Attenuating strategies of the Insecticidal Effect and Life history Traits of Chaitophorus leucomelas (Koch, 1854) (Insecta: Aphididae): Case of the Aqueous Extracts of Asteraceae Dittrichia viscosa and a Synthetic product of the neonicotinoids / pyrethroids Family

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Abstract

Methods used to control natural enemies; insects in particular, have been mainly chemical. Given the irritations associated with the use of pesticides, a search for alternatives is required, particularly through the use of plant extracts. The present study focused on comparing the insecticidal power of aqueous extracts of *Dittrichia viscosa* in combination with a bio-adjuvant *Silene fuscata* and a synthetic pesticide Thiamethoxam/ Lambda-cyhalothrin on the abundance, biochemical life traits and demographic parameters of the winter phenotype of *Chaitophorus leucomelas*. The results show a strong effect of the aqueous extracts of *Dittrichia viscosa* on the abundance of *Chaitophorus leucomelas*, with a well pronounced insecticidal activity under the effect of the aqueous extract ratio *Dittrichia viscosa*/ *Silena fuscata*. Lipid and carbohydrate energy biomarkers of *Chaitophorus leucomelas* sexuparae undergo strong changes depending on the products used, with a very significant disturbing action of the synthetic product compared to aqueous extracts. The fecundity of *C. leucomelas* shows a remarkable disturbance under the action of the active ingredient Thiamethoxam/ Lambda-cyhalothrin compared to aqueous extracts. The results also confirm that the products applied cause a disturbance in the growth rate (r_m) and reproduction (R_0) of *C. leucomelas* females, with the chemical treatment having the strongest effect. The full dose of the active ingredient causes remarkable disturbances on the multiplication rate (λ) and the mean generation time (T) of the sexuparae compared to the other applied molecules. Some stability is reported for the doubling time (DT) of treated females compared to the control ones.

Introduction

The green aphid, *Chaitophorus leucomelas* (Hemiptera: Aphididae, Chaitophorinae), is one of the most important pests of black poplar *Populus nigra* (Chararas 1972; Nef and Janssens 1982; Barrios-San et al. 2014). It causes direct damage to the host plant by extracting their sap, which leads to a growth restriction of up to 15%, and indirect damage by transmitting viruses and excreting honeydew which is a favorable environment for fungal development (Sauvion 1995; Ramîrez et al. 2004; Ramîrez and Verdugo 2008; Dedryver et al. 2010; Rubio-Melendez et al. 2011). Like most aphids, *C. leucomelas* is mainly controlled by synthetic pesticides. However, their massive use leads to multiple dysfunctions in agro-ecosystems including the emergence of resistance (Powles 2008; Van Den Bosch and Gilligan 2008; Hazarika et al. 2009; Dângelo et al. 2021), resurgence or appearance of new pests (Hardman et al. 1991; Hazarika et al. 2009) and the destruction of beneficial organisms (Desneux et al. 2007; Marins et al. 2021). To restore this situation, operators in the field of plant protection have turned to biological control (Hågvar and Hofsvang 1991). In this context, the potential of botanical insecticides nowadays represents an effective and non-polluting means of control. Substances of plant origin have always been a major source to develop new substances with biocidal properties (Gomez et al. 1997). Several authors suggest that essential oils or plant extracts are promising for the control of insect pests (Ngamo and Hance 2007). *Dittrichia viscosa* is an invasive plant belonging to the Asteraceae family (Omezzine et al. 2011), it has been frequently used in traditional pharmacopoeia for various therapeutic purposes. Many compounds have been identified and isolated from this plant such as flavonoids (Hernández et al. 2007), monoterpenes (Pérez-Alonso et al. 1996), condensed tannins (Rhimi W et al. 2017), triterpenoids, lactones and sesquiterpene acids including ilicic acid (Fontana et al. 2007) and polyphenols (Danino et al. 2009). Extracts of *D. viscosa* have shown a phytotoxic (Levizou et al. 2002), antifungal (Cohen et al. 2006), nematocidal (Oka et al. 2001), acaricidal (Mansour et al. 2004) and insecticidal effect (Alexenizer and Dorn 2007). Perdikis et al. (2007), report that *D. viscosa* has a major biocidal activity on aphids.

In addition to reducing the availability, the intrinsic toxicity of active ingredients can affect the energy balance and demographic parameters of pests. This toxicity can produce biochemical, histological or morphological disturbances, resulting in specific alterations of an organ, a system or a function, otherwise of a biochemical or biological process (Moussavou Moudouma 2010; Louat 2013). Some authors argue that the observed variations in the major life characteristics of organisms can be explained by life history theory. This theory is based more specifically on the characters having a direct link with reproduction or survival, such as size at birth, size and age at sexual maturity, the number of offspring produced, their ability to survive, etc. (Stearns 1992; Roff 2002 in Giron 2006).

Facing the different stressful conditions, the organism must make an arrangement between the different life history traits involved based on the available energy level (Levins 1968). Molven and Goksoyr (1993) report that organisms use different means likely to restore homeostasis and that all responses seem to technically cost the organism metabolic resources, especially sugar and lipids. Besides, Lagadic et al. (1997), confirm that the assessment of disturbances at the organism level requires the study of biomarkers. This latter makes it possible to describe, explain and even predict disturbance effects of different stressful states.

