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Evaluation of *Agave angustifolia* as a Sustainable Alternative to Synthetic Pesticides: Phytochemical Composition and Multi-Trophic Bioactivity

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ABSTRACT

Plant diseases caused by fungi, bacteria, nematodes, and viruses contribute significantly to annual crop losses and economic hardship. Reliance on chemical pesticides, over a thousand used globally, raises major environmental and health concerns. This study evaluates *Agave angustifolia* (accession no. 1624) as an eco-friendly alternative for managing pests and pathogens. Plant extracts were prepared using hexane (AAHE), chloroform (AACE), and methanol (AAME), with AAME showing the highest yield (5.16%). In vitro bioassays assessed nematicidal, fungicidal, bactericidal, antifeedant, insecticidal, and acetylcholinesterase (AChE) inhibitory activities. AAME was most effective against *Meloidogyne incognita*, inhibiting egg hatching and increasing larval mortality, followed by AACE and AAHE. AAME also showed strong antifungal activity against *Sclerotinia sclerotium*, *Fusarium oxysporum*, and *Macrophomina phaseolina*, with *Sclerotinia sclerotium* being the most sensitive. AAME exhibited the highest antibacterial efficacy against *Xanthomonas oryzae* and *Dickeya zeae*, while *Escherichia coli* was insensitive. Antifeedant tests against tobacco caterpillar (*Spodoptera litura*) revealed AACE (95%) as most effective, followed by AAME (93%) and AAHE (72.4%). Insecticidal activity against white grubs (*Holotrachia* spp.) showed 100% mortality on Day 7 by AACE, 80%, and 60% by AAME and AAHE, respectively. GC–MS analysis revealed bioactive compounds: AAME contained esters, fatty acids, and sugars; AACE had sesquiterpenoids, terpenes, and steroids; and AAHE comprised ketones, aldehydes, and hydrocarbons. AChE inhibition was highest with AAME (71.10%), indicating potential neurotoxic effects on pests. Overall, *Agave angustifolia* demonstrates promise as a sustainable, low-cost alternative to synthetic pesticides, particularly for marginal farmers. Further research is needed to isolate active constituents, determine mechanisms of action, and develop formulations for practical agricultural use.

1 | Introduction

Crop pests and diseases significantly impact global food production, causing yield losses of 20%–40% and economic damages amounting to billions of dollars annually [1–3]. Among biotic stressors, soil-borne pathogens—including plant-parasitic nematodes (PPNs), fungi, and bacteria—are major threats, with potential crop losses reaching 50%–75% in key agricultural crops [4, 5]. PPNs alone account for over 30% of global yield reductions [6], while pathogenic fungi and bacteria contribute to 70%–80% of biotic crop diseases, resulting in over $1 billion in losses annually [7–9]. Additionally, insect pests such as white

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grubs (*Holotrachia* spp.) and the tobacco caterpillar (*Spodoptera litura*) cause severe damage, with the latter leading to 10%–30% crop loss [10, 11].

The excessive use of synthetic pesticides raises concerns regarding environmental toxicity, non-target effects and persistence in ecosystems. Consequently, there is growing interest in botanical pesticides as sustainable alternatives for pest management [12, 13]. More than 2500 plant species from 235 families have been reported to possess pesticidal properties, as they leverage a wide range of bioactive compoundssuch as alkaloids, flavonoids, tannins, and saponins to disrupt pest physiology through repellency, toxicity, feeding deterrence and growth inhibition, many pesticides act as neurotoxins by inhibiting the enzyme acetylcholinesterase (AChE), leading to the accumulation of acetylcholine at synaptic junctions. This disrupts nerve signal transmission, resulting in paralysis and death of the target pests [14, 15].

Agave angustifolia, a member of the Agavaceae family, is widely recognised for its economic and ecological significance. Traditionally used for fibre, food, beverages and medicine, it also plays a crucial role in soil conservation and carbon sequestration [16]. Agave species are rich in secondary metabolites, including terpenes, glycosides, saponins and flavonoids, which exhibit antimicrobial, insecticidal and nematicidal properties [17]. Despite its diverse applications, its potential for plant protection remains largely unexplored. This study aims to evaluate the pesticidal potential of *Agave angustifolia* by assessing its anti-nematic, antimicrobial and insecticidal activities, alongside phytochemical profiling. Understanding its bioactive properties could provide a sustainable, plant-based alternative for integrated pest management in agriculture.

2 | Material and Methods

2.1 | Plant Material

The plant was collected from various locations of Pantnagar, GBPUA&T, Uttarakhand during August 2023 (latitude: 29.02° N; longitude: 79.4° E; altitude: 243.8 m above mean sea level (MSL); mean annual precipitation: 1434.5 mm; mean annual maximum and minimum temperatures: 42.5°C ± 1.5°C and 1.5°C ± 1.0°C, respectively). The plant sample was verified by a plant taxonomist from the Department of Biological Sciences at Govind Ballabh Pant University of Agriculture and Technology, confirming it as *Agave angustifolia*. The specimen was assigned accession number GBPUH-1624 on May 8, 2024, under the G.B. Pant University Herbarium (GBPUH).

2.2 | Extraction

Agave angustifolia whole aerial parts were collected, dried and pulverised to form powder. The extraction was carried out using different polarity solvents such as hexane (AAHE), chloroform (AACE), and methanol (AAME) by using cold percolation method [18]. The extract obtained (Percent yield) from the above method is calculated using formula [19].

$$\text{Percent yeild} = \frac{\text{weight of dried extract}}{\text{weight of dried plant material}} \times 100$$

2.3 | GC–MS Analysis

The phytochemical composition of the phyto-extracts was identified by adopting an analysing technique; that is, GC–MS using GCMS-QP 2010 Ultra DB-5 and GCMS-QP 2010 Ultra Rtx-5MS column. The analysis was conducted using the following settings: the column oven was maintained at 50°C, while the injector temperature was set at 260°C, utilising a split injection mode. Flow control was operated under linear velocity conditions, with a pressure of 69 kPa, a total flow rate of 28.4 mL/min, a column flow rate of 1.21 mL/min, a linear velocity of 39.9 cm/s and a purge flow of 3 mL/min. The split ratio was adjusted to 20.0 and the high-pressure injection, carrier gas saver and splitter hold options were deactivated. The oven temperature program began at 50°C with a 2 min hold, followed by an increase at a rate of 3°C/min to reach 210°C (held for 2 min) and then further ramped at 6°C/min to a final temperature of 280°C, which was maintained for 11 min. For the mass spectrometer, the ion source temperature was kept at 220°C and the interface temperature at 270°C. The constituents of the extracts were tentatively identified based on retention times, and matching against mass spectral libraries (NIST version 2.1 and WILEY 7th edition) and by comparing the mass fragmentation patterns with those reported in the literature.

2.4 | Nematicidal Activity

2.4.1 | Mortality of Second Stage Juveniles of *Meloidogyne incognita*

In vitro nematicidal activity was carried out following the method outlined. The in vitro experiment assessed the nematicidal effects of AAHE, AACE, and AAME extracts on second-stage juveniles (J2) of *Meloidogyne incognita*. Root-knot infected brinjal plant samples were obtained from the Nematology lab, Department of Plant Pathology, G.B. Pant University of Agriculture and Technology, Pantnagar. Galled roots and egg masses from these samples were used for the isolation of J2. The eggs were incubated at 28°C ± 1°C using the Modified Baermann funnel technique to obtain J2. In 40 mm Petri dishes, 100 J2 per plate were exposed to 1 mL of each extract at 100, 250, 500, 750 and 1000 ppm with 15 treatments replicated three times. The concentrations were made by dissolving the extracts in 1% Tween-20 solutions (1 mL Tween-20 solution/100 mL distilled water) for evaluating the efficacy of extracts against root-knot nematode. Distilled water served as the negative control and Nimitz at 250 ppm (a.i. Fluensulfone) commercial nematicide was the positive control. The setup was randomised and maintained at 27°C ± 1°C in a BOD incubator. Mortality was observed at 24, 48, 72, and 96 h using a stereo-binocular microscope; straight J2 were considered dead, while mobile or winding J2 as alive. The percentage of mortality was calculated using Abbott's formula [20]:

$$\text{Percent mortality} = \frac{(\text{Mortality in treatment}) - (\text{Mortality in control})}{(100 - \text{Mortality in control})} \times 100$$

2.4.2 | Inhibition of Egg-Hatching of *Meloidogyne incognita*

The experiment was carried out using gridded Petri dishes of 40 mm diameter with 1 mL egg suspension of *Meloidogyne incognita* (approximately 100 eggs/mL). Eggs were sourced from a pure culture maintained in the glasshouse. Each Petri dish received 1 mL of extract at concentrations of 100, 250, 500, 750, and 1000 ppm forms five treatments with distilled water as negative and Nimitz at 250 ppm as the positive control. The experiment was replicated thrice. Treatments were randomised and incubated at 27°C ± 1°C. Hatchability was assessed at 24, 48, 72, and 96 h by counting hatched eggs under a 4× magnification microscope [21]. The percentage of egg hatching inhibition was calculated as:

$$\text{Percent egg hatching inhibition} = \frac{(\text{Egg hatching in control}) - (\text{Egg hatching in treatment})}{(\text{Egg hatching in control})} \times 100$$

2.5 | Antifungal Activity

An experiment was conducted for evaluating the effects of AAHE, AACE and AAME on three soil-borne plant pathogenic fungi—*Fusarium oxysporum*, *Sclerotinia sclerotiorum* and *Macrophomina phaseolina* using the Poison Food Assay [22]. Pure cultures were maintained in the department of Plant Pathology, GBPUAT, Pantnagar. The efficacy of AAHE, AACE and AAME was tested at 1, 5, 10, 15, and 20 mg/mL concentrations with positive and negative control each in CRD design with three replications each. Petri plates were kept in a BOD incubator at a temperature of 27°C ± 1°C and the observations were taken on alternate days till the full plate growth in the negative control plate. The percentage of growth inhibition over control was estimated using Vincent's [23] formula.

$$I = \frac{(C - T)}{C} \times 100$$

where *I*, percent inhibition of mycelial growth. C, fungal mycelial growth in control. *T*, growth of fungal mycelium in treatments.

