JEE Journal of Ecological Engineering

Journal of Ecological Engineering, 2025, 26(1), 14–29
https://doi.org/10.12911/22998993/195185
ISSN 2299–8993, License CC-BY 4.0

Received: 2024.10.16
Accepted: 2024.11.15
Published: 2024.12.01

Eco-friendly extract derived from *Dittrichia viscosa* (L.) Greuter 1973, from Algerian arid region, antioxidant evaluation and biopesticide use

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ABSTRACT

To investigate the effects of *Dittrichia viscosa* L. Greuter 1973 extract as a biopesticide on beneficial entomofauna in a greenhouse setting in the Biskra region, the study was conducted at the experimental site (CRSTRA). The aerial parts of *D. viscosa* were collected, dried, and analyzed in laboratory. The antioxidant potential of the plant extract was assessed by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH), alkaline DMSO superoxide, O-phenanthroline chelating (Phen) and iron reducing power (RP) methods. Enzyme inhibitory was studied using the iodine/potassium iodide method. A 200 m² greenhouse was established in November 2021 and planted with tomato (Cecilia), divided into four blocks, each equipped with sticky traps for monitoring entomofauna from May 1st to June 1st. The pulverized *D. viscosa* extract was applied at different concentrations (D1 = 5 ml/l; D2 = 10 ml/l; D3 = 15 ml/l) every 10 days. Analysis of the hydro-methanolic extract of *D. viscosa* revealed significant antioxidant activities and effective α-amylase inhibition, indicating its potential as a free radical scavenger and a useful therapeutic agent for addressing radical-related pathological damage. Additionally, the biopesticidal effects of the extract on beneficial insects were evaluated. Results showed a total richness of 75 species across 7 orders and 44 families, with the most abundant species belonging to Hymenoptera (37 species), Coleoptera (14 species), and Diptera (11 species). The predominant categories included parasitoids (29%), predators (24%), pests (15%), and pollinators (11%). As a biopesticide, the extract proved effective in reducing pest populations by 50% at the highest concentration (15 ml/L). The Pielou evenness index values ($H_{5\ ml/L}$ = 0.8787, $H_{10\ ml/L}$ = 0.8506, $H_{15\ ml/L}$ = 0.836, compared to the control = 0.4179) indicated a nearly homogeneous distribution of individuals among species. The Shannon diversity index suggested that increasing concentrations of *D. viscosa* extract ($E_{5\ ml/L}$ = 0, $E_{10\ ml/L}$ = 1.33, and $E_{15\ ml/L}$ = 0.5623) did not significantly impact the presence of pollinator species in the tomato greenhouse in the Biskra region.

Keywords: naturel ennemis, biopesticides, *Dittrichia viscosa* (L.) Greuter 1973, antioxidant, arid regions.

INTRODUCTION

Plants have long been vital to human life, serving as sources of food and medicine. The Mediterranean basin, with its diverse plant species, is of significant interest for scientific research [Davis and Patrick, 2024]. Algeria's diversified geography and varied climate provide an ideal setting for the development of numerous plant species with diverse chemical profiles. One such plant is the sticky inula from the Asteraceae family, notable for its sticky, aromatic leaves and early summer blooms. In Biskra (Algeria), this plant is recognized for its strong fragrance and thrives in humid, rocky environments as well as uncultivated areas of the Mediterranean region. It is used to treat various ailments, including bronchitis, diabetes, and wounds, and serves as a poultice for back pain or migraines. Despite its rich medicinal potential, many of its therapeutic properties

remain unexplored. Recently, plant extracts have garnered increasing interest as potential sources of bioactive natural compounds, with studies investigating their potential as alternatives for plant protection [Boukemaya, 2016]. Due to the drawbacks of synthetic pesticides, biopesticides (phytopesticides) are effective and safer means of controlling pests. cheap; environment friendly, specific in their target, sustainable and not associated with the release of greenhouses gases and have lesser risks to human health, environment and other organisms. in comparison to the synthetic pesticides [Saberi et al., 2020; Borges et al., 2021; Malahlela et al., 2021; Idris et al., 2022].

The extensive use of synthetic pesticides, which harms both the environment and human health, has led governments, organizations, and farmers to seek more eco-friendly agricultural practices [Konda et al., 2002]. Biochemical or biopesticides, which are natural compounds or their synthetic counterparts with active ingredients, offer an alternative for pest control. These compounds are effective on the target pests and designed to be safe for the environment and humans [Kumar, 2012; Reddy and Chowdary, 2021]. However, it is important to note that while biopesticides can effectively manage pests, they may also unintentionally impact beneficial organisms [Khater, 2012; Giroux et al., 1994; Roger et al., 1995]. Biopesticides derived from various sources such as bacteria, fungi, viruses, nematodes, and plant extracts [Lengai and Muthomi, 2018; Kumar et al., 2021] work well in conjunction with traditional biological control methods, like introducing predators or parasites. To explore this further, the presented study examined the effects of biopesticides, specifically plant extracts from spontaneous species collected from arid regions in southern Algeria on various organisms, including pests, predators, parasitoids, and pollinators under greenhouse.

MATERIAL AND METHODS

Study area

The study area is located in the southern part of Biskra (34° 48’ N, 5° 44’ E), a wilaya in southern Algeria (Fig. 1a). It is bordered to the north by the province of Batna, to the northwest by the provinces of M’Sila and Ouled Djellel, to the northeast by Khenchela, and to the south by El Oued and Ouargla [Deghiche Diab et al., 2022]. Its vast territory, coupled with distinct geological, and climatic features, harbors a variety of ecosystems comprising forests, mountains, plateaus, steppes, and oasis habitats, thereby fostering a rich diversity of flora [Deghiche Diab and Deghiche, 2016].

The areal part of *Dittrichia viscosa* (L.) Greuter 1973 specimens (Fig. 1b), were collected from several natural sites (Fig. 1c) in Djemmorah (35° 04′ 10″ N, 5° 50′ 39″ E, 403 m a.s.l.), located at 36 km to the north-east of the wilaya of Biskra, bounded to the north by the commune of Ain Zaatout, to the south by the commune of Branis, to the east by the commune of Tigharghar and Manaa, and to the west by the commune of El Outaya. The area has a semi-continental climate with hot, arid summers and cold winters. Summer temperatures range from a minimum of 3 °C to a maximum of 45 °C, while annual rainfall during the study year (2021) varied between 200 and 300 mm.

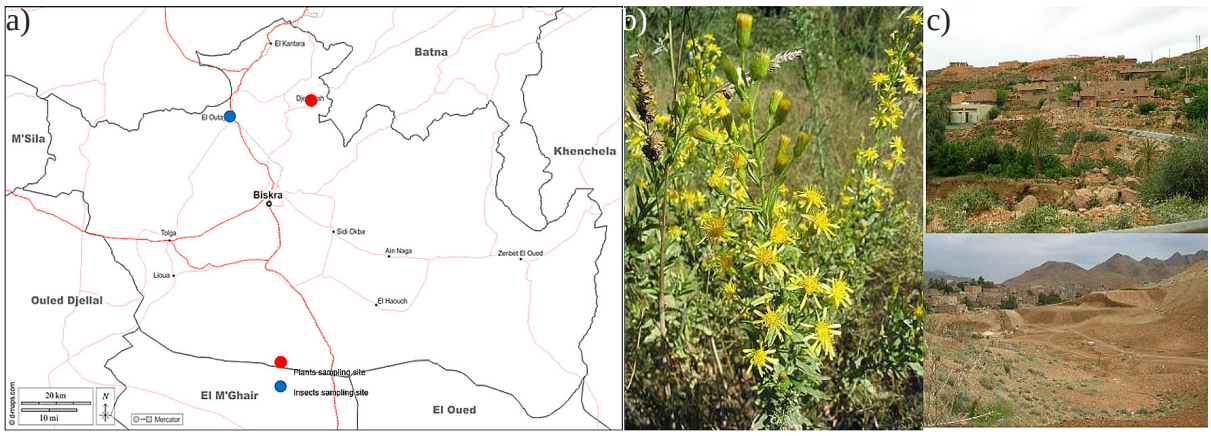

Figure 1. Study area: a) situation, b) *D. viscosa* (L.) Greuter 1973 areal part, c) sampling site