The general context of the present study aims to find ways to improve the efficacy and estimate the biocidal activity of phytopreparations through formulations that extend persistence in the field or incorporate synergistic products which, being themselves non-toxic at the used doses, enhance the performance of the active ingredients particularly by allowing reducing the dose used, thus limiting their impact on the fauna and flora and ultimately lessening loss problems during the use of bioproducts. The objective of the present study is to evaluate the effect of aqueous extracts of *D. viscosa* in comparison with an active ingredient Thiamethoxam/Lambda-Cyhalothrin, on the abundance, conditioning, energy balance and on certain demographic parameters such as fecundity, net reproduction rate (R_0), intrinsic rate of increase (r_m), finite rate of increase (λ), mean life time (T) and doubling time (DT) of *Chaitophorus leucomelas*.

Materials And Methods

Geographical location of the study region

The present study was carried out in 2012 in the middle of Mitidja plain in Soumâa region (Algeria) at the piedmont of the Atlas mountains of Blida province, at an altitude of 80 to 100 m, a longitude of 2°45'E and a latitude of 36°35'N (Loucif and Bonafonte 1977). Precipitation largely fluctuates, varying between 380 mm and 787.88 mm and occurs during winter and spring. The coldest months are January and February with average temperatures of 4.49°C and 4.48°C, respectively. The hottest months are July and August with respective average temperatures of 37.2°C and 37, 00°C for the period from 1997 to 2012. For the study year 2012, the cold temperature was recorded in December with an average value of 11°C, while the hottest temperature is that of July with 33.2°C. Emberger's Bioclimatic Quotient Q2 (Sauvage 1963), classifies the Soumâa zone in the humid bioclimatic stage with mild winters. In this region, two artificial poplar groves with 8-year-old black poplar *Populus nigra* alignment species were chosen for the study.

Preparation of the aqueous extracts

The plant material selected for the aqueous extract preparation was only of two common spontaneous plants in the Mediterranean region *Silene fuscata* (Caryophyllaceae) and *Dittrichia viscosa* (Asteraceae). The first species *Dittrichia viscosa* was used as an active ingredient; it was collected in the flowering stage of the sub-littoral region of Soumâa during the summer period. The second species, used as a bio-adjuvant for the first time, was collected at the flowering stage during the autumnal period in the mountainous region of Chrea located at an altitude of 980 m, a longitude 2°52'36"E and a latitude of 36°25'N. The precipitation, mainly in winter and spring, is characterized by a great interannual and intermonth irregularity varying between 1225.38 and 360.83 mm. Regarding temperature, the coldest month recorded is January with an average minimum temperature of 1.01°C, while the hottest month is July with an average maximum temperature of 31.37°C. Emberger's Bioclimatic quotient Q2 (Q2 = 83.44), classifies Chrea region in the sub-humid bioclimatic stage with mild winters (Sauvage 1963). The collected plants were cleaned and dried in the open air, away from light and moisture. The plant material after drying in the shade is crushed into powder by a propeller mixer (MOULINEX). An aqueous maceration was carried out with 20 g of vegetable powder added to 250 ml of distilled water. The bottles are horizontally agitated for 72 hours at room temperature. The homogenates were first filtered with compresses, then through whatman paper (N° 1). The aqueous extracts obtained were preserved aseptically in 25 cm^3 Roux bottles, surrounded by aluminum foil in order to avoid any degradation of the molecules by light and then stored in a refrigerator at + 4°C for later use (De Souza et al. 1995).

The synthetic Material

The pesticide used to compare efficacy is a product based on two active ingredients (Thiamethoxam/Lambda-cyhalothrin), belonging to two different chemical families neonicotinoids/pyrethroids, and with the crude chemical formulas $C_8 H_{10} ClN_5 O_3$ / $C_{23} H_{19} ClF_3 NO_3$. The molecule has three modes of action (contact, ingestion and systemic) by blocking membrane permeability and the opening of sodium channels (Couteux and Lejeune 2012).

Sampling and Treatment

Treatments were focused on the different stages of *Chaitophorus leucomelas* (Aphididae, Homoptera) growing on *Populus nigra* leaves during the autumn-winter period. Two poplar groves were set aside for the efficacy tests. Two supply modes of aqueous extracts were applied, namely: aqueous extracts of the whole plant *Dittrichia viscosa* and aqueous extract ratio of *Dittrichia viscosa* and *Silene fuscata* (1:1). For the chemical product (Thiamethoxam/Lambdacyhalothrin), we used the prescribed dose (4ml/l) and the half dose (2ml/l). As for the control, a spray of running water was applied. A linear device with observation plots proposed by Frontier (1983) was used. From the 21 trees obtained through the observation plots, we took five leaves from each cardinal direction at a rate of one sample per day from the eight trees randomly taken from the different blocks. The samples were taken during a period of 11 days, from November 30th 2012 to December 9th 2012. All samples were taken at man height (170 cm). Samples taken from the field will undergo an abundance assessment in the laboratory. Living females are weighed and placed in 1.5 ml Eppendorf tubes, then stored at -20°C for a quantification of the energy balance.

Estimation of the abundance

The counting technique of individual aphids obtained through plant transects consists of taking leaves from each cardinal point during the period of investigation which lasted over one month. The different life stages (larvae and adults) were then identified and counted under a magnifying binocular microscope (G × 40).

Estimation of the conditioning

From the residual populations, we were interested in estimating the weight of females. Each ten females were put in a 1.5 ml eppendorf (previously tared), then weighed. The weight measurements of the females were done using an accuracy balance (Princeton Instruments, Model YP402N).

Estimation of the energy balance

The preserved females were subjected to a quantification of lipid and carbohydrate energy biomarkers following the protocols established by Van Brummelen and Suijfzand (1993) and Win Decoen (2000 *in* Mostefaoui et al. 2014), respectively.

