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Effect of Glebionis segetum (corn marigold) extracts: Insecticidal and nematicidal activities against agricultural pests *Thrips tabaci*, *Ditylenchus dipsaci*, and *Sitophilus oryzae*

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ABSTRACT

This research aims to evaluate the biological insecticidal activity of the plant from the Asteraceae family *Chrysanthemum* (*Glebionis segetum*) on crop pest species: *Thrips tabaci* (thrips), *Ditylenchus dipsaci* (nematode), and *Sitophilus oryzae* (rice weevil). Materials and Methods: The flowers were dried in the shade, ground, then subjected to ultrasound-assisted extraction (40 kHz, 60 min, 50 °C) using a water/ethanol mixture design with a solid-liquid ratio of 1:10 (w/v). The obtained extracts were filtered, then lyophilized (72 h, 0.04–0.2 mbar). The tests were conducted according to a completely randomized design. Mortality was monitored over a period ranging from 1 to 144 hours. The results obtained show variable effectiveness depending on the extract and the target pest. Extract E5 showed strong effectiveness against both *Thrips tabaci* and *Ditylenchus dipsaci*, with mortality rates reaching (MR48h = 94.4%) and (MR48h = 90.0%), respectively, and corresponding LC50 values of 13.0 and 10.3 mg/mL. This extract also showed moderate activity against *Sitophilus oryzae*, while extracts E7 and E4 exhibited better activity against the latter, with mortalities of (MR48h = 70.8%) and (MR48h = 66.7%), respectively. In contrast, extract E2 showed a balanced activity spectrum, exceeding 50% mortality for each of the three pests, making it particularly suitable for crops affected by multiple pest species. Toxicological analyses allowed for the estimation of lethal concentrations (LC50), providing key parameters to compare the effectiveness of the extracts. conclusion: the exploration of ethanolic extracts from the wild plant *Glebionis segetum* (L) demonstrated significant potential underutilized for crop protection. However, as this study was limited to in vitro tests, field research is necessary to validate the efficacy of the extracts and identify the active compounds responsible for the observed effects.

Keywords: biopesticides, botanical insecticides, nematicidal activity, *Asteracae*, ultrasound-assisted extraction, agricultural pests.

INTRODUCTION

Crop pests are among the major biotic constraints limiting agricultural production worldwide. Estimates indicate that annual yield losses due to pests (insects, nematodes, pathogens) range between 20 and 40% depending on the region and cropping system, representing a major challenge for food security in the face of a growing global population (FAO, 2022, 2025; Savary et al., 2019; Sbai et al., 2024). Among these bio-aggressors, phytophagous insects *Thrips tabaci* (thrips) Lindeman *(Thysanoptera : Thripidae),* is a pest of citrus, cereals, and especially onion (*Allium cepa*), leek, tomato, cucumber (Diaz-Montano et al., 2011; Jandricic et al., 2024); peppers, chili peppers, and garlic (Boualam et al., 2024; Laamari and Houamel, 2015; Razi, 2017). Another key pest is *Sitophilus oryzae* (L.) (*Coleoptera: Curculionidae*), commonly known as the rice weevil, a frequent pest of stored cereals such as rice, wheat, barley, and occasionally peas Finally, the phytoparasitic

nematode *Ditylenchus dipsaci Filipjev* (*Tylenchida: Anguinidae*) is particularly feared in onion, garlic, strawberry, pea, alfalfa, sugar beet, and oat crops, as reported in studies conducted in different regions of Morocco, (Andaloussi and Bachikh, 2001; Filali Alaoui et al., 2021; Tanji et al., 1995). These genera are the most widespread and rank among the most damaging groups, causing considerable economic losses in both crop production and post-harvest stages in the country (Deutsch et al., 2018; Nicol et al., 2011). The management of these pests still relies largely on conventional control methods, including pyrethroids, organophosphates, and carbamates, which are becoming increasingly ineffective (Wang and Wang, 2024), sometimes insufficient, and have persistent negative impacts such as environmental contamination, non-target effects, and resistance (Abbou et al., 2024; Ahmad et al., 2024; Bouzaidi et al., 2020; Desneux et al., 2007; Goulson et al., 2015). Consequently, international agricultural policies encourage a reduction in the use of chemical pesticides and the development of sustainable alternative solutions (Alami et al., 2023; Dunan et al., 2023a; Elazzouzi et al., 2022; Ngamo and Hance, s. d.; Praneetvatakul et al., 2024, 2024; Saravanan, 2022), moving towards control using alternative methods, particularly those based on plants with insecticidal and nematicidal activity. In this context, botanical insecticides represent a promising alternative. Essential oils and plant extracts are attracting renewed interest due to their richness in secondary metabolites (alkaloids, terpenoids, flavonoids, phenolics). These compounds exhibit various modes of action (neurotoxicity, repellency, growth inhibition) that limit the risk of resistance development in target pests (Gagnon et al., 2024; Isman, 2006; Pavela and Benelli, 2016). Numerous studies have documented the insecticidal and nematicidal activity of plant extracts from the *Meliaceae, Lamiaceae, Asteraceae* and *Rutaceae* families (Ainane et al., 2019; El Abdali et al., 2022; Gounssa et al., 2018; Hansen et al., 2021; H. Li et al., 2025; Mokhtari et al., 2020; Pérez et al., 2003; Valant-Vetschera et al., 2003). Furthermore, plant-derived biopesticides have the advantage of rapid environmental degradability and generally low toxicity to mammals and beneficial crop organisms, making them compatible with the principles of integrated pest management (Regnault-Roger et al., 2012; Isman, 2020). These extracts show promising potential thanks to their active components, which possess antifungal, nematicidal, herbicidal, and insecticidal properties. (Abbou et al., 2024; Astapchuk et al., 2021; El Abdali et al., 2022; El Ajjouri et al., 2008; Kasmi et al., 2017; Maache et al., 2024; Naamane et al., 2020; Ngamo and Hance, 2007; Praneetvatakul et al., 2024; Saravanan, 2022).The genus *Chrysanthemum* (family Asteraceae) has been studied for its insecticidal properties as a source of natural pyrethrins (Kadala, 2011), which are used as biopesticides against many crop pests (Dunan et al., 2023a; Kumar et al., 2005a). In contrast, *Glebionis segetum* (corn marigold), also belonging to the Asteraceae family like *chrysanthemums* and *pyrethrums*, is described as a "serious weed" for cereal crops in Morocco (Zidane et al., 2009) and Ethipia (Hundessa et al., 2023b, 2023a) and can cause significant yield losses (Hundessa et al., 2023b). However, wild species that are naturally rich in secondary metabolites are considered the most promising (Sassi et al., 2014). Indeed, *Glebionis segetum* (L) demonstrates a high capacity to accumulate bioactive compounds such as monoterpenes, alkaloids, flavonoids, flavanol glycosides, saponins, coumarins, tannins, and steroids in its leaves and flowers, compounds that have also been reported in the aerial parts of *C. coronarium* (Alvarez-Castellanos and Pascual-Villalobos, 2003; Gounssa et al., 2018; Harborne et al., 1970; Keliat et al., 2024; Li et al., 2012; Ochocka et al., 1995; Valant-Vetschera et al., 2003). This richness in secondary metabolites explains their wide use for their biological properties, including antibacterial, antifungal, insecticidal, and antiviral activities. (Haouas et al., 2011a; Kumar et al., 2005b; Lograda et al., 2013). Despite numerous studies on plant-derived biopesticides from several *Chrysanthemum* species (Asteraceae family), including *Chrysanthemum cinerariaefolium* (Touré, 2018; Wandahwa et al., 1996), the essential oil of *Anacyclus pyrethrum* recognized for its insecticidal and larvicidal properties particularly against *mosquito larvae* (Elazzouzi et al., 2022; Mokhtari et al., 2020), and extracts of *Chrysanthemum macrotum* (Haouas et al., 2011b) active against *Spodoptera littoralis* caterpillars, comparative data regarding the efficacy of the same plant species against taxonomically distinct pest groups remain scarce. Another study on *Chrysanthemum coronarium* demonstrated in vitro nematicidal activity (aqueous extract) against the nematode *M. javanica* (Bar-Eyal et al., 2006). In contrast, *Glebionis segetum* (L) flowers, mixed with other *Asteraceous species*, showed demonstrated activity

