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Comparative Toxicity and Genotoxicity of Four Insecticide Classes on *Spodoptera littoralis* (Lepidoptera: Noctuidae)

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ABSTRACT

The Egyptian cotton leafworm, *Spodoptera littoralis,* is a polyphagous and destructive pest worldwide, infesting many plant families, leading to significant crop losses. In this study, we have used leaf-dip and comet assays to test the toxicity and genotoxicity of four insecticides, "Lambda® 10% EC, Emafel® 4% ME, Xentari® 54% WG, and peppermint oil" *on* fourth-instar larvae of The Egyptian cotton leafworm. Regarding toxicity, Emafel® was the most potent, with LC_{50} values dropping from 0.691 µg/mL on the first day to 0.199 µg/mL by the fourth day. In contrast, Xentari® worked more slowly but steadily, with LC_{50} values decreasing from 45.713 to 4.766 µg/mL over ten days. Lambda® demonstrated moderate effectiveness, whereas peppermint oil had little impact on larval death. Transitioning to DNA damage results, the comet assay showed that Lambda® caused the most DNA damage, followed by the Egyptian cotton leafworm. Notably, peppermint oil caused very little DNA damage, even though it contains dl-menthol and l-menthone, as found by GC-MS. In conclusion, Emafel offers rapid control but with significant genotoxicity, while Xentari provides effective, environmentally safer pest management. Peppermint oil, despite its lower toxicity, presents minimal genotoxic risk and potential for integrated pest management applications.

INTRODUCTION

The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), is a highly polyphagous pest widely distributed across Africa, the Middle East, and southern Europe, posing a significant threat to agricultural productivity due to its extensive host range spanning over 40 plant families, including economically critical crops like cotton, tomatoes, and soybeans (Salama *et al.,* 1984; Alford, 2000). Its status as an A2 quarantine pest underscores its severity, with larval feeding causing up to 50-70% yield losses in untreated fields, exacerbating food security challenges in subtropical regions (Fargues & Rodriguez-Rueda, 1980; Alford, 2000). The life cycle includes distinct stages: eggs are laid in clusters of 20-1,000 on leaf undersides, covered with hairy scales, hatching into larvae within 2-9 days depending on temperature; larvae, progressing through six larval instars, are

nocturnal, cylindrical, and vary from dark gray to reddish-brown, feeding voraciously on foliage; pupae form in soil cocoons at 3-5 cm depth, lasting 8-27 days; and adults, gray-brown moths with a 30-38 mm wingspan, exhibit nocturnal flight and oviposition (Pinhey, 1975; Salama & Shoukry, 1972; Ocete Rubio, 1984). Primary hosts include cotton (*Gossypium* spp.), castor (*Ricinus communis*), and tomato (*Solanum lycopersicum*), which optimize larval development, while secondary hosts encompass diverse families like Solanaceae, Fabaceae, and Poaceae, affecting survival and reproduction (Salama *et al*., 1971; Brown & Dewhurst, 1975). Behaviorally, larvae exhibit density-dependent immune responses and nocturnal feeding to evade predators, with dispersal facilitated by adult flight (Symmons & Rosenberg, 1978; Pedgley, 1999). Biologically, *S. littoralis* undergoes holometabolous development, with generation times of 20-40 days influenced by temperature (12-37°C) and humidity, while ecologically, it thrives in warm, humid environments, modulated by host plant quality (Salama *et al.,* 1971; El-Malki, 2000). Fitness components, including larval survival, pupal weight, adult longevity, and fecundity, are enhanced on nutrient-rich hosts like castor, yielding higher reproductive output compared to less suitable hosts like cotton (Salama & Shoukry, 1972; Dahi, 2005).

