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Phytochemical composition and insecticidal effects of native plant extracts against the mango mealybug, *Rastrococcus invadens* Williams (Homoptera: Pseudococcidae)

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Abstract

The mango mealybug *Rastrococcus invadens* Williams causes enormous damage to fruit trees in Burkina Faso, with losses of up to 90% for mango trees and 53% for citrus fruits. The objective of this study was to evaluate the insecticidal effects of native plant extracts against this insect pest. The insecticidal activity and biological effectiveness of the extracts were evaluated on Petri dishes and mango leaves previously contaminated by spraying them with the native plant extracts. The experimental was a completely randomized Fischer block, and compared six doses of the hydroalcoholic extracts of *A. conyzoides* and *H. suaveolens* with abamectin 18 EC and water (control). These treatments were repeated up to ten times. The determination of the efficiency coefficients was achieved using Abbott's formula (1925). Both extracts of *A. conyzoides* and *H. suaveolens* were effective, but the one based on *A. conyzoides* at 50 gl $^{-1}$ was the most effective with 100% mortality in 48 hours compared to 95.00% for *H. suaveolens'* extract in 72 h. Their respective LC50 were 12.50 gl $^{-1}$ and 17.49 gl $^{-1}$, showing that *A. conyzoides'* extract is more toxic to *R. invadens*. The high content in phenols, flavonoids, alkaloids, etc. of leafy stems of *A. conyzoides* and of *H. suaveolens* could help to sustainably control *R. invadens* in an integrated approach and effectively protect mango orchards.

Keywords: *Rastrococcus invadens, Ageratum conyzoides, Hyptis suaveolens,* bioinsecticide, biological effectiveness.

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INTRODUCTION

The mealybug *Rastrococcus invadens* Williams (Homoptera: Pseudococcidae) is a major insect pest of fruit trees including mango and citrus. This mealybug causes enormous damage to the mango tree, up to 90% loss of mangoes and 53% loss of fruit yield in citrus fruits (FAO, 2022) . Currently, this insect pest is distributed in over 27 countries in Africa, Asia, and French Guiana in Southern America (CABI, 2022). Nymphs and females feed on fruit and leaves leading to severe damage. These produce honeydew which causes sooty mold, leading to a reduction in production. *Rastrococcus invadens* is a polyphagous insect pest attacking more than forty-five plant species in twenty-two families, including economically important crops such as mango and citrus, *Citrus* spp. (Nébié *et al.,* 2018, Fall *et al*. 2019).

The means of control undertaken throughout Africa, such as the destruction of the attacked organs or insecticide treatments, have gradually proven to be ineffective with toxicological risks and more growing invasion due to the great dispersion of host plants and to climate change (Germain *et al*., 2015, Azrag *et al.,* 2023). Treatment with synthetic insecticides is not always effective and safe and leads the mango orchards owners to alternative practices, mainly the usage of botanicals.

Synthetic insecticides, such as Thiametoxam (neonicotinoid) and Chlorpyrifos-ethyl (organophosphorus) evaluated in real environments, have proven effective against the insect pest, but remain incompatible with the action of parasitoids associated with *R. invadens* (Nébié, 2011). Furthermore, the resistance to insecticides has been demonstrated for many years by several authors and the use of these pesticides is proving to be dangerous for ecosystems and human health (Ahmad *et al.,* 2010; Abd El-Rahman *et al.*, 2021). These pesticides even annihilate the action of parasitoids used in biological control programs against this insect pest in West Africa (Mano *et al.,* 2022, Mohamed *et al.,*2022).

However, organic insecticides based on extracts and essential oils from indigenous plants could constitute a sustainable alternative. Numerous studies have previously revealed the insecticidal potential of plant extracts which, according to Mano and Nana (2022) also preserve natural enemies. For example, neem and Mahogoni extracts were reported to exhibit good control of the mango leafhopper *Idioscopus clypealis,* (Shawan *et al*., 2018). The same was shown with neem oil against the same insect pest on the mango tree (Ferdous and Jahan, 2020). The use of plant extracts in plant protection is non-polluting and less toxic compared to synthetic insecticides. This justifies the importance of the present study, which aimed at evaluating the insecticidal effects of native plant extracts against the mango mealybug, *R. invadens*.