in pot experiments (soil amendment) against *M. artiella*, significantly reducing reproduction rates (Pérez et al., 2003). Therefore, phytochemical research on the *Glebionis segetum* (L), not only for its flowers but also for its leaves, essential oils, aqueous extracts, and their derivatives (active components), is attracting considerable interest. However, published studies focus exclusively on how to control this plant in the field, rather than on the use of its extracts. Furthermore, the richness of this plant in bioactive metabolites explains its widespread use against various agricultural pests. In particular, there is a lack of integrated studies evaluating multi-pest activity, especially for *Glebionis segetum* (L), which has received limited attention in the context of multi-target control. To fill this gap, the present study systematically evaluates, under standardized in vitro conditions, the insecticidal and nematicidal potential of nine hydroethanolic flower extracts of *Glebionis segetum* (L) against three pests of agronomic importance in Morocco: *Thrips tabaci*, *Ditylenchus dipsaci*, and *Sitophilus oryzae*. The specific objectives are to quantify and compare (i) the mortality responses induced by each extract at 48 hours under standardized in vitro conditions, (ii) the temporal profiles of biological activity, and (iii) the median lethal concentrations (LC50) among the different pest groups, in order to identify extracts with either broad-spectrum or specific efficacy that are relevant for the future development of plant-based phytosanitary control tools.

MATERIALS AND METHODS

Plant material

The plant studied, *Glebionis segetum* (L.) by Fourr.(Fourreau, 1869; Stroh et al., 2023), commonly known as corn marigold, was chosen based on three criteria: its wide distribution in the Mediterranean basin and in Morocco, its use for aromatic and medicinal purposes, and the limited number of in-depth studies devoted to this wild species.

Glebionis segetum (L) is an annual plant of the Asteraceae family, present throughout Morocco except the Sahara. It is characterized by high frequency, wide geographical distribution, and a strong ability to adapt to various edaphic conditions, easily colonizing unstable and diverse biotopes. The plant is distinguished by its capitula with bright yellow ligulate flowers, erect stems 20 to 60 cm tall, slightly fleshy and glaucous, simple or branched, and simple leaves 2 to 8 cm long, with acute lobes at the apex.

Plants were collected on May 23, 2024, in the Kenitra region (Morocco), during their flowering period. Botanical identification was carried out by Professor Lahcen Zidane, and a specimen was deposited at the Herbarium of the Faculty of Sciences, Ibn Tofail University, Kenitra, under number 96/05/24. After harvest, the whole plant (stems, leaves, flowers, and roots) was air-dried duration of 14 days in the shade to preserve thermosensitive compounds. The flowers were then separated, dried, and ground using an electric grinder to obtain a fine powder with particles ranging from 70 to 300 mesh. Although most scientific studies focus on aerial parts, flowers were selected for this study due to their presumed richness in bioactive metabolites (Derouiche et al., 2018; Han et al., 2019; Howarth and Williams, 1972; Kennouche et al., 2016; Li et al., 2012; Lograda et al., 2013; Ochocka et al., 1995). The 9 dried plant extracts, and the *in vitro* tests on the three pests were entirely carried out in parallel at the National Agency of Medicinal and Aromatic Plants (ANPMA) and the laboratory of ZIRAATY company. Despite technical difficulties related to pest rearing and sample handling, the high number of 9 extracts required a significant time investment to ensure a high quality of finished work (Figure 1).

Extraction

Nine extraction experiments were carried out according to a mixture plan using different ethanol/water ratios: extract 1 (100% water), extract 2 (50% water / 50% ethanol), extract 3 (75% water / 25% ethanol), extract 4 (25% water / 75% ethanol), extract 5 (66.66% water / 33.33% ethanol), and extract 6 (33.33% water / 66.66% ethanol). extrait 7 (50% water / 50% ethanol), extrait 8 (50% water / 50% ethanol), extrait 9 (75% water / 25% ethanol), For each trial, 25 g of flower powder was introduced into an Erlenmeyer flask containing the hydro-ethanolic mixture according to the defined proportions. For all extraction experiments, a constant solid-to-liquid ratio of 1:10 (w/v) was used, corresponding to 25 g of plant powder in 250 mL of hydro-ethanolic solvent at the specified proportions. The initial concentration of plant material was the same for all extracts (250 mg/mL)

Figure 1. Preparation stages of the plant material, from the collection of Glebionis segetum to the processing of dried flowers into a fine botanical powder

Ultrasound-assisted extraction and filtration

To maximize bioactive compounds yield, ultrasound-assisted extraction was performed for each mixture for 60 min at 50 °C using an ultrasonic bath (Ultrasons-HD model, JP Selecta) at a frequency of 40 kHz with a generator power of 180 W. After the extraction process, the resulting solutions were collected for subsequent analyses. To remove insoluble residues, each extract passed through filtration Whatman No. 1 paper. The filtered extracts were transferred into dark glass containers, and maintained at refrigerated temperatures (2–8 °C) until lyophilization processes.

Lyophilization

The liquid extracts *Glebionis segetum* (L) flowers were sent to the CIRAD center in Rabat for lyophilization. Lyophilization of the samples was carried out using a Labconco FreeZone 6 freeze dryer to ensure complete removal of residual solvents. The samples were first frozen at −80 °C and then subjected to drying for 72 hours under controlled conditions. The condenser temperature was maintained between approximately −30 °C and −50 °C to promote efficient solvent removal. A vacuum pressure ranging from 0.04 to 0.2 mbar was applied throughout the process. This method enabled complete drying and yielded a fine powder extract suitable for subsequent analyses. The obtained dried extracts showed varying appearances and colors depending on the solvent proportions used (Figure 2).

Biological material

Populations of *Thrips tabaci* (adults and larvae) were sampled from *Allium cepa* in the Meknes region (Morocco) and acclimated on pesticide-free onion plants under controlled conditions (25 ± 2 °C, 16:8 h photoperiod). Larvae of *Ditylenchus dipsaci* were isolated from infected onion bulbs using the maceration and sieving method described by (Hooper et al., 2005; Southey et al., 1986). Adults of *Sitophilus oryzae* (1–3 days old) were obtained from a laboratory colony maintained on wheat and rice grains at 28 ± 1 °C and 75 ± 5% relative humidity.

Experimental design (Bioassays)

The bioassays were conducted according a completely randomized design. To ensure statistical power, each treatment was replicated five times. The experimental unit consisted of a Petri dish (9 cm) containing 20 individuals for *T.*

Figure 2. Sequential stages of the phytochemical extraction process, from ultrasound-assisted extraction to final lyophilization of dry botanical extracts

Table 1. Dry extract mass (g) after lyophilized aqueous extracts

Sample	Water	Ethanol	Dry extract mass (g)
Extract 1	100%	--	16.232 g
Extract 2	50%	50%	17.135 g
Extract 3	75%	25%	16.203 g
Extract 4	25%	75%	21.141 g
Extract 5	66.66%	33.33%	16.581 g
Extract 6	33.33%	66.66%	16.875 g
Extract 7	50%	50%	16.002 g
Extract 8	50%	50%	17.061 g
Extract 9	75%	25%	16.118 g

tabaci, D. dipsaci, and *S. oryzae*. Plant extracts (initial concentration of 1 mg/mL) were applied on Whatman No. 1 filter paper discs placed in Petri dishes (9 cm diameter) before the introduction of the pests. For each pest, a control (distilled water or 5% ethanol) for the negative control and a positive control (chemical standard) Abamectin (*T. tabaci*), Oxamyl (*D. dipsaci*), and Deltamethrin (*S. oryzae*), to validate the strain sensitivity were included. Individuals were considered dead if no movement was observed after mechanical stimulation (Figure 3).