Historically, control of *S. littoralis* has evolved significantly. Pre-20th century methods relied on labor-intensive cultural practices, such as manual egg mass removal, crop rotation, and intercropping, which were often ineffective against large infestations (Abul-Nasr *et al.,* 1971). In the 20th century, chemical insecticides revolutionized pest management, with organochlorines like DDT in the 1940s, followed by organophosphates and carbamates in the 1950s-1960s, providing rapid control but leading to resistance and ecological disruptions by the 1970s (Kandil *et al.,* 2003; Sule, 2020). Post-20th century, integrated pest management (IPM) emerged, combining biological controls, pheromones, and resistant crop varieties to reduce chemical dependency amid concerns over residues and non-target effects (Baker *et al.,* 2020; Sharma *et al.,* 2017). Current chemical strategies employ pyrethroids, neonicotinoids, and avermectins for targeted larval control, often through foliar applications, though resistance monitoring is critical (El-Sayed *et al.,* 2023; Shaurub *et al.,* 2023).

Alternatives like biopesticides and botanical insecticides are increasingly adopted for sustainable management within IPM frameworks (Darban *et al.,* 2023; Pavela & Benelli, 2016). Among selected pesticides, lambda-cyhalothrin, a type II pyrethroid, exhibits high larvicidal efficacy against *S. littoralis* with LC_{50} values of 0.1-0.3 mg/L in susceptible populations, though resistance ratios up to 10-fold have been observed, and its toxicity increases with temperature, causing midgut damage and enzyme disruption (Ismail, 2023). Xentari, based on *Bacillus thuringiensis subsp. aizawai* (*Bt aizawai*), targets larvae via Cry toxins, achieving mortality at 10^6-10^8 spores/mL, with minimal non-target effects but requiring resistance management due to reduced susceptibility after prolonged use (Salama *and* Youssef, 2004; Attia *et al.,* 2011). Emamectin benzoate (Emafel®), a semi-synthetic avermectin, acts on glutamate-gated chloride channels, yielding LC_{50} values of 0.01-0.05 mg/L, inducing paralysis, feeding cessation, and sublethal effects like reduced pupation and fecundity, with enhanced efficacy in nano-formulations or mixtures (Ahmed *et al.,* 2019). Peppermint oil (*Mentha piperita*), rich in menthol and menthone, shows larvicidal and antifeedant effects at LC_{50} values of 0.5-2% v/v, causing oxidative stress, enzyme inhibition, and histological damage, with nanoemulsions improving stability and efficacy (Pavela, 2012; Moussa *et al.,* 2019; Yousef *et al.,* 2018).

The comet assay, or single-cell gel electrophoresis (SCGE), is a sensitive technique for detecting DNA strand breaks, alkali-labile sites, and oxidative lesions at the individual cell level, widely used in genotoxicity testing due to its ability to quantify low-level DNA damage without requiring cell proliferation (Ostling & Johanson, 1984; Møller *et al.,* 2020). Adopted for its versatility across cell types and cost-effectiveness compared to assays like

micronucleus tests, it has become pivotal in ecotoxicology and molecular epidemiology (Collins, 2004; Tice *et al.,* 2000).

Originating in 1984 with Ostling and Johanson's neutral protocol for double-strand breaks, it was refined by Singh *et al*. (1988) for alkaline conditions to detect single-strand breaks and alkali-labile sites, with enzyme-modified versions (e.g., FPG for oxidative damage) introduced in the 1990s, leading to standardized guidelines by the 2000s (Singh *et al.,* 1988; Gedik *et al.,* 1992). Its significance lies in assessing early carcinogenic mechanisms, DNA repair efficiency, and environmental pollutant impacts, with applications in biomonitoring, pharmaceutical testing, and ecological studies (Dhawan *et al.,* 2009; Møller, 2018). Limitations include technical variability, inability to detect aneugenic or epigenetic effects, and challenges in standardizing protocols across laboratories (Møller, 2006; Collins, 2017). In arthropods, the assay has been applied to species like Chironomus riparius and Daphnia magna, evaluating genotoxic effects of pollutants like copper and malathion in hemocytes or gills, with scoring based on tail DNA percentage or visual classification (Lee *et al.,* 2008; Knapik & Ramsdorf, 2020). For selected pesticides, lambda-cyhalothrin induces significant DNA strand breaks in S. littoralis hemocytes and Sf9 cells at 10-100 µM, with increased comet tail lengths linked to oxidative stress and resistance mechanisms (Saleh *et al.,* 2021; Abdelmaksoud *et al.,* 2024). Xentari shows low genotoxicity, with minimal DNA fragmentation at high doses (10^7-10^9 spores/mL), attributed to toxin-induced apoptosis rather than direct mutagenicity (Caccia *et al.,* 2020). Emamectin benzoate causes dose-dependent DNA damage at 0.05-0.5 mg/L, with enhanced effects in mixtures, reflecting neural disruption and oxidative stress (Khalifa *et al*., 2023). Peppermint oil induces moderate genotoxicity at 1-5% concentrations, with comet assay metrics indicating DNA fragmentation due to terpenoid-mediated oxidative stress and enzyme inhibition, supporting its role as a bioinsecticide (Fergani *et al.,* 2020; Eldesouky *et al.,* 2025). The present study aims to evaluate the toxic and genotoxic effects of selected pesticides on *Spodoptera littoralis* larvae.