Biological material

Native to the Mediterranean basin, *D. viscosa* (L.) *Greuter* (Fig. 1b), known by its homotypic synonym *Inula viscosa* (L.). *Dittrichia viscosa* (L.) Greuter 1973 is a perennial ruderal plant belonging to the Asteraceae family (Parolin et al., 2014). Owing to its high seed production and spreading, good adaptability, and resistance to adverse conditions, it is considered as an important environmental weed. Widespread in the Mediterranean basin, this plant has been extensively used in traditional medicine since the Roman times. The species has spreading branches and toothed leaves that have a sticky resin. It blooms during summer period with small yellow flowers to produce tiny achenes [Brullo and Marco, 2000; Deghiche Diab, 2019].

Plant preparation

During flowering period in October 2021, plant samples (including leaves, flowers, and stems) were washed and subsequently air-dried at room temperature (25 °C). Once completely dry (H% < 10%), the organs were finely ground using a Sayona Electric Coffee Spices Grinder and stored in a refrigerator at 4 °C for analysis.

Plant extraction

Powdered dried aerial parts (60 g) were mixed with methanol: water (1:3; v/v, 400 mL) and macerated under heating for 3 h at 50 °C, then the material mixture was filtered and evaporated. The crude extract obtained was stored for further analysis. This procedure was repeated in triplicate.

Biological activities evaluation

Total phenols content (TPC)

The total phenolic content of the extract of *Dittrichia viscosa* was measured using Folin-Ciocalteu reagent. Firstly, 100 µL of Folin-Ciocalteu (10-fold dilution) and 75 µL of sodium carbonate (Na_2CO_3, 7.5%) were added to 20 µL of studied extract in distilled water (1 mg/mL). Then, this mixture was incubated for 2h at room temperature in the dark. The absorbance was measured at 765 nm using a 96-well microplate reader (Thermo ScientificTM Multiskan Sky). Contents results were expressed in micrograms of gallic acid equivalents per milligrams of dry extract (µg GAE/mg DE). The calibration curve was performed using gallic acid at different concentrations up to 200 µg/mL [Muller et al., 2010].

Total flavonoid content (TFC)

The total content of flavonoids was evaluated using the aluminum chloride colorimetric method [Topçu et al., 2007]. Firstly, 50 µL of extract were mixed with 50 µL of aluminum chloride ($AlCl_3$, $6H_2O$, 2%) and 150 µL of 5% sodium acetate in wells of a 96-well microplate. The microplate was then incubated in dark at room temperature for 2.5 h. After incubation, absorbance was determined at 440 nm. The results are expressed in µg equivalent of quercetin/mg (µg EQ/mg) of the extract with reference to the calibration curve for quercetin.

DPPH radical scavenging assay

The scavenging ability of *Dittrichia viscosa* hydroalcoholic extract was determined according to Bochoukh et al. [2019]. Briefly, 40 µL of extract at different concentrations were added to 160 µL of 0.1 mM DPPH radical solution in methanol. The decrease of DPPH absorption was measured at 517 nm after 30 min of incubation in the dark. Butylhydroxytoluene (BHT) was used as a positive control. The inhibition percentage of DPPH radical was calculated as the efollowing equation:

$$DPPH\ Se\ \% = \frac{Abs\ C - Abs\ S}{Abs\ C} \times 100 \quad (1)$$

where: *Se* – scavenging effect, *Abs C* and *Abs S* are the absorbances of control and samples at 30 min, respectively.

This activity was also expressed as IC_{50} (µg/mL) which corresponds to the dose of sample that causes 50 % of inhibition of DPPH radical.

Alkaline DMSO superoxide activity

Superoxide radical scavenging activity by alkaline dimethyl sulfoxide (DMSO) was performed according to the method of Kunchandy and Rao [1990]. Firstly, 40 µL of extract at different doses was mixed with 130 µL of alkaline DMSO (20 mg of NaOH was dissolved in 1 mL of H_2O and supplemented with DMSO at 100 mL), then 30 µL of NBT (nitrobluetetrazolium, 1 mg/mL). The absorbance of the solution was then determined at 560 nm, (BHT) was used as positive control. The scavenging activity of sample was calculated according to Equation 4:

$$SRSA = \frac{Abs\ C - Abs\ E}{Abs\ C} \times 100 \quad (2)$$

where: *SRSA* – superoxide radical scavenging activity, *Abs C* and *Abs E* are the absorbance of control and sample, respectively.

O-Phenanthroline chelating activity (Phen)

By following the method of Szydłowska-Czerniaka et al. [2008], the phenanthroline assay was determined and assisted. At first, 10 µL of each sample at different concentrations was added to 50 µL of $FeCl_3$ (0.2%), 30 µL O-Phenanthroline (0.5%) and finally 110 µL of methanol. Then, the absorbance was measured at 510 nm after 20 min incubation at 30 °C. BHT was used as positive control. This activity was expressed as IC_{50} (µg/mL).

Iron reducing power (RP)

This effect was assessed according to the method of Oyaizu [1986]. Firstly, 10µL of samples were added to 40µL of phosphate buffer (pH = 6.6) and 50µL of potassium ferricyanide (1%). This mixture was incubated for 20 min at 50 °C, then 50 µL of trichloroacetic acid (10%), 40 µL of distilled water and 10 µL of ferric chloride ($FeCl_3$, 0.1%) were added to the first mixture. For comparison, BHT was used as positive control. Absorbance was measured at 700 nm using a microplate reader. In this activity, $A_{0.5}$ was also determined.

α-Amylase inhibitory activity

The α-amylase inhibitory activity of hydroalcoholic extract of *D. viscosa* was investigated according to [Zengin et al., 2014; Randhir and Shetty, 2007], using the iodine/potassium iodide method, with slight modifications. The reaction mixture was prepared in a 96-well microplate by adding 25 µL of the extract at various concentrations with amylase solution in 1 U of sodium phosphate buffer (pH = 6.9 with 6 Mm NaCl). After incubation at 37 °C for 10 min, 50 µL of 0.1% starch solution was added. A control was simultaneously prepared without the enzyme solution. Re-incubation for 10 min at 37 °C was performed, followed by the addition of 25 µL HCl (1M) and 100 µL of iodine-potassium iodide solution (IKI) in order to stop the reaction. For comparison, acarbose was used as positive control The absorbance was measured at 630 nm. The % inhibition of α-amylase was estimated as follows (5):

$$\% \text{ inhibition} = 1 - [(AbsC - AbsE) - (AbsS - AbsB)/(AbsC - AbsE)] \quad (3)$$

where: *AbsC* = absorbance (volume of solvent, enzyme, starch, HCl, IKI), *AbsE* = absorbance (sample, enzyme, starch, HCl, IKI), *AbsS* = absorbance (sample, starch, Enzyme, IKI, HCl), *AbsB* = absorbance (sample, sodium phosphate buffer, IKI).