MATERIALS AND METHODS

Insects and insecticides

The mealybugs were collected from untreated mango tree plants using bags in the city of Bobo Dioulasso in April 2023. They were bred in the laboratory (T°: 38 ± 2 °C; RH: 70%; PP : 12/12h) until the adults of the F1 generation were obtained. Identification was made by possible using the Williams (1989) and Williams and Granara De Willink (1992) identification keys. The native insecticide used were abamectin 18 EC and hydroalcoholic extracts of leafy stems of *Ageratum conyzoides* and *Hyptis suaveolens.*

Preparation of extracts

Powders from the leafy *stems* of *A. conyzoides* and *H. suaveolens* were used as solid-liquid extraction substrate. For this purpose, 50 g of each leafy stem powder were extracted by maceration with 0.5 L of 80% ethanol in 1 L Erlenmeyer flasks under constant mechanical stirring using a laboratory shaker for 24 h. An additional volume of 125 ml of the extracting solvent was added under a percolator and filtered through cotton. The total extracts obtained were dried in an oven (40 ± 1 °C) to 100 gl^{-1} and used as insecticide products.

Evaluation of insecticidal activity

The host mango tree leaves were used as a trophic support during the various tests in the laboratory from April to June 2023. The insecticidal activity of the extracts was evaluated under laboratory conditions in a Fischer block with four treatments [Abamectin (manufactured by the company SAVANA based in Burkina Faso), water control, extracts from *A. conyzoides* and *H. suaveolens*] repeated ten times. Mango leaves were previously cut into leaf discs and placed in Petri dishes. Each box received 10 adult mealybugs. and foliar contamination of 0.6 ml of extract.The number of dead or weakened individuals was counted under a binocular microscope after 24; 48 and 72 hours of exposure. As for dose effects, concentrations of 12.5; 25 and 50 gl^{-1} of the most active extracts were tested on Petri dishes as previously.

Evaluation of the biological effectiveness of insecticides

Four treatments including the extracts of *A. conyzoides* 50 SL and *H. suaveolens* 50 SL were compared with abamectin 18 EC and water as a control in the same device above. The infestation was recorded from the average number of *R. invadens* per mango leaf. The mortalities' means of the mealybug after 72 hours of treatment were calculated and submitted to the Abbott formula (1925) for the determination of the efficiency coefficient.

Phytochemical screening

The chemical screening of the extracts was carried out by the qualitative method described by Ciulei (1982). These are general and/or specific chemical reaction tests for coloring or complexation in a liquid medium. The aim is to highlight the bioactive phytochemical groups involved in the insecticidal activities observed. Quantitative analysis was performed following the standard procedure for phenols (Ainsworth and Gillespie, 2007), flavonoids (Chang *et al.,* 2002) and alkaloids (Puro *et al.,* 2018).

Statistical analysis of data

R software version 4.0.3 was used for data analysis. The Chapiro test verifying the normality of data and that of Fligner-Killeen attesting the homogeneity of variances allowed the analysis of variance of mortalities. The means were compared using the Tukey test at 5% threshold. Lethal concentrations LC50 and LC90 were determined using logistic regression by probit analysis.

RESULTS

Extraction yield

The yields of hydroalcoholic extracts were higher in *A. conyzoides.* A mass of extract of 16.50 g or 8.25% was obtained with *A. conyzoides* compared to 12.86 g or 6.43% for *H. suaveolens*.

Insecticidal activity of the extracts on *R. invadens*

As demonstrated by the analysis of variance (Table I), the organic extracts studied induced mortality on adults of *R. invadens* with very highly significant differences (P<.001; F ≥ 244.10). The extracts increased mortality proportionally to their doses. The mortalities induced by the extract of *A. conyzoides* varied from 50.00% with 12.5 g/L to 92.50% with 50 g/L in 24 hours and 50.11% with 12.5 g/L to 100% with 50 g/L in 72 hours. As for those of *H. suaveolens extracts,* the transition from 12.5 g/L to 50 g/L resulted in mortality rates varying from 37.50 to 90.00% in 24 hours, from 47.50% to 92.50% in 48 hours and 50.00% to 95.00% in 72 hours.

Table I: Analysis of mortality of *R. invadens* caused by treatments

Treatments	**Concentrations (g/L)**	**Means ± SE of mortality (%)**		
		24h	**48 hours**	**72 hours**
Control (water)	-	0.00 ± 00 f	0.00 ± 00d	4.75 ± 0.63 f
Abamectin 18 EC	-	62.50 ± 2.03 bc	75.00 ± 2.17 b	80.00 ± 2.09c
	12.5	37.50 ± 1.90 e	47.50 ± 198c	50.00 ± 2.04 e
H. suaveolens	25	70.00 ± 2.61 b	85.00 ± 2.03 a	87.50 ± 2.23 b.c.
	50	90.00 ± 2.04 a	92.50 ± 2.05 a	95.00 ± 2.11 ab
A. conyzoides	12.5	50.00 ± 2.00 d	50.02 ± 2.04 c	50.11 ± 2.33 e
	25	55.00 ± 2.08 cds	55.00 ± 1.89c	65.00 ± 2.21 d
	50	92.50 ± 2.05 a	92.50 ± 2.06 a	100.00 ± 0.01 a
F. value (24)		244.10	266.70	267.40
Pr		2.2e-16	2.2e-16	2.2e-16
Meaning		***	***	***