2.6 | Antibacterial Activity

The experiment was conducted at the Department of Plant Pathology, College of Agriculture, G.B. Pant University of Agriculture and Technology. Three plant pathogenic bacteria, *Xanthomonas oryzae*, *Dickeya zeae*, and *Escherichia coli*, were used for the experimental studies and pure cultures were maintained. Disc diffusion agar [24] method with five different concentrations, that is, 1, 5, 10, 15, and 20 mg/mL of AAHE, AACE, and AAME, along with negative and positive checks, was performed. All the treatments were in CRD design with three replications each. Petri plates were incubated in a BOD incubator at 24°C ± 1°C for 24 h. The infused extract in the discs inhibits the growth of bacteria and forms a zone of inhibition, which was calculated by measuring the diameter of the cleared zone after 24 h.

$$\text{Zone of inhibition formulae} = \frac{\text{A test} - \text{A neg}}{\text{A pos} - \text{A neg}} \times 100$$

2.7 | Insecticidal Activity

2.7.1 | Insect Antifeedant Activity

Insect antifeeding activity of phytoextracts was assessed using the protocol of Vattikonda and Sangam [25]. Tobacco caterpillar larvae (*Spodoptera* sp.) were collected from maize fields at CRC, GBPUA&T, Pantnagar, in March–April 2023. The larvae were reared under controlled conditions (27°C, 75%–80% RH) and fed fresh castor leaves. Second-generation, day-old larvae were starved for 24 h before the experiment. Leaves (25 cm^2) were dipped in AAME, AACE and AAHE at concentrations of 1, 5, 10, 15 and 20 mg/mL with three replications each, then air-dried and placed in Petri dishes with moisture paper. Each dish contained starved larvae and leaf consumption was measured at 6, 12, 18 and 24 h using the graph paper method. The parameters assessed included:

- Feeding percentage [26]

$$\frac{(\text{initial leaf area provided for feeding}) - (\text{leaf area left after feeding})}{(\text{initial leaves are provided})} \times 100$$

- Antifeedant activity [27]

$$\frac{(\text{leaf area protection in treated disc}\,(\%)) - (\text{leaf area protection in control disc})}{(100 - \text{leaf protection in control disc})} \times 100$$

$$\text{Leaf area protection}\,(\%) = \frac{(\text{leaf area provided}\,(\%)) - (\text{mean leaf area consumed})}{(100 - \text{leaf protection in control disc})} \times 100$$

- Feeding inhibition [28]

$$\frac{(\text{consumption of control disc}) - (\text{consumption of treated disc})}{(\text{consumption of control disc}) + (\text{consumption of treated disc})} \times 100$$

- Preference index (C-VALUE) [29]

$$\frac{2 \times \text{eaten area of treated leaf disc}}{(\text{eaten area of treated leaf disc}) + (\text{eaten area of control leaf disc})} \times 100$$

The antifeedant activity of test samples was worked out based on preference indices (C-value) according to the following scale given by [30].

2.7.2 | In Vitro Insect Mortality Activity

AAHE, AACE, and AAME were assessed for their insecticidal activity against white grubs (*Holotrichia* sp.) using a soil treatment bioassay. Larvae were collected from the soil beneath the mango trees, Pantnagar, Uttarakhand, in May 2024. The grubs were reared in glass jars filled with soil, under laboratory conditions (27°C, 75%–80% RH), with regular soil moistening. Soil was sieved through a 20 mm mesh, autoclaved at 121°C for 60 min at 15 psi, and mixed with pre-sterilised cow dung manure (1:3 ratio) to enhance porosity. In a plastic plant cell tray (48 × 30 × 6 cm) with aeration holes, 40 g of soil mixture was placed in each cell, and one larvae was introduced per cell; thus, each treatment consisted of 15 larvae. After sealing the tray with polythene sheets overnight, cells were treated with 5 mL of phytoextract and distilled water. An additional 1 mL of extract was applied after 3 days. Mortality was recorded over 7 days; dead insects did not move when prodded with a fine brush [31].

$$\text{Mortality}\,(\%) = \frac{\text{No. of insects dead}}{\text{Total No. of insects treated}} \times 100$$

2.8 | Acetylcholinesterase (AChE) Inhibition Activity

Following the evaluation of nematicidal and insecticidal activities against plant-parasitic targets, the in vitro acetylcholinesterase (AChE) inhibition assay was conducted to further investigate the potential neurotoxic effects of the extracts. The acetylcholinesterase inhibitory activity of the tested extracts was measured according to the slightly modified spectrophotometric method developed by Ellman et al. [32] and Alimi et al. [33]. The substrate used was acetylthiocholine iodide (ATChI) (HIMEDIA, CAS number 1866–()5) at a concentration of 1.0 mM. AAHE, AACE and AAME were tested at five concentrations viz. 100, 250, 500, 750 and 1000 ppm that were obtained by the multiple dilution method. For the acetylcholinesterase inhibition assay, all extracts (AAHE, AACE, and AAME), irrespective of their extraction solvent, were re-dissolved in sodium phosphate buffer solution (0.1 M, pH 8.0), and 20 μL of AChE (0.1 U/mL) (HIMEDIA, CAS number 9000-81-1) containing methanol (final methanol concentration < 10%, v/v) prior to use. The incubation was carried out at 25°C for 15 min. Subsequently, 10 μL of Di-thiodinitrobenzoicacid (DTNB) (CDH) (10 mM) was added, and the reaction was initiated by the addition of the substrate (10 μL of ATChI, 1 mM). Following this procedure, ATChI was broken down by the enzyme into thiocholine and acetate, and thiocholine reacted with DTNB to produce a yellow colour. Reactions were conducted for 30 min, with absorbance recorded at 405 nm every 5 min using a UV–Vis Spectrophotometer (Thermo Fisher Scientific, Evolution-201, USA). The blank consisted of methanol (10% MeOH in buffer), the negative control used phosphate buffer in place of acetylcholine, and physostigmine salicylate (HIMEDIA, CAS number ()64-7) served as the positive control. Assays were performed in triplicates, and the results were presented as IC_{50} values (μg/mL) representing the concentration causing 50% inhibition. The percentage of AChE inhibition was determined by comparing the reaction rates of the samples relative to a blank sample using a specific formula

$$\text{Inhibitory activity}\,(\%) = \left(A_o - A_t\right) A_o \times 100$$

where A_o, absorbance values of control and A_t, absorbance values of tested samples.

Percent inhibition was plotted against concentrations and the equation for the line was used to obtain the IC_{50} (half-maximal inhibitory concentration) values.

2.9 | Statistical Analysis

The results were expressed as the mean of three repetitions ± standard deviation. Mycelial growth inhibition, anti-bacterial, and anti-feeding were assessed using an analysis of variance (ANOVA) followed by a Tukey's test. A value of $p < 0.05$ was considered statistically significant. The statistical package OPSTAT was used for ANOVA, and ORIGIN PRO software was used for Tukey's test. Percentage data were angularly transformed using arcsine square-root transformation before analysis to meet the assumptions of ANOVA.

3 | Results and Discussion

3.1 | GC–MS Analysis of AAHE, AACE, and AAME

GC–MS analysis of AAHE, AACE, and AAME identified a diverse range of bioactive compounds contributing to their pesticidal potential, as listed in Table 1. AAHE major compounds include primarily 3,3-dimethyl-2-hexanone (31.51%), hexadecanoic acid, ethyl ester (12.33%), and *cis*, *cis*, *cis*-7,10,13-hexadecatrienal (11.52%). AACE comprised ß-sitosterol (17.25%), sarsasapogenin (11.44%), and curdione (7.62%) as predominant compounds. AAME contained succinic acid, 8-chlorooctyl phenethyl ester (35.55%), glycerol (32.34%), and α-D-galactopyranoside, methyl (13.49%) as major constituents. Being the most polar extract, AAME (Methanol) predominantly contained polar compounds such as esters (succinic acid derivatives), glycerol, glycosides, and monosaccharides. In contrast, AACE (chloroform extract) was rich in moderately non-polar constituents including steroids (β-sitosterol, sarsasapogenin), sesquiterpenoids (curdione), diterpenes (neophytadiene, phytol), and alkanes. Meanwhile, AAHE (hexane extract), the most non-polar fraction, mainly comprised low polarity compounds such as ketones (3,3-dimethyl-2-hexanone), aldehydes (*cis*, *cis*, *cis*-7,10,13-hexadecatrienal), fatty acids (9,12-octadecadienoic acid), hydrocarbons, and phthalates. Figure 1 represents the structures of major compounds identified from AAHE, AACE, and AAME.

Comparative analysis with previous studies Rizwan et al. [34] performed a GC–MS analysis on *Agave attenuata* and found that primary components were mono-ethylhexyl phthalates (11.37%), 1, benzene dicarboxylic acid (6.33%), n-docosane (6.30%) and eicosane (6.02%). Many of the n-alkanes and fatty acids identified in the *A. sisalana* fibre were also present in the volatile fraction of *A. attenuata* leaves. Olvera-García et al. [35] conducted a GC–MS analysis on extracts of *Agave atrovirens*. Their findings revealed compounds, including carboxylic and fatty acids, n-alkanes, phenolic compounds and steroidal sapogenins. The analysis identified several

TABLE 1 | Major compounds found in AAME, AACE, and AAHE.