Experimental site

Greenhouse preparation

The experiment was set up under greenhouse of 200 m^2 at the bio-resource station situated at 12 far from the Scientific and Technical Research Center on the Arid regions**.** 200 tomato plants (Cecelia var.) were transplanted in November 2021 on two bands 2 m apart, with each band consisting three lines with 50 plants. For the experiments, a split plot design was implemented, consisting of four blocks, each containing 21 tomato plants. Three repeated treatments were applied in addition to the control, from May 1st to June 1st. To prevent the dispersal of ground-up products, the four blocks were separated by plastic, with a buffer zone of three rows of tomato plants (Fig. 2a, 2d).

Collecting technic and application of bioproduct

Natural enemies of insect species associated with tomato cultivation in greenhouses were collected using four sticky traps [Franck, 2013]. These traps were installed in each of the four separate blocks and were collected and replaced every 10 days, from May 1st to June 1st, 2022 (Fig. 2b).

To assess the impact of the bioproduct on beneficial fauna, tomato plants were sprayed every 10 days with different doses of plant extracts [Roy et al., 2016; Vernner and Beauer, 2007]. The first dose (D_1 = 5 ml/l) consisted of 5 ml of the mother formulation (10% extract + 90% bio-adjuvants) diluted in 1 liter of water. The second dose (D_2 = 10 ml/l) was made up of 10 ml of the mother formulation diluted in 1 liter of water, while the third dose (D_3 = 15 ml/l) included 15 ml of the same formulation diluted in 1 liter of water (Fig. 2c).

Identification species

The species collected from the sticky traps in each block were taken to the laboratory for counting and identification under a binocular magnifier. The identification was carried out by one of our team members, an expert at CRSTRA.

Data management and statistical analysis

The antioxidant data are presented as mean ± standard deviation (SD). Statistical analysis was conducted using one-way ANOVA followed by Tukey's HSD for multiple comparisons between

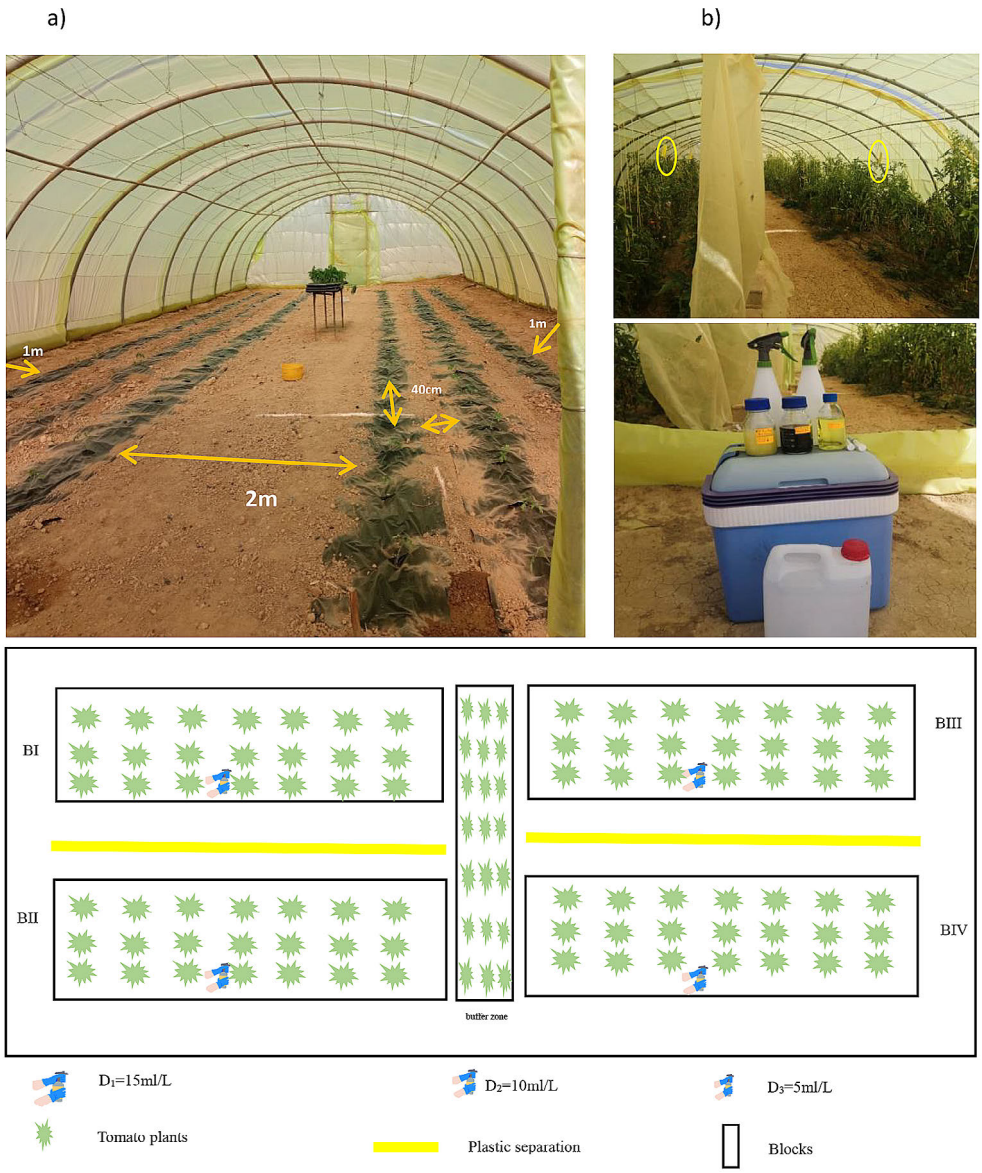

Figure 2. a) Experimental greenhouse, b) used sticky traps and separated blocs, c) materials from extract plants used for treatment, d) work plan diagram and treatment application

the groups. Results were statistically significant if P-values were less than 0.05. Statistical tests were carried out using Statistica software (version 6.0, 2001).

Insects data treatment and analysis

The free software R (R version 4.0.0, 2020-04-24) was used to carry out the insects' statistical analyses, the graphs and figures were made by Venn Diagram and Inext" packages. The figures and tables are included in the PAST program (V. 2.17) that allow characterizing data statistics, as well as generating graphs and calculating differences, ecological indicators and statistics [Hammer et al., 2001; Dieumegard, 2010]. The rarefaction method was used to adjust the samples and facilitate comparisons of alpha diversity. It involves producing diversity metrics calculated to compare ecosystems, regardless of differences in sample sizes (Weiss et al., 2017).

RESULTS AND DISCUSSION

Antioxidant activity evaluation

The obtained results for the experimental part in laboratory related to the antioxidant activity of the used *D. viscosa* hydro-methanolic extract are presented in Figure 3 and Table 1 to be discussed.