Mortality assessment and Statistical analysis

Incubation was carried out under controlled conditions (25–28 °C, 70% RH). Mortality counts were performed at 1, 4, 24, and 48 hours. The data were then subjected to Abbott's correction before statistical analysis.

Mortality in the control group (Mc) was 5% (5 deaths out of 100), and corrected mortality (CM) was calculated using Abbott's formula (Abbott, 1925) to account for natural mortality observed in control batches:

$$MC = 100 \times \frac{M\ treated - M\ \text{control}}{100 - M\ \text{control}} \quad (1)$$

where: $M_{treated}$ and $M_{control}$ represent the percentage mortalities in treaed and control batches, respectively.

To assess the toxic impact of the tested extracts, statistical analysis of the data was performed using SPSS, Excel, and R software. For the most active extracts (mortality > 70% at 48 h), a range of five concentrations was tested to estimate the median lethal concentrations (LC50). Probit regression was used to estimate the LC50 values with their 95% confidence intervals, as well as the slopes of the dose-response curves. Multiple comparisons between extracts were conducted using one-way analysis of variance (ANOVA) in R, followed by a Tukey post-hoc test ($\alpha = 0.05$) to identify significant differences.

RESULTS

Evaluation Table 2, of the biological activity of nine hydro-ethanolic extracts of *Glebionis segetum*, applied at a dose of 1 mg/mL, revealed significant yet contrasting levels of toxicity depending on both the target pest species and the polarity of the extraction solvent. The validity of the bioassays is confirmed by very low natural mortality in the control groups (5%) and remarkable consistency of results across the five replicates, highlighting the precision and reliability of the experimental protocol. A marked specificity was observed: whereas certain extracts (e.g., E5 type) demonstrated broad-spectrum insecticidal and nematicidal activity, other extracts exhibited a more targeted action according to the biological model tested.

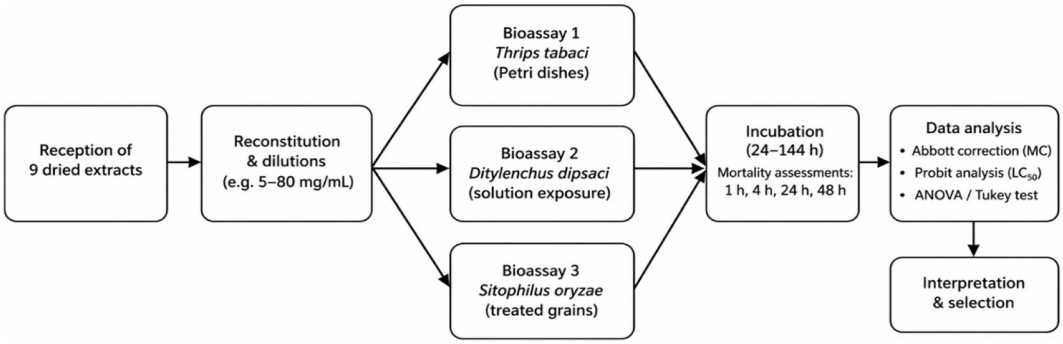

Figure 3. Simplified diagram of the experimental design (rearing conditions, exposure, and reading times)

Table 2. Results for *Thrips tabaci, Ditylenchus dipsaci*, and *Sitophilus oryzae* with Abbott's corrected mortality (MC)

Treatment	R1	R2	R3	R4	R5	Total dead	Mortality (%)	MC (Abbott)	R1	R2	R3	R4	R5	Total dead	Mortality (%)	MC (Abbott)	R1	R2	R3	R4	R5	Total dead	Mortality (%)	MC (Abbott)
Control	1	1	1	1	1	5	5%	-	1	1	1	1	1	5	5%	-	1	1	1	1	1	5	5%	-
E1	9	10	9	9	9	46	46%	43.2%	15	16	15	16	15	77	77%	75.8%	8	8	7	8	8	39	39%	35.8%
E2	16	17	16	16	16	81	81%	80.0%	12	12	12	12	12	60	60%	57.9%	11	12	11	11	11	56	56%	53.7%
E3	12	12	12	12	12	60	60%	57.9%	9	9	10	9	9	46	46%	43.2%	8	7	8	7	8	38	38%	34.7%
E4	7	8	8	8	8	39	39%	35.8%	7	6	7	6	7	33	33%	29.5%	14	13	14	13	14	68	68%	66.3%
E5	19	19	19	19	19	95	95%	94.7%	18	18	19	18	18	91	91%	90.5%	9	10	9	9	9	46	46%	43.2%
E6	5	5	6	5	5	26	26%	22.1%	11	11	10	11	11	54	54%	51.6%	4	4	4	4	4	20	20%	15.8%
E7	17	18	17	18	17	87	87%	86.3%	8	7	8	7	8	38	38%	34.7%	14	15	14	15	14	72	72%	70.5%
E8	3	3	3	4	3	16	16%	11.6%	4	4	5	4	4	21	21%	16.8%	4	3	4	3	4	18	18%	13.7%
E9	10	10	10	9	10	49	49%	46.3%	17	17	17	17	17	85	85%	84.2%	6	6	7	6	6	31	31%	27.4%

Corrected mortality at 48 hours: Comparative analysis

The analysis of variance Table 3 revealed statistically significant differences and highly variable efficacy depending on the extract and the pest. On *Thrips tabaci*, mortalities range from 11.3% (E8) to 94.4% (E5). For *Ditylenchus dipsaci*, performances are overall higher, ranging from 16.4% (E8) to 90.0% (E5). In contrast, on *Sitophilus oryzae*, mortalities are lower, ranging from 13.9% (E8) to 70.8% (E7). Examination of standard deviations indicates good reproducibility for several combinations (notably E5 and E9 on *D. dipsaci*), while some show greater variability (E1 and E3 on *S. oryzae*). Three extracts stand out: E5, exceptional on *T. tabaci* and *D. dipsaci*; E7, very active on *T. tabaci* and *S. oryzae*; and E2, with a balanced spectrum across the three pests. Conversely, E8 consistently shows the lowest mortalities.

The data illustrated in Figure 4 show highly contrasting efficacy of the nine plant extracts depending on the target pest.

For *Thrips tabaci*, extracts E5 (94.4 ± 2.6%), E7 (85.9 ± 5.0%), and E2 (80.3 ± 5.2%) stand out for their high efficacy, while E8 (11.3 ± 6.5%) and E6 (22.5 ± 5.9%) are very poorly active. Extracts E3 (57.7 ± 3.1%), E9 (46.5 ± 5.7%), and E1 (43.7 ± 5.0%) show moderate activity. The heat map (Figure 4) and the bar graph (Figure 5) confirm the superiority of E5, E7, and E2, with low intra-extract variability indicating test reproducibility.

For *Ditylenchus dipsaci*, E5 is the most active (90.0 ± 0.7%), followed by E9 (84.4 ± 1.2%) and E1 (75.5 ± 1.4%). E2 (57.8 ± 1.7%) and E6 (51.7 ± 3.4%) show moderate efficacy, while E3 (42.7 ± 1.0%), E7 (34.8 ± 3.3%), and E4 (29.8 ± 2.0%) are less effective. Figure 4 shows a more balanced distribution, and the very narrow error bars for E5 and E9 Figure 5 indicate excellent reproducibility.