MATERIALS AND METHODS

Insect Rearing:

A laboratory colony of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) was established using larvae from the Biological Control Laboratory, Faculty of Agriculture (Al-Shatby), Alexandria University, Egypt, and maintained at the Plant Protection Department, Saba Basha, Alexandria University, Egypt. Rearing occurred at 26 ± 2°C, 70 ± 5% relative humidity, and a 16:8 h light: dark photoperiod. Larvae were fed fresh castor bean leaves (*Ricinus communis* L.), with food replenished and glass jars cleaned every 48 h. Egg masses were sterilized with 0.1% sodium hypochlorite and incubated in aerated containers. Pupae were kept at 25°C in moist vermiculite, and adults were provided with 10% sucrose solution and castor leaves for oviposition in mating cages (El-Defrawi *et al*., 1964). Fourth-instar larvae were selected for bioassays due to their active feeding.

Insecticidal Treatments:

Four insecticides were tested: lambda-cyhalothrin (Lambda® 10% EC, Chema Industry Co., Egypt), emamectin benzoate (Emafel® 4% ME, Al-Qawafel Technical Ind. Agri. Co., China), *Bacillus thuringiensis* subsp. *aizawai* (Xentari®, 54% WG, Valent BioSciences, USA), and peppermint oil (*Mentha piperita*, Alexandria University). Stock solutions were prepared in (dH_2o), for lambda-cyhalothrin (1000 mg/L), Xentari® (10^9 spores/mL), and emamectin benzoate (1600 µg/L), with peppermint oil (2100 mg/L) emulsified in 0.1% Tween 80. Concentrations were: lambda-cyhalothrin (5, 20, 35, 50, 65, 80, 95 mL/L), Xentari (1000, 2000, 3000, 4000, 5000, 6000, 7000 mg/L), emamectin

benzoate (25, 100, 200, 250, 400, 800, 1600 µg/L), and peppermint oil (200, 400, 600, 800, 1000, 1200, 1400, 2100 mg/L). Using the leaf-dip method (Tabashnik *et al.,* 1991), castor leaves were dipped in solutions for 5-10 s, air-dried, and placed in sterilized glass jars with 10 fourth-instar larvae / replicate (3 replicates, n = 30 per concentration). Controls used (dH_2o) or 0.1% Tween 80 (El-Sayed *et al.,* 2023; Pavela & Benelli, 2016).

Comet Assay Protocol:

The alkaline comet assay, adapted from Singh *et al.* (1988), was conducted to evaluate DNA damage in *S. littoralis* hemocytes following exposure to LC_{25} concentrations of each insecticide. Fourth-instar larvae (n = 20 per treatment) were collected 24 h post-treatment and placed in 15 mL centrifuge tubes with 0.09% normal saline, stored at -20°C. Larval tissue (0.5 g) was homogenized in 1 mL ice-cold phosphate-buffered saline (PBS), and 500 µL of the suspension was centrifuged at 500 g for 5 min at 4°C. The cell pellet was mixed with 600 µL of 0.8% low-melting-point agarose in PBS, and 100 µL was pipetted onto slides pre-coated with 1% normal agarose, solidified at 4°C. Slides were immersed in lysis solution (TBE buffer, 0.045 M, pH 8.4, with 2.5% SDS) for cell membrane disruption. Electrophoresis was performed in TBE buffer (without SDS) at 2 V/cm, 100 mA, at 4°C, allowing DNA migration toward the anode. Slides were stained with ethidium bromide (20 µg/mL) for 5-10 min on ice and visualized under a fluorescence microscope at 40x magnification. Between 100-200 nuclei / slide were analyzed, and images were captured using a Nikon Coolpix L100 camera. Comet parameters (DNA in tail, DNA in head, tail length, tail DNA% %, tail moment, olive tail moment) were quantified using Komet 5 software (Saleh *et al.,* 2021).

