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Phytochemical dosages of aqueous and ethanolic extracts of Indian verbena in comparison of their larvicidal efficacy on *Hellula undalis* and *Plutella xylostella*

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AbstractCabbage, like all other vegetable crops, is generally attacked by a number of pests. These pests are often controlled by synthetic chemical insecticides, which are a real danger to human health and the environment. Thus, this study is part of the search for alternatives based on plant extracts. In the present study, phytochemical dosages of aqueous and ethanolic extracts of Indian verbena were carried out, with determination of their larvicidal efficacy on *Hellula undalis* and *Plutella xylostella*. Phytochemical characterization revealed the presence of gallic tannins and saponins in the two extracts analyzed. Flavonoid content was 0.042 ± 0.016 mg/g in the aqueous extract and 0.050 ± 0.001 mg/g in the ethanolic extract. Total phenol levels were 0.046 ± 0.110 and 0.052 ± 0.110 mg/g for aqueous and ethanolic extracts respectively. Larvicidal tests revealed higher mortality rates for both extracts on *H. undalis* than on *P. xylostella* at all concentrations. LC50 calculations showed a higher toxicity of the ethanolic extract on *H. undalis* (34.33±0.022 mg/ml) and *P. xylostella* (49.74±0.056 mg/ml) than the aqueous extract (42.49±0.01 mg/ml on *H. undalis* and 53.98±0.00 mg/ml on *P. xylostella*). This study revealed the larvicidal activity of Indian verbena extracts on *H. undalis* and *P. xylostella*.**KEYWORDS :**Cabbage, *Plutella xylostella*, *Hellula undalis*, *Cymbopogon schoenanthus*, larvicide, extracts

1. Introduction

At a time when the world's urban population has surpassed the rural population for the first time, the supply of available, healthy food is one of the major challenges for the coming decades (Argenti, 2010). This challenge is particularly acute on the African continent, as a quarter of the world's undernourished population lives in sub-Saharan Africa, a region where food production has continued to fall since 1970 (Williamson, 2002 ; FAO, 2012). Market gardening makes a significant contribution to the food security of most of the world's and Africa's urban populations. It employs a significant proportion of urban dwellers, who often drop out of the school system too early, and provides them with an income (Delamarche, 2007 ; Mondédji, 2010). In Africa, they play an important role not only in improving the diet of the population, but also in significantly reducing unemployment (Olanrewaju et al., 2004).

Among cultivated vegetable crops, leafy vegetables such as cabbage (*Brassica oleracea*) are better represented than vegetables produced for their fruits, roots, bulbs or tubers (Muzingu, 2007 ; Kanda et al., 2014). Brassicaceae cultivation represents an important food resource, with over 70 million tonnes produced worldwide (FAOSTAT, 2013). It is one of the most widely grown and consumed vegetables, due to its relatively short cycle (60-90 days after transplanting) and the possibility of being grown all year round.

However, as with all vegetable crops, cabbage production is limited by multiple biotic and abiotic constraints. This crop is vulnerable to several pests, the most important of which are cabbage borer (*Hellula undalis*) and cabbage moth (*Plutella xylostella*) (Fening et al., 2020). These pests and diseases contribute exponentially to yield reduction and loss of cabbage quality. This high parasitic pressure leads market gardeners to overuse synthetic pesticides, especially on cabbage (Dovlo, 2007). This inadequate chemical control of crop pests poses serious environmental problems, leading to the destruction of useful species and a major risk of human and animal poisoning (Djaneyé-Boundjou et al., 2000 ; Toé et al., 2001). The quantity of active ingredient that reaches the targets remains very low, and most researchers estimate it at less than 0.3%, meaning that 99.7% of the substances spilled go elsewhere (Pimentel, 1995). Furthermore, the uncontrolled use of synthetic insecticides is leading to the development of resistance within pest and pathogen populations (Kranthi et al., 2001 ; Anstead et al., 2005). And in the case of cabbage, this overuse has led to the development of resistance to several classes of insecticides in *P. xylostella* (Kim et al., 2001 ; Beak et al., 2005).