For *Sitophilus oryzae*, performances are overall lower. E7 (70.8 ± 6.0%), E4 (66.7 ± 4.0%), and E2 (54.2 ± 3.5%) are the most effective, while E5 (43.1 ± 5.1%), E1 (36.1 ± 7.1%), and E3 (34.7 ± 6.4%) are moderate, and E9

Table 3. Corrected mortality at 48 h (mean ± standard error)

Extract	*Thrips tabaci* CM48h (%)	*Ditylenchus dipsaci* CM48h (%)	*Sitophilus oryzae* CM48h (%)
E1	43.7 ± 5.0	75.5 ± 1.4	36.1 ± 7.1
E2	80.3 ± 5.2	57.8 ± 1.7	54.2 ± 3.5
E3	57.7 ± 3.1	42.7 ± 1.0	34.7 ± 6.4
E4	35.2 ± 2.6	29.8 ± 2.0	66.7 ± 4.0
E5	94.4 ± 2.6	90.0 ± 0.7	43.1 ± 5.1
E6	22.5 ± 5.9	51.7 ± 3.4	15.3 ± 2.6
E7	85.9 ± 5.0	34.8 ± 3.3	70.8 ± 6.0
E8	11.3 ± 6.5	16.4 ± 3.0	13.9 ± 5.6
E9	46.5 ± 5.7	84.4 ± 1.2	27.8 ± 2.8

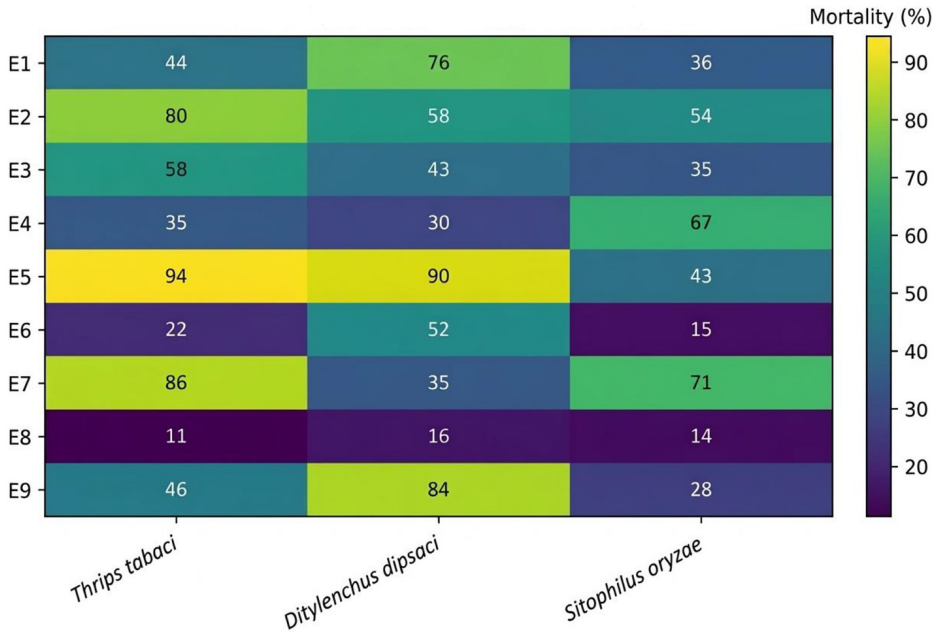

Figure 4. Heat map of corrected mortalities at 48 h

(27.8 ± 2.8%), E6 (15.3 ± 2.6%), and E8 (13.9 ± 5.6%) are poorly active. Figure 4 reveals less frequent red intensity, reflecting lower susceptibility of this species.

Analysis of activity profiles (Figures 4 and 5) allows distinguishing:

- E5: broad spectrum, exceptional on *T. tabaci* (94.4%) and *D. dipsaci* (90.0%), moderate on *S. oryzae* (43.1%).
- E7: complementary, very active on *T. tabaci* (85.9%) and *S. oryzae* (70.8%), moderate on *D. dipsaci* (34.8%).
- E2: broad balanced spectrum (80.3%, 57.8%, 54.2%).
- E9: specific to *D. dipsaci* (84.4%), moderate on *T. tabaci* (46.5%), low on *S. oryzae* (27.8%).
- E1, E3, E4: intermediate profiles, with good activity of E4 on *S. oryzae* (66.7%).
- E6 and E8: consistently the least effective (mortalities < 25% except E6 on *D. dipsaci* at 51.7%).

The heat map Figure 4 groups extract into three categories: broad spectrum (E5, E2), intermediate spectrum (E7, E9, E1, E4), and low activity (E3, E6, E8). The error bars (Figure 5) show low variability for E5 and E9 on *D. dipsaci* (standard deviations of 0.7 and 1.2), and greater variability for *S. oryzae* (E1: 7.1; E7: 6.0), reflecting heterogeneity in susceptibility within this species.

Comparative study of temporal kinetics: Best plant extracts vs. control

Mortality kinetics on Thrips tabaci

The biocidal kinetics against *Thrips tabaci* revealed a dose- and time-dependent efficacy for all tested extracts. An intense activity phase was observed between 4 and 24 hours, during which mortality exceeded the critical threshold of 50%, while the control group consistently remained below 5%. Although E7 exhibited a slightly faster initial knockdown effect within the first 4 hours, extract E5 emerged as the most potent treatment, achieving a final mortality approximately 94.7% higher at 48 hours. At this time point, a statistically significant divergence was observed compared to E7 (86.3%) and E2 (80.0%). This difference in kinetic profiles between E5 and the other two extracts may reflect variations in phytochemical composition and, consequently, in the mode of action (Figure 6).

Mortality kinetics on Ditylenchus dipsaci

Figure 7 presents the mortality kinetics for extracts E5, E9, and E1, the most active against *Ditylenchus dipsaci*. Extract E5 shows exceptional efficacy, exceeding 70% mortality as early as 24 hours and reaching 90% at 48 hours. Extract E9 follows a similar trend, with 84% mortality at 48 hours, while E1 progresses more slowly to reach 75% at 48 hours. The very low standard deviations observed for E5 (± 0.7) and E9 (± 1.2) at

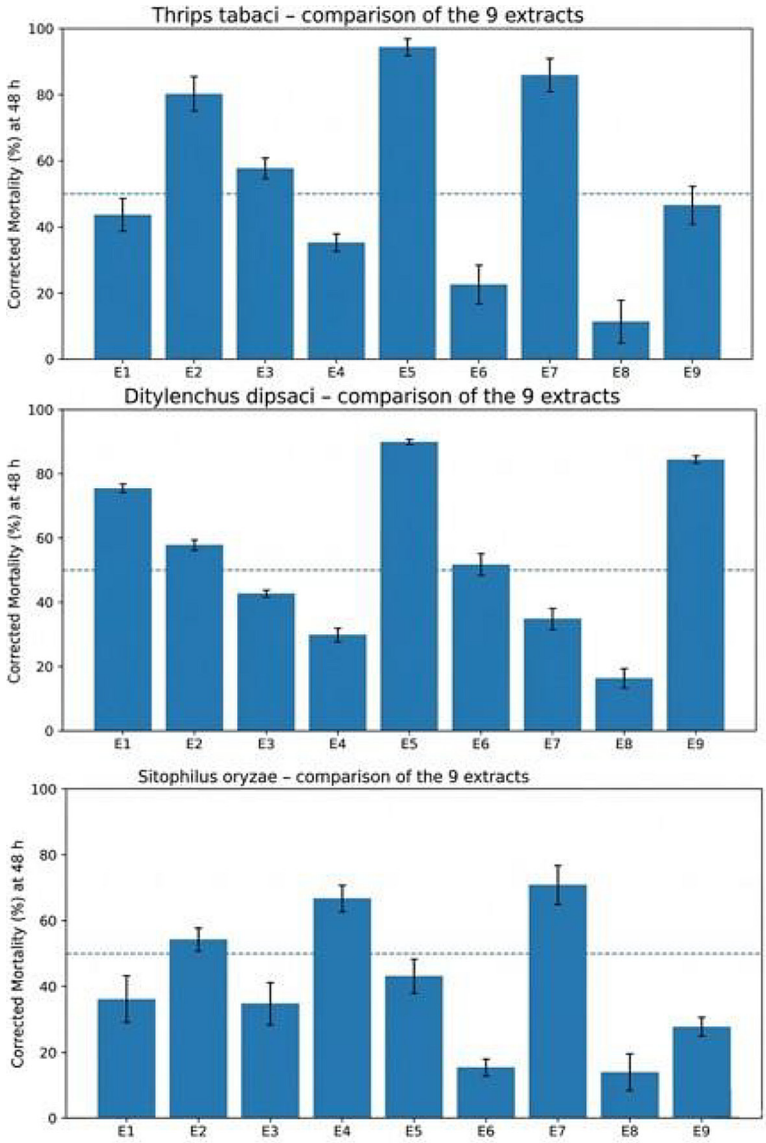

Figure 5. *Thrips tabaci*, *Ditylenchus dipsaci*, and *Sitophilus oryzae* – corrected mortality at 48 h by extract