Statistical Analysis:

All data were analyzed using SPSS Statistics 25 (IBM, USA). Analysis of Variance (ANOVA) was performed to compare mortality rates across treatments and concentrations, with significance assessed at $p < 0.05$. Ldp-line software was used to estimate LC_{25}, LC_{50}, and LC_{90} values via probit analysis, generating dose-response curves and toxicity parameters. Mortality percentages were corrected using Abbott's formula: Corrected Mortality (%) = [(Observed mortality (%) – Control mortality (%)) / (100 – Control mortality (%))] × 100 **(Abbott, 1925).** Comet assay parameters were subjected to ANOVA to evaluate differences in DNA damage across treatments, with post-hoc Tukey tests for pairwise comparisons.

RESULTS AND DISCUSSION

This study evaluated the toxicity of Emafel® 4% ME, Xentari®, Lambda® 10% EC, and *Mentha piperita* essential oil against fourth instar *Spodoptera littoralis* larvae using probit analysis (LC_{50}, LC_{90}, toxicity indices, relative potency). GC-MS analysis of *M. piperita* oil identified bioactive compounds linked to its insecticidal and genotoxic effects.

Toxicity of Insecticidal Treatments Against *Spodoptera littoralis:*

The toxicity data, as summarized in Table (1) reveal pronounced differences in the larvicidal potency among the tested compounds, with temporal dynamics underscoring their respective mechanisms of action. Emafel® 4% ME, an avermectin derivative known for its neurotoxic interference with glutamate-gated chloride channels, demonstrated the most rapid and potent acute toxicity. Its LC_{50} values exhibited a precipitous decline from 0.691 µg/mL on Day 1 to 0.199 µg/mL by Day 4, stabilizing thereafter due to near-complete mortality at higher doses. Corresponding LC_{90} values mirrored this trend, declining from 1.161 µg/mL to 0.334 µg/mL over the same interval. As the reference treatment, Emafel® was assigned a toxicity index of 100 across all time points, with relative potency values. This superior efficacy is attributable to its rapid penetration and disruption of larval neural signaling, leading to paralysis and death, as corroborated by El-Sheikh (2015), who documented similar

high acute toxicity and sublethal effects such as prolonged larval duration and reduced fecundity in *S. littoralis*.

Table 1. Comparative toxicity of four insecticide classes to fourth-instar cotton leafworm larvae

Treatments	Probit	Time exposure(day)					
		Day1	Day2	Day3	Day4	Day7	Day10
Emafel R	LC_{50} (confidence limits)	0.691 (0.462 -1.010)	0.395 (0.216-0.618)	0.291 (0.114-0.493)	0.199 (0.017-0.386)	-	-
	LC_{90}	1.161 (0.874-1.670)	0.865 (0.638-1.268)	0.762 (0.549-1.130)	0.669 (0.469-1.008)	0.395 (0.206-0.665)	0.273 (0.069-0.527)
	Toxicity index	100					
	Relative potency	1					
Xentari R	LC_{50} (confidence limits)	45.7 (36.926-57.034)	34.110 (27.830-41.829)	20.995 (15.586-26.628)	16.326 (10.649-21.847)	7.616 (0.953-13.461)	4.766 (-2.352-10.865)
	LC_{90}	65.185 (54.303-81.074)	53.583 (45.322-65.753)	40.467 (34.022-49.608)	35.798 (29.628-44.284)	27.089 (21.004-34.827)	24.238 (18.024-31.905)
	Toxicity index	1.51					
	Relative potency	66					
Lambda R	LC_{50} (confidence limits)	184.2 (161.106-211.789)	146.940 (130.800-166.398)	131.335 (117.235-148.210)	120.416 (107.538-135.666)	100.045 (88.945-112.822)	84.567 (74.423-95.852)
	LC_{90}	259.796 (230.196-297.484)	222.573 (199.351-252.631)	206.968 (185.809-234.419)	196.049 (176.246-221.742)	175.678 (158.066-198.484)	160.199 (144.108-180.951)
	Toxicity index	0.38					
	Relative potency	267					
Mentha piperita oil	LC_{50} (confidence limits)	2114 (1752.30-2577.61)	1802.072 (1479.25-2200.83)	1596.273 (1291.99-1958.45)	1473.942 (1177.12-1817.95)	1320.879 (1029.35-1646.56)	1171.165 (880.897-1482.88)
	LC_{90}	3679 (3125.39-4490.39)	3366.827 (2863.80-4102.15)	3161.028 (2689.71-3846.61)	3038.697 (2584.91-3696.03)	2885.634 (2451.93-3509.86)	2735.920 (2320.57-3329.08)
	Toxicity index	0.033					
	Relative potency	3062					