The market-growing sector, having become aware of the negative repercussions of the use of these synthetic chemical products, is increasingly advocating and encouraging integrated crop management, one of the parameters of which is the use of biological products to defend the various crops against pests and diseases. Plant extracts can be an ecological alternative for preserving crops. Thus, the aim of this study is to contribute to the sustainable management of cabbage pests and more specifically, to evaluate the larvicidal efficacy of aqueous and ethanolic extracts of Indian verbena (*Cymbopogon schoenanthus*) on *H. undalis* and *P. xylostella* with the determination of their phytochemical dosages.

2. Materials and methods

2.1. Experimental site

Aqueous and ethanolic extractions, phytochemical dosages and larvicidal tests were carried out at the Applied Agronomic and Biological Sciences Laboratory (LaSABA) of the University of Kara.

2.2 Materials

2.2.1. Plant material

The Sultana cabbage variety was used for the experiment. This variety grows best in subtropical regions and is highly appreciated in northern Togo. The Indian verbena plants were harvested at the Agropole of the University of Kara, where it is already grown.

2.2.2. Entomological material

H. undalis and *P. xylostella* larvae from mass rearing were used for insecticide testing. Adult egg masses were collected from infested cabbage plots and maintained on their natural support (cabbage leaves). These were incubated in rearing boxes measuring 9 cm in diameter by 5.5 cm in height. After emerging, the first-stage larvae used for the tests were fed healthy, fresh, tender cabbage leaves.

2.3 Method

2.3.1. Phytochemical tests

Aqueous and ethanolic extracts were subjected to qualitative and quantitative analyses of certain secondary metabolites, the latter being based on staining and/or precipitation reactions.

Qualitative analyses

Identification of tannins

Tannins were detected by mixing 1.5 ml of each extract (aqueous and ethanolic) with a few drops of ferric chloride. After stirring, the mixture turned blue-black in the presence of gallic tannins and greenish-brown in the presence of catechic tannins.

Identification of saponins

Saponins were detected by shaking small quantities (15ml) of aqueous and ethanolic extracts placed in tubes. After a resting period, the height of the persistent foam, greater than 1 cm, indicates the presence of saponins according to Koffi et al. (2009).

Quantitative analysis

Dosage of total phenols

The total phenol content of extracts was determined by mixing 120 µl of each extract with 1 ml of diluted Folin-Ciocalteu reagent and 0.8 ml of 7.5% sodium carbonate (Singleton and Rossi, 1965). The mixture was incubated at room temperature for 30 min, and the absorbance was read using a spectrophotometer at 760 nm.

Flavonoid dosage

The aluminum trichloride (AlCl3) colorimetric method was used to determine the flavonoid content of aqueous and ethanolic extracts. A 150 µl volume of each extract was mixed with 0.03 ml of a 5% NaNO2 sodium nitrite solution. After 5 min, 0.02 ml of a 10% AlCl3 solution was added. After a while, 0.2 ml of a sodium carbonate solution mixed with distilled water was added to the extract mixtures. The mixture was vortexed and absorbance measured at 490 nm (Kim et al, 2003).

2.3.2. *In vitro* larvicidal test

Preparation of aqueous and ethanolic extracts of *C. schoenanthus*

Aqueous extracts of *C. schoenanthus* were obtained by grinding 200g fresh leaves in a blender. A quantity of water (1000 ml), previously brought to the boil, was added to the crushed material, and the resulting mixture, after homogenization, was left to macerate for 1 hour at room temperature, under stirring, before being filtered. The filtrate was concentrated using a rotavapor and freeze-dried to provide the solid extracts. Concentrations of 10, 20, 40 and 50 mg/ml were prepared from these extracts. For the ethanolic extracts, fresh *C. schoenanthus*

leaves were macerated in 70% ethanol under agitation for 48 hours at room temperature. The resulting liquid was evaporated in a rotary evaporator at 60°C under pressure, frozen at -15°C and freeze-dried to form the ethanolic extract. The same concentrations of ethanolic extract are also used for larvicidal tests.