48 hours demonstrate excellent test reproducibility and a homogeneous response of the exposed nematode populations. The rapid action of E5 and E9 is particularly interesting from the perspective of controlling this endoparasitic nematode, whose damage occurs quickly after penetration into plant tissues.

Mortality kinetics on Sitophilus oryzae

Figure 8 illustrates the temporal evolution of corrected mortality for the most active extracts against *Sitophilus oryzae* (E7, E4, and E2), compared to the control. Unlike the kinetics observed for *Thrips tabaci* and *Ditylenchus dipsaci*, mortality in the rice weevil progresses more slowly, reaching its maximum only at 48 hours of exposure. At this point, extract E7 shows the highest efficacy with 70.8% mortality, followed by extract E4 (66.7%) and extract E2 (54.2%). At earlier times, mortality remains moderate: at 4 hours, it does not exceed 12% for all extracts, and at 24 hours, it ranges between 25% (E2) and 48% (E7). The control remains below 5% throughout the experiment, confirming the absence of significant natural mortality.

Determination of median lethal concentrations (LC50) by probit modeling

Table 4 presents the estimated median lethal concentrations (LC50) obtained by probit regression for the most active extracts against each pest. These values allow quantifying the relative toxicity of the extracts and establishing a precise hierarchy of their efficacy.

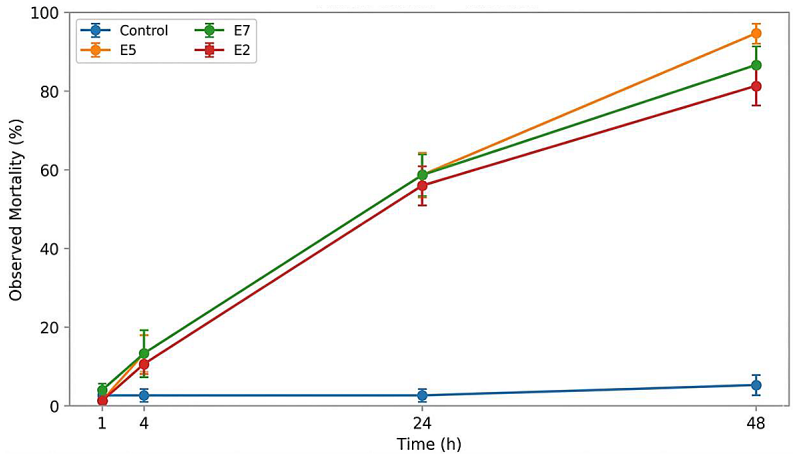

Figure 6. Mortality kinetics – *Thrips tabaci* (most active extracts vs. control)

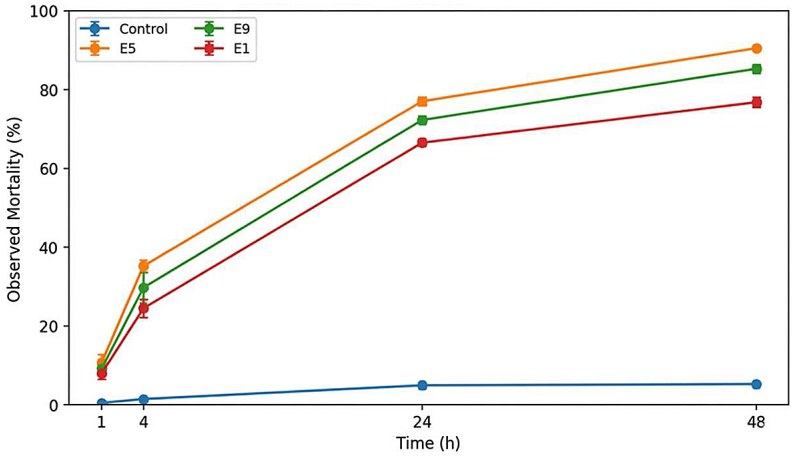

Figure 7. Mortality kinetics – *Ditylenchus dipsaci* (most active extracts vs. control)

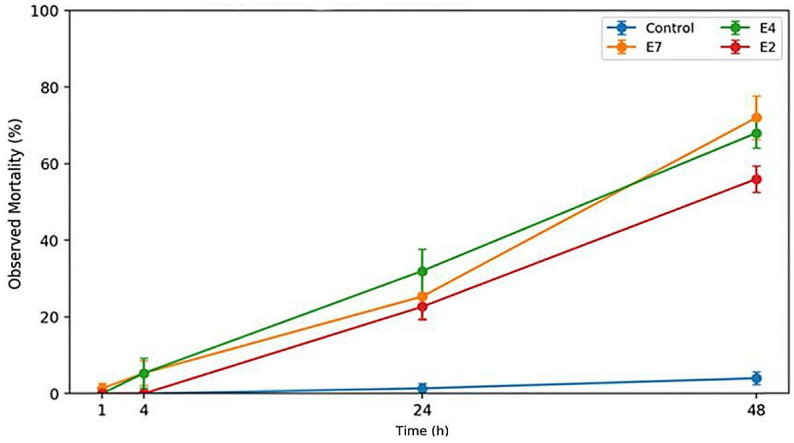

Figure 8. Mortality kinetics – *Sitophilus oryzae* (most active extracts vs. control)

All probit analyses allow ranking the extracts according to their toxicity and anticipating potential resistance risks.

- E5 proves to be the most potent extract, with the lowest LC50 values against *T. tabaci* (13.0 mg/ml) and *D. dipsaci* (10.3 mg/ml). Its high slopes (≥ 2.7) indicate a homogeneous response of the exposed populations, which is favorable for use in integrated pest management as it limits the risk of rapid selection of resistant individuals.

Table 4. LC50 by probit analysis

Pest	Extract	LC50 (mg/ml) – 95% CI	Slope (b)
Thrips tabaci	E5	13.0 [11.2–15.0]	2.86
Thrips tabaci	E7	19.6 [16.8–23.0]	2.50
Thrips tabaci	E2	21.9 [18.6–25.7]	2.39
Ditylenchus dipsaci	E5	10.3 [9.4–11.3]	2.70
Ditylenchus dipsaci	E9	14.3 [13.1–15.6]	2.53
Ditylenchus dipsaci	E1	20.5 [18.8–22.4]	2.51
Sitophilus oryzae	E7	29.8 [22.4–39.5]	1.29
Sitophilus oryzae	E4	29.8 [22.8–39.0]	1.37
Sitophilus oryzae	E2	47.9 [36.4–63.0]	1.64

- E9 ranks as the second-best extract against *D. dipsaci* (LC50 = 14.3 mg/ml), also with a high slope (2.53) and excellent reproducibility.
- E7 and E4 are the most effective against *S. oryzae* (LC50 = 29.8 mg/ml), but their lower slopes (≤ 1.37) suggest greater heterogeneity in susceptibility. In this context, the use of these extracts should be associated with rotation or combination strategies with other control methods to prevent the emergence of resistance.
- E2 presents a balanced action spectrum with intermediate LC50 values against the three pests (21.9 mg/ml on *T. tabaci*, 47.9 mg/ml on *S. oryzae*), but its moderate slopes (2.39 on *T. tabaci*, 1.64 on *S. oryzae*) place it in an intermediate position.