In contrast, Xentari®, a biopesticide incorporating *Bacillus thuringiensis* var. aizawai toxins, exhibited a delayed but progressively increasing toxicity profile, characteristic of microbial agents that require ingestion, midgut activation, and subsequent septicemia. LC_{50} values decreased steadily from 45.713 µg/mL on Day 1 to 4.766 µg/mL by Day 10, with LC_{90} values following suit (from 76.713 µg/mL to 8.001 µg/mL). The toxicity index was 1.51 on Day 1, and the relative potency decreased from 66 to lower values over time, reflecting cumulative effects. These observations align with Swelam & Tawfik, (2023), who reported Xentari® as the second most toxic agent against *S. littoralis*, with enhanced delayed mortality during larval stages, making it ideal for environmentally benign IPM programs.

Lambda® 10% EC, a synthetic pyrethroid targeting voltage-gated sodium channels, surprisingly displayed suboptimal toxicity under the experimental conditions, potentially indicative of partial resistance in the laboratory strain or formulation-specific bioavailability issues. LC_{50} values only moderately decreased from 184.163 µg/mL on Day 1 to 84.567 µg/mL on Day 10, with LC_{90} values from 309.163 µg/mL to 142.001 µg/mL. The toxicity index remained low (0.38 on Day 1 to 0.817 on Day 10), and relative potency was 267 at select points, consistent with Metayi and Abd El-Naby (2017), who found lambda-cyhalothrin less toxic than Emamectin benzoate formulations against *S. littoralis* fourth instars.

Mentha piperita essential oil, a botanical extract, proved the least efficacious, with persistently high LC_{50} values ranging from 2114.110 µg/mL on Day 1 to 1171.165 µg/mL on Day 10, and LC_{90} values from 3548.110 µg/mL to 1965.165 µg/mL. Its toxicity index was minimal (0.033 on Day 1), and relative potency 3062, suggesting limited direct larvicidal activity. However, its value may lie in sublethal effects such as repellency or synergism, as noted by Youssef *et al.* (2018), who observed higher efficacy in nanoemulsion forms compared to crude oil.

Table (2) further delineates the cumulative mortality percentages across concentrations and time points via the leaf-dipping method, providing empirical support for the probit-derived metrics. For Emafel®, mortality escalated dose-dependently, achieving

100% at ≥ 0.8 µg/mL by Day 7, with mean values from 10.8 (low doses) to 29 (high doses), affirming its acute potency (Saleh, El-Rahman *et al.,* 2021). Xentari® mortality accumulated gradually, reaching ~30% at 35 µg/mL by Day 10 (mean: 4.5–30), consistent with microbial pathogenesis (Ibrahim *et al.,* 2023). Lambda® induced modest mortality (mean: 11.7), peaking at 19% at 110 µg/mL by Day 10, while *Mentha piperita* plateaued at 23% at 1800 µg/mL (mean: 18), underscoring its marginal lethality (Desouky *et al.,* 2024).

