Bioactivity evaluation of lemongrass-derived flavonoids and its formulation against *Spodoptera littoralis* and some plant pathogenic fungi

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Abstract: Aromatic plants are rich in phenolic compounds which known as safe alternatives to chemical pesticides. This study is made for the evaluation of insecticidal and antifungal activities of flavonoid compounds extracted from lemongrass (*Cymbopogon citratus*) leaves and its formulation. The high performance liquid chromatographic (HPLC) analysis identified 15 compounds.The abundance of them were chlorogenic acid (52.62 μg/ml), methy gallate (22.78 μg/ml), gallic acid (21.32 μg/ml), rutin (11.10 μg/ml), ellagic acid (7.43 μg/ml), and coumaric (6.89 μg/ml), with respect to their standards. The local formulation overruns all of the laboratory investigations as soluble liquid (SL) formulation. The effectiveness of different concentrations of the flavonoid extract of *C. citrates* on the neonates of *Spodoptera littoralis* was evaluated before and after formulation. The flavonoids formulation (SL) have more effects than flavonoid extract (a.i), where LC_{50} values were 3074.2 and 5689.3 ppm, respectively. In addition both compounds exhibit the significant reductions in all developmental stages of *S. littoralis* larval survival, pupation, adult emergence, coupled with prolonged developmental durations and negatively affect reproductive activity. On the other hand, the antifungal effects of the same extract and its formulation were evaluated on *F.. oxysporum*, *F. solani* and *A.solani in vitro*. Flavonoid extract (a.i) or formulation (SL) had an inhibitory impact on the linear growth of fungal mycelial at all tested concentrations. The formulation (SL) gave completely inhibition for *F. oxysporium* at concentration of 8000 ppm, the EC_{50} value was 1860.90 ppm. Finally from this study and the previous results flavonoid extract formulation (SL) could be used as safe alternative of chemical pesticides against *S. littoralis* and *F. oxysporum, F. solani* and *A. solani* after complete the needed studies.

Keywords: lemongrass; flavonoids; extraction; HPLC; formulation; insecticidal; antifungal activities.

1. Introduction:

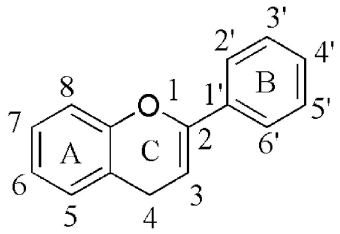
In recent years, the studies have been focused on biological control programs and the use of natural products as a safe alternative to chemical pesticides which affect human, animal and environment (**Alotibi *et al.*, 2020**). Most plants are rich sources of medical compounds and have a major impact on human health.

Phytochemicals are bioactive substances derived from plants. We refer them as a secondary metabolites because the plants that produces them might be not need them, but they play a crucial role in mediating interactions between plants and their biotic environment, serving as effective models of active defense against insect and pathogen attacks **(Pandey *et al.,* 2017)**. Every part of the plant, including the bark, leaves, stem, roots, flowers, fruits, and seeds are naturally synthesized with them and contain active ingredients (a.i). The chemical components of plants should be studied as they are used to produce complex. Secondary metabolites that have many pharmacological characteristics are due to their content of: phenolic, alkaloids, flavonoids, saponins, tannins, terpenoids, glycosides, gum, resin and volatiles oils **(Umar *et al.,* 2016)**.

Phenolic compounds represent a diverse group of secondary metabolites found in plants. Such compounds can be categorized into several groups based on their structures: simple phenols, phenolic acids, hydroxyl cinnamic acid derivatives, and flavonoids. Each category has characteristics and

properties, showing the diverse profile of phenolic compounds. These classes of bioactive substances are used as anti-aging, anti-cancer, anti-inflammatory, antidiabetic, antiviral, cardioprotective and neuroprotective, antioxidant, antibacterial, antifungal and pest control **(Ali *et al.,* 2022)**, as well as consumed daily as a dietary supplements including tea, red wine, apples, onions and tomatoes (**Rakers *et al.,* 2014).**

In addition, flavonoids have been categorized based on variations in aglycone structures, sugar components, inter-sugar linkages, and glycosylation positions. Their fundamental structure consists of a C_6-C_3-C_6 framework, which includes ring A (an aromatic ring connected to a pyran ring), ring B (a separate aromatic ring), and ring C (the pyran ring).



C_6-C_3-C_6

These structural variations lead to a diverse range of sub-class compounds **(Tian