The DPPH assay measures the antioxidant capability of substances according to their ability

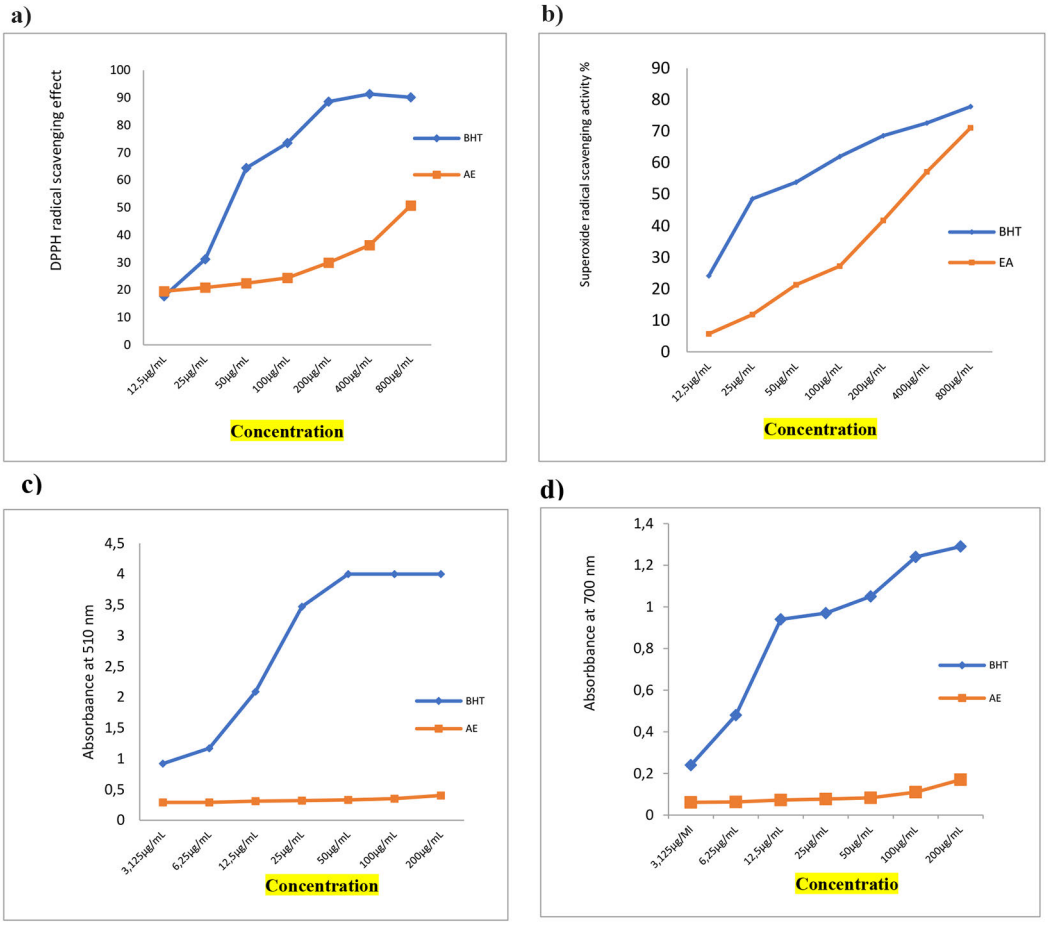

Figure 3. Inhibition percentage of free radicals, absorbance values and superoxide radical scavenging activity by aqueous extract of *Dittrichia viscosa*: a) DPPH activity, b) alkaline DMSO superoxide activity, c) phenanthroline activity, d) reducing power. AE: hydro-methanolic

Table 1. Phenolic content, IC_{50} and $A_{0.5}$ values of antioxidant activity of *D. viscosa* hydro-methanolic extract

	TPC (µg GAE/mg)	TFC (µg EQ/mg)	DPPH (IC_{50})	DMSO (IC_{50})	Phen ($A_{0.5}$)	RP ($A_{0.5}$)
BHT	–	–	38.87±0.007[a]	40.21±0.3[a]	<3.125[a]	6.52±0.07[a]
AE	15.96±0.2	1.74±0.2	781.47±7.11[b]	321.95±4.2[b]	410.25±1.6[b]	745.27±6.7[b]

Note: Data are shown as mean ± DS, n = 3, Means in each column followed by different letters are considered significant $p < 0.05$ (ANOVA followed by Tukey test), AE: hydromethanolic extract

to scavenge free radicals. The *D. viscosa* hydro-alcoholic extract exhibited a significant capacity of scavenging the radical DPPH. According to the results presented in Table 1, AE (IC_{50}: 781.47 ± 7.11 µg/mL) showed moderate antioxidant activity in the DPPH method, in which the IC_{50} was relatively low to the IC_{50} stated by the BHT (IC_{50}: 38.87 ± 0.007 µg/mL).

The *D. viscosa* hydro-methanolic extract has the ability to capture the superoxide anion radicals (Fig. 1b). This extract has a better scavenging activity (IC_{50} = 321.95 ± 4.2 µg/mL) than in the DPPH method. Meanwhile, BHT exhibit a high capacity by $A_{0.5}$ = 40.21 ± 0.3 µg/ml compared to that of the tested extract.

Regarding the phenanthroline test, the hydro-methanolic extract of *D. viscosa* showed a significant capacity ($A_{0.5}$ = 410.25 ± 1.6 µg/ml) compared to standard. The results showed that BHT recorded better activity ($A_{0.5}$ = < 3.125 µg/ml) than hydro-methanolic extract (Fig. 1c).

The obtained results of reducing power of *D. viscosa* hydro-methanolic extract (Fig. 1d). With the increasing concentration, the absorbance in 700 nm of the extract is increasing and the growth rate is slowing down. The result obtained revealed

that hydro-methanolic extract of *D. viscosa* has low activity ($A_{0.5}$ = 745.27 ± 6.7 µg/ml) compared to the standard BHT which exhibit a high antioxidant activity by $A_{0.5}$= 6.52 ± 0.07 µg/ml.

The α-amylase inhibitory activity of extract plant *D. viscosa* was tested, from the obtained results it appears to be dose-dependent, since sample concentration clearly affects the amount of enzyme inhibited (Table 2).

The analysis indicates that the tested extract demonstrates significant and strong inhibition potential, with an IC_{50} value 33.25 ± 0.5 µg/ml. In comparison, Acarbose, used as a reference standard, exhibited 94.9 ± 0.4% inhibition of α-amylase activity at a concentration of 1000 µg/ml, while its IC50 value was determined to be less than 15.625 µg/ml.

In addition to the actives of *D. viscosa* extract plant obtained in this study, its effect as a biopesticide was also tested against the different guilds from insects' class (Pollinators pests and auxiliaries).

Table 2. Inhibitory effect of hydromethanolic extract of *D. viscosa against* α-amylase

Concentration	Acarbose	AE
15.625 µg/ml	54.4±0.001^{b}	42.67±0.6^{a}
31.25 µg/ml	56.9±0.007^{b}	49.44±0.5^{a}
62.5 µg/ml	65.66±0.02^{a}	61.73±0.2^{a}
125 µg/ml	77.033±0.01^{a}	81.97±0.3^{b}
250 µg/ml	97.96±0.005^{b}	86.99±0.5^{a}
500 µg/ml	100±0.009^{b}	92.53±0.4^{a}
1000 µg/ml	100±0.009^{b}	94.9±0.4^{a}
IC_{50} (µg/ml)	<15.625^{a}	33.25±0.5^{b}

Note: Data are shown as mean ±DS, n = 3, column followed by different letters are considered significant $p < 0.05$, AE: hydromethanolic extract.

Biopesticide effect evaluation

Total collected species under greenhouse

During the experimental period (May 1st to June 1st, 2022), which focused on assessing the effects of *Dittrichia viscosa* (L.) Greuter 1973 extract on pests, beneficial insects, and pollinators in greenhouse tomato cultivation at the El Outaya site (Biskra), it was possible to identify 75 species belonging to 7 orders and 44 families. The most important order was Hymenoptera with 37 species and 19 families, the second order was Coleoptera order with 14 species and 4 families, in the third position there is the Diptera order with 11 species and 7 families followed by the Hemiptera order with 8 species and 6 families (Fig. 4a).