Extract	Compound	Relative area (%)	Class
AAHE	3,3-dimethyl-2-hexanone	31.51	Ketone
	cis,*cis*,*cis*-7,10,13-hexadecatrienal	11.52	Aldehyde
	Hexadecanoic acid, ethyl ester	12.33	Fatty acid ester
	2,2-dimethyl-pentanal	9.34	Aldehyde
	9,12-octadecadienoic acid (Z,Z)—	8.67	Fatty acid
	Decane	2.70	Hydrocarbon
	Di-n-octyl phthalate	2.53	Phthalate
	2-hexanone, 3,3-dimethyl—	2.30	Ketone
	Phytol	2.20	Diterpene
	Eicosane	2.16	n-Alkane
	Octacosane	2.01	Hydrocarbon
AACE	ß-Sitosterol	17.25	Steroid
	Sarsasapogenin	11.44	Steroidal sapogenin
	Curdione	7.62	Sesquiterpenoids
	Neophytadiene	5.82	Diterpene
	2(3H)-Benzofuranone, hexahydro-4,4,7a-trimethyl- (tetrahydroactinidiolide)	5.44	Benzofuran
	Docosane	5.25	n-alkane
	Pentadecane	5.11	Alkane hydrocarbon
	Phytol	4.33	Diterpenoid
	Hexadecanoic acid, ethyl ester	3.70	Fatty acid ester
	1,16-Hexadecanedichloro	3.09	Halogenated hydrocarbon
AAME	Succinic acid, 8-chlorooctyl phenethyl ester	35.55	Esters
	1,2,3-Propanetriol (Glycerol)	32.34	Glycerol
	α-D-Galactopyranoside, methyl	13.49	Glycoside
	Benzeneacetic acid, 2-phenylethyl ester	6.34	Aromatic hydrocarbon
	Succinic acid, dodec-2-en-1-yl phenethyl ester	3.37	Ester
	1,2,3,4-Butanetetrol, [S-(R*, R*)]- (Tetritol)	2.16	Monosaccharide (Tetrose)

prominent compounds including hexadecanoic acid, (Z)-octadecenoic acid, tetracosane, pentacosane, 2, bis (methylphenyl ethyl)-phenol, 2,4 bis (dimethylbenzyl)-t-butylphenol and steroidal sapogenins. Shah et al. [36] confirmed the presence of key bioactive classes such as carboxylic acids, fatty acids, n-alkanes, phenolic compounds and steroidal sapogenins, including hexadecanoic acid, octadecenoic acid, phenols, eicosane, oleic acid, docosane and phthalates, which are associated with insecticidal activity. Similarly, Omale and Alawa [37] investigated the antimicrobial potential of *Agave sisalana* by preparing a crude methanol extract of its leaves, which was subsequently fractionated into hexane, chloroform and aqueous layers. Phytochemical screening revealed the presence of saponins, flavonoids, tannins, glycosides and steroids across the fractions. The methanol extract exhibited the strongest antimicrobial activity, producing inhibition zones of 28–32 mm against various bacterial strains. The hexane and chloroform fractions also showed moderate antibacterial effects. The minimum inhibitory concentrations (MICs) ranged from 10 to 20 mg/mL and the minimum bactericidal concentrations (MBCs) ranged from 20–40 mg/mL, highlighting the significant role of both polar and non-polar bioactive constituents in the antimicrobial properties of *A. sisalana*. These findings align with previous reports on *Agave* species and underscore the potential of AAHE, AACE and AAME as promising bio-insecticidal agents for sustainable pest management.

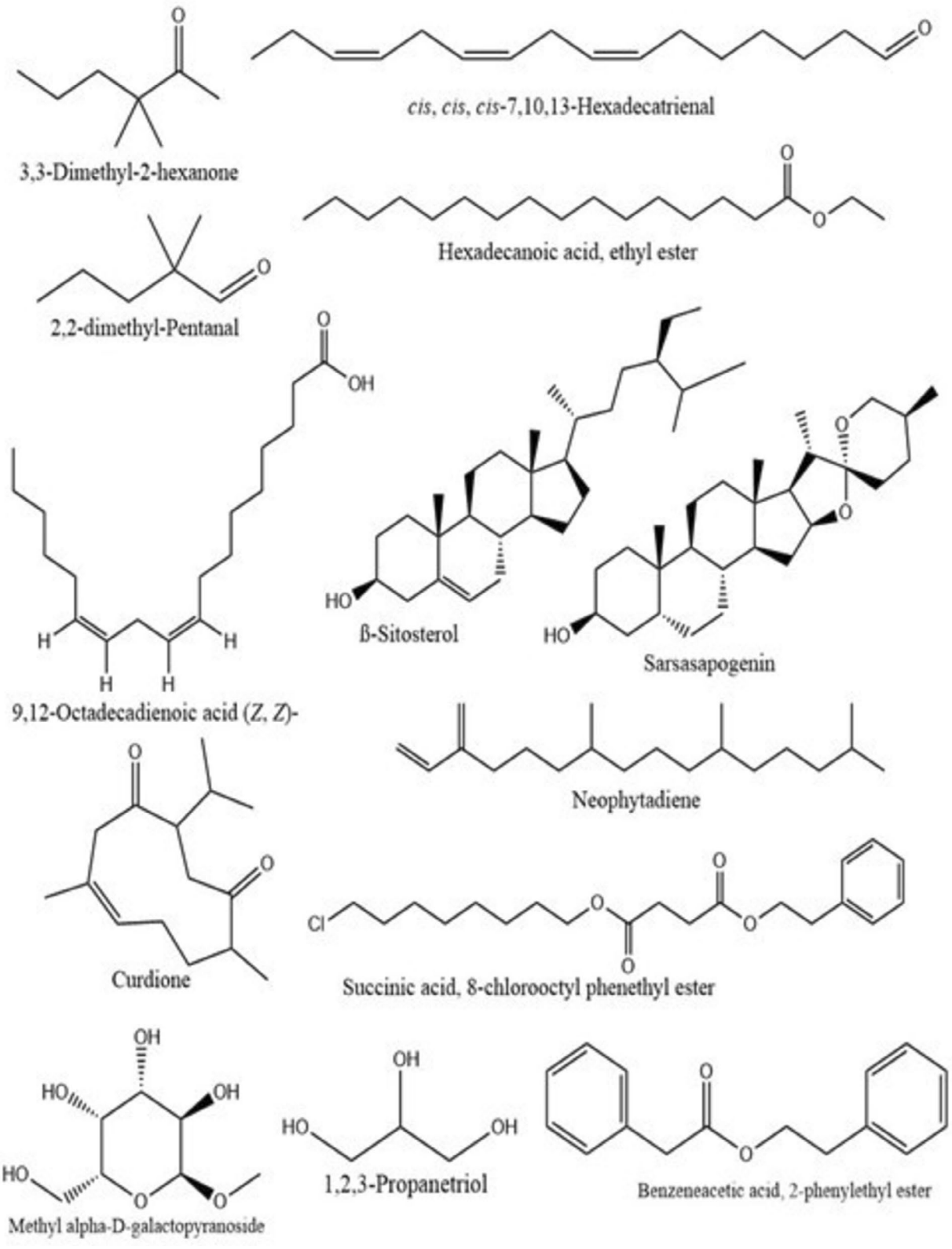


FIGURE 1 | Structures of major compounds from AAHE, AACE, and AAME.

TABLE 2 | Mortality of second stage juveniles of *Meloidogyne incognita*.

Treatments	Concentration (ppm)	Mortality of J2 at different exposure periods (hours)			
		24 h	48 h	72 h	96 h
AAHE	100	0.00 ± 0.00^{m} (0.00)	6.00 ± 1.41^{klm} (14.00)	$12.66 \pm 1.70^{fghijklm}$ (20.80)	$14.66 \pm 1.25^{efghijklm}$ (22.49)
	250	0.00 ± 0.00^{m} (0.00)	6.66 ± 0.94^{jklm} (14.92)	$17.00 \pm 1.41^{defghijkl}$ (24.32)	$18.33 \pm 1.25^{defghijk}$ (25.33)
	500	0.00 ± 0.00^{m} (0.00)	9.00 ± 1.63^{hijklm} (17.38)	$20.33 \pm 1.25^{defghijk}$ (26.78)	22.33 ± 2.05^{cdefghi} (28.16)
	750	0.00 ± 0.00^{m} (0.00)	$14.33 \pm 0.47^{efghijklm}$ (22.23)	22.66 ± 1.25^{cdefgh} (28.41)	24.00 ± 1.63^{cdefg} (29.30)
	1000	0.00 ± 0.00^{m} (0.00)	$16.00 \pm 2.16^{defghijkl}$ (23.52)	25.66 ± 0.94^{bcdefg} (30.42)	26.33 ± 2.05^{bcdef} (30.84)
	Regression eq.		$y = 0.01x + 4.08$	$y = 0.01x + 12.62$	$y = 0.0124x + 14.69$
	R^2		$R^2 = 0.95$	$R^2 = 0.96$	$R^2 = 0.94$
	Correlation		0.97	0.98	0.97
AACE	100	0.00 ± 0.00^{m} (0.00)	$11.66 \pm 2.49^{fghijklm}$ (19.86)	$15.00 \pm 2.16^{defghijkl}$ (22.72)	$18.00 \pm 2.16^{defghijk}$ (25.05)
	250	0.00 ± 0.00^{m} (0.00)	$16.00 \pm 3.27^{defghijkl}$ (23.46)	$17.33 \pm 2.05^{defghijkl}$ (24.55)	$21.00 \pm 2.16^{cdefghij}$ (27.23)
	500	0.00 ± 0.00^{m} (0.00)	$17.33 \pm 2.87^{defghijkl}$ (24.52)	$20.33 \pm 0.47^{defghijk}$ (26.79)	25.33 ± 2.05^{cdefg} (30.19)
	750	0.00 ± 0.00^{m} (0.00)	$20.33 \pm 2.05^{defghijk}$ (26.76)	23.00 ± 1.41^{cdefgh} (28.63)	28.66 ± 1.25^{bcde} (32.35)
	1000	0.00 ± 0.00^{m} (0.00)	22.00 ± 2.16^{cdefghi} (27.93)	28.00 ± 0.82^{bcde} (31.93)	35.33 ± 2.87^{bc} (36.44)
	Regression eq.		$y = 0.0106x + 11.93$	$y = 0.01x + 13.5$	$y = 0.01x + 16.08$
	R^2		$R^2 = 0.93$	$R^2 = 0.98$	$R^2 = 0.98$
	Correlation		0.96	0.99	0.99
AAME	100	0.00 ± 0.00^{m} (0.00)*	6.66 ± 2.05^{jklm} (14.77)	$11.00 \pm 0.82^{fghijklm}$ (19.35)	$13.00 \pm 1.63^{fghijklm}$ (21.08)
	250	0.00 ± 0.00^{m} (0.00)	7.00 ± 0.82^{jklm} (15.31)	$15.66 \pm 0.94^{defghijkl}$ (23.29)	$20.00 \pm 2.45^{defghijk}$ (26.51)
	500	0.00 ± 0.00^{m} (0.00)	7.66 ± 0.47^{ijklm} (16.06)	$17.66 \pm 2.05^{defghijk}$ (24.80)	22.66 ± 1.70^{cdefgh} (28.40)
	750	0.00 ± 0.00^{m} (0.00)	8.33 ± 0.47^{hijklm} (16.76)	$21.33 \pm 1.25^{cdefghij}$ (27.48)	29.66 ± 2.05^{bcd} (32.97)
	1000	0.00 ± 0.00^{m} (0.00)	11.33 ± 0.47^{ghijklm} (19.66)	29.00 ± 2.94^{bcde} (32.54)	40.33 ± 3.68^{b} (39.39)
	Regression eq.	—	$y = 0.0047x + 5.7483$	$y = 0.0181x + 9.545$	$y = 0.0279x + 10.626$
	R^2	—	$R^2 = 0.85$	$R^2 = 0.95$	$R^2 = 0.96$
	Correlation		0.92	0.97	0.98
Control	(–)	0.00 ± 0.00^{m} (0.00)	0.00 ± 0.00^{m} (0.00)	0.00 ± 0.00^{m} (0.00)	2.66 ± 1.70^{lm} (8.92)

(Continues)

TABLE 2 | (Continued)

Treatments	Concentration (ppm)	Mortality of J2 at different exposure periods (hours)			
		24 h	48 h	72 h	96 h
Control	(+)	0.00 ± 0.00[m] (0.00)	59.66 ± 10.50[a] (50.65)	64.66 ± 13.27[a] (53.83)	74.33 ± 4.92[a] (59.63)
Factors	**C.D.**	**SE (d)**	**SE (m)**	***p***	**Significance**
Factor A (treatments)	1.83	0.92	0.65	< 0.00001	Significant
Factor B (concentrations)	1.63	0.83	0.58	< 0.00001	Significant
Factor C (exposure time)	1.83	0.92	0.65	< 0.00001	Significant
Interaction A × B	3.65	1.85	1.31	0.00233	Significant
Interaction A × C	4.09	2.07	1.46	0.00233	Significant
Interaction B × C	3.65	1.85	1.31	0.02239	Significant
Interaction A × B × C	N/A	4.14	2.93	0.98841	Non-significant

Note: Control (−) sterilised distilled water. Control (+)Nimitz@250 ppm. Different letters in the same column indicate significant differences ($0.05 \geq p$) according to Tukey's multiple range test.
*Angular transformed value.