NB: Means in the same column, assigned with the same letter, are not significantly different at the 5% threshold (Tukey test); ***: Highly significant

Table II: Analysis of extracts toxicity on *R. invadens*

	Concentration (gl $^{-1}$)				
Extract	**LC50 ± SE**	**95% CI**	**CL90 ± SE**	**95% CI**	**R^2**
A. conyzoides	12.50 ± 1.71	11.35 - 15.00	47.67 ± 1.01	43.12 – 50.56	0.863
H. suaveolens	17.49 ± 2.01	14.83 – 19.69	50.00 ± 0.14	47.78 – 58.21	0.998

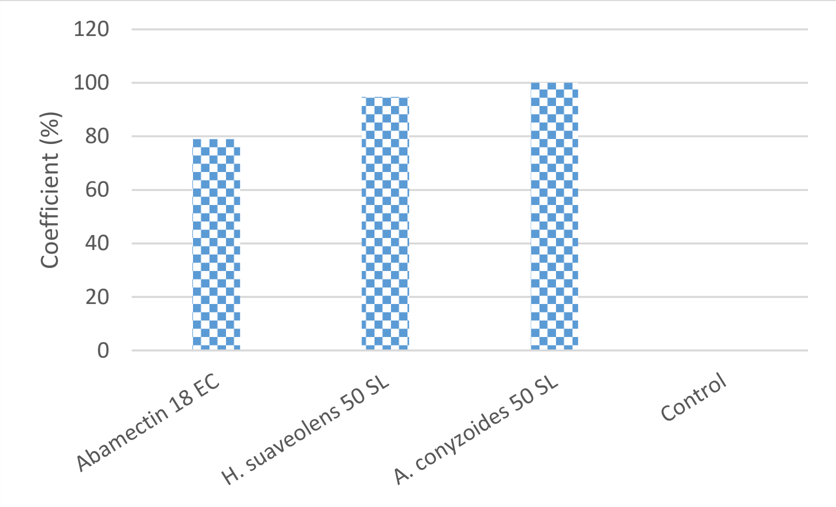

Figure 1: Biological effectiveness of treatments using the Abbott formula

Extracts toxicity on *R. invadens*

The toxicity analysis (Table II) shows that the extract of *A. conyzoides* is more toxic on *R. invadens* with LC 50 of 12.50 ± 1.71 gl $^{-1}$ and CL 90 of 47.67 ± 1 .01 gl $^{-1}$ (R^2= 0.998) against LC 50 of 17.49 ± 2.01 gl $^{-1}$ and an LC 90 of 50.00 ± 0.14 gl $^{-1}$ (R^2= 0.863) for *H. suaveolens*.

Biological effectiveness of plant extracts on *R. invadens*

The data presented in Figure 1 showed variable biological effectiveness coefficients following the different treatments. The highest biological effectiveness (100.00%) was recorded with the extract of *A. conyzoides* 50 SL, followed by that of *H. suaveolens* 50 SL (94.75%) which is in close proximity to Abamectin 18 EC (79.00%).

Phytochemical composition of extracts

The chemical characterization test of the two active extracts showed the presence of polyphenols, flavonoids, leucoanthocyanins, tannins, alkaloids, saponins, quinones and anthraquinones and essential oils (Table III). Leucoanthocyanins, quinones and anthraquinones were absent in *A. conyzoides* while sterols and terpenes were not detected in *H. suaveolens.* Quantitative analysis indicated phenolic, flavonoid and alkaloid contents respectively of 0.50; 143.29; 125.48 mg/g of dry matter of *H. suaveolens* versus 7.54; 29.25 and 2.33 mg/g of dry matter of *A. conyzoides.*

Table II: Chemical composition of extracts

	Chemical groups	Hydro alcoholic extracts _	
		A. conyzoides	***H. suaveolens***
Qualitative analysis	Sterols And terpenes	+	(-)
	Polyphenols	+	+
	Flavonoids	+	+
	Leucoanthocyanins	(-)	+
	Catechical tannins	+	+
	Tannins gallic	+	+
	Alkaloids	+	+
	Saponins	+	+
	Quinones and anthraquinones	(-)	+
	Essential oil	+	+
Quantitative analysis (mg/g)	Phenols	7.5 4± 0.36	0.50 ± 0.06
	Flavonoids	29.25 ± 0.87	125.48 ± 3.66
	Alkaloids	2.33 ± 0.90	143.29 ± 2.80