The LC50 values obtained fall within ranges compatible with practical applications, subject to validation under real conditions and optimization of formulations. Combining these toxicity data with action kinetics allows guiding the choice of extracts according to the priority target and the intended use conditions.

Figures 9, 10 and 11 present the fitted probit regression curves for each active extract, visually illustrating the relationship between concentration and mortality.

Dose-response curves (Probit) for Thrips tabaci

Figure 9 shows the fitted probit regression curves for the three most active extracts against *Thrips tabaci*: E5, E7, and E2. The abscissa represents the extract concentration (mg/ml) on a logarithmic scale, while the ordinate represents the corrected mortality at 48 h expressed as a percentage.

The curve for E5 is distinctly positioned furthest to the left. This location reflects the lowest LC50 (13.0 mg/ml), meaning that this extract reaches 50% mortality at a lower concentration than the other two. Visually, the E5 curve crosses the 50% mortality threshold at approximately 13 mg/ml, while E7 and E2 only reach this same threshold at higher concentrations (19.6 mg/ml and 21.9 mg/ml, respectively).

This leftward shift indicates that, for a given concentration, E5 consistently induces higher mortality than E7 and E2. For example, at a concentration of 15 mg/ml, mortality from E5 exceeds 60%, while that from E7 and E2 is around 45% and 40%, respectively.

E5 stands out as the most potent and reliable extract against *T. tabaci*. Its dose-response curve indicates that a well-chosen concentration will achieve maximum efficacy with low response variability. E7 and E2 remain very active, with respective LC50 values of 19.6 mg/ml and 21.9 mg/ml; their slopes, although lower than that of E5, remain above b=2.3, which is considered a good indicator of response homogeneity. They constitute interesting alternatives, particularly in a rotation strategy to prevent the development of resistance.

Dose-response curves (Probit) for Ditylenchus dipsaci

Figure 10 shows the probit regression curves for the three most active extracts against *Ditylenchus dipsaci*: E5, E9, and E1. The curve for E5 is clearly shifted to the left, confirming its lowest LC50 (10.3 mg/ml). That of E9 is positioned between E5 and E1 (LC50 = 14.3 mg/ml), while E1 is shifted to the right (LC50 = 20.5 mg/ml), reflecting a clear toxicity hierarchy.

The three curves have comparable and high slopes (E5: b = 2.70; E9: b = 2.53; E1: b = 2.51),

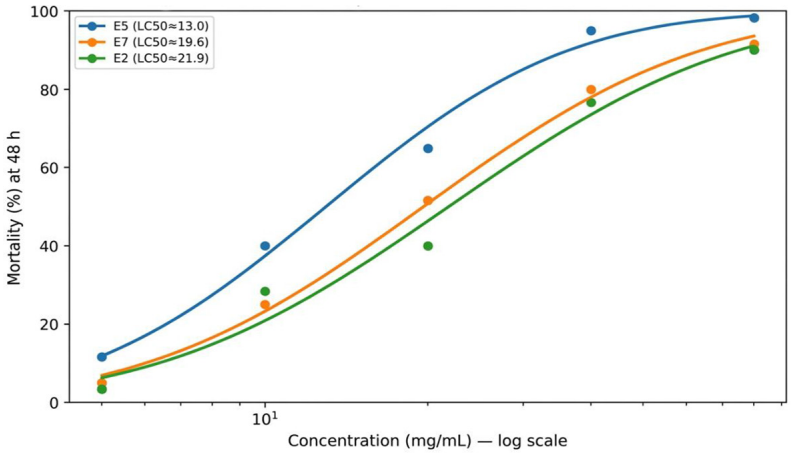

Figure 9. Dose-response curve (probit) – *Thrips tabaci*

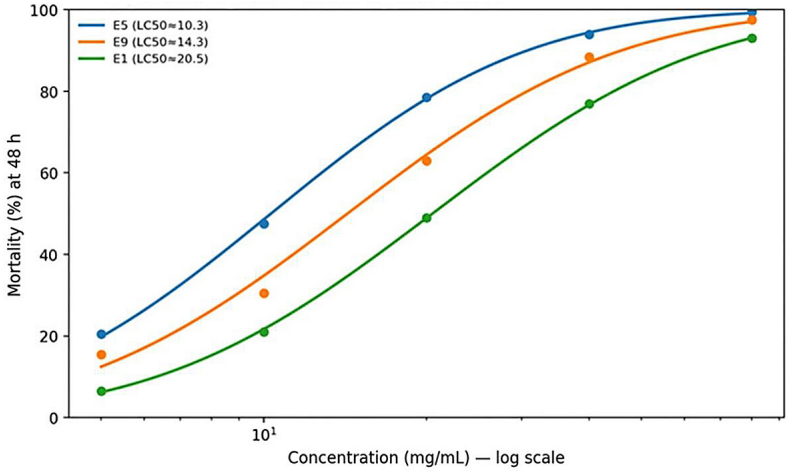

Figure 10. Dose-response curve (probit) – *Ditylenchus dipsaci*

consistent with the values reported in Table 3. These slopes above 2.5 indicate a very marked dose-response relationship: a slight increase in concentration leads to a rapid increase in mortality, which is favorable for practical use. The small difference between slopes suggests homogeneity of response within the tested population, likely related to its low genetic variability.

The experimental points fit closely to the regression lines, with minimal scatter, validating the reliability of the estimates. E5 stands out as the most potent extract, combining the lowest LC50 and the highest slope. E9 constitutes an excellent alternative, while E1, though less potent, remains active.

The high slopes observed (≥ 2.5), consistent with Table 3, indicate a low risk of rapid resistance emergence, which is favorable for sustainable use. These results provide a solid basis for guiding validation trials under field conditions.

Dose-response curves (Probit) for Sitophilus oryzae

The curves for E7 and E4 are almost superimposed, with identical LC50 values (29.8 mg/ml). The curve for E2 is clearly shifted to the right, reflecting a higher LC50 (47.9 mg/ml). At a concentration of 30 mg/ml, E7 and E4 reach about 50% mortality, while E2 only reaches 35%. The slopes from Table 3 are noticeably lower than for the other two pests (E7: b = 1.29; E4: b = 1.37; E2: b = 1.64). These slopes below 2 indicate a less pronounced dose-response relationship: an increase in concentration produces a more moderate increase in mortality. This characteristic reflects greater heterogeneity in susceptibility within the tested *S. oryzae* population. The experimental points show greater scatter than for the other pests, particularly for E7 and E4, resulting in wider confidence intervals (95% CI: 22.4–39.5

for E7). The fit remains acceptable but indicates increased biological variability.

E7 and E4 are the most active extracts against *S. oryzae*, with identical LC50 values (29.8 mg/ml). Their low slopes (1.29 and 1.37) suggest a heterogeneous population response. E2 is less active (LC50 = 47.9 mg/ml) but has the highest slope in the group (1.64), indicating a slightly more homogeneous response. Slopes below 2 indicate a potential risk of resistance emergence with repeated use. For this species, an integrated pest management strategy combining multiple modes of action (extract rotation, physical methods) is recommended.

The LC50 values obtained (29.8–47.9 mg/ml) are higher than for the other two pests, confirming the lower susceptibility of *S. oryzae* to the tested extracts. Higher concentrations or optimized formulations will be necessary for application in grain storage. This interpretation highlights the specificity of *S. oryzae*: lower susceptibility, reduced slopes, and increased variability, implying a different management approach than for the other two pests (Figure 11).