Trophic guild under greenhouse

The most important group of insects was represented by the Parasitoid species with 29% (21 species) of the total collected species, 24% (18 species) were predators and 15% represent pest species (11 species), whereas Phytophagous accounted only 14% (10 species). The category of Pollinators was represented by 11% (8 species) and 7% (5 species) were coprophagous species of the existing entomofauna (Fig. 4b).

Extract plant effect on pest species

The effect of different doses of *Dittrichia viscosa* plant extract (D_1 = 5 ml/L, D_2 = 10 ml/L, D_3 = 15 ml/L) on insect species in a cultivated tomato

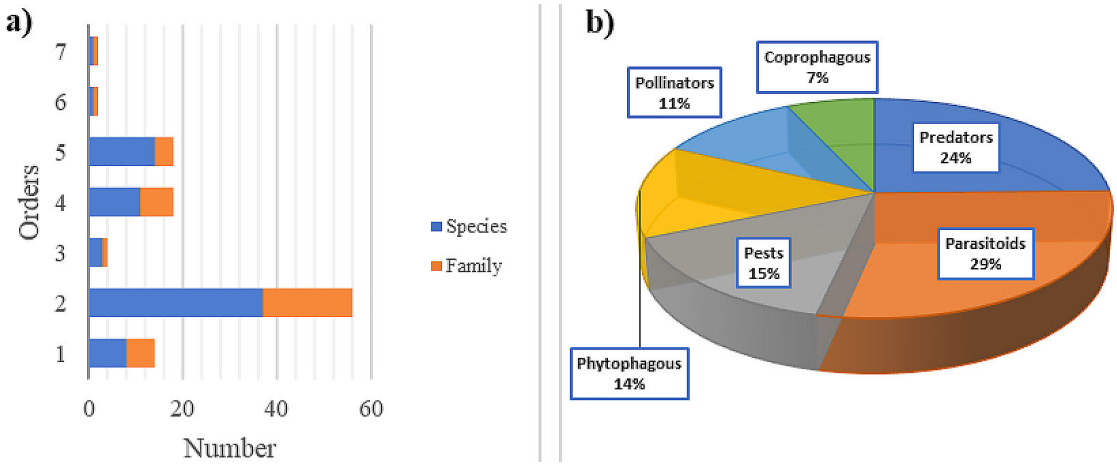

Figure 4. Importance of: a) species, orders, families and b) trophic guilds under greenhouses at El Outaya site-Biskra region

greenhouse at the El Outaya site was observed from May 1 to June 1, along with their distribution patterns. The results showed that, compared to the control with 9 pest species collected, the plant extract had a positive impact on species richness. At the highest concentration of 15 ml/L, only 4 (50%) out of the 9 species from the control persisted. The 1st dose (5 ml/L) resulted in 6 species, and the 2nd dose (10 ml/L) led to 5 species, reflecting a slight decrease in the number of species (Fig. 5).

The ranges of the Shannon diversity index were 0.9249, 1.358 and 1.143, from the different applied extract plant doses; 5 ml/L, 10 ml/L, and 15 ml/L respectively (Fig. 5b). The highest impact of the extract and the lowest species richness for pest control were observed with the highest dose (15 ml/L), which resulted in only 4 species under greenhouse conditions. Significant differences in Shannon index scores were noted between the various treatments. The lower Shannon index value for the 5 ml/L dose indicated reduced species diversity in this area compared to the other treatments and the control.

The values of the Pielou evenness index (Fig. 5c) varied from 0.4203, 0.5554, 0.7843 for the different applied doses; 5 ml/L, 10 ml/L, and 15 ml/L and in comparison, to the control (0.5802). That translate into the effect of the 3rd extract concentration (15 ml/L = 4 species) on the dominance of some species on the others. However, these values, which they approached the maximum value (p = 1), indicated the homogeneous and equitable distribution of individuals under greenhouse. According to this observation, it can be said that the extract plant *Dittrichia viscosa* with different doses can have an effect on some species in comparison to the others, significant loss of insects' species was observed at the highest concentration 15 ml/L.

The impact of an extract concentration on pest species (Fig. 6a) indicated that a repeated application of 15 ml/L extract is expected to significantly reduce both number of individuals (< 50) and species (< 4 species). Lower concentrations of 5 ml/L and 10 ml/L are anticipated to support a greater diversity, with approximately 6 species and fewer than 100 individuals persisting. In contrast, the control group is expected to maintain a similar diversity with 9 species and approximately 230 individuals. Both Shannon-Weiner diversity and Simpson diversity indices (Fig. 6b; Fig. 6c) were expected to remain stable with a total sample size of fewer than 50 individuals.

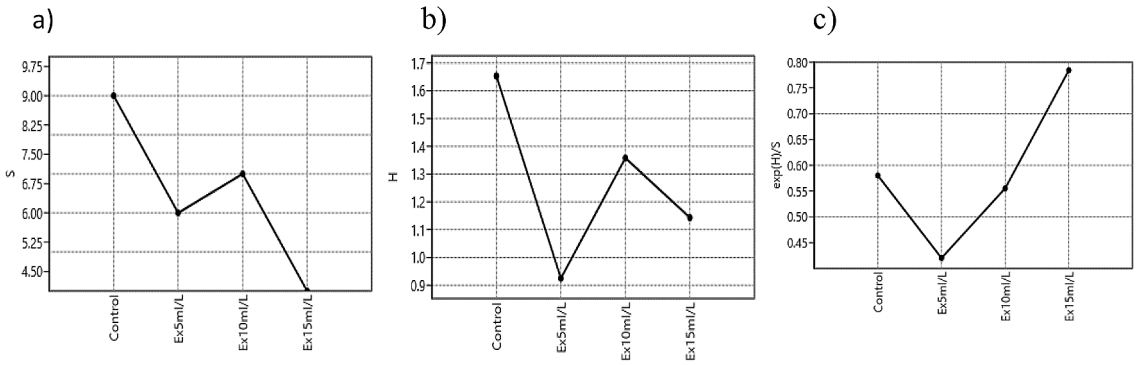

Figure 5. *Dittrichia viscosa* extract plant effect on pest species distribution using ecological indices: a) Richness (S), b) Shannon diversity index (H), c) Pielou evenness index (E)

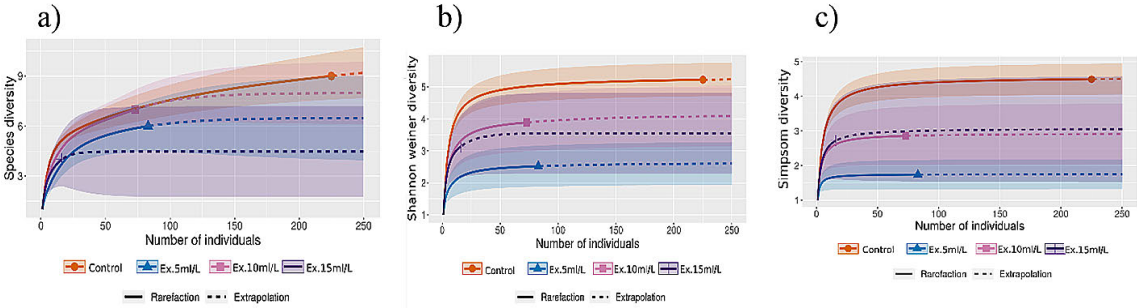

Figure 6. Sample-based rarefaction and extrapolation of : a) species diversity, b) Shannon diversity index, c) Simpson diversity for three extract plant concentrations on pest insects species

Extract plant effect on auxiliary insect species

The highest species richness scores for natural enemy species were observed with the 3rd used plant extract concentration (15 ml/L) under greenhouse (Fig. 7), where 15 species were observed in comparison to the 1st extract concentration (5 ml/L) and 2nd one (10 ml/L) were 11 species and 12 species were observed respectively and in comparison, to the control (12 species). It is important to note that axillary species were found in high numbers across all the applied treatments. Furthermore, species from the control group remained present, species present in the control group were still observed, with others that likely entered during the opening of the greenhouse.