3.2 | Nematicidal Activity

3.2.1 | Mortality of Second-Stage Juveniles of *Meloidogyne incognita*

All extracts tested at various concentrations demonstrated activity against *Meloidogyne incognita* second stage juvenile (J2) compared to the control. AAME was the most effective with 40.33% larval mortality at 1000 ppm and 96 h of exposure, followed by AACE with 35.3% and AAHE with 26.33% mortality in Table 2.

No mortality was observed at 24 h, but effects were noted at 48 h for all concentrations. Larval mortality positively correlated with both extract concentration and exposure time. ANOVA revealed highly significant interactions between these factors.

3.2.2 | Egg-Hatching Inhibition of *Meloidogyne incognita*

All three extracts at five concentrations inhibited egg hatching of *M. incognita* compared to the control. AAME at 1000 ppm and 96 h exposure was the most effective, followed by AACE and AAHE at the same concentration and exposure time. AAME consistently outperformed the other extracts. Egg hatching rates decreased from 25.3% to 16.7% as AAME concentration increased from 100 to 1000 ppm. AACE and AAHE showed reductions from 29.3% to 24.0% and from 33.3% to 24.3%, respectively. A negative correlation was found between extract concentration and egg hatching, while exposure time positively correlated with hatching. ANOVA indicated that the interactions between factors were highly significant in Table 3.

Our results were following previous studies [38] examined aqueous extracts of *Agave angustifolia* at 20% concentration, showed 100% mortality of *Panagrellus redivivus*. Aqueous extract of *Agave tequilana* at concentrations of 3%–12% resulted in mortality rates of 62%–100%, respectively for *Panagrellus redivivus*, while its ethyl acetate crude extract exhibited it at 0.4% only [39]. *Agave sislana* aqueous extract showed 100% mortality of *Scutellonema bradys* J2 [40]. Similarly, against *Radopholus similis*, fresh and fermented residues of *Agave sislana* at 25% showed a mortality rate of 100% and an immobility rate of 72.8% for fermented residue [41]. Joshi [42] examined the effects of aqueous leaf extract from *Agave americana* on egg hatching and larval mortality and found that after 96 h at 10%, the mean egg hatching rate was reduced to 12.67; furthermore, the highest larval mortality was 79.33%. Our study found that extract activity was evident at 48 h, with increased concentrations and exposure times leading to higher *M. incognita* larval mortality, consistent with patterns observed for other *Meloidogyne* species. The nematicidal effects are attributed to secondary metabolites such as alkaloids, saponins, terpenes, flavonoids, and glycosides in *Agave* liquid residue, which have anti-parasitic properties.

The presence of homo isoflavonoids and saponins in *Agave* residue is associated with its nematicidal effects, while saponins in sisal residue contribute to nematode repelling properties [43, 44].

3.3 | Antifungal Activity

AAHE, AACE, and AAME effectively inhibited *Sclerotinia sclerotiorum, Fusarium oxysporum*, and *Macrophomina phaseolina* at 1–20 mg/mL under in vitro conditions, with AAME demonstrating the highest efficacy. *Sclerotinia sclerotiorum* was the most sensitive, while *Macrophomina phaseolina* exhibited the least susceptibility as shown in Tables 4–6. These findings are consistent with Maharshi et al. [45], who reported 22.27%–39.34% inhibition of *Fusarium solani* and 48.6%–64.75% inhibition of *Macrophomina phaseolina* for crude extract of *Agave*. Similarly, 46%–53% inhibition with *Agave lechuguilla* extracts at 1000 mg/mL and 90% inhibition with ethanolic extracts at 200 mg/mL.

TABLE 3 | Effect of AAHE, AACE and AAME on egg-hatching of *Meloidogyne incognita*.

Treatments	Concentration (ppm)	Egg hatching at different exposure periods (time taken in hours)			
		24 h	**48 h**	**72 h**	**96 h**
AAHE	100	$16.33 \pm 0.47^{qrstuvwxyza1}$ (21.95)	$21.00 \pm 2.45^{klmnopqrs}$ (25.05)	31.00 ± 0.82^{def} (30.83)	33.33 ± 1.25^{de} (32.74)
	250	$13.66 \pm 1.25^{vwxyza1b1c1d1e1}$ (20.22)	$19.33 \pm 2.62^{nopqrstuv}$ (22.49)	27.33 ± 1.25^{fghij} (30.40)	30.33 ± 0.47^{defg} (31.71)
	500	$12.00 \pm 0.82^{xyza1b1c1d1e1f1g1}$ (18.37)	$18.33 \pm 1.25^{pqrstuvw}$ (21.65)	$25.00 \pm 0.82^{ghijklmn}$ (27.95)	28.33 ± 1.25^{efghi} (29.98)
	750	$9.33 \pm 1.25^{b1c1d1e1f1g1h1i1j1}$ (16.72)	$16.00 \pm 1.63^{stuvwxyz}$ (20.82)	$23.00 \pm 1.63^{ijklmnopq}$ (26.30)	26.66 ± 1.25^{fghijk} (29.30)
	1000	$7.33 \pm 1.25^{f1g1h1i1j1k1}$ (13.26)	$13.66 \pm 1.89^{vwxyza1b1c1d1e1}$ (18.07)	$20.00 \pm 1.63^{lmnopqrst}$ (24.31)	$24.33 \pm 0.47^{hijklmno}$ (26.49)
	Regression equation	$y = -0.0091x + 14.659$	$y = -0.008x + 17.87$	$y = -0.0108x + 27.74$	$y = -0.0097x + 30.244$
	R^2	$R^2 = 0.98$	$R^2 = 0.91$	$R^2 = 0.99$	$R^2 = 0.97$
	Pearson correlation	−0.99	−0.95	−0.99	−0.98
AACE	100	$14.00 \pm 0.82^{uvwxyza1b1c1d1}$ (21.95)	18.00 ± 2.16^{qrstuvw} (25.05)	26.33 ± 2.87^{fghijk} (30.83)	29.33 ± 3.30^{efgh} (32.74)
	250	$12.00 \pm 1.41^{xyza1b1c1d1e1f1g1}$ (20.22)	$14.66 \pm 1.25^{tuvwqyza1b1}$ (22.49)	25.66 ± 2.49^{fghijkl} (30.40)	27.66 ± 1.70^{efghij} (31.71)
	500	$10.00 \pm 1.63^{a1b1c1d1e1f1g1h1i1}$ (18.37)	$13.66 \pm 1.70^{vwxyza1b1c1d1e1}$ (21.65)	$22.00 \pm 0.82^{jklmnopqr}$ (27.95)	$25.00 \pm 0.82^{ghijklmn}$ (29.98)
	750	$8.33 \pm 1.25^{d1e1f1g1h1i1j1k1}$ (16.729)	$12.66 \pm 0.94^{wxyza1b1c1d1e1f1}$ (20.828)	$19.66 \pm 1.25^{mnopqrstu}$ (26.304)	$24.00 \pm 1.63^{hijklmnop}$ (29.309)
	1000	5.33 ± 1.25^{h1i1j1k1} (13.26)	$9.66 \pm 1.25^{b1c1d1e1f1g1h1i1j1}$ (18.07)	17.00 ± 1.63^{rstuvwx} (24.31)	$20.00 \pm 2.94^{lmnopqrst}$ (26.49)
	Regression equation	$y = -0.0091x + 14.659$	$y = -0.008x + 17.87$	$y = -0.0108x + 27.74$	$y = -0.0097x + 30.244$
	R^2	$R^2 = 0.98$	$R^2 = 0.91$	$R^2 = 0.99$	$R^2 = 0.97$
	Pearson correlation	−0.99	−0.95	−0.99	−0.98

(Continues)

TABLE 3 | (Continued)