DISCUSSION

Variability of extract efficacy according to pests

The results highlight variable efficacy, depending both on the extract considered and the target pest. This interspecific variability, frequently observed in biopesticide studies, reflects the diversity of modes of action of secondary metabolites (Alvarez-Castellanos et al., 2001; Alvarez-Castellanos and Pascual-Villalobos, 2003; Kennouche et al., 2016; Pavela and Benelli, 2016). Extract E5 stands out for exceptional activity against *T. tabaci* (94.4%) and *D. dipsaci* (90.0%), with LC50 values of 13.0 and 10.3 mg/ml, comparable to those reported for azadirachtin (5–20 mg/ml) or *Tagetes* spp. extracts (8–25 mg/ml) (Hooks et al., 2010; Rind et al., 2025). This high activity could be due to broad-spectrum compounds like saponins or alkaloids (Wink, 2018). Its moderate efficacy against *S. oryzae* (43.1%) is explained by that cuticular sclerotization in Coleoptera remains a major barrier for non-formulated crude extracts, which limits the penetration of active compounds (Harmouzi et al., 2024; Liang et al., 2025)

Extract E7 shows high activity against *T. tabaci* (85.9%) and *S. oryzae* (70.8%), but limited efficacy against *D. dipsaci* (34.8%). This profile, complementary to that of E5, illustrates the interest in a range of extracts with differentiated spectra (Regnault-Roger et al., 2012). Its efficacy against *S. oryzae* is remarkable, as extracts active against stored grain beetles are less numerous (Isman, 2020). With an LC50 of 29.8 mg/ml, E7 falls within the efficacy range of extracts like clove (20–40 mg/ml) (Li et al., 2025).

Extract E2 presents a balanced spectrum, with mortalities above 50% for all three pests (80.3%, 57.8%, and 54.2%). This versatility is interesting for onion crops where *T. tabaci* and *D. dipsaci* frequently coexist (Diaz-Montano et al., 2011). The high slopes of its dose-response curves (b = 2.39 and 2.51) indicate a homogeneous population response and a low risk of resistance (Al Naggar et al., 2025).

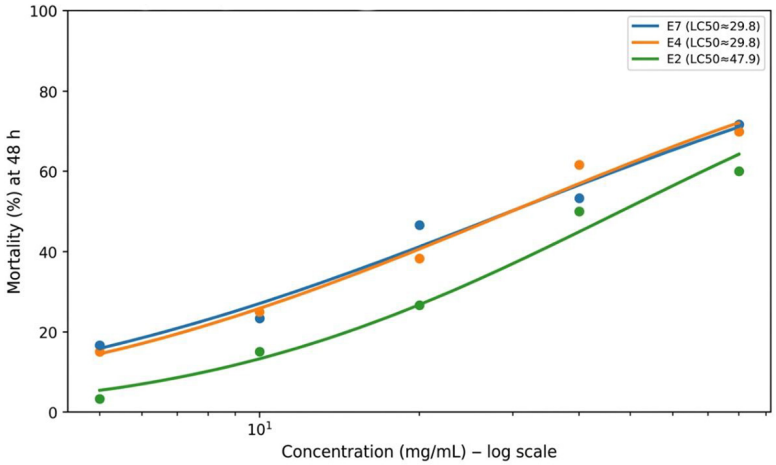

Figure 11. Dose-response curve (probit) – *Sitophilus oryzae*

Less Active Extracts: Conversely, E8 consistently shows the lowest mortalities (< 20%), suggesting a lack of significant biocidal activity, likely related to insufficient concentration of active compounds or their degradation (Isman, 2006)

The diversity of activity profiles underscores the importance of rational extract selection. In an integrated pest management strategy, the use of complementary extracts such as E5 and E7 – by combining these extracts – could target different physiological sites of action (e.g., acetylcholinesterase inhibition (the nervous system) for one, membrane disruption for another), thereby reducing the likelihood of the pest developing resistance (Haouas et al., 2011b; Minto and Blacklock, 2008; Regnault-Roger et al., 2012; Sparks and Nauen, 2015). The versatility of E2 makes it a candidate of choice for organic farming, where curative solutions are often limited (Pretty and Bharucha, 2015a). Its interest lies in the simultaneous management of thrips and nematodes on a crop such as onion, thereby reducing the number of required interventions(Gagnon et al., 2024)

Action kinetics and practical implications

The rapid action observed for E5 and E9 against *Ditylenchus dipsaci* (mortality > 70% at 24 h) is a major advantage, as rapid action limits damage and reduces the risk of resistance development by decreasing the probability of transmission of resistance genes (Al Naggar et al., 2025; Regnault-Roger et al., 2012). This characteristic is crucial for endoparasitic nematodes like *D. dipsaci*, whose damage (necrosis, deformations) occurs quickly after penetration into plant tissues(Andaloussi and Bachikh, 2001; Dash et al., 2023). The aqueous extract of C. coronarium demonstrated nematostatic activity against M. incognita egg-masses in a dose-dependent manner, with an LC_{50} value of 16,207.6 mg L^{-1} after 24 h of exposure against root-knot nematodes. This in vitro activity was heat-resistant and was attributed to thermostable bioactive compounds present in the extract (Abbassy et al., 2015; Alvarez-Castellanos et al., 2001; Pérez et al., 2003). Similarly, the rapid action of E5 against *Thrips tabaci* (85% at 24 h) is a considerable asset, as thrips transmit phytopathogenic viruses like TSWV and IYSV within minutes of feeding (Riley et al., 2011); rapid mortality thus reduces the probability of viral transmission, reinforcing the interest of these extracts against associated diseases (Diaz-Montano et al., 2011). In contrast, the slower kinetics on *Sitophilus oryzae* is explained by the weevil's morphology: the thick, waxy chitinous integument of Coleoptera limits the penetration of active compounds, explaining their lower susceptibility to botanical insecticides (Isman, 2020; Liang et al., 2025), and their feeding mode (grain borers) partially protects them from surface treatments, requiring prolonged exposure (at least 48 h) for optimal efficacy (Kim et al., 2024; Machuca-Mesa et al., 2023). These differences in kinetics should guide application methods: for *D. dipsaci* and *T. tabaci*, the rapid action of E5 and E9 allows for curative treatments (bulb dipping, foliar spraying); for *S. oryzae*, prolonged exposure is necessary, achievable by fumigation or application to grains before bagging (Harmouzi et al., 2024) Combining extracts with complementary kinetics (E5 and E7) could optimize temporal efficacy. Finally, rapid action reduces environmental impacts: shorter, more effective treatments decrease the quantities needed and limit exposure of non-target organisms (Desneux et al., 2007). From a sustainable development perspective, fast-acting extracts like E5 and E9 therefore offer an additional advantage.

Interpretation of probit slopes and resistance management

The slopes of the dose-response curves (Table 4) constitute a valuable indicator for assessing the risk of resistance emergence. In probit analysis, a high slope (b > 2) reflects a homogeneous population response and a low risk of rapid selection of resistant individuals, while a slope below 2 suggests heterogeneity in susceptibility favoring resistance emergence (Al Naggar et al., 2025; Sparks and Nauen, 2015).