The obtained results from the Shannon diversity index analysis were 2.269, 2.477 and 2.529 for the different applied concentrations: 5 ml/L, 10 ml/L, and 15 ml/L respectively (Fig. 7a). The low value of the Shannon index for the used plant extract with 5 ml/L application reflected the low species diversity in this greenhouse compared to the others concentration and to the control. Even with increasing of extract concentration a high number of species persist, reflecting height Shannon diversity index (H = 2.529)

The values of the Pielou evenness index (Fig. 7b) varied from 0.8787, 0.8506, 0.836 for 5 ml/L, 10 ml/L and 15 ml/L of spread extract concentrations, respectively, and in comparison, to the control (0.4179). However, these values, which approached the maximum value (E≈1), indicated the homogeneous and equitable distribution of individuals within species under greenhouse that reflect maintaining the same number of species even with the height concentration of biopesticide.

Thus, the effect of different extract plant (*D. Viscosa*) concentrations on auxiliary species using the rarefication curve (Fig. 8) indicated that, with a repeated application of extract plant concentration, even using 15 ml/L extract concentration sprayed, it is anticipated to support a greater diversity in both number of individuals (< 40) and species (< 20). The lower concentrations of 5 ml/L and 10 ml/L are expected to have less diversity

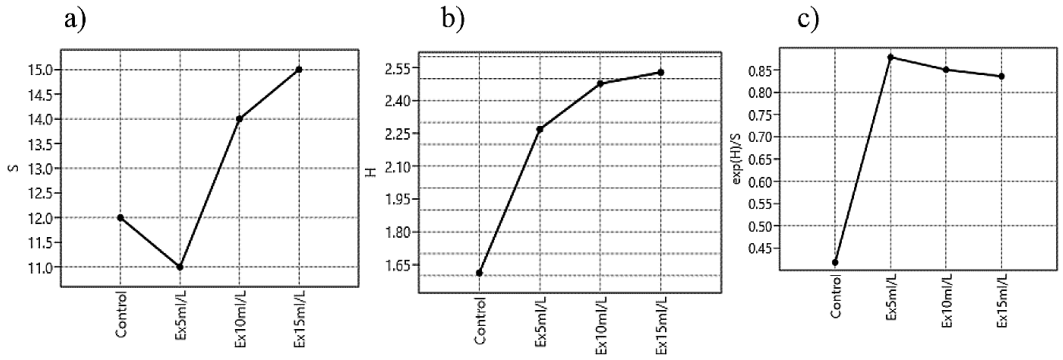

Figure 7. Effect use of extract plant on the auxiliary's species distribution using ecological indices: a) Richness (S), b) Shannon diversity index (H), b) Pielou evenness index (E)

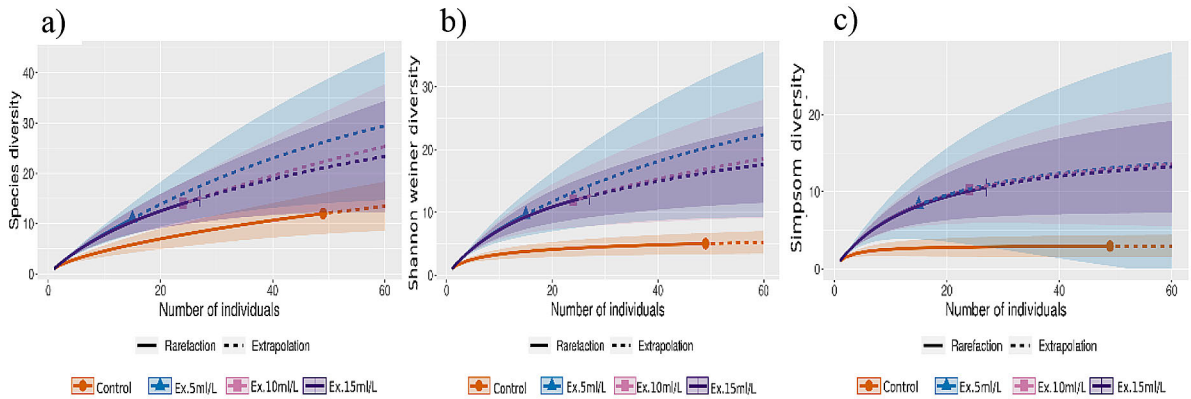

Figure 8. Sample-based rarefaction and extrapolation of: a) species diversity, b) Shannon diversity index, c) Simpson diversity index estimated for three extract plants doses on auxiliaries species under greenhouse at El Outaya site

in the number of individual and species (< 20) in comparison to the control and to the highest concentration, with approximately less than 20 species and fewer than 60 individuals persisting. In contrast, the control group is expected to maintain a similar diversity with >10 species and approximately 50 individuals. Both Shannon-Weiner diversity and Simpson diversity indices were expected to remain stable with a total sample size of fewer than 60 individuals.

Extract plant effect on pollinator insects

Under greenhouse, pollinator species richness was higher with D_2 = 10 ml/L and D_3 = 15 ml/L plant extract concentrations. In contrast, the D_1 = 5 ml/L treatment led to a significant decline in species diversity, with only one species observed compared to four species in the control. This suggests that increasing the biopesticide concentration did not affect the presence of pollinator species (Fig. 9a). The obtained results of Shannon diversity index ranged from 0 at 5 ml/L, to 1.33 at 10 ml/L, and 0.5623 at 15 ml/L (Fig. 9b). The low Shannon index value at 5 ml/L (0) indicates reduced species diversity and the dominance of a single species, while the highest Shannon index value at 15 ml/L (0.5623) signifies greater species diversity with this treatment.

The Pielou evenness index values were 1, 0.9449, and 0.8774 for the treatments of 5 ml/L, 10 ml/L, and 15 ml/L, respectively, compared to 0.9473 for the control. While these values are close to the maximum (E≈1), indicating that one species dominated, they approached the maximum value (E≈1), suggesting a more equitable distribution of species in the greenhouse (Fig.9c).

By using the rarefication curve, the different extract plant of *D. viscosa* concentration effect on pollinator species indicated that with a repeated application of extract plant concentration (5 ml/L), it is expected to have less diversity in number of individual and species (< 5) in comparison to the control and to the highest concentration (15 ml/L) where it is anticipated to support a greater diversity in both number of individuals (> 1) and species (> 10). The lower concentrations of 5 ml/L and 10

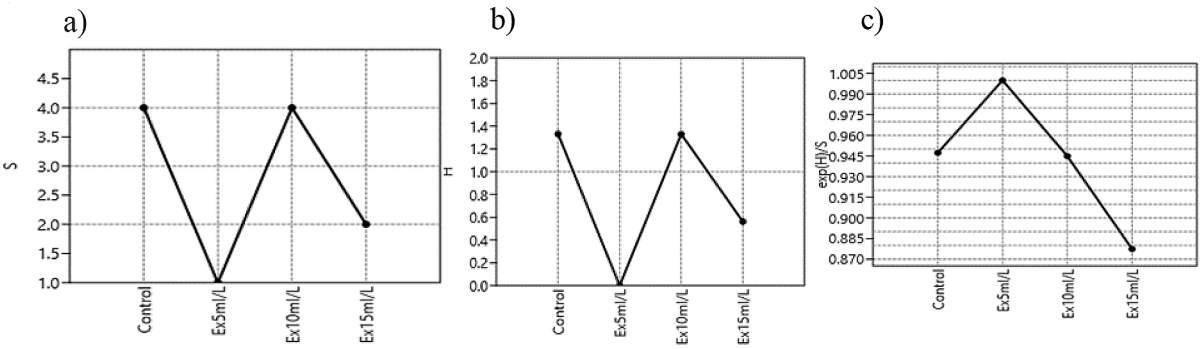

Figure 9. Effect of extract plant use on the pollinator species distribution using ecological indices: a) Richness (S), b) Shannon diversity index (H), c) Pielou evenness index (E)