Ingestion test

In vitro d′ingestion tests were carried out according to the adapted Insecticide Resistance Action Commitee method (IRAC, 2015). Fresh healthy cabbage leaves collected were soaked for 5 seconds in the different concentrations of aqueous and ethanolic extracts of *C. schoenanthus*. The zero dose or absolute control was distilled water, and the positive control, a synthetic chemical insecticide, Cypercal 50EC (50g/l), was also used. The soaked healthy cabbage leaves were air-dried for 5 to 10 minutes before being placed in boxes containing ten (10) *H. undalis* or *P. xylostella* larvae for 24 hours. The boxes with their contents were placed under laboratory conditions (ambient temperature : 28°C ; relative humidity 80%) for the various observations.

Mortality larvae were counted after 24 h of exposure. This operation was repeated five (5) times for each concentration. Average mortality rates were determined using the Abbott formula (1) recommended by the FAO and WHO for insecticide testing, as shown below (Abbott, 1925) :

$$Mc = \frac{Mob - Mcb}{100 - Mcb} \times 100$$

Mc = Corrected mortality in % ; Mcb = Mortality observed in the control box ; Mob = Mortality observed in in the other boxes.

Determination of 50% lethal concentrations (LC50) of extracts tested on pests

The Finney (1952) method, based on the regression of mortality probits against the logarithms of product concentrations, was used to determine the 50% lethal concentration (LC50) of each extract estimated after 24 hours exposure of *H. undalis* and *P. xylostella* larvae to the various concentrations tested. This method, integrated into Biostat® 5.8.4 software, enabled the LC50 of each extract to be determined from logarithmic curves.

2.3.3. Data analysis

Statistical analysis of average mortality rates was carried out using STATISTICA 6.0 software. Duncan's and Student's t-tests at the 5% threshold were used to discriminate homogeneous groups of means for the different data. Histograms describing the data were produced using Excel spreadsheets.

3. Results and discussion

3.1. Phytochemical dosages

Phytochemical dosage results showed that the aqueous and ethanolic extracts of *C. schoenanthus* all contained gallic tannins. No catechic tannins were identified in either extract. However, they all contain saponins (Table 1). These secondary metabolites are present in other plants of the *Cymbopogon genus*, notably in the species *Cymbopogon citratus*, where Kasmi et al. in 2017 also revealed the presence of tannins and saponins. These molecules, which are all families of bioactive compounds, are recognized for their potential to control other organisms (Maniepi et al. 2022).

Table 1 : Composition of tannins and saponins

Extracts of *C. schoenanthus*	Tannins		Saponins
	Gallic tannins	*Catechic tannins*	
Aqueous	+	-	+
Ethanolic	+	-	+

– : negative test ; + : positive test

The flavonoid contents were statistically different, with a higher content in the ethanolic extract of *C. schoenanthus* (0.050 ± 0.001 mg/g) than in the aqueous extract (0.042 ± 0.016 mg/g) (Table 2). Also in terms of total phenol content, the results showed that there was a significant difference between the content of the aqueous extract (0.046 ± 0.110 mg/g) and that of the ethanolic extract (0.052 ± 0230 mg/g). The ratio of flavonoids/total phenols (Fc/Tpc) was calculated as 91.30% for the aqueous extract and 96.15% for the ethanolic extract (Table 2). According to Hayaouni et al. 2007, the use of polar solvents for extraction results in a high phenolic compound content. This result of high contents of both compounds with the ethanolic extract compared to the aqueous extract is confirmed by other work such as that of Koffi et al., 2010 on the ethanolic and aqueous extraction of twenty-three Ivorian plants, where a higher yield of polyphenols was obtained with the extraction.

Table 2 : Total flavonoid and phenol content

C. schoenanthus extracts	Flavonoid content (mg catechin equivalents/g)	Total phenol content (mg gallic acid equivalents/g)	(Fc/Tpc) ×100

Aqueous	0,042 ± 0,016a	0,046 ± 0,110a	91,30%
Ethanolic	0,050 ± 0,001b	0,052 ± 0230b	96,15%

Fc : Flavonoid content ; Tpc : Total phenol content ; Within the same column, averages with the same letter do not differ statistically from one another ($p \leq 0.05$).