The extraction of lipid reserves is carried out after crushing of *Chaitophorus leucomelas* (10 individuals per tube) with a monophasic mixture 1: 2: 0.8 (chloroform: methanol: double distilled water). The tubes are centrifuged for 5 minutes at 14 000 revolutions/min at 4°C. Adding chloroform to the tubes separates the mixture into two phases. The chloroform solutions containing the lipids are recovered and pooled, then dried over sodium sulfate. The lipids are recovered after rinsing the sodium sulfate with chloroform. The tubes are evaporated to dryness under a stream of nitrogen. Sulfuric acid is added to the dry residue, then heated for 10 minutes at 100°C. After cooling, the vanillin reagent is added to each sample. The solution then takes on a pink color, and the optical density is read at 540 nm after 10 minutes. The white color is obtained from a series of concentrations of cholesterol mixed with sulfuric acid and reagent vanillin.

Regarding the extraction and quantification of carbohydrate reserves, *Chaitophorus leucomelas* individuals are homogenized in double-distilled water with a grinder, then trichloroacetic acid (TCA 15%) is added in order to precipitate the proteins. Precipitation is facilitated by a centrifugation for 10 minutes at 3000 revolutions/min at 4°C. The sugar-containing supernatant is recovered and the pellet is redissolved in a solution of T.C.A. 5%. A solution of 250 µl containing the supernatants is poured into a test tube; to which 250 µl of 5% phenol and 1 ml of H_2SO_4 are quickly added. The mixture is placed in a well of a microplate in the light and at room temperature. Sample adsorption is measured after 30 minutes at 490 nm. The white color is obtained from a stock solution of glucose at 0.5 mg/ml (5 mg of glucose in 10 ml of distilled water).

Estimation of the fecundity

Fecundity is measured by the ratio of the number of larvae to the number of female adults, according to the formula (Carey 1982):

FER = NL / NF

Where:

NL: number of larvae;

NF: number of females.

Estimation of the reproduction parameters

The sexuparae of *C. leucomelas* are only represented by females in this phase of their development cycle. Before applying the active molecules, 20 leaves were randomly taken from which we kept one nymph and one adult per leaf (Tahriri Adabi et al. 2010). After 24 hours of treatment, the nymph and the adult on each leaf were checked daily and their survival was recorded at each of the different experimental units. The presence of exuviae was adopted to determine the moulting period. When nymphs developed into adults, daily monitoring was carried out to estimate survival and reproduction. We took the measures to remove all the neonate larvae from the leaves after being counted. These observations were maintained until the 10th day after applying the treatments. Demographic parameters of *C. leucomelas* such as Net Reproductive Rate (R_0), Intrinsic Rate of Increase (r_m), Finite Rate of Increase (λ), Mean Life Time (T) and Doubling Time (DT) were calculated according to the formulas proposed by Carey (1982) and Gul et al (2021).

Data analysis

The statistical analysis involved the evaluation of the insecticidal activity of phytopreparations based on aqueous extracts *Dittrichia/Silene* on the different biological forms of *Chaitophorus leucomelas*. Variance analyses are made on homogeneous means adopted on the basis of a coefficient of variance (C.V. <15%). The comparisons of the mean abundances and energy biomarkers of the trapped aphids are followed by different variance tests (WILCOXON; MONTE CARLO). The cross-correlation test was used to explore the order of inflowl under the different stressful conditions. Under the effect of different applications, the statistical description of the temporal variation trends of fecundity and demographic parameters was established by the ANOVA test. The significant contributions retained are at the threshold of a probability of 5% and the calculations were carried out by the PAST software vers. 1.37 (Hammer et al. 2001).

Results

Toxicity of the aqueous extracts on the abundance of Chaitophorus leucomelas **sexuparae under the effect of different applied molecules**

Table 1 shows the abundance of *Chaitophorus leucomelas* under the effect of the different treatments applied (aqueous extracts ratio *Dittrichia viscosa*/*Silena fuscata*, the aqueous extract of the whole plant *D. viscosa* and the synthetic product Thiamethoxam/Lambdacyhalothrin). With reference to the abundances, the results indicate that the aqueous extracts have a validated toxicity on *C. leucomelas* individuals compared to the control group (p < 5%). The low median values recorded in the treated samples confirm the pronounced insecticidal activity of the aqueous extracts in the phytopreparation ratio (p < 5%). The cross-correlation test is done in advance in order to verify the effect of phytopreparations on the overall dynamics of *C. leucomelas*. Barycenter values (Maximum Abundance) of treated and control populations show a non-significant time lag that does not exceed two days. The same test shows that the reestablishment of maximum abundances indicates a significant time lag following effect of phytopreparations ($p < 1\%$).

The insecticidal activity of the dose and half-dose of Thiamethoxam/Lambdacyhalothrin treatment is very distinctive in terms of abundance reduction of *C. leucomelas* sexuparae compared to the control group ($p < 5\%$), whereas the confrontation of the abundances under the dose and the half-dose effect indicated no significant difference ($p > 5\%$). The medians show the lowest values under the synthetic product effect ($p < 5\%$). The cross-correlation test indicates a significant temporal shift (3 day Lag) between the treated and the control groups ($p < 5\%$).