Treatments	Concentration (ppm)	Egg hatching at different exposure periods (time taken in hours)			
		24 h	48 h	72 h	96 h
AAME	100	$10.66 \pm 3.09^{za1b1c1d1e1f1g1h1}$ (18.88)	$14.33 \pm 0.47^{tuvwqyza1b1c1}$ (22.23)	$22.33 \pm 3.09^{jklmnopqr}$ (28.13)	$25.33 \pm 1.25^{fghijklm}$ (30.20)
	250	$8.66 \pm 1.25^{c1d1e1f1g1h1i1j1}$ (17.071)	$13.66 \pm 2.87^{vwxyza1b1c1d1e1}$ (21.577)	$20.00 \pm 3.56^{lmnopqrst}$ (26.474)	$25.00 \pm 1.63^{ghijklmn}$ (29.976)
	500	$7.00 \pm 0.82^{f1g1h1i1j1k1}$ (15.31)	$12.00 \pm 0.82^{xyza1b1c1d1e1f1g1}$ (20.24)	17.00 ± 2.16^{rstuvwx} (24.30)	$21.33 \pm 1.25^{klmnopqrs}$ (27.488)
	750	5.66 ± 0.94^{h1i1j1k1} (13.72)	$11.00 \pm 0.82^{yza1b1c1d1e1f1g1h1}$ (19.35)	$15.00 \pm 0.82^{tuvwxyza1b1}$ (22.77)	$19.00 \pm 0.82^{opqrstuv}$ (25.82)
	1000	4.00 ± 0.82^{j1k1} (11.47)	$8.00 \pm 2.16^{e1f1g1h1i1j1k1}$ (16.26)	$12.00 \pm 0.82^{xyza1b1c1d1e1f1g1}$ (20.24)	$16.66 \pm 2.87^{rstuvwxy}$ (24.00)
	Regression equation	$y = -0.007x + 10.846$	$y = -0.0067x + 15.293$	$y = -0.0111x + 23.032$	$y = -0.0102x + 26.78$
	R^2	$R^2 = 0.97$	$R^2 = 0.96$	$R^2 = 0.99$	$R^2 = 0.98$
	Pearson correlation	−0.98	−0.98	−0.99	−0.99
Control	(−)	35.66 ± 0.94^{d} (36.65)	44.00 ± 1.41^{c} (41.53)	51.33 ± 2.62^{b} (45.74)	68.00 ± 2.16^{a} (55.53)
Control	(+)	2.66 ± 1.25^{k1} (9.08)	4.33 ± 1.25^{i1j1k1} (11.89)	$6.33 \pm 0.94^{g1h1i1j1k1}$ (14.52)	$7.33 \pm 1.25^{f1g1h1i1j1k1}$ (15.65)

Factors	C.D.	SE (d)	SE (m)	*p*	Significance
Factor A (treatments)	0.73	0.37	0.26	< 0.00001	Significant
Factor B (concentrations)	0.65	0.33	0.23	< 0.00001	Significant
Factor C (exposure time)	0.73	0.37	0.26	< 0.00001	Significant
Interaction A × B	1.46	0.74	0.52	< 0.00001	Significant
Interaction A × C	1.63	0.85	0.58	< 0.00001	Significant
Interaction B × C	N/A	0.74	0.52	0.92727	Non-significant
Interaction A × B × C	N/A	1.65	1.17	1.00000	Non-significant

Note: Control (−) sterilised distilled water. Control (+) Nimitz@250 ppm. Different letters in the same column indicate significant differences ($0.05 \geq P$) according to Tukey's multiple range test.

TABLE 4 | Effect of different extracts on mycelial growth (cm) of *Sclerotinia sclerotiorum* on the 4th day of incubation.

Concentration (mg/mL)	AAHE	AACE	AAME
1	7.10 ± 0.32^{b} (15.44)	4.61 ± 0.90^{ghi} (12.34)	1.30 ± 1.13^{t} (5.23)
5	6.98 ± 0.29^{bc} (15.31)	3.93 ± 1.37^{ijk} (11.23)	0.00 ± 0.00^{u} (0.00)
10	4.71 ± 0.21^{ghi} (12.53)	2.75 ± 0.35^{nopqr} (9.52)	0.00 ± 0.00^{u} (0.00)
15	3.68 ± 1.27^{jklm} (10.90)	2.56 ± 0.41^{pqr} (9.18)	0.00 ± 0.00^{u} (0.00)
20	2.23 ± 0.18^{qrs} (8.58)	1.45 ± 0.51^{st} (6.79)	0.00 ± 0.00^{u} (0.00)
Control (–)	8.00 ± 0.00^{a} (16.42)	8.00 ± 0.00^{a} (16.43)	8.00 ± 0.00^{a} (16.43)
Control (+)	0.00 ± 0.00^{u} (0.00)	0.00 ± 0.00^{u} (0.00)	0.00 ± 0.00^{u} (0.00)

Note: IC_{50}: AAHE = 12.74; AACE = 3.687; AAME = 0.075.

TABLE 5 | Effect of different extracts on mycelial growth (cm) of *Fusarium oxysporum* on 4th day of incubation.

Concentration (mg/mL)	AAHE	AACE	AAME
1	4.33 ± 0.12^{hij} (12.00)	3.56 ± 0.12^{jklmn} (10.88)	3.50 ± 0.08^{jklmn} (10.77)
5	3.70 ± 0.08^{jkl} (11.08)	3.40 ± 0.08^{klmnop} (10.62)	3.23 ± 0.12^{klmnop} (10.35)
10	3.43 ± 0.17^{klmno} (10.67)	3.16 ± 0.12^{klmnop} (10.22)	2.83 ± 0.05^{mnopqr} (9.68)
15	3.10 ± 0.16^{klmnop} (10.13)	2.83 ± 0.12^{mnopqr} (9.68)	2.57 ± 0.09^{pqr} (9.21)
20	2.90 ± 0.08^{lmnopq} (9.80)	2.63 ± 0.12^{opqr} (9.33)	2.03 ± 0.05^{rst} (8.19)
Control (–)	5.00 ± 0.00^{efgh} (12.91)	5.00 ± 0.00^{efgh} (12.91)	5.00 ± 0.00^{efgh} (12.91)
Control (+)	0.00 ± 0.00^{u} (0.00)	0.00 ± 0.00^{u} (0.00)	0.00 ± 0.00^{u} (0.00)

Note: IC_{50}: AAHE = 24.084; AACE = 22.402; AAME = 14.637.

TABLE 6 | Effect of different extracts on mycelia growth (cm) of *Macrophomina phaseolina* on the 4th day of incubation.

Concentration (mg/mL)	AAHE	AACE	AAME
1	7.017 ± 0.13^{bc} (15.354)	7.483 ± 0.35^{ab} (15.866)	6.183 ± 0.64^{cd} (14.380)
5	7.000 ± 0.09^{bc} (15.335)	7.350 ± 0.08^{ab} (15.724)	5.850 ± 0.48^{de} (13.984)
10	6.983 ± 0.19^{bc} (15.316)	7.167 ± 0.27^{ab} (15.519)	5.783 ± 0.15^{de} (13.909)
15	6.900 ± 0.46^{bc} (15.218)	5.850 ± 0.43^{de} (13.982)	5.650 ± 0.33^{def} (13.741)
20	6.867 ± 0.33^{bc} (15.182)	5.433 ± 0.22^{defg} (13.471)	4.867 ± 0.28^{fgh} (12.736)
Control (–)	8.000 ± 0.00^{a} (16.423)	8.000 ± 0.00^{a} (16.423)	8.000 ± 0.00^{a} (16.423)
Control (+)	0.000 ± 0.00^{u} (0.000)	0.000 ± 0.00^{u} (0.000)	0.000 ± 0.00^{u} (0.000)

Note: IC_{50}: AAHE (–) not reached; AACE = 32.775; AAME = 38.408. Control (–): in sterilised distilled water. Control (+): Mancozeb at 0.05 mg/mL. Different letters in the same column indicate significant differences ($0.05 \geq p$) according to Tukey's multiple range test. Values are mean ± SE of three replications. Figures in parentheses indicate percent inhibition over control. Means followed by different superscript letters differ significantly at $p \leq 0.05$ (ANOVA, Tukey's HSD test).

TABLE 7 | Anti-bacterial properties against *Xanthomonas oryzae*, *Dickeya zeae*, and *Escherichia coli*.

Zone of inhibition (mm)									
24 h	**Dickeya zeae**			**Xanthomonas oryzae**			**Escherichia coli**		
Conc.	**AAHE**	**AACE**	**AAME**	**AAHE**	**AACE**	**AAME**	**AAHE**	**AACE**	**AAME**
1 mg/mL	0.00 ± 0.00^{n} (0.00)*	0.00 ± 0.00^{n} (0.00)	11.16 ± 0.58^{kl} (19.51)	0.00 ± 0.00^{n} (0.00)	0.00 ± 0.00^{n} (0.00)	11.16 ± 0.76^{kl} (19.50)	0.00 ± 0.00^{n} (0.00)	0.00 ± 0.00^{n} (0.00)	0.00 ± 0.00^{n} (0.00)
5 mg/mL	0.00 ± 0.00^{n} (0.00)	6.66 ± 2.36^{m} (14.78)	12.83 ± 0.76^{jk} (20.97)	0.00 ± 0.00^{n} (0.00)	11.33 ± 0.29^{kl} (19.66)	16.83 ± 1.04^{fg} (24.20)	0.00 ± 0.00^{n} (0.00)	0.00 ± 0.00^{n} (0.00)	0.00 ± 0.00^{n} (0.00)
10 mg/mL	0.00 ± 0.00^{n} (0.00)	10.83 ± 0.29^{kl} (19.20)	15.16 ± 0.76^{fghij} (22.90)	0.00 ± 0.00^{n} (0.00)	13.50 ± 2.29^{hijk} (21.50)	20.50 ± 0.50^{de} (26.91)	0.00 ± 0.00^{n} (0.00)	0.00 ± 0.00^{n} (0.00)	0.00 ± 0.00^{n} (0.00)
15 mg/mL	0.00 ± 0.00^{n} (0.00)	13.16 ± 0.58^{ijk} (21.26)	15.50 ± 0.50^{fghij} (23.17)	0.00 ± 0.00^{n} (0.00)	14.33 ± 1.26^{ghij} (22.22)	22.33 ± 0.58^{cd} (28.18)	0.00 ± 0.00^{n} (0.00)	0.00 ± 0.00^{n} (0.00)	0.00 ± 0.00^{n} (0.00)
20 mg/mL	10.00 ± 0.50^{l} (18.42)	15.66 ± 0.76^{fghi} (23.30)	17.83 ± 0.76^{ef} (24.96)	0.00 ± 0.00^{n} (0.00)	16.00 ± 0.50^{fgh} (23.56)	24.50 ± 0.87^{bc} (29.65)	0.00 ± 0.00^{n} (0.00)	0.00 ± 0.00^{n} (0.00)	0.00 ± 0.00^{n} (0.00)
Control (−)	0.00 ± 0.00^{n} (0.00)	0.00 ± 0.00^{n} (0.00)	0.00 ± 0.00^{n} (0.00)	0.00 ± 0.00^{n} (0.00)	0.00 ± 0.00^{n} (0.00)	0.00 ± 0.00^{n} (0.00)	0.00 ± 0.00^{n} (0.000)	0.00 ± 0.00^{n} (0.00)	0.00 ± 0.00^{n} (0.00)
Control (+)	24.00 ± 0.50^{bc} (29.32)	24.00 ± 0.50^{bc} (29.32)	24.00 ± 0.50^{bc} (29.32)	29.16 ± 3.01^{a} (32.65)	29.16 ± 3.01^{a} (32.65)	29.16 ± 3.01^{a} (32.65)	25.83 ± 1.26^{b} (30.53)	25.83 ± 1.26^{b} (30.53)	25.83 ± 1.26^{b} (30.53)
IC-50 Value	88.72	49.63	43.6933	—	50.98	41.79	—	—	—
Regression equation	$y = 0.4246x — 2.331$	$y = 0.7795x + 1.3161$	$y = 0.6611x + 11.112$	—	$y = 0.7104x + 3.787$	$y = 0.6626x + 12.308$	—	—	—
R^2 value**	$R^2 = 0.5201$	$R^2 = 0.9271$	$R^2 = 0.963$	—	$R^2 = 0.7126$	$R^2 = 0.9242$	—	—	—