3.2 Larvicidal test on *P. xylostella* and *H. undalis*

3.2.1. Treatment with aqueous extract of *C. schoenanthus*

The *in vitro* ingestion test with aqueous extract on both pests revealed after 24 h that larval mortality varied according to extract concentrations. Mortality rates increased proportionally with concentration (Figure 1). In boxes containing solutions with a concentration of 10mg/ml extract, we observed a mortality rate of 2.10 ± 1.15% for *H. undalis*, and 1.31 ± 0.25 for *P. xylostella*. These lowest mortality rates increase as the concentration of extracts increases. Mortality rates ranged from 18.84 ± 4.99 to 66.42 ± 8.00 for *H. undalis* and 10.66 ± 2.70 to 53.94 ± 8.10 for *P. xylostella* for extracts with concentrations of 20, 40 and 50mg/ml (Figure 1). The maximum mortality rate achieved with the aqueous extract was therefore 66.42 ± 8.00 for *H. undalis* and 53.94 ± 8.10 for *P. xylostella*. However, testing with the commercial insecticide Cypercal produced mortality rates of 100 ± 0.00 for *H. undalis* and 99 ± 2.23 for *P. xylostella* (Figure 1). Mean discrimination of mortality rates using Duncan's test at the 5% threshold revealed that the effects of the different concentrations were quite distinct for *H. undalis* ($p= 0.00117$) and *P. xylostella* ($p= 0.00021$).

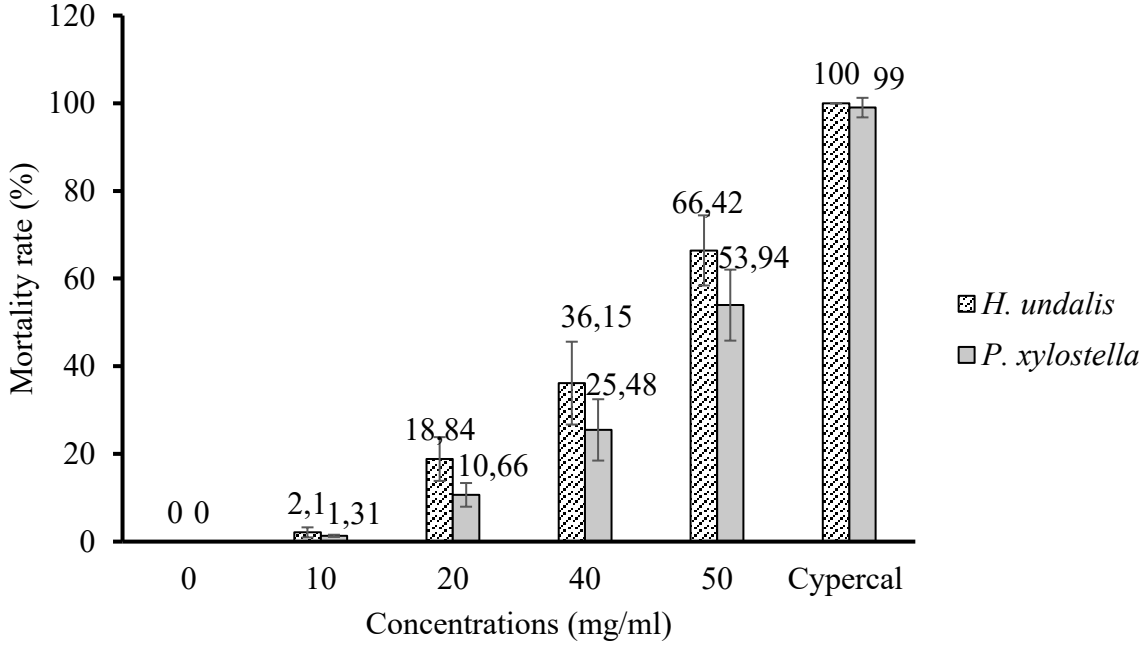

Figure 1 : Comparison of the effect of *C. schoenanthus* aqueous extract on *P. xylostella* and *H. undalis* mortality