Table 1
Phytopreparation effect of *Dittrichia viscosa*, *Silene fuscata* and Thiamethoxam/Lambdacyhalothrin on the abundance of *Chaitophorus leucomelas* sexuparae

Abundance	Treatments	N	Mean ± S.E.	Median	Wilcoxon test	Monte Carlo test	Cross-correlation test		
							Barycentre	Lag	p-value
	Control	11	219,72 ± 46,07^a	138	**0,007****	**0,004****	4,73	1,38	0,337 Ns
	Aqueous extract of plant		55 ± 9,27^b	26			3,35		
	Control	11	219,72 ± 46,07^a	138	**0,005****	**0,003***	4,73	1,79	0,337 Ns
	Aqueous extract of ratio		47,54 ± 10,61^b	14			2,94		
	Aqueous extract of plant	11	55 ± 9,27^a	26	**0,029***	**0,026***	3,35	0,41	0,001**
	Aqueous extract of ratio		47,54 ± 10,61^b	14			2,94		
	Control	11	469,45 ± 71,88^a	465	**0,007****	**0,004****	4,62	2,92	0,010*
	Half-dose		144,81 ± 14,47^b	15			1,64		
	Control	11	469,45 ± 71,88 ^{a}	465	**0,007****	**0,004****	4,62	2,95	0,025*
	Dose		126,36 ± 06,70^b	19			1,67		
	Half-dose	11	144,81 ± 14,47	15	**0,202**^{Ns}	**0,234** ^{Ns}	1,64	0,3	0,081^{Ns}
	Dose		126,36 ± 06,70	19			1,67		

NS: Non-Significant • *: Significant at 5% • **: Significant at 1‰ • ***: Significant at 1‰.

Different letters indicate differences among groups Control, Aqueous extract of plant, aqueous extract of ratio, Half-dose of active ingredient, Dose of active ingredient.

Estimates of the effect of aqueous extracts and pesticide on demographic parameters

The graphic representation of the temporal fluctuation of *C. leucomelas*' fecundity under the effect of two treatment types shows a remarkable drop in the biotic potential of the exposed females compared to the control group (Fig. 1). Following exposure to Thiamethoxam/Lambdacyhalothrin, fecundity undergoes a gradual reduction until the fifth day, with a striking disturbing action of the prescribed dose compared to the half-dose. Beyond this period, a gradual recovery of biotic potential is notified, and it continues until the end of the experiment (Fig. 1a). On the other hand, the biotic potential better tolerates the effect of phytopreparations, whose aqueous extracts ratio engages a very pronounced disturbance of fecundity compared to aqueous extracts of the whole plant (Fig. 1b).

The evolution of the demographic parameters of *Chaitophorus leucomelas* under the effect of the different applications are presented in Table 2. The results of the ANOVA-type variance analysis, supported by the post-hoc test, demonstrate that the Net reproductive rate (R_0) of the females exposed to phytopreparations and to Thiamethoxam/Lambdacyhalothrin shows significantly low values compared to the control group.

Insignificant values are indicated by the comparison of the reproduction rate under the effect of aqueous extracts of the whole plant *D. viscosa* and the ratio *D. viscosa / S. fuscata* and the dose and half-dose of Thiamethoxam/Lambdacyhalothrin. In regard to the intrinsic rate of increase (rm) of *C. leucomelas* populations, analyzes indicate a significant increase between the treated and the controls. This increase is very pointed under the effect of Thiamethoxam/Lambdacyhalothrin compared to the effect of aqueous extracts of phytopreparations. Moreover, the Thiamethoxam/Lambdacyhalothrin applied, the homologous dose and the aqueous extract of the *D. viscosa*/ *S. fuscata* ratio clearly reduced the multiplication rate (λ). The same results show that the homologous dose of Thiamethoxam/Lambdacyhalothrin continues as a treatment, significantly affecting the mean generation time (T) of *Chaitophorus leucomelas* populations. Finally, the different types of treatment barely affect the doubling time (DT) of populations (Table 2).

The effects of molecules on biochemical and weight traits of Chaitophorus leucomelas

Table 3 shows the lipid-carbohydrate energy balance and the weight variation of *C. leucomelas* after applying phytopreparations and Thiamethoxam/Lambdacyhalothrin. The results plainly demonstrate the disruption of the lipid reserves of *C. leucomelas* females exposed to the phytopreparation ratio and to doses of the synthetic product Thiamethoxam/Lambdacyhalothrin compared to the control group ($p < 1\%$). However, carbohydrate stores are severely affected by the whole plant phytopreparation of *D. viscosa* and the homologous dose of the synthetic product ($p < 5\%$). At the same time, weight measurements show a significant difference following the two types of treatment ($p < 5\%$). Tests concluded that Thiamethoxam/Lambdacyhalothrin made significant disturbances on the lipid-carbohydrate energy balance of the exposed populations compared to those exposed to phytopreparations.

Table 2
Demographic parameters of *Chaitophorus leucomelas* under the effect of biological and chemical treatments