In our study, the slopes observed for *Thrips tabaci* (b = 2.39 to 2.86) and *Ditylenchus dipsaci* (b = 2.51 to 2.70) are all above 2.5, indicating a limited risk of resistance development and homogeneous susceptibility of the tested populations, likely related to their rearing origin, but also potential for sustainable use within integrated pest management strategies. It is important to note, however, that studies on field populations are necessary to confirm these results, as wild populations may exhibit greater genetic variability (Isman, 2020). In contrast, the slopes

observed for *Sitophilus oryzae* (b = 1.29 to 1.64) reflect greater heterogeneity in susceptibility, associated with an increased risk of resistance emergence. Several factors may explain this heterogeneity: higher natural genetic variability in Coleoptera, developed enzymatic detoxification mechanisms, and a thick cuticular barrier modulating the penetration of active compounds. Given these low slopes, prudent management is necessary for *S. oryzae*: rotation of extracts with different compositions, association with physical methods (atmospheric control, thermal management), use of synergistic formulations (adjuvants like piperonyl butoxide), and preventive treatments on grains before storage. For *T. tabaci* and *D. dipsaci*, the high slopes allow consideration of simpler control strategies, but an extract rotation approach (E5, E9, E2) is recommended to preserve long-term efficacy and maintain diversified selection pressure. Probit analyses constitute a first step in assessing resistance risk; complementary studies are necessary to characterize the genetic basis of susceptibility and monitor the evolution of tolerance under field conditions, through periodic sensitivity tests on populations collected from agricultural environments.(Arich et al., 2024; Machuca-Mesa et al., 2023)

Comparison with literature data

The LC50 values obtained for the most active extracts fall within ranges compatible with practical applications, with notable differences according to the pest. For *Thrips tabaci*, E5 has an LC50 of 13.0 mg/ml, comparable to that of azadirachtin (5–20 mg/ml), a reference biopesticide (Rind et al., 2025). while extracts of *Nicotiana tabacum* (15–25 mg/ml) and essential oils of *Thymus vulgaris* (8.5 mg/ml) confirm that E5 is in the high range of plant biopesticides (Li et al., 2025; Wang and Wang, 2024) For *Ditylenchus dipsaci*, E5 has an LC50 of 10.3 mg/ml, comparable to the best botanical nematicides: *Tagetes erecta* (8.5 mg/ml), *Ruta graveolens* (15.2 mg/ml), and *Azadirachta indica* (12–25 mg/ml)(Dash et al., 2023; Filali Alaoui et al., 2021; Hooks et al., 2010; Hooper et al., 2005); E9 (14.3 mg/ml) and E1 (20.5 mg/ml) also show interesting performance. For *Sitophilus oryzae*, the LC50 of E7 and E4 (29.8 mg/ml) are higher than those of essential oils (5–15 mg/ml), such as clove (5.8 mg/ml) or cinnamon (8.2 mg/ml)(H. Li et al., 2025; Pavela and Benelli, 2016). However, dry extracts like E7 and E4 offer better stability and more flexible applicability, and their LC50 remain promising with optimized formulations. Several formulation strategies can improve extract efficacy: encapsulation protects active compounds from degradation and can reduce LC50 by 30 to 50% (Isman, 2020; Pavela and Benelli, 2016); synergy between compounds, by combining extracts with complementary modes of action (e.g., E5 + E7), can broaden the action spectrum and reduce the required concentrations; the addition of adjuvants (Tween 80, DMSO) improves cuticular penetration, while synergists like piperonyl butoxide inhibit detoxification enzymes. The LC50 obtained are compatible with common uses: bulb dipping for *D. dipsaci* (10–50 mg/ml), foliar sprays for *T. tabaci* (5–30 mg/ml) (Dash et al., 2023; Rind et al., 2025); for *S. oryzae*, higher concentrations (30 mg/ml) can be achieved by fumigation or application before bagging (Machuca-Mesa et al., 2023). Cross-study comparisons should be interpreted cautiously due to variations in experimental conditions, but our results fall within ranges consistent with the literature, validating their robustness. The LC50 of E5, E7, E9, E4, and E2 are therefore compatible with practical applications; E5 and E9 show performance comparable to the best botanical biopesticides, and for *S. oryzae*, formulation efforts could further improve efficacy. These results provide a solid basis for the development of plant-based biopesticides.

Study limitations and perspectives

This study has several limitations that must be considered for a nuanced interpretation of the results and to guide future work, concerning both experimental conditions, extract characterization, and safety assessment. The tests were conducted under *in vitro* conditions, which do not fully reflect the complexity of field conditions, where biotic and abiotic interactions (climatic factors, interactions with the host plant, presence of beneficial organisms, pest behavior) modulate the efficacy of biopesticides.(Dunan et al., 2023b; Gupta et al., 2023; Praneetvatakul et al., 2024; Regnault-Roger et al., 2012). Greenhouse and field trials are therefore necessary to validate the efficacy of the extracts under realistic conditions, with monitoring over several crop cycles to assess persistence of efficacy, cumulative effects, and impact on yields (Regnault-Roger et al., 2012).

Furthermore, the precise phytochemical composition of the extracts was not characterized, which constitutes an important limitation. Identification of active molecules is essential (Derouiche et al., 2018; Han et al., 2019; Hundessa et al., 2023a; Kennouche et al., 2016; Li et al., 2012), for standardization (ensuring reproducible efficacy), understanding mechanisms of action (guiding research and resistance management), optimizing formulations, and toxicological evaluation. Analyses by GC-MS (gas chromatography-mass spectrometry) and HPLC (high-performance liquid chromatography), accompanied by bio-guided tests, are essential to identify the active fractions and molecules responsible for the observed effects.

Finally, non-target toxicity studies are necessary to evaluate the safety of the extracts towards beneficial crop organisms and the environment. Although plant biopesticides are generally safer than synthetic pesticides, they are not exempt from adverse effects. Priority targets include pollinators (*Apis mellifera*), natural predators and parasitoids, soil organisms (*Eisenia fetida*), and aquatic organisms (*Daphnia magna*) (Goulson et al., 2015; Isman, 2020)

Standardized ecotoxicological studies (OECD guidelines) are necessary for the most promising extracts (E5, E7, E9, E2), including environmental persistence tests.

Beyond these limitations, several research perspectives emerge: optimization of extraction conditions to enrich extracts in active compounds, synergy studies between extracts (e.g., E5 + E7) to reduce required concentrations and broaden the action spectrum histological and physiological studies to understand mechanisms of action, and in the longer term, orientation of varietal selection programs to strengthen natural plant defenses (Pretty and Bharucha, 2015b).

The identified limitations do not invalidate the results obtained but underline the need for further research. The perspectives are numerous: validation under real conditions, phytochemical characterization, non-target toxicity studies, formulation optimization, and understanding of mechanisms of action. This work will enable the transformation of the promising extracts identified in this study into effective, safe, and sustainable biopesticides, integrable into integrated pest management strategies.

CONCLUSIONS

This study assessed the in vitro insecticidal and nematicidal activity of *Chrysanthemum segetum* extracts against *Thrips tabaci*, *Ditylenchus dipsaci*, and *Sitophilus oryzae.* The results appear promising regarding the toxicity of our hydro-ethanolic extracts, based on the mortality rates observed in the pests. Notably, extract E5 showed the highest insecticidal activity against tobacco and onion thrips (*Thrips tabaci*) and nematicidal activity against the stem endoparasite (*Ditylenchus dipsaci*), with mortality rates of 94.4% and 90.0%, and LC_{50} values of 13.0 and 10.3 mg/mL, respectively. Meanwhile, the cereal weevil (*Sitophilus oryzae*) was more resistant to extract E5 but proved more susceptible to extracts E7 and E4. The LC_{50} value for the insecticidal activity of these two extracts was determined to be 29.8 mg/mL, with mortality rates of 70.8% and 66.7%, respectively. Extract E2 showed consistent activity (>50% mortality) against all three pests. Given that bioactive compounds also play an important role in biological activities, the extracts richest in these compounds generally exhibited the highest toxicity levels, corroborating literature data. Dose-response analysis confirmed the toxic potential of the extracts.

Mortality kinetics varied according to the pest species. A steep slope was observed for *Thrips tabaci* (b = 2.86) and *Ditylenchus dipsaci* (b = 2.70), indicating a low risk of resistance emergence, compared to *S. oryzae*, which showed shallower slopes (b = 1.29 and 1.37). Furthermore, significant differences in susceptibility were observed among the tested species, with some being more vulnerable while others showed relative resistance. Significant susceptibility differences were noted among species. While this study highlights the potential of these extracts as bioinsecticides or nematicides, further research on bioactive compounds and toxicity is needed to validate the sustainable use of this species as a biopesticide.

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