Factors	**C.D.**	**SE (d)**	**SE (m)**	***p***	**Significance**
Factor (A) (bacteria)	0.32	0.16	0.11	< 0.0001	Significant
Factor (B) (treatment)	0.41	0.20	0.14	< 0.0001	Significant
Factor (C) (concentrations)	0.41	0.20	0.14	< 0.0001	Significant
Interaction A × B	0.71	0.36	0.25	< 0.0001	Significant
Interaction A × C	0.71	0.36	0.25	< 0.0001	Significant
Interaction B × C	0.92	0.46	0.33	< 0.0001	Significant
Interaction A × B × C	1.60	0.81	0.57	< 0.0001	Significant

Note: Control (−): sterilised distilled water. Control (+): Streptomycin at 0.25 mg/mL. Different letters in the same column indicate significant differences ($0.05 \geq P$) according to Tukey's multiple range test.
*Angular transformed values.
**Coefficient of determination.

TABLE 8 | Effect of AACE, AAME and AAHE on the anti-feedant activity of tobacco caterpillar (*Spodoptera* sp.)

EXTRACT	CONCENTRATION (mg/mL)	MLAC (cm^2)	Antifeedant activity (%)	Preference index (C-value)	Antifeedant category
AACE	1	14.00 ± 0.00	44.00	0.72	Moderately antifeedant
	5	9.50 ± 0.41	62.00	0.55	Moderately antifeedant
	10	7.57 ± 0.42	69.73	0.46	Strongly antifeedant
	15	1.54 ± 0.03	93.85	0.12	Extremely antifeedant
	20	1.20 ± 1.20	95.20	0.09	Extremely antifeedant
AAME	1	14.63 ± 0.45	41.47	0.74	Moderately antifeedant
	5	12.02 ± 0.06	51.93	0.65	Moderately antifeedant
	10	9.50 ± 0.41	62.00	0.55	Moderately antifeedant
	15	1.95 ± 0.04	92.21	0.14	Extremely antifeedant
	20	1.66 ± 0.03	93.35	0.12	Extremely antifeedant
AAHE	1	17.50 ± 0.41	30.00	0.82	Slightly antifeedant
	5	15.80 ± 0.16	36.80	0.77	Slightly antifeedant
	10	11.77 ± 0.21	52.93	0.64	Moderately antifeedant
	15	8.80 ± 0.24	64.80	0.52	Moderately antifeedant
	20	6.90 ± 0.08	72.40	0.43	Strongly antifeedant
CONTROL	00.00	25 ± 0.00	0.00	1.00	Preferred plant

Note: Control: Sterilised distilled water.
Abbreviation: MLAC, mean leaf area consumed (in cm).

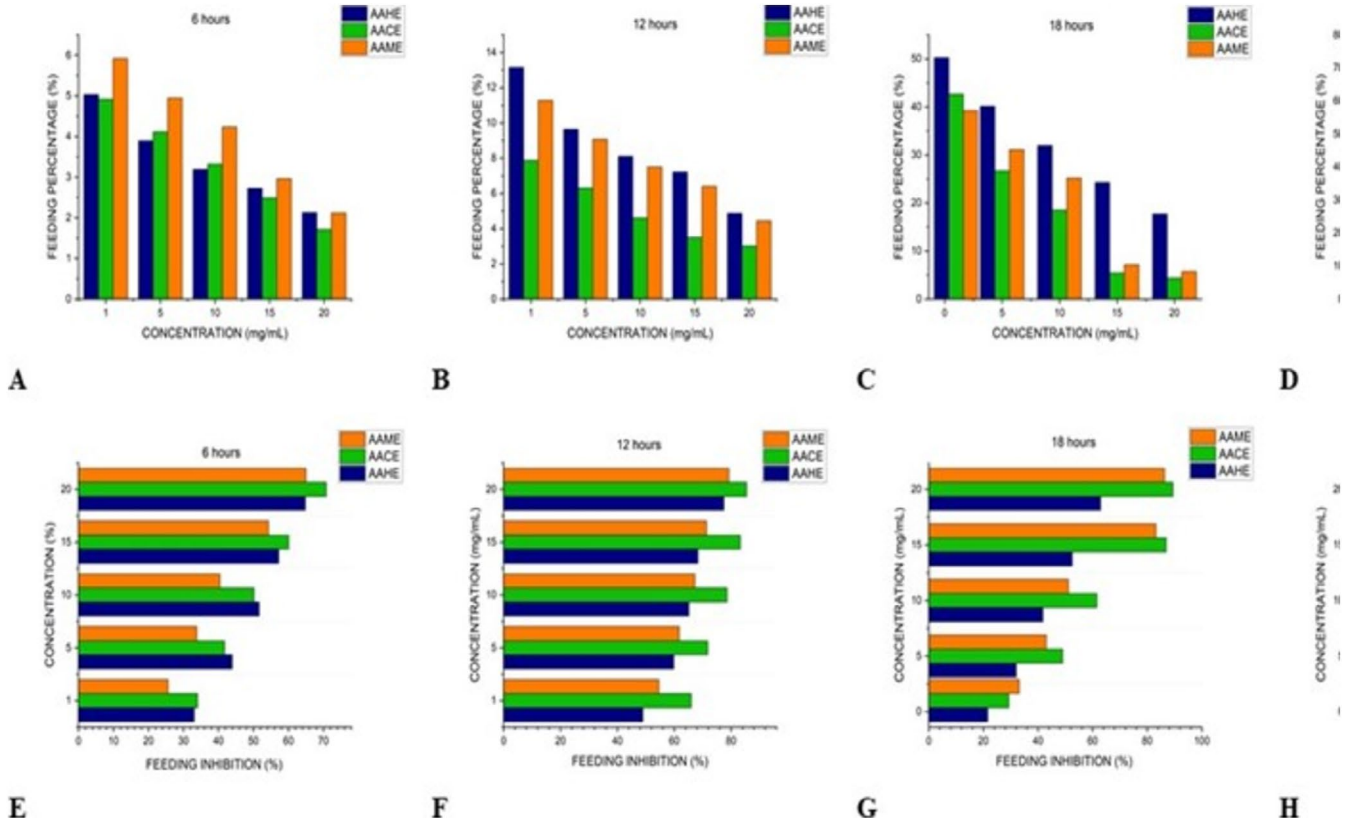


FIGURE 2 | Effect of AAHE, AACE, and AAME on feeding percentage: A,B,C, D and Feeding inhibition: E,F, G, H of tobacco caterpillar (*Spodoptera* sp.) at different concentrations and exposure time of 6, 12, 18, and 24 h.

Agave americana extracts strongly inhibited *Penicillium digitatum* (87.73%) and *Sclerotium rolfsii* (80.81%), while Almazán-Morales et al. [46] reported complete or significant inhibition of other fungal pathogens. Phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, and saponins, known for their antifungal properties. Alkaloids disrupt fungal proteins, flavonoids penetrate membranes, tannins inhibit enzyme activity, and saponins interfere with membrane integrity [47].

3.4 | Antibacterial Activity

The antibacterial potential of *Agave angustifolia* extracts—AAHE, AACE, and AAME—was assessed against plant pathogens *Dickeya zeae*, *Xanthomonas oryzae* and the human pathogen *Escherichia coli*. Among the extracts, AAME exhibited the strongest inhibitory effect, especially at 20 mg/mL, showing inhibition zones of 24.50 mm for *X. oryzae* and 17.83 mm for *Dickeya zeae*. AACE demonstrated moderate activity, while AAHE showed minimal effect. None of the extracts suppressed *Escherichia coli* growth, which is consistent with earlier reports [48, 49]. Statistical analysis (ANOVA) revealed significant interactions among extract type, concentration levels and bacterial species, with IC_{50} values highlighting *X. oryzae* as the most sensitive strain (Table 7). The resistance of *E. coli* may be due to its human pathogenicity and lower susceptibility to phytochemicals. The observed antibacterial effects of the extracts could be attributed to secondary metabolites such as saponins and flavonoids [50, 51]. These findings reinforce the targeted efficacy of *Agave* extracts against phytopathogenic bacteria and mark the first documented evidence of their bioactivity against *D. zeae* and *X. oryzae*.

TABLE 9 | Effect of AAHE, AACE, and AAME on the percent mortality of white grub (*Holotrichia* sp.)

Extract	Day	No. of dead insects in treatment	No. of dead insects in control	% Mortality
AAHE	3	3	0	20
	5	6	0	40
	7	9	0	60
AACE	3	9	0	60
	5	9	0	60
	7	15	0	100
AAME	3	6	0	40
	5	9	0	60
	7	12	0	80
Control		—	0	0

Note: Control: sterilised distilled water. Total number of white grub larvae = 15.

3.5 | Antifeedant Activity

The antifeedant activity of *Agave angustifolia* extracts (AAHE, AACE, AAME) was evaluated against day-old *Spodoptera* larvae. AACE showed the highest activity at 20 mg/mL (95% antifeedant, 4.8% feeding), followed closely by AAME (93.35%), both categorised as extremely antifeedant, while AAHE exhibited 72.4% activity, making it strongly antifeedant. Activity increased with concentration across all extracts. Control showed 0% antifeedant effect, confirming high feeding (Table 8; Figure 2). The efficacy order was AACE > AAME > AAHE. These findings align with Chowdhury et al. [52], who reported 40% feeding inhibition by *Agave sisalana* extract on *Spilosoma obliqua*. Similarly, Saba [53] found that DCM extract of *Agave attenuata* significantly reduced Russian wheat aphid settling, attributing activity to saponins and steroids. The strong antifeedant action observed here may similarly be due to such bioactive allelochemicals.