3.2.2. Treatment with ethanolic extract of *C. schoenanthus*

The average insect mortality rates as a function of the concentration of the substances tested are shown in Figure 2. In all cases, the mortality rates increased with increasing ethanolic extract concentration (Figure 2). Average mortality rates rose from 0% (absolute control) to 73.8 ± 6.4% with ethanolic extract of *C. schoenanthus* on *H. undalis*. These rates ranged from 0% to 58.14 ±5.6% on *P. xylostella*. The maximum mortality rate induced with ethanolic extract of *C. schoenanthus* was 73.8 ± 6.4 and 58.14 ± 5.6% respectively on *H. undalis* and *P. xylostella* at a concentration of 50mg/mL. The synthetic insecticide caused high mortality rates of 100% for *H. undalis* and 99 ±2.23% for *P. xylostella*. Analysis of variance showed a significant difference in the lethal effects of the different extract concentrations at the 5% threshold according to Duncan's test on *H. undalis* (p= 0.00002) and *P. xylostella* (p= 0.00015).

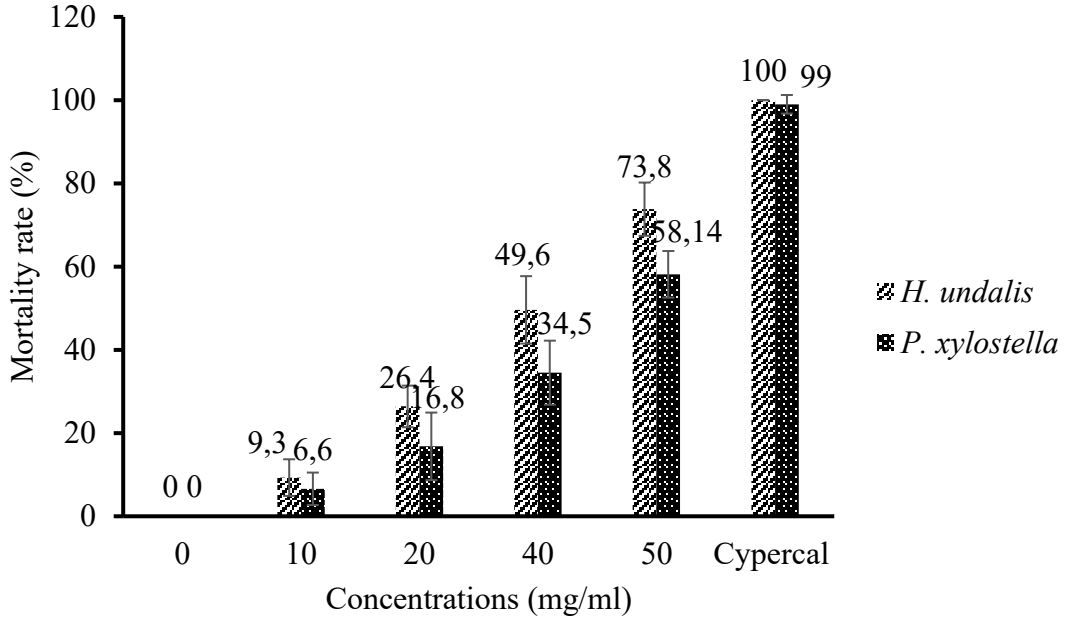

Figure 2 : Comparison of the effect of ethanolic extract of *C. schoenanthus* on mortality of *P. xylostella* and *H. undalis*

3.2.3. Lethal concentrations at 50% mortality of extracts tested on pests

The calculated LC50 of the aqueous extract were 42.49 ±0.014 and 53.98 ±0.00mg/ml on *H. undalis* and *P. xylostella* respectively (Table 3). They were 34.33±0.022 mg/ml on *H. undalis* and 49.74±0.056 mg/ml on *P. xylostella* with ethanolic extract (Table 3). The lowest LC50 values were obtained with the ethanolic extract on both pests, thus demonstrating the high

toxicity of this extract on the larval types. Also contrary to *P. xylostella*, *H. undalis* is more sensitive to both types of extract tested.