Parameters / Treatments		Net reproductive rate (*R0*)		Intrinsic rate of increase (r_m)		Finite rate of increase (λ)		Mean life time (T)		Doubling time (DT)	
		Mean ± SE	p-value	Mean ± SE	p-value	Mean ± SE	p-value	Mean ± SE	p-value	Mean ± SE	p-value
Biological	**Control**	5,481 ± 0,271[a]	**0,000*****	0,021 ± 0,012	**0,293 Ns**	0,691 ± 0,012	**0,212 Ns**	2,632 ± 0,071	**0,996 Ns**	2,171 ± 0,131	0,654 Ns
	Aqueous extract of D. viscosa **plant**	2,862 ± 0,252[b]		0,011 ± 0,011		0,634 ± 0,011		2,601 ± 0,072		2,443 ± 0,072	
	Control	5,481 ± 0,271 [a]	**0,000*****	0,021 ± 0,012 [a]	**0,051***	0,691 ± 0,012[a]	**0,025***	2,632 ± 0,071	**0,276 Ns**	2,171 ± 0,131	0,864 Ns
	Aqueous extract of D. viscosa/ S. fuscata **ratio**	2,085 ± 0,463 [b]		0,039 ± 0,011 [b]		0,611 ± 0,010[b]		2,121 ± 0,353		2,012 ± 0,341	
	Aqueous extract of D. viscosa **plant**	2,862 ± 0,252	**0,260 Ns**	0,011 ± 0,011 [a]	**0,054***	0,634 ± 0,011	**0,185 Ns**	2,601 ± 0,072	**0,232 Ns**	2,443 ± 0,072	0,355 Ns
	Aqueous extract of D. viscosa/S. fuscata **ratio**	2,085 ± 0,463		0,039 ± 0,011 [b]		0,611 ± 0,010		2,121 ± 0,353		2,012 ± 0,341	
Chimical	**control**	5,081 ± 0,561 [a]	**0,010***	0,091 ± 0,022 [a]	**0,021***	0,701 ± 0,011	**0,14 Ns**	2,603 ± 0,071	**0,818 Ns**	2,154 ± 0,152	0,998 Ns
	Half-dose	2,702 ± 0,471 [b]		0,132 ± 0,013 [b]		0,692 ± 0,012		2,341 ± 0,272		2,171 ± 0,251	
	Control	5,081 ± 0,561 [a]	**0,000*****	0,091 ± 0,022 [a]	**0,025***	0,701 ± 0,011 [a]	**0,009****	2,603 ± 0,071	**0,072 Ns**	2,154 ± 0,152	0,352 Ns
	Dose	1,603 ± 0,541 [b]		0,118 ± 0,011 [b]		0,601 ± 0,011 [b]		1,629 ± 0,441		1,553 ± 0,421	
	Half-dose	2,702 ± 0,471	**0,321**	0,132 ± 0,013	**0,115 Ns**	0,692 ± 0,012	**0,07 Ns**	2,341 ± 0,272	**0,223 Ns**	2,171 ± 0,251	0,322 Ns
	Dose	1,603 ± 0,541		0,118 ± 0,011		0,601 ± 0,011		1,629 ± 0,441		1,553 ± 0,421	

NS: Non-Significant • *: Significant at 5% • **: Significant at 1‰ • ***: Significant at 1‰.

Different letters indicate differences among groups Control, Aqueous extract of plant, aqueous extract of ratio, Half-dose of active ingredient, Dose of active ingredient.

Table 3

Estimation of the toxicity of chemical and biological molecules on lipido-carbohydrate energy reserves and weight measurements of *Chaitophorus leucomelas*

Treatments		Energy reserves								weighabl measure			
		Lipid				**Carbohydrate**							
		N	Mean ± E.S.	Wilcoxon test	Monte Carlo test	N	Mean ± E. S.	Wilcoxon test	Monte Carlo test	N	Mean ± **S.E**	Wilcoxon test	Monte Carlo test
Biological	**Control**	11	9,041 ± 0,740	0,722 Ns	0,748 Ns	11	0,202 ± 0,014 a	0,005*	0,003**	11	3,69 ± 0,259 a	0,007*	0,004*
	Aqueous extract of plant	11	9,153 ± 1,693			11	0,166 ± 0, 025 b			11	3,245 ± 0,149 b		
	Control	11	9,041 ± 0,740 a	0,003**	0,001***	11	0,202 ± 0,014	0,878Ns	0,911 Ns	11	3,69 ± 0,259 a	0,007*	0,004*
	Aqueous extract of ratio	11	12,611 ± 2,896 b			11	0,197 ± 0,035			11	3,133 ± 0,400 b		
	Aqueous extract of plant	11	9,153 ± 1,693a	0,003**	0,001***	11	0,166 ± 0,025	0,052*	0,063 Ns	11	3,245 ± 0,149 a	0,005*	0,002**
	Aqueous extract of ratio	11	12,611 ± 2,896b			11	0,197 ± 0,035			11	3,133 ± 0,400 b		
Chemical	**Control**	11	29,783 ± 12,103 a	0,041*	0,051 *	11	0,214 ± 0,029	0,325 Ns	0,343 Ns	11	3,367 ± 0,641	0,413 Ns	0,373 Ns
	Half-dose	11	38,391 ± 2,876 b			11	0,223 ± 0,022			11	3,289 ± 0,798		
	Control	11	29,783 ± 12,103 a	0,010*	0,020 *	11	0,214 ± 0,029 a	0,046*	0,049*	11	3,367 ± 0,641 a	0,040*	0,041*
	Dose	11	39,688 ± 4,089 b			11	0,240 ± 0,022 b			11	3,779 ± 0,678 b		
	Half-dose	11	38,391 ± 2,876	0,070 Ns	0,050*	11	0,223 ± 0,022	0,063 Ns	0,058*	11	3,289 ± 0,798 a	0,040*	0,042*
	Dose	11	39,688 ± 4,089			11	0,240 ± 0,022			11	3,779 ± 0,678 b		

NS: Non-Significant • *: Significant at 5% • **: Significant at 1‰ • ***: Significant at 1‰.

Different letters indicate differences among groups Control, Aqueous extract of plant, aqueous extract of ratio, Half-dose of active ingredient, Dose of active ingredient.