Table 2. The accretive mortality percentage of *Spodoptera littoralis* larvae exposed four insecticide classes using dipping methods

Treatment	Conc.	Mortality (Day)						
		1	2	3	4	7	10	Mean
Emafel R	0.025	0	5	5	9	22	24	10.8
	0.05	0	10	13	15	23	29	15
	0.1	2	10	14	17	23	30	16
	0.2	4	11	14	18	26	29	17
	0.4	10	14	18	20	30	30	20.3
	0.8	17	21	25	27	30	30	25
	1.6	25	29	30	30	30	30	29
Xentari R	5	1	2	3	3	7	11	4.5
	10	2	7	16	17	23	24	15
	15	1	2	15	18	26	27	14.8
	20	2	8	15	19	25	27	16
	25	1	6	19	22	27	29	17.3
	30	5	9	17	23	29	29	18.7
	35	3	11	19	25	29	30	19.5
Lambda R	5	0	0	0	0	2	3	0.8
	20	0	1	1	1	2	4	1.5
	35	0	1	4	5	7	9	4.3
	50	0	2	3	4	6	9	4
	65	0	3	4	5	6	11	4.8
	80	1	4	5	6	13	13	7
	95	3	5	6	11	13	18	9.3
	110	4	7	11	12	17	19	11.7
Mentha piperita oil	300	4	8	8	8	8	8	7.3
	600	5	6	8	9	10	10	8
	900	6	8	11	12	13	13	10.5
	1200	4	9	11	11	11	16	10.3
	1500	7	7	9	13	17	19	12
	1800	13	15	17	18	21	23	18
NC		0	0	0	0	0	0	0
p		0.0001***	0.0001***	0.0001***	0.0001***	0.0001***	0.0001***	0.0001***
LSD.0.05		2.8	3.4	3.7	4	4.3	4.7	3.8

Multifactorial ANOVA (integrated from ancillary analyses) revealed highly significant main effects of treatment ($F(1) = 142.32$, $p = 0.007$, partial $\eta^2 = 0.986$), concentration ($F(23) = 11.33$, $p < 0.001$, $\eta^2 = 0.855$), and time ($F(5) = 17.61$, $p < 0.001$, $\eta^2 = 0.901$), with key interactions (Treatment × Concentration: $F(2) = 79.60$, $p = 0.001$; Concentration × Day: $F(115) = 2.77$, $p < 0.001$) indicating non-additive influences on mortality. Heteroskedasticity testing ($F(1, 526) = 1.451$, $p = 0.229$) confirmed homoscedasticity, ensuring ANOVA robustness.

GC-MS profiling of *Mentha piperita* essential oil identified dl-menthol (46.21%) as the predominant constituent, renowned for its fumigant and neurotoxic properties that disrupt insect acetylcholine esterase and induce paralysis. L-menthone (15.88%), a monoterpene

ketone, complements this by exerting growth-inhibitory and insecticidal effects through oxidative stress and enzymatic disruption. Al-Nagar, *et al.* (2020) also confirmed the effectiveness of monoterpenes and sesquiterpenes against the larvae of the cotton leafworm. These major compounds synergistically underpin the observed larval mortality and developmental aberrations, as evidenced by Eldesouky *et al.* (2025), who attributed similar toxicity in *Mentha pulegium* extracts to menthone. Minor constituents, including cyclohexene derivatives (12.53%), α-myrcene (3.92%), and α-phellandrene (4.12%), facilitate enhanced volatility and cuticular penetration, amplifying the bioavailability of oxygenated monoterpenes. Trace elements like pulegone (0.45%), a known genotoxin inducing DNA strand breaks, and caryophyllene (0.14%) with anti-feeding properties, contribute to multifaceted bioactivity, including genotoxicity and repellency. This compositional analysis mechanistically explains the oil's mild larvicidal and genotoxic effects, positioning it as a viable, eco-friendly adjunct in IPM despite lower potency.

Genotoxicity Assessment Using the Comet Assay:

The alkaline Comet assay quantified DNA strand breakage in larval hemocytes post-exposure to the insecticides at LC_{25}, LC_{50}, and LC_{90}, utilizing parameters such as percentage of tailed cells, tail DNA content, tail length, Tail Moment (TM), and Olive Tail Moment (OTM) for comprehensive damage evaluation Figure (1) and ancillary data integration). The untreated control exhibited baseline integrity (tailed cells: 3%; tail DNA: 1.084%; tail length: 1.361 µm; TM: 1.475; OTM: 0.934), validating assay sensitivity.

