other extracts. We do not yet know the chemical compounds responsible for the decrease in leaf consumption activity or the possible extract with sublethal effects. However, rotenoids are known to be responsible for insecticidal activities of *D. utilis* (Dos Santos et al. 2000, Lawson et al. 2010, Zubairi et al. 2016), and essential oils composed predominantly by monoterpene for *Cymbopogon* insecticidal activities (Setiawati et al. 2011, Solomon et al. 2012).

Other authors reported the toxicity of secondary metabolites of several plant species from the same genera. For example, Antonious et al. (1992) showed feeding deterrence effects of *Dieffenbachia* sp. on the second- and fourth-instar larvae of *Spodoptera littoralis*, leading to mortality; *W. solanacea* and *Witheringia coccoloboides* (Dammer) were shown to contain cytotoxic compounds, whereas *Clibadium* sp. contains tannins, glycosides, sesquiterpenes, carotenoids, terpenoids, essential oils, and alkaloids (Filgueiras et al. 2011), which may explain the decreased leaf consumption by larvae. The latter two species also contain inorganic salts such as oxalic acid, which results in the formation of oxalate (Sefa-Dedeh and Agyir-Sackey 2004, Chávez and Enriquez 2013) in *Xanthosoma poeppigii* and calcium oxalate (Finley 1999) in *D. costata*.

Field and Semifield Studies

Diamondback Moth Field Studies

In our field studies, we expected that the number of diamondback moth larvae would only decrease either due to natural mortality or due to a combination of natural mortality and mortality caused by an insecticide or a plant extract. However, the diamondback moth population fluctuated over time, regardless of the botanical treatment applied. The synthetic insecticides (positive controls) controlled the population of diamondback moth larvae as expected. Although in some cases an insecticidal effect of the extracts was evident, surprisingly, we found no clear explanation for the pattern of the effect. The species *D. utilis* and *C. citratus* are all well known for their insecticidal activities, but the significant effects observed under laboratory conditions and reported in the literature do not always translate into field conditions.

A possible explanation for the lack of clear effects under field conditions could be the rapid degradation of the active chemical compounds present in the botanical extracts. The levels of solar radiation in the mountains of Ecuador are among the highest in the world (CONELEC 2008), and this could easily result in the decomposition of *D. utilis* rotenone and *C. citratus* oils in the field. Rotenone is only effective for a short period after application because the chemical decomposes quickly in the presence of light and air (Cheng et al. 1972, Guleria and Tiku 2009). In a previous study, *C. citratus* oils were affected by environmental conditions (Kongkaew et al. 2011).

Other possible explanations are that the extraction time may lead to biodegradation of the active substances or that the plants used to make the extracts vary in their effectiveness due to genetic or environmental differences (Belmain et al. 2012, Stevenson et al. 2012), resulting in chemotypes under the absence or at low concentrations of rotenone/rotenoids. To ensure the effectiveness of the insecticide under field conditions, it would be necessary to know the ecological and chemical conditions that maximize the quality and quantity of insecticidal components present in the plant. Additionally, detailed studies of the fate of rotenone and insecticidal oils in the environment (i.e., its stability) over a range of environmental conditions would be highly desirable.

Finally, the observed fluctuations in the population size of *P. xylostella* may be a response to environmental factors such as rainfall or temperature or biotic factors such as the presence of natural predators or the possibility of multiple infestations (Wakisaka et al. 1992). For example, during the dry season, the abundance of larvae per plant in all treatments (except synthetic chemicals) was higher in weeks 3–5, when precipitation was lower (INAMHI 2010). In the rainy season, the population of diamondback moth larvae remained under control except during weeks 6 and 7; in those months, precipitation was lower (INAMHI 2010). A previous study has confirmed that precipitation controls diamondback moth populations (Wakisaka et al. 1992). These uncontrolled parameters, such as rainfall, may explain the field trial results. We therefore conclude that, based on our results, botanical extracts failed to control the pest population in the field conditions.

Brevicoryne brassicae Semifield Studies

Our results show that, except for *D. utilis*, all botanicals failed to control aphid *B. brassicae* in the semifield trail. *Degulia utilis* contains rotenoids, whereas *C. citratus* is known for its bioactive essential oils. It is therefore surprising that *C. citratus* did not work as botanical insecticide. *Cymbopogon citratus* is a tropical grass species introduced and acclimated as an unknown variety to the Ecuadorian Amazon (De la Torre and Macia 2008). It can be that Ecuadorian *C. citratus* variety is a chemotypes with low concentrations of Terpenoids. Terpenoids are not really water-soluble compounds, and consequently, water was not an effective extraction agent. An interesting alternative in this sense could be the use of water of industrial essential oil hydrodistillation processes, which contains more terpenoids.

In our experiments, degradation of the active substances happened relatively quickly. Extracts of *Clibadium* sp. and *D. costata* were as effective as the chemical insecticide Imidacloprid and *D. utilis* in controlling the abundance of *B. brassicae* at day 3 after the applications. This faculty was subsequently lost at 7 and 15 d after the application.

Concluding Remarks

This is the first study conducted on aqueous extracts from six plant species from the Ecuadorian Amazon that are used by natives as traditional medicine or as natural products. *Degulia utilis*, *X. purpuratum*, *Clibadium* sp., *W. solanacea*, *C. citratus*, and *D. costata* were investigated in laboratory, semifield, and field trials to evaluate their potential use as botanical insecticides. We conclude that the highest level of insecticidal activity under laboratory conditions was the deterrence of oviposition, with the *D. costata* extract being 40 times more effective than the synthetic insecticide. However, 3 d after the application in a semifield enclosure, all botanical extracts except *D. utilis* lost their ability to control *B. brassicae*, and in an open-field broccoli crop trial, none of the extracts controlled the abundance of *P. xylostella* larvae, including the extract from *D. utilis*, which contains rotenone. Finally, our results regarding the move from laboratory to semifield enclosures and open-field conditions were negative, as the tested extracts did not show insecticidal activity under these conditions.

Botanical insecticides are considered a promising tool for the control of insect pests, and there is an explosion of publications on this subject, most of which show positive results under laboratory conditions (Isman 2008, 2014; Miresmailli and Isman 2014). As our work has shown, the results of laboratory studies on insecticidal activity are not always applicable under semifield and field conditions, but they offer an indispensable starting point for further studies to identify chemicals, to evaluate the effect/efficiency of different extraction methods (Chiasson et al. 2001, Sajfrtová et al. 2008, Pavela et al. 2009), and to assess the influence of environmental parameters on chemical stability and chemotype biodiversity, thereby broadening our understanding whether and how to move from laboratory to field applications.

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