Discussion

Natural products are and always will be an inexhaustible source of complex and diverse structures (Laurençon 2013). Several authors have shown that plants are capable of producing a wide variety of active substances involved in the defense against pests (Deravel et al. 2013). These substances usually come as cocktails of metabolic compounds with different activities (Alexenizer and Dorn 2007). In this context, this preliminary study aims to look for methods to improve the effectiveness of new bioactive molecules with biocidal activity by incorporating synergistic products.

Assessment of the insecticidal potential of phytopreparations and of the active ingredient on the abundance of Chaitophorus leucomelas

The results of biological treatments by applying aqueous extracts of the whole plant *Dittrichia viscosa*, aqueous extract ratio *Dittrichia viscosa* / *Silene fuscata* and the active ingredient Thiamethoxam/Lambda-cyhalothrin showed an explicit knock-down effect on the abundance of *Chaitophorus leucomelas* sexuparae compared to the control group. This reported shock effect on abundances shows an upward rating of toxicity starting from the aqueous extract of the whole plant *Dittrichia viscosa*, then the aqueous extracts to the *Dittrichia* / *Silene* ratio and finally the active ingredient. Considering the results obtained by the cross-correlation test, we find that the aphid populations settle first in the block treated with the aqueous extracts and then in the block treated with the active ingredient. In accordance with these results, it can be assumed as a hypothesis that the synthetic product generates a moderately persistent repressive shock effect on the population of *Chaitophorus leucomelas*, which is ephemeral under the effect of phytopreparations.

Studies have shown that chemicals have the ability to disrupt the normal functioning of exposed organisms (Jean and Benmarhnia 2011). In other words, the impact of pesticides on harmful organisms targets the integrity of the individual, therefore a dysfunction of all of his biological parameters where each parameter plays a role in his survival.

Very satisfactory results have been found following the use of the aqueous extract of the entire plant *Dittrichia viscosa* on the population structure of *Chaitophorus leucomelas*. These results allow us to suggest that the obtained aqueous extracts contain a wide variety of bioactive components that have been released during the extraction process and that act in synergy. This hypothesis is supported by a fairly rich literature which states that *Dittrichia viscosa* contains natural defensive substances that have been used as a very diverse therapy and have been known for a long time (Cafarchia et al. 2002; Kattouf et al. 2009). *D. viscosa* is known for its antihypertensive (Kattouf et al. 2009), anti-inflammatory and antioxidant (Lounis et al. 2009), antidiabetic, antipyretic, wound healing, antiseptic and antiphlogistic activities (Lauro and Rolih 1990; Omezzine et al. 2011) and its antiulcerogenic action is attributed to its flavonic composition. Moreover, extracts of *D. viscosa* were tested for thier antiviral (Abad et al. 2000), antifungal (Mamoci et al. 2011), antimicrobial (Maoz and Neeman, 1998), antibacterial (Squalli et al. 2007), herbicidal (Muehlchen et al. 1990) nematicidal (Oka et al. 2006), acaricidal (Mansour et al. 2004) and insecticidal activities (Alexenizer and Dorn 2007).

The aqueous extract ratio *Dittrichia viscosa* / *Silene fuscata* expressed a remarkable toxic action compared to unformulated aqueous extracts. The use of ratios increased the insecticidal efficacy of *Dittrichia viscosa* and reduced the incidence of side effects on population recovery. Presuming that the bio-adjuvant *Silene fuscata* accelerated the penetration of the bioactive molecule, this assumes that the distribution of the biomolecules to the sensitive sites of the pest is done in a relatively short period of time. These results are consistent with the results of other researchers such as Hayes et al. (2006), who also showed that the adjuvant is used primarily to increase the quantity and penetration speed of the product into organisms, therefore to increase its speed of action, to expand its functions and to offer it a better adhesion. According to Hernandez Ochoa (2005), adjuvants improve the performance of active ingredients by notably allowing a reduction in the usable doses, thus limiting their impact on the flora and fauna and helping protect the environment.

Evaluation of the insecticidal potential of Dittrichia viscosa **aqueous extract-based phytopreparations on the demographic parameters**

The results relating to the application of the different treatments on *C. leucomelas* allowed us to clearly see that fecundity is remarkably disturbed following the action of the active ingredient Thiamethoxam/Lambda-cyhalothrin compared to aqueous extracts. These results are comparable to those discussed by Jean and Benmarhnia (2011) expressing that xenobiotic substances have the capacity to act on a broad spectrum of animal or plant species and interrupt their normal functioning. Bernard (1992), had shown that most pesticides act on the fecundity of contaminated organisms through partial or total sterilization and by reducing the number of eggs laid. Dallaire (2003), shows that tebufenozide affects the development as well as some aspects of chemical communication and of the reproductive success of insects such as Lepidoptera. This product causes a deceleration in ovarian maturation and consequently a decrease in the fecundity of females. In addition, the effects of tebufenozide on fecundity and fertility were found to vary widely depending on the development stage at the time of treatment. In the same aspect, bioproducts have insecticidal and anti-appetizing effects, thus affecting the growth, moulting, development and fecundity of insects (Konstantopoulou et al. 1992; Keane and Ryan 1999).