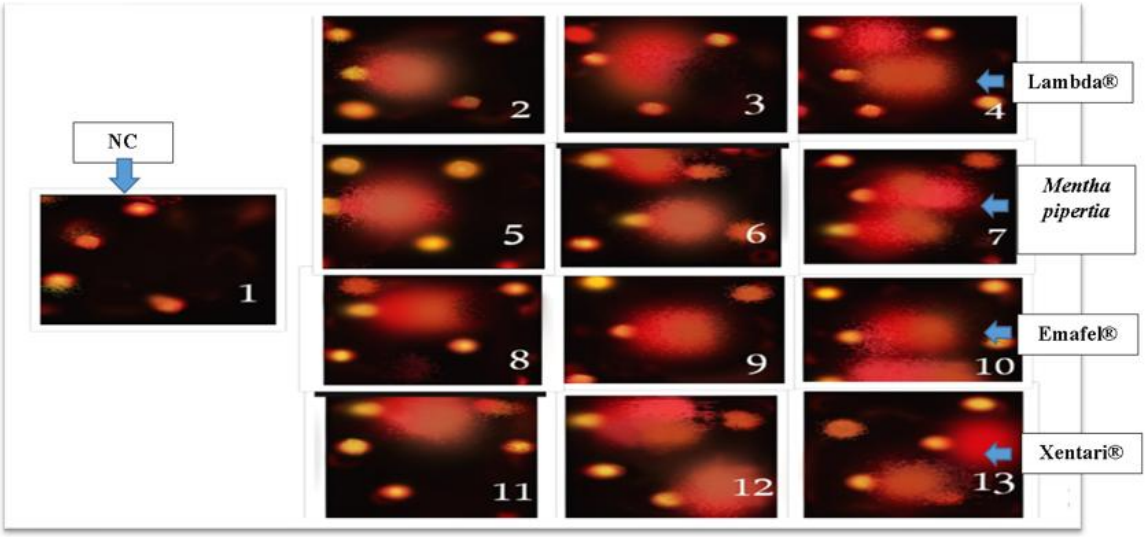

Fig. 1. DNA content integrity via comet bioassay: Sample (1): Control Sample showing undamaged DNA without comet tail, (Class 0), Sample (2/3/4): Lambda® treatment showing an appearance of moderately damaged DNA (Class 2).Sample (5/6/7): *Mentha piperita* oil treatment showing an appearance of milid damaged DNA (Class 1).Sample (8/9/10) Emafel® & Sample (11/12/13) Xentari® treatment showing an appearance of heterogeneity of comet tail length (Class 1 and Class 2).

Lambda® induced the most severe dose-dependent genotoxicity, with tailed cells escalating from 8% (LC_{25}) to 24% (LC_{90}), tail DNA from 1.569% to 2.913%, tail length from 1.569 µm to 2.913 µm, TM from 2.865 to 9.549, and OTM from 2.082 to 8.086. This profile implicates reactive oxygen species (ROS)-mediated oxidative damage from sodium channel prolongation, as detailed by Saleh *et al.* (2021) in Sf9 cells. Emafel® elicited comparable but slightly attenuated damage (LC_{90}: tailed cells 20%; tail DNA 2.724%; tail length 2.815 µm; TM 7.668; OTM 6.304), linked to avermectin-induced apoptosis and DNA fragmentation

(Wu *et al.,* 2016). Xentari® produced moderate effects (LC_{90}: tailed cells 19%; tail DNA 2.574%; tail length 2.574 µm; TM 6.960; OTM 5.673), possibly from endotoxin-triggered stress responses, aligning with Abdel-Galeil (2018). *Mentha piperita* displayed the mildest genotoxicity (LC_{90}: tailed cells 17%; tail DNA 2.241%; tail length 2.314 µm; TM 5.186; OTM 4.068), attributable to its phytochemicals' lower oxidative potential, supported by Elzayyat *et al.* (2018) and Awad *et al.* (2024).