3.6 | In Vitro Insecticidal Activity

The in vitro insecticidal activity of *Agave angustifolia* extracts (AAHE, AACE, AAME) against third instar larvae of white grub (*Holotrichia* sp.) showed promising results. AACE was the most effective, achieving 100% mortality at 20 mg/mL on the 7th day, followed by AAME (80%) and AAHE (60%). Mortality rates increased with exposure time, while no mortality was observed in

TABLE 10 | Percent inhibition of AChE by AAHE, AACE, and AAME.

S.No.	Extract		% inhibition of Acetylcholinesterase (AChE) at 405 nm				
			100 ppm	250 ppm	500 ppm	750 ppm	1000 ppm
1.	AAHE	Average	29.13	32.89	35.66	42.64	49.83
		Std	±0.56	±0.72	±0.31	±0.83	±0.27
2.	AACE	Average	35.33	41.09	48.95	55.92	62.68
		Std	±0.16	±0.56	±0.31	±0.83	±0.56
3.	AAME	Average	39.87	47.73	55.92	65.89	71.10
		Std	±0.47	±0.56	±0.68	±0.83	±0.72
4.	Standard	Average	52.05	57.59	75.08	84.61	90.70
		Std	±0.31	±0.41	±0.27	±0.31	±0.27

Note: Standard: physostigmine salicylate.

TABLE 11 | IC_{50} values for percent inhibition of AChE activity by AAHE, AACE, and AAME.

S.No.	Extract	Regression equation	R^2 value	IC_{50} Value
1	AAHE	$y = 0.0223x + 26.419$	0.9773	1057.444
2	AACE	$y = 0.0301x + 33.144$	0.9963	554.0259
3	AAME	$y = 0.0348x + 38.022$	0.9846	344.195
4	Standard	$y = 0.046x + 48.27$	0.9736	37.6086

the control (Table 9). These findings align with Herbert-Doctor et al. [39], who reported 100% mortality of *Bemisia tabaci* using *Agave tequilana* extract and de Oliveira et al. [54], who observed cytotoxic effects of *A. sisalana* on insect hemocytes. The insecticidal potential may be attributed to phytochemicals like saponins, flavonoids, phenols, and n-alkanes, also noted by Costa et al. [55] and Poonsri et al. [56]. AACE's superior performance may be due to the combined effect of active compounds and chloroform's inherent bioactivity. To our knowledge, this is the first report of such effects on *Holotrichia* sp. using these *Agave* extracts.

3.7 | Acetylcholinesterase Inhibition Activity

The study evaluated the acetylcholinesterase (AChE) inhibitory activity of three *Agave* extracts—AAHE, AACE and AAME—at concentrations from 100 to 1000 ppm using the Ellman method [32]. Among them, AAME showed the highest inhibition (71.10% at 1000 ppm), followed by AACE (62.68%) and AAHE (49.83%), with inhibitory activity increasing with concentration (Table 10). The standard, Physostigmine, showed the strongest effect (91.03% at 1000 ppm) and the lowest IC_{50} value (37.51), compared to AAME (344.19), AACE (554.03) and AAHE (1057.44), indicating AChE inhibition in the order: standard > AAME > AACE > AAHE (Table 11). The trend in AChE inhibition was reflected in nematode mortality, suggesting that the mechanism of action involves disruption of acetylcholine signalling essential for nematode motor function. Similarly, Maribel et al. [57] reported that extracts from *Agave tequilana*, *A. angustifolia* and *A. americana* significantly reduced AChE activity in a neuroinflammatory mouse model, supporting the neuroactive potential of Agave species. Given the health and environmental risks of chemical pesticides [58], such plant-derived AChE inhibitors offer promising eco-friendly alternatives for pest and nematode control.

4 | Conclusion

GC–MS profiling of *Agave angustifolia* revealed a rich diversity of bioactive secondary metabolites, providing a biochemical basis for its pesticidal potential. This study demonstrates, for the first time, the strong bio-efficacy of *A. angustifolia* extracts against the phytopathogenic bacterium *Dickeya zeae* and the insect pest *Holotrichia* sp., in addition to nematodes, fungi, and other bacterial and insect targets. Among the evaluated extracts, the methanolic extract (AAME) consistently demonstrated the highest antifungal, antibacterial, nematicidal, insecticidal, and antifeedant activities. These pronounced bioactivities may be attributed to the presence of major bioactive constituents within the extract or to the possible synergistic interactions among these compounds, including succinic acid, chlorooctyl phenethyl ester, and 1,2-propanetriol (glycerol). Notably, AAME showed maximum inhibition of acetylcholinesterase (AChE) activity, indicating a neurotoxic mode of action that likely contributes to nematode and insect mortality. This mechanistic insight strengthens the biological relevance of the observed bioactivity and supports AChE inhibition as a key pathway underlying the pesticidal effects of *A. angustifolia*. Overall, the findings establish *A. angustifolia* as a promising, eco-friendly botanical resource with broad-spectrum pesticidal activity. Future studies should focus on isolation of active compounds, formulation development, field-level validation, and safety assessments to facilitate the integration of *A. angustifolia* based products into sustainable and integrated pest management programs.

Author Contributions

Rashika Tamta: writing – original draft, methodology, investigation, experimental work, formal analysis, conceptualization. Shilpi Rawat: review, editing, supervision, conceptualization, validation. Himani Karakoti: validation, methodology, formal analysis. Tannu Rani: writing – original draft, formal analysis, data curation. Pooja Bargali: validation, methodology, formal analysis. Ravendra Kumar: writing – review and editing, supervision, conceptualization, validation. Stefania Garzoli: formal analysis, writing – review and editing, visualisation. Renu Pandey: methodology, resources.

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The authors declare no conflicts of interest.

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Data will be made available on request.

References

1. S. Chatterjee, Y. Kuang, R. Splivallo, P. Chatterjee, and P. Karlovsky, "Interactions Among Filamentous Fungi *Aspergillus niger*, *Fusarium verticillioides* and *Clonostachys rosea*: Fungal Biomass, Diversity of Secreted Metabolites and Fumonisin Production," *BMC Microbiology* 16 (2016): 1–13.

2. M. D. Junaid and A. F. Gokce, "Global Agricultural Losses and Their Causes," *Bulletin of Biological and Allied Science Research* 2024, no. 1 (2024): 66.

3. S. Savary, L. Willocquet, S. J. Pethybridge, P. Esker, N. McRoberts, and A. Nelson, "The Global Burden of Pathogens and Pests on Major Food Crops," *Nature Ecology & Evolution* 3, no. 3 (2019): 430–439.

4. P. Dutta, A. Kumari, M. Mahanta, et al., "Nanotechnological Approaches for Management of Soil-Borne Plant Pathogens," *Frontiers in Plant Science* 14 (2023): 1136233.

5. M. Mihajlović, E. Rekanović, J. Hrustić, M. Grahovac, and B. Tanović, "Methods for Management of Soilborne Plant Pathogens," *Pesticidi i Fitomedicina* 32, no. 1 (2017): 9–24.

6. R. A. Sikora, J. Helder, L. P. Molendijk, J. Desaeger, S. van den Eves-Akker, and A. K. Mahlein, "Integrated Nematode Management in a World in Transition: Constraints, Policy, Processes, and Technologies for the Future," *Annual Review of Phytopathology* 61, no. 1 (2023): 209–230.

7. V. R. Kannan, K. K. Bastas, and S. D. Rajendran, "Scientific and Economic Impact of Plant Pathogenic Bacteria," in *Sustainable Approaches to Controlling Plant Pathogenic Bacteria*, eds. V. R. Kannan and K. K. Bastas (CRC Press, 2015), 369–392.

8. J. Li, F. Gu, R. Wu, J. Yang, and K. Q. Zhang, "Phylogenomic Evolutionary Surveys of Subtilase Superfamily Genes in Fungi," *Scientific Reports* 7, no. 1 (2017): 454–456.

9. J. Mansfield, S. Genin, S. Magori, et al., "Top 10 Plant Pathogenic Bacteria in Molecular Plant Pathology," *Molecular Plant Pathology* 13, no. 6 (2012): 614–629.

10. S. Kolla, P. V. R. Reddy, S. Singh, B. Bhattacharyya, and S. Bhagawati, "White Grubs (Coleoptera: Scarabaeidae) on Fruit Crops: Emerging as Pests of Economic Importance," *Pest Management in Horticultural Ecosystems* 25, no. 2 (2019): 263–267.

11. B. Sundar, V. Rashmi, H. K. Sumith, and S. Sandhya, "Study the Incidence and Period of Activity of *Spodoptera litura* on Soybean," *Journal of Entomology and Zoology Studies* 6, no. 5 (2018): 331–333.

12. L. Chengala and N. Singh, "Botanical Pesticides—A Major Alternative to Chemical Pesticides: A Review," *International Journal of Life Sciences* 5, no. 4 (2017): 722–729.

13. P. M. Ngegba, G. Cui, M. Z. Khalid, and G. Zhong, "Use of Botanical Pesticides in Agriculture as an Alternative to Synthetic Pesticides," *Agriculture* 12, no. 5 (2022): 600.

14. D. Fischer, C. Imholt, H. J. Pelz, M. Wink, A. Prokop, and J. Jacob, "The Repelling Effect of Plant Secondary Metabolites on Water Voles, Arvicola amphibius," *Pest Management Science* 69, no. 3 (2013): 437–443.

15. P. C. Stevenson, M. B. Isman, and S. R. Belmain, "Pesticidal Plants in Africa: A Global Vision of New Biological Control Products From Local Uses," *Industrial Crops and Products* 110 (2017): 2–9.

16. A. Garcia-Mendoza and R. Galván, "Richness of Agavaceae and Nolinaceae Families in Mexico," *Boletín de la Sociedad Botánica de México* 56 (1995): 7–24.

17. S. S. El-Hawary, H. A. El-Kammar, and R. S. El-Dine, "Antimicrobial Activity and Genetic Variability Between *Agave* Cultivars Using ISSR and SCoT Analyses," *Egyptian Journal of Chemistry* 63, no. 10 (2020): 3817–3822.

18. S. Manandhar, S. Luitel, and R. K. Dahal, "In Vitro Antimicrobial Activity of Some Medicinal Plants Against Human Pathogenic Bacteria," *Journal of Tropical Medicine* 2019 (2019): 1895340.

19. S. N. H. M. Azmin and M. S. M. Nor, "Chemical Fingerprint of *Centella asiatica* Bioactive Compounds in the Ethanolic and Aqueous Extracts," *Advances in Biomarker Sciences and Technology* 2 (2020): 35–44.