The net reproductive rate (R_0) of *C. leucomelas* females exposed to phytopreparations and to Thiamethoxam/Lambda-cyhalothrin underwent a notable decline compared to the control, this decline is very clear under the homologous dose of the active ingredient Thiamethoxam/Lambdacyhalothrin compared to the aqueous extracts applied. Our results are confirmed by those found by Tron et al. (2015), that showed an important link between exposure to pesticides and some reproductive and developmental disorders. According to Forbes and Forbes (1997), reproduction is often closely related to environmental variables that delay the reproductive period, which can impair reproductive success. The same author indicates that the impact of pollutants at the individual level is more often related to growth and reproduction than to population abundance. Studies have shown that insecticides can have subtle effects, particularly in impairing recognition of the breeding partner and identification of the spawning site. Therefore, the products cause an alteration in the expression of genes important for reproduction. This work has demonstrated a considerable susceptibility of the effects of insecticides on the ability of insects to reproduce (Amichot 1999). Devault (2007), shows that the behavior, reproduction and survival of adults as well as the number and properties of their offspring can be affected by exposure to pesticides.

Furthermore, the results obtained allowed us to perceive that the aqueous extract exerts a moderately important pressure effect on the reproduction rate of *C. leucomelas*. This result reminds us of the statements of Delimi et al. (2013), who found that the biopesticide can disturb adult reproduction by extending the preoviposition period and reducing the egg deposition period given that fertilized females cannot live longer than one or two days, which reduces the number of eggs laid.

Regarding the growth rate (rm) of *C. leucomelas* cohorts, the results demonstrate a significant increase between the treated and the controls. This increase is much pronounced following the action of the active ingredient Thiamethoxam/Lambdacyhalothrin compared to that of the aqueous extracts. The results show that the prescribed dose of the active ingredient Thiamethoxam/Lambdacyhalothrin and the aqueous extract of *D. viscosa* / *S. fuscata* ratio cause a significant decrease in the multiplication rate (λ) of the populations studied. A strong decrease in the mean life time (T) is reported in the population of *C. leucomelas* following the use of the homologous dose of the active ingredient compared to other treatments that remain close to the control. At the end, the various treatment systems hardly affect the doubling time (DT) of the populations. Toxic effects of pesticides on the demographic parameters are scarce in the scientific literature.

Evaluation of the insecticidal potential of phytopreparations and of the active ingredient on the biochemical life traits of Chaitophorus leucomelas

Studies show that chemicals can reach all the intracellular organelles and change their number, structure and location in the cell and that they can also act on intracellular energy reserves (lipids and glycogen) (Gernhöfer et al. 2001; Triebskorn et al. 2002). Calow (1991) proves that energy reserves are mobilized following stress. The aim of this investigation is to show the role of energy biomarkers in understanding the behavioral or physiological strategies that allow *C. leucomelas* females to partially or totally circumvent bioactive or active materials. This study describes the metabolic reactions and weight measurements of *C. leucomelas* under the effect of phytopreparations and the synthetic product Thiamethoxam/Lambda-cyhalothrin. The results reveal a significant quantitative change between lipid and carbohydrate reserves stored in the tissues of biological model females, where lipid reserves are clearly distinguished from carbohydrate reserves. Moreover, it is very important to coordinate the strong positive correlations existing between the reorganization of lipid reserves and the chemical treatment under the different applied doses (homologous dose or half-dose). The dominance of energetic lipid biomarkers can probably be explained by a change in the biochemical life traits of females exposed to the different applications, especially the active ingredient Thiamethoxam/ Lambda-cyhalothrin. This hypothesis can be explained by the fact that the synthetic product has a stimulating effect on the physiology or the behavior of an organism after exposure. The weight measurements show a slight disturbance under the effect of the two treatment types compared to the control.

Several authors point out that exposure to chemical stress can disrupt the energy balance of living organisms as a direct consequence of the tolerance means adopted (e.g. defense mechanisms, damage repair) and this at the expense of the energy allocated to reproduction and to growth (Amiard and Amiard-Triquet 2008; Palais et al. 2011). This energy balance can also be negative under certain environmental conditions, generating consumption of energy reserves to activate and/or set up tolerance and defense mechanisms.

Our results are consistent with the results of other studies showing that organisms exposed to chemical contamination will use energy to limit the physiological alteration caused by substances present in the environment. Thus, the amount of energy available to ensure the body's vital functions will be lower than that in unexposed organisms. The dosage of energy reserves (proteins, glycogen and lipids) allocated to the various functions of the body will then provide information on the overall physiological state of living organisms (Poisson et al. 2011). The results demonstrate a strong accumulation of lipid reserves in the cohort exposed to the synthetic product. This lipid accumulation indicates that the treated females are in fact subjected to a stressful action which could stimulate a high production and a greater accumulation of lipids. The explanation most often described in the literature is that lipids generally accumulate in organisms exposed to organic contaminants (Köhler 1989; Pelosse 2008). Hence, an increase in lipid metabolites promotes the storage of the toxic substance. According to Abdoulaye (2007), lipids are necessary for maintaining good health, they contribute to the formation of cell membranes, to the synthesis of hormones; without disregarding that they represent a concentrated source of energy that is twice as much as carbohydrates or proteins. The lipid content is closely related to survival, which means that the decrease in lipid stores could be responsible for the death of individuals. However, it should be noted that the measured lipid level corresponds to the amount of lipids contained in the entire body of the insect. Lipids are involved in various functions in insects, which may play a role in the survival, dispersal or even constitute a crucial source of energy for egg production (Pelosse 2008).

The results show a relatively significant disturbance of the carbohydrate energy balance of *C. leucomelas* females after applying the dose of the active ingredient Thiamethoxam/ Lambda-cyhalothrin. Carbohydrate biomarkers are very low but stable, which suggests that the low amount of sugar is related to the detoxification action (Amiard and Amiard-Triquet 2008).