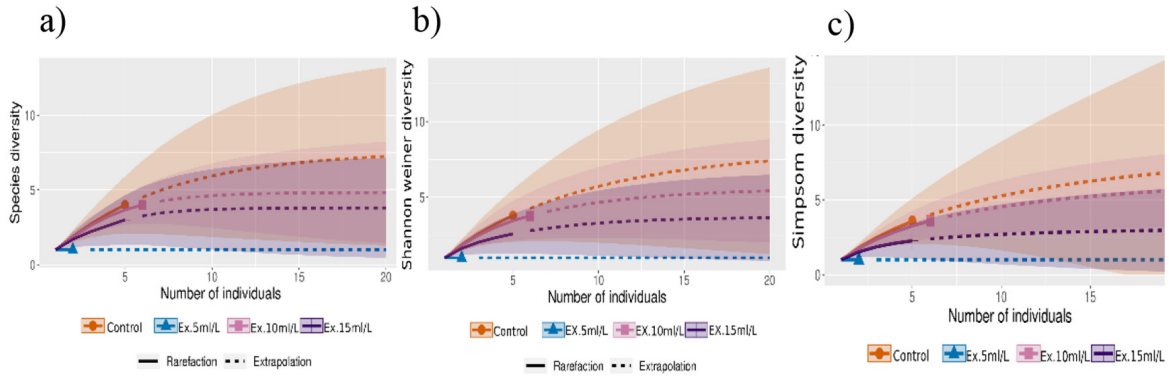

Figure 10. Sample-based rarefaction and extrapolation of: a) species diversity (S), b) Shannon diversity index, c) Pielou evenness index

ml/L are expected to have less diversity in number of individual and species (< 5) in comparison to the control and to the highest concentration, with approximately less than 10 species and fewer than 10 individuals persisting.

Similarity analysis between plants extract effect on species

The obtained Venn diagram showed the efficiency of spread biopesticide on pest insect species, where 4 species were affected by using the different doses (D_1 = 5 ml/L; D_2 = 10 ml/L; D_3 = 15 ml/L) including *Liothrips olea, Frankliniella occidentalis, Mayetiola destructor* and *Liriomyza trifolii* from the total pest species collected during the experiment (Fig. 11a). With eleven (11) species shared among the group of naturel enemies and present under greenhouse even with the application of different doses of applied biopesticide (D_1 = 5 ml/L; D_2 = 10 ml/L; D_3 = 15 ml/L) 90% of existing species were maintained of the total collected present species (12 species) including *Trimorus* sp., *Omalus biaccinctus*, *Ceraphron* sp. *Acmotemnus* sp, *Scymnus suturalis*, …(Fig. 11b). There was no exclusive effect on the eight (8) present pollinators species in the whole study or even on the 4 species present in the control, effected by the different applied doses (D_1 = 5 ml/L; D_2 = 10 ml/L; D_3 = 15 ml/L) of prepared biopesticides from plant extract of *D. viscosa* (Fig. 11c).

DISCUSSION

Antioxidant activity evaluation

Plant-based substances have always been a major source for creating new substances with therapeutic properties. Many previous studies in Algeria have proved extract plants antioxidant activities of the studied species *D. viscosa* [Chahmi et al., 2014; Tchaker et al., 2016]. Mssillou et al. (2022), reported that ethanolic extract showed high radical scavenging activity with an IC_{50} equal to 12.54 ± 0.2 µg/mL in DPPH test. In addition, and based on the findings of Rhimi et al. [2019], the ethanolic *D. viscosa* exhibited a strong antioxidant activity explained by the high phenolic content, particularly by the highest caffeoylquinic acid content.

The α-amylase inhibitory activity, *D. viscosa*, was tested during the first time study from an arid region in Algeria. The calculated IC_{50} showed that the tested extract inhibition potential is significant and strong, with an IC_{50} of 33.25 ± 0.5 µg/ml.

Biopesticide effect evaluation

Total collected species under greenhouse

Many recent studies have shown that plant extracts can be also considered as biopesticides [Rahuman et al., 2009] proving to be highly effective against pest species while having a reduced impact on beneficial organisms [Belien et al., 2021; Chavana and Joshi, 2024]. The conducted work aimed to underscore the valorization of phytogenetic resources of Algeria and the reduction of pesticide and synthetic chemical use in agriculture, with the goal of mitigating their impact on the environment and biodiversity a specially the beneficial one.

Trophic guild under greenhouse

Greenhouses can create or maintain relatively stable climatic conditions that are ideal for certain pests to enhance their reproductive and invasive capacities that explain the importance of pest species under the greenhouse. Insects infesting agriculture can lead to significant economic losses each year and threaten global food security. The lack of natural predators and the abundance

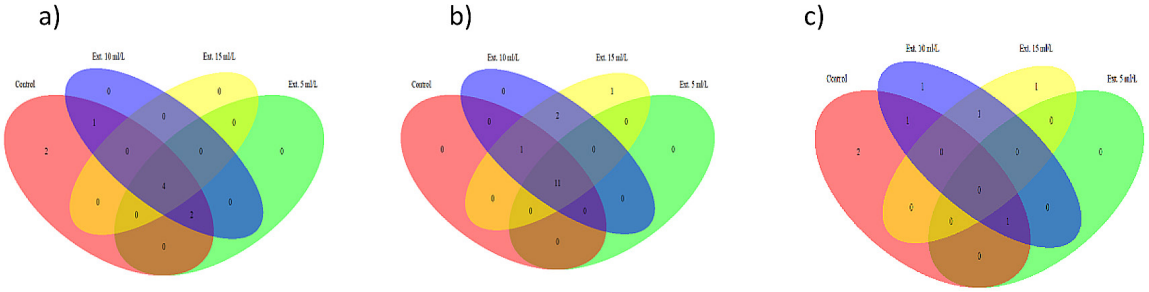

Figure 11. Venn diagram displaying extract plant doses effect; a) on pest insect species, b) auxiliary species, c) pollinator species

of food in greenhouses may also make them an ideal habitat for pests, which explains the observed diversity of orders and species within this controlled area. Therefore, it is crucial to monitor the presence of beneficial species while managing pests. It was reported that sticky traps are effective for capturing various types of insects and are useful for estimating the populations of both beneficial insects and pests [Franck, 2013].