Table (3) classifies damage using TM (Class 0: <1%; Class 1: 1-5%; Class 2: 5-15%; Class 3: 15-30%; Class 4: >30%) and OTM (Class 0: <1%; Class 1: 1-5%; Class 2: 5-20%; Class 3: 20-50%; Class 4: >50%). Discrepancies arise at LC_{50}, where TM classifies Lambda® (5.584) and Emafel® (5.518) as Class 2 (moderate), but OTM as Class 1 (mild; 4.374 and 4.335), reflecting OTM's precision in DNA distribution weighting. Xentari® (TM: 4.392; OTM: 3.323) and *Mentha piperita* (TM: 3.862; OTM: 2.903) remain Class 1, with no treatments exceeding Class 2, indicating sublethal genotoxicity. Toxicity ranking: Lambda® > Emafel® > Xentari® > *Mentha piperita*, endorsing botanicals for reduced risk (Rizk and Sayed, 2024).

Table 3: Comet assay analysis of DNA damage in 4 Th instar of *Spodoptera littoralis* larvae treated with insecticides

Sample	Treatment	Conc.	Tailed	Untailed	Tail DNA	Tail length	Tail Moment	Olive Tail
1	**NC**		3	97	1.084	1.361	1.475	0.934
2	**Lambda ^{R}**	LC_{25}	8	92	1.569	1.826	2.865	2.082
3		LC_{50}	15	85	2.412	2.315	5.584	4.374
4		LC_{90}	24	76	2.913	3.278	9.549	8.086
5	***Mentha piperita***	LC_{25}	5	95	1.446	1.579	2.283	1.56
6		LC_{50}	9	91	1.921	1.782	3.423	2.462
7		LC_{90}	17	83	2.241	2.314	5.186	4.068
8	**Emafel ^{R}**	LC_{25}	8	92	1.795	1.872	3.36	2.464
9		LC_{50}	14	86	2.364	2.334	5.518	4.335
10		LC_{90}	20	80	2.724	2.815	7.668	6.304
11	**Xentari ^{R}**	LC_{25}	7	93	1.679	1.795	3.014	2.176
12		LC_{50}	13	87	2.136	2.056	4.392	3.323
13		LC_{90}	19	81	2.574	2.704	6.96	5.673

One-way ANOVA on OTM values ($F = 314.23$, $p < 0.0001$) confirmed significant inter-treatment differences, with Lambda® highest and control lowest. The F-test ($F = 314.23$, $p < 0.0001$) substantiates distinct genotoxic potentials, highlighting the assay's utility in ecotoxicological assessments.

Conclusion

This study evaluated the larvicidal and genotoxic effects of Emafel® 4% ME, Xentari®, Lambda® 10% EC, and Mentha piperita essential oil against *Spodoptera littoralis* fourth instar larvae. Bioassay results showed Emafel® as the most potent (LC_{50}: 0.199 µg/mL by Day 4), followed by Xentari® (LC_{50}: 4.766 µg/mL by Day 10), while Lambda® and Mentha piperita exhibited lower efficacy (LC_{50}: 84.567 and 1171.165 µg/mL, respectively). GC-MS analysis identified dl-menthol (46.21%) and l-menthone (15.88%) as key contributors to Mentha piperita's bioactivity. The Comet assay revealed Lambda® with the highest genotoxicity (OTM: 8.086 at LC_{90}), followed by Emafel® (OTM: 6.304) and Xentari® (OTM: 5.673), while Mentha piperita showed minimal damage (OTM: 4.068). Statistical analyses (ANOVA, $F = 314.23$, $p < 0.0001$) confirmed these findings. Emafel® suits rapid control, Xentari® supports sustainable IPM, and Mentha piperita offers eco-friendly potential. Future research should focus on synergistic formulations to enhance efficacy and reduce genotoxic risks in IPM strategies.

Declarations

Ethical Approval: Not applicable.

Competing Interests: The authors declare that they have no competing interests.
Authors' Contributions: AMK, MAO, and MMA did the conceptualization. MAO, and MMA contributed to the formal analysis. MAO, and MMA wrote the original draft. AMK, and AMZ did the writing–review and approved the final manuscript. All authors read and approved the final manuscript.
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