Table 3 : Lethal concentrations at 50% mortality of tested substances

	Mean LC50 values (mg/ml)	
Type of *C. schoenanthus* extracts	***H. undalis***	***P. xylostella***
Aqueous extracts	42,49 ±0,014	53,98±0,00
Ethanolic extracts	34,33±0,022	49,74±0,056

Treatments with various aqueous and ethanolic extracts of *C. schoenanthus* on *H. undalis* and *P. xylostella* larvae produced results confirming the larvicidal effect of this plant on these pests. Treatment with aqueous extract was less effective than ethanolic extract on both pests, and its efficacy was concentration-dependent. The insecticidal properties of both extracts were more effective on *H. undalis* than on *P. xylostella*. The extracts were less effective than the commercial insecticide, but showed insecticidal efficiencies in excess of 50% mortality with their 50 mg/ml concentrations, which is satisfactory for sustainable, environmentally respectful agriculture. The lowest LC50 values were for the ethanolic extract (34.33±0.022 mg/ml for *H. undalis* and 49.74±0.056 mg/ml for *P. xylostella*), and so the latter is confirmed to be more toxic to pests than the aqueous extracts. According to these *in vitro* test results, both extracts exert an insecticidal effect on *H. undalis* and *P. xylostella*. This larvicidal activity is due to the fact that these extracts contain natural organic compounds with insecticidal activity. This action could be attributed to secondary metabolites such as polyphenols and terpenoids present in *C. schoenanthus* extracts (Koba et al., 2003). This insecticidal activity of *C. schoenanthus* plant extracts was demonstrated by the work of Kolani et al, (2016), who found in their study that *C. schoenanthus* essential oil had a lethal and inhibitory effect on the emergence of *P. xylostella* adults. Also cabbage plants treated with aqueous extracts of *C. schoenanthus* leaves showed a reduction in the level of *P. xylostella* damage to less than 5% with a dosage of 2 g/l (Laba et al., 2012). This study on the phytosanitary efficacy of *C. schoenanthus* extracts in the field confirms the results of our *in vitro* tests. Referring to the results of phytochemical tests, we note that both extracts contain saponins which are generally composed of terpenoids, the main components of essential oils. Several studies also carried out on *C. schoenanthus* essential oils have confirmed the in vitro insecticidal properties of this aromatic plant. These include work

by Agossou (2001) ; Ketoh et al., (2005) ; Sanda et al., (2006) ; Nyamador (2009) ; Nadio et al., (2013), etc. on *Callosobruchus maculatus*, *P. xylostella*, *Aphis spiraecola*, *Callosobruchus subinnotatus* and *Dysdercus voelkeri* respectively. Saponins are known for their activity against plant pests and their ability to interact with sterols, proteins and phospholipids in cell membranes, causing a loss of cell membrane structural integrity and an increase in ionic permeability (Gruiz and Biacs, 1989). The two extracts of *C. schoenanthus* proved larvicidal against *H. undalis* and *P. xylostella*, probably due to their content of bioactive molecules revealed by phytochemical tests and possibly other metabolites present in these extracts which can be found at low doses.

Conclusion

The results of our studies revealed that ethanolic and aqueous extracts of *C. schoenanthus* do indeed contain tannins and saponins, with high levels of flavonoids and total phenols in the ethanolic extracts. *In vitro* mortality tests showed that *C. schoenanthus* extracts have a significant larvicidal action on *H. undalis* and *P. xylostella*, particularly the ethanolic extracts which proved more toxic with low LC50 values (34.33±0.022 mg/ml for *H. undalis* and 49.74±0.056 mg/ml for *P. xylostella*). These results suggest that *C. schoenanthus* extracts have development potential as a biopesticide against cabbage pests. In a dynamic move towards sustainable agriculture, these extracts could be used as raw materials for phytosanitary products as an alternative to synthetic chemical insecticides. However, tests in semi-controlled or on-farm environments could be envisaged to determine the phytosanitary efficacy of these extracts.

Competing interests

The authors declare that they do not have any conflicts of interest.

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