Extract plant effect on auxiliary insect species

The impact of *D. viscosa* on pest species has been documented globally [Rotundo, 2019; Lampiri et al., 2020] and in Algeria [Salima et al., 2016; Benddine, 2018; Gueribis et al.,2019]. In our experiment testing the effect of D. viscosa extract on insect species under greenhouse conditions, nine pest species were identified: *Nysius graminicola, Liothrips olea, Frankliniella occidentalis, Thrips tabaci, Bradysia tilicola, Bradysia ocellaris, Liriomyza trifolii, Thaumatomyia notata*, and *Tuta absoluta*. Application of the *D. viscosa* extract at different concentrations (5, 10, and 15 ml/L) resulted in a significant reduction in the number of species, with only four species observed at the highest concentration (15 ml/L). The extract also specifically affected certain species, leading to reductions in the numbers of individuals; *Bradysia tilicola, Bradysia ocellaris, Liothrips olea* and *Thrips tabaci*. Notably, some species such as *Liriomyza trifolii, Thaumatomyia notata*, and *Tuta absoluta* continued to be problematic for tomatoes even at the highest concentration, while *Liothrips olea, Frankliniella occidentalis*, and *Thrips tabaci* were completely eliminated with the same dose.

In general, it is known that the main pest species under greenhouse were the western flower thrips *Frankiniella occidentalis* and the onion thrips *Thrips tabaci* (Howard et al., 1994; Portree, 1996). The species where present under greenhouse all the period of spraying extract and were the most effected by different applied dose. These results were confirmed also by Oudjiane et al. [2018].

The effectiveness of plant extracts on pest species has been demonstrated multiple times. Bambara and Tiemtoré [2008] showed the efficacy of Euphorbia-based extracts against thrips in Burkina Faso. More recently, Lokbani [2018], found that aqueous extracts of Inula viscosa, whether used alone or in combination, yielded significant results against black aphids on broad beans. Conversely, Diouf et al. [2022] reported that other plant treatments did not reduce whitefly infestation on tomato leaves. Additionally, Ouahchia et al. [2017] demonstrated that Inula viscosa leaves and flowers did not exhibit acute or sub-chronic toxicity.

Although aphids are not typically common pests in tomato greenhouses, they can appear early, even during the seedling stage of the plants. The *Macrosiphum euphorbiae* aphid was observed and reported to be present in the greenhouse at an early stage. Following the application of the plant extract, it was noted that this aphid species completely disappeared.

The efficiency of *D. viscisa* extract plant against aphids was also confirmed by Monteiro dos Santos *et al.* [2004] and Muzemu *et al.* [2011]. The results obtained in the presented study are also in line with those of Tchacker, 2016 Benddine [2018] and Gueribis et al., [2019] in reducing the number of *Tuta absoluta*.

Extract plant effect on auxiliary insect species

Due to its significance in Mediterranean agro-ecosystems for reducing pests on various crops [Perdikis et al., 2007], *D. viscosa* has also demonstrated a strong potential for conserving and increasing the presence of predators. In the greenhouse, several predator species were available for biological control, including *Orius insidiosus, Macrolophus* sp., *Macrolophus pygmaeus, Nesidiocoris tenuis, Coccinella septempunctata, Coccinella algerica, Brumus quadrimaculatus, Scymnus flavicollis, and Scymnus suturalis*. The impact of the biopesticide (*D. viscosa*) on these predators was evaluated. At the highest concentration (D_3 = 15 ml/L), species such as *Nesidiocoris tenuis, Trimorus* sp., *Larina* sp., *Pemphredon* sp., *Chrysoperla carnea, Necremnus tutae, Nomada* sp., *Omalus biaccinctus, Torymus* sp., and *Ceraphron* sp. were still present. However, it notably affected and caused the complete disappearance of *Scymnus suturalis, Cremastus* sp., *Diaphorencyrtus aligarhensis*, and *Aphidius colemani* after the plant extract application.

The results obtained were consistent with the findings reported by Cemagref [2007], which indicated that biopesticides negatively impact most arthropods. Similarly, numerous studies have highlighted the potential for unintended effects on natural enemies [Burel and Garnier, 2009], with impacts varying depending on the specific molecules used and the life cycle stages of the species involved [Burel and Garnier, 2009].

Extract plant effect on pollinator insects

Bees, which belong to the Hymenoptera order, are the main group of pollinating insects, with approximately 20,000 described species globally [Santos et al. 2014]. They show the greatest biodiversity in Mediterranean and arid climates. Over recent decades, biopesticides have emerged as a key source of health and sustainability. With their eco-friendly, safe, and environmentally compatible approach, biopesticides play a crucial role in agriculture and environmental management. Numerous studies actively explored their use for controlling crop pests and managing non-target organisms.

The effect of used biopesticides represented by the extract plant of *D. viscosa* on the pollinator species under greenhouse was performed using different extract doses (5, 10 and 15 ml/L). From the total represented species in the control (8 species). it was noted that during the program of application, a number of species; *Lasioglossum pallens, Andrena* sp1*, Andrena* sp2*, Apis andreniformis,* was present with an application of lowest dose (5 ml/L), Even with increasing the dose (15 ml/L) of the spread product, some other species *Chelostoma florisomne, Andrena aglissima* and *Apis epeoloides* also were observed.

Flower pollinators from insects play a crucial role in plant reproduction and the preservation of ecosystem biodiversity Memmott et al., [2007]. They are considered as the most vital for both natural ecosystems and agriculture. As reported by Belien et al., [2021], the disappearance of pollinators could be related to the direct contact to the biopesticide via application. This can easily contaminate other pollinator species when visiting flowers and leaves. Pollinators may be target of biopesticides through contact with foragers, ingestion, or from application around or inside their nests for parasite control [Cappa et al., 2022; Belien at al., 2021].

The negative effects of biopesticides on bees and other pollinators for cultivated ecosystem, remained poorly investigated over the world. The azadirachtin biopesticide implemented against the honey bee ectoparasite Varroa mite shown negative effects on honey bees [Cappa et al., 2022]. This observation was confirmed also by Peng et al. [2015] where the concentrations > 18 μg/mL significantly increased mite and honey bee mortality. Challa et al. [2019], indicated in their report that biopesticides have proven to be selective for bees; however, considering their effectiveness in pest control and increased yield, they could be used effectively in an Integrated Pest and Pollinator Management Program, while avoiding their use during the flowering period. However, Chavana and Joshi [2024], reported that all obtained results are based on studies involving a limited number of key pollinator species specific to a region, and may not apply to the many other species that contribute to crop pollination. Since each biopesticide affects different pollinator species in varying ways, it would be risky to assume their non-toxicity across different taxa and environmental contexts. In addition to the direct toxicity of biopesticides, they can also lead to a range of more subtle harmful effects in both solitary and social pollinator species [Capp et al., 2022]. Secondary metabolites, phenols, resins, steroids, terpenes, alkaloids, flavonoids, and tannins included in biopesticides have often been reported to exhibit antifungal, antimicrobial, antioxidant, or insecticidal activities [Muddanuru et al., 2019; Xing et al., 2019]. Thus, all the potential side effects need to be properly evaluated.

CONCLUSIONS

Recent studies suggest that plant extracts can be effective biopesticides, demonstrating significant efficacy against pest species while reducing harm to beneficial organisms. To test this hypothesis, the valorization of spontaneous bioresources from Algeria's arid regions has highlighted the *D. viscosa* extract as a promising biopesticide. It offers antioxidant properties and effectively reduces pests from various orders, including Diptera and Thysanoptera (*Bradysia tilicola, Bradysia ocellaris, Liothrips olea,* and *Thrips tabaci*), while safeguarding beneficial fauna, including auxiliary species (*Nesidiocoris tenuis, Trimorus* sp., *Larina* sp., *Pemphredon* sp., *Chrysoperla carnea, Necremnus tutae, Nomada* sp., *Omalus biaccinctus, Torymus* sp., and *Ceraphron* sp.) as well as pollinators. This potentially positions it as a valuable tool for sustainable agricultural practices and natural integrated pest management.

Acknowledgements

The authors would like to kindly acknowledge DGRSDT for supporting them.

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