Finally, many stresses (physical and/or chemical) can lead to the mobilization of energy reserves. In addition to variations related to exposure to toxicants, the variability of the energy reserve concentrations in organisms depends on several biotic and/or abiotic factors. These energy reserves can be mobilized to supply defense mechanisms (Storage, elimination, detoxification of contaminants). In this toxic situation, energy reserves can provide vital information on the maintenance, growth and reproduction capacities of individuals (Amiard and Amiard-Triquet 2008).

Conclusion

This study was carried out as part of the evaluation of the efficacy of aqueous extracts of *Dittrichia viscosa*/*Silena fuscata* on the green poplar aphid *Chaitophorus leucomelas*. The results seem to be very promising and confirm the biocidal activity of different extracts on the studied target. The use of the aqueous extract ratio *D. viscosa*/*S. fuscata* allowed to amplify the toxic capacity of bioactive molecules, the expression of which is manifested by a significant mortality and an acceptable duration of phytosanitary coverage compared to unformulated aqueous extracts. This shock action reported on the abundance of *C. leucomelas* shows an upward rating of toxicity ranging from aqueous extracts of unformulated *D. viscosa*, to aqueous extracts of *D. viscosa*/*S. fuscata* ratios and finally to the synthetic product. The results show that Thiamethoxam/Lambdacyhalothrin has a significant disruptive action on the lipid-carbohydrate reserves of exposed populations compared to phytopreparations. The temporal assessment of fecundity is shown to be remarkably disturbed after applying the active ingredient compared to the bioactive material. A slight disturbance was recorded in the growth rate of the populations exposed to the two treatments compared to the control group. Futhermore, the female's reproduction rate is influenced by both treatments, with a stimulating disturbance of the active ingredient compared to the phytopreparations. Likewise, the prescribed dose of the active ingredient generates a considerable average disturbance on the multiplication rate and the average generation time of the studied populations compared to the other products used. The results also show that the different treatments applied do not affect the doubling time of *Chaitophorus leucomelas* populations. *Dittrichia viscosa*/*Silena fuscata* extracts may be of a highly promising potential source of bioactive molecules against insects.

Authors' Contributions The two authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Fatma Zohra TCHAKER. Writing, review & editing supervised by Fatma Zohra TCHAKER and Zahr-Eddine DJAZOULI. The two authors read and approved submission of the final manuscript.

Declarations

Authors' Contributions The two authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Fatma Zohra TCHAKER. Writing, review & editing supervised by Fatma Zohra TCHAKER and Zahr-Eddine DJAZOULI. The two authors read and approved submission of the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

Informed consent Informed consent was obtained from all individual participants included in the study

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Figures

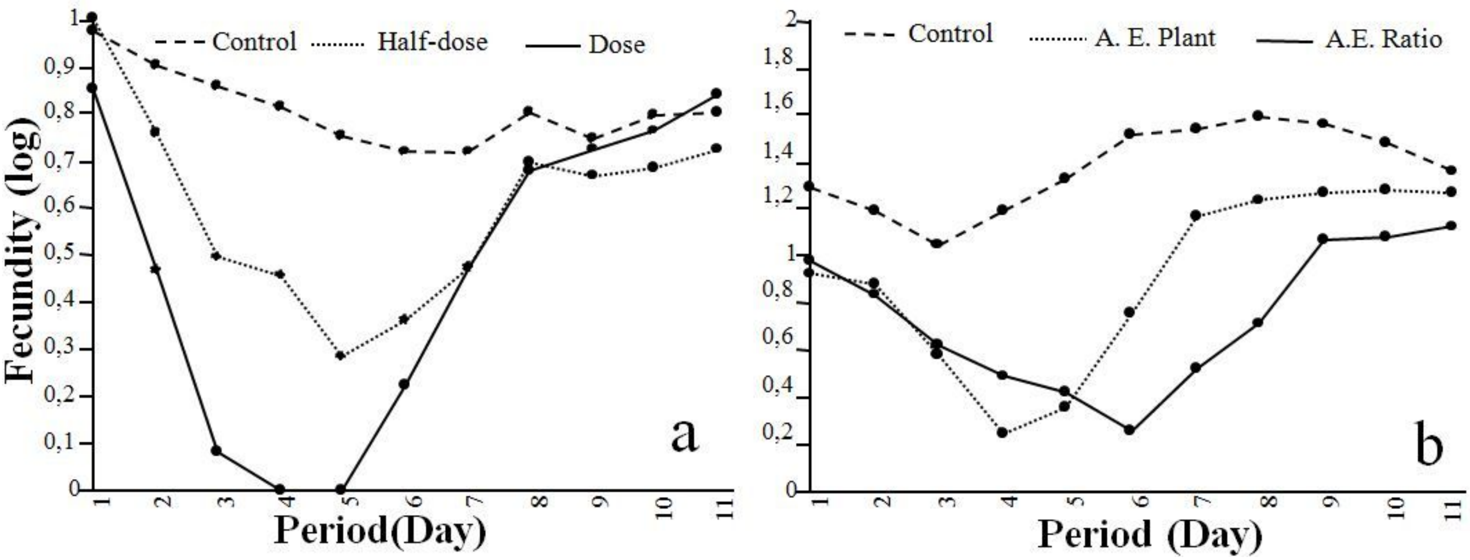

Figure 1

Temporal variation of the fecundity of *Chaitophorus leucomelas* under the effect of chemical and biological treatments

(a) Active ingredient Thiamethoxam/Lambdacyhalothrin; (b) Phytopreparation of *Dittrichia viscosa* and *Silene fuscata*