Note: += present; (-) = absent

DISCUSSION

An insecticidal activity causing mortalities of 50.00% to 95.00% of *R. invadens* in 72 hours was obtained with the hydroalcoholic extracts of *H. suaveolens.* Toxicological studies also reported an LC 50 of 17.49 gl^{-1}. These results confirmed those of several authors. According to Thiam *et al* ., (2017) the aqueous extract of *H. suaveolens* at 187.5 gl $^{-1}$ is effective against *H. armigera.* Extracts from this plant help to reduce losses (Johnson *et al.*, 2018) . Some authors exhibited larvicidal activity against the larvae of the yellow fever mosquito *Aedes aegypti* (L) and *Aedes albopictus* (Conti *et al.,* 2012 and Tennyson *et al.,* 2018). But what explains these biological activities detected in *H. suaveolens?* In this study, a wide range of chemical compounds were isolated from this plant. These were polyphenols, flavonoids, tannins,alkaloids,saponins, leucoanthocyanins, quinones and anthraquinones and essential oil. Previous studies indicated that the biological activities are due to compounds such as alpha-pinene, beta-pinene, sabinene, terpinolene, betacaryophyllene, and 4-terpineol (Conti *et al.,* 2012). Phenolic compounds for example, which are the most abundant secondary metabolites synthesized by plants subjected to biotic and abiotic stresses to adapt and survive, are potentially toxic for many pests (Dai and Mumper, 2010). Furthermore, this study reports that *extracts* of *A. conyzoides* caused mortalities of 50.11% and 100% in 72 hours at 50 gl -1 with [a] CL 50 of 17.49 gl^{-1}. These data confirmed the results of several studies which have shown that the variety of *Ageratum* species has excellent biological properties. Arya *et al.* (2011) and Vasantharani *et al.* (2022) reported various biological properties, such as antifungal, cytotoxic, nematicidal, antifeedant and larvicidal activities. These occurring activities could be related to the presence of various phytochemicals, such as bromophenols, polysaccharides, tannins, flavonoids, phenols, phenolic acids and carotenoids (Chandraker *et al.,* 2019; Puro *et al.,* 2018).

Phytochemical screening of leafy stem powder of *A. conyzoides* indicated the presence of secondary metabolites such as sterols and terpenes, polyphenols, flavonoids, catechic and gallic tannins, alkaloids and saponins. These results are not very similar to those recently reported by Chabi-Sika *et al.* (2023) but very similar to those of Okereke *et al.* (2017) and Ndacnou *et al.* (2020). These authors demonstrated in *A. conyzoides* the presence of various phytochemical compounds such as terpenoids, alkaloids, flavonoids, chromenes, coumarin, saponins, tannins, glycosides, phenols and resins. Variations in phytochemical profile would be due to the screening methodology, the nature, polarity and concentration of solvents, the collection area, the nature of the soil and the stage and organs of the plant used (Nawaz *et al.,* 2020, Lefebvre *et al.*, 2021). Phenolic compounds produced by plants contribute to their antioxidant capacity compared to other natural resources. Polyphenolic compounds such as flavonoids highlighted in this study, possess various biological activities including the formation of complexes with the extracellular wall (Ichino *et al.,* 2014).

CONCLUSION

This study aimed at evaluating the insecticidal effects of native plant extracts against the the mango mealybug, *R. invadens*. Hydroalcoholic extracts based on leafy *stems* of *A. conyzoides* and *H. suaveolens* at 50 gl $^{-1}$ have effective control against *R. invadens.* The one from *A. conyzoides* is more toxic to the mealybug. These plant extracts are rich in phytochemical compounds such as phenols, flavonoids, alkaloids, etc. which are natural resources with insecticidal properties. These active ingredients could contribute in effectively controlling mango mealybug in an integrated approach. Their application in mango orchards could increase fruit yields while preserving the ecosystems and environment of tropical areas. Not having been able to handle the young stages of the mealybug and deepen the chemical analysis, further studies should focus on the mechanism of action of each extract. Knowledge of the mechanism of action will help in the development of complexes of irresistible insecticide principles against *R. invadens* and other related homopteran insect pests.

Author contribution

Conceived, designed, and critically revised the study (M.E., S.F., S.A., D.A.R., N.S.); Collection and culture maintenance (S.A.K.D.D., S.A.); data analysis, interpretation and manuscript drafting (O.R.A., S.P.A.E.D.).

Conflict of interest

The authors declare that they have no conflict of Interests.

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