20. W. S. Abbott, "A Method of Computing the Effectiveness of an Insecticide," *Journal of Economic Entomology* 18 (1925): 265–267.

21. R. S. Hussey and K. R. Barker, "A Comparison of Methods of Collecting Inoculum of Meloidogyne spp., Including a New Technique," *Plant Disease Report* 57 (1973): 1025–1028.

22. Q. Abdullah, A. Mahmoud, and A. Al-Harethi, "Isolation and Identification of Fungal Post-Harvest Rot of Some Fruits in Yemen," *PSM Microbiology* 1, no. 1 (2016): 36–44.

23. J. M. Vincent, "The Esters of 4 Hydroxybenzoic Acid and Related Compounds. Part 1. Methods for the Study of Their Fungistatic Properties," *Journal of the Society of Chemical Industry* 66, no. 5 (1974): 149–155.

24. A. W. Bauer, D. M. Perry, and W. M. Kirby, "Single-Disk Antibiotic-Sensitivity Testing of Staphylococci: An Analysis of Technique and Results," *Archives of Internal Medicine* 104, no. 2 (1959): 208–216.

25. S. R. Vattikonda and S. R. Sangam, "Assessment of Antifeedant Potential of Azadirachtin, a Plant Product of *Azadirachta indica* Against *Papilio demoleus* L. (Lepidoptera: Papilionidae) Larvae," *Journal of Entomology and Zoology Studies* 4, no. 5 (2016): 956–959.

26. J. P. Purwar and R. P. Srivastava, "Toxicity and Antifeedant Activity of Diflubenzuron (Dimilin 25 WP) Against *Spodoptera litura*," *Indian Journal of Applied Entomology* 17, no. 1 (2003): 28–32.

27. R. P. Singh and N. C. Pant, "Lycorine—A Resistance Factor in the Plants of Subfamily Amaryllidoideae (Amaryllidaceae) Against Desert Locust, *Schistocerca gregaria* F," *Experientia* 36, no. 5 (1980): 552–553.

28. D. Pande and R. P. Srivastava, "Toxicity and Antifeedant Activity of Indoxacarb (Avaunt 14.5 SC) Against Tobacco Caterpillar, *Spodoptera litura* (Fabricius)," *Insect Environment* 9, no. 2 (2003): 69–70.

29. M. Kogan and R. D. Goeden, "The Host-Plant Range of *Lema trilineata daturaphila* (Coleoptera: Chrysomelidae)," *Annals of the Entomological Society of America* 63, no. 4 (1970): 1175–1180.

30. R. K. Sharma and R. S. Bisht, "Antifeedant Activity of Indigenous Plant Extracts Against *Spodoptera litura* Fabricius," *Journal of Insect Science* 21, no. 1 (2008): 56–60.

31. M. A. Sheikh, M. Yaqoob, F. J. Wani, T. A. Bhat, M. Gani, and M. A. Bhat, "Insecticidal Activity of Different Doses of *Acorus calamus* Essential Oil Against *Sitophilus oryzae*," *Journal of Scientific Research and Reports* 30, no. 6 (2024): 666–670.

32. G. L. Ellman, K. D. Courtney, V. Andres, Jr., and R. M. Featherstone, "A New and Rapid Colorimetric Determination of Acetylcholinesterase Activity," *Biochemical Pharmacology* 7, no. 2 (1961): 88–95.

33. D. Alimi, A. Hajri, S. Jallouli, and H. Sebai, "Acaricidal and Anthelmintic Efficacy of *Ocimum basilicum* Essential Oil and Its Major Constituents Estragole and Linalool, With Insights on Acetylcholinesterase Inhibition," *Veterinary Parasitology* 309 (2022): 109743.

34. K. Rizwan, M. Zubair, N. Rasool, M. Riaz, M. Zia-Ul-Haq, and V. De Feo, "Phytochemical and Biological Studies of *Agave attenuata*," *International Journal of Molecular Sciences* 13, no. 5 (2012): 6440–6451.

35. V. Olvera-García, S. T. M. del Campo, J. A. Gutiérrez-Uribe, and A. Cardador-Martínez, "GC–MS and HPLC–MS-TOF Characterization of *Agave atrovirens* Extracts: A Preliminary Study," *Industrial Crops and Products* 78 (2015): 39–47.

36. S. M. Shah and T. Z. Abdul-Jalil, "Qualitative and Quantitative Estimation of Chemical Constituents From Leaves and Roots of Iraqi *Agave attenuata* by GC-MS and RP-HPLC," *Iraqi Journal of Pharmaceutical Sciences* 31, no. Suppl (2022): 75–85.

37. O. E. Omale and C. B. I. Alawa, "Phytochemical and Antimicrobial Screening of Methanol and Aqueous Extracts of *Agave sisalana*," *Pharmacologia* 2, no. 8 (2011): 553–560.

38. C. Lubian, D. D. Martinha, R. Portz, et al., "Anthelmintic Activity of Plant Aqueous Extracts Against *Panagrellus redivivus* In Vitro," *Arquivos do Instituto Biológico* 86 (2019): e0672018.

39. L. A. Herbert-Doctor, M. Saavedra-Aguilar, M. L. Villarreal, A. Cardoso-Taketa, and O. Vite-Vallejo, "Insecticidal and Nematicidal Effects of *Agave tequilana* Juice Against *Bemisia tabaci* and *Panagrellus redivivus*," *South West Entomology* 41, no. 1 (2016): 27–40.

40. J. F. D. Santos, C. D. S. Sousa, A. C. F. Soares, F. D. S. Lima, and D. H. S. G. Barbosa, "Actinobacteria and Organic Fertilizers for Management

of the Nematode *Scutellonema bradys* in Yam Plants," *Revista Caatinga* 29 (2016): 548–558.

41. F. N. Jesus, J. C. Damasceno, D. H. Barbosa, R. Malheiro, J. A. Pereira, and A. C. Soares, "Control of the Banana Burrowing Nematode Using Sisal Extract," *Agronomy for Sustainable Development* 35 (2015): 783–791.

42. A. Joshi, "Studies on the Nematicidal Potential of Weeds for the Sustainable Management of *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 in Tomato (*Solanum lycopersicum* L.)," Master's thesis, GB Pant University of Agriculture & Technology, 2023.

43. M. B. Botura, J. D. G. dos Santos, G. D. da Silva, et al., "In Vitro Ovicidal and Larvicidal Activity of *Agave sisalana* Perr. (Sisal) on Gastrointestinal Nematodes of Goats," *Veterinary Parasitology* 192, no. 1–3 (2013): 211–217.

44. G. Francis, Z. Kerem, H. P. Makkar, and K. Becker, "The Biological Action of Saponins in Animal Systems: A Review," *British Journal of Nutrition* 88, no. 6 (2002): 587–605.

45. A. R. Maharshi and V. S. Thaker, "Antifungal Activity of *Agave* Species From Gujarat, India," in *Microbial Diversity and Biotechnology in Food Security* (Springer India, 2014), 423–430.

46. A. Almazán-Morales, M. E. Moreno-Godínez, E. Hernández-Castro, et al., "Phytochemical Profile and In Vitro Activity of *Agave angustifolia* and *A. cupreata* Extracts Against Phytopathogenic Fungi," *Revista Mexicana de Fitopatología* 40, no. 2 (2022): 169–187.

47. A. Scalbert and G. Williamson, "Dietary Intake and Bioavailability of Polyphenols," *Journal of Nutrition* 130, no. 8 (2000): 2073S–2085S.

48. A. M. Farhan, P. C. Lee, S. E. How, and A. G. Jualang, "Antibacterial Activities of *Agave angustifolia* and *Pittosporum ferrugineum*," *Journal of Environmental Microbiology and Toxicology* 1, no. 1 (2013): 15–17.

49. M. T. J. Khan, K. Ahmad, M. N. Alvi, et al., "Antibacterial and Irritant Activities of Organic Solvent Extracts of *Agave americana* Linn., *Albizzia lebbek* Benth., *Achyranthes aspera* Linn., and *Abutilon indicum* Linn.: A Preliminary Investigation," *Pakistan Journal of Zoology* 42, no. 1 (2010): 93–97.

50. M. Alcázar, T. Kind, A. Gschaedler, et al., "Effect of Steroidal Saponins From *Agave* on the Polysaccharide Cell Wall Composition of *Saccharomyces cerevisiae* and *Kluyveromyces marxianus*," *LWT* 77 (2017): 430–439.

51. A. E. Osbourn, "Saponins in Cereals," *Phytochemistry* 62, no. 1 (2003): 1–4.

52. H. Chowdhury, C. S. Kar, S. K. Sarkar, and M. K. Tripathi, "Feeding Inhibitory Effect of Some Plant Extracts on Jute Hairy Caterpillar (*Spilosoma obliqua*)," *Indian Journal of Agricultural Sciences* 82, no. 1 (2012): 59.

53. L. Saba, "Initiation of Defence Responses by Plant Extracts and Their Insecticidal Role Against the Russian Wheat Aphid in Wheat," Master's thesis, University of the Free State, 2015.

54. L. H. G. de Oliveira, P. A. P. S. de Sousa, F. F. Hilario, et al., "*Agave sisalana* Extract Induces Cell Death in *Aedes aegypti* Hemocytes Increasing Nitric Oxide Production," *Asian Pacific Journal of Tropical Biomedicine* 6, no. 5 (2016): 396–399.

55. M. F. Costa, J. T. A. Osuna, H. N. Brandão, M. Haraguchi, and C. D. S. Ledo, "Chemical Composition and Foliar Toxicity of Extracts From the Waste Liquid of Sisal," *Magistra* 26, no. 3 (2014): 376–388.

56. W. Poonsri, W. Pluempanupat, P. Chitchirachan, V. Bullangpoti, and O. Koul, "Insecticidal Alkanes From *Bauhinia scandens* Var. *Horsfieldii* Against *Plutella xylostella* L. (Lepidoptera: Plutellidae)," *Industrial Crops and Products* 65 (2015): 170–174.

57. H. R. Maribel, J. F. Enrique, Z. Alejandro, J. A. A. Ruperto, A. O. M. Lucía, and M. B. Nayeli, "Acetylcholinesterase Inhibition, Nootropic and Antioxidant Effect of Extracts From *Agave* Species," *University Journal of Pharmaceutical Research* 8, no. 4 (2023): 9–16.

58. C. S. Pundir and A. Malik, "Bio-Sensing of Organophosphorus Pesticides: A Review," *Biosensors & Bioelectronics* 140 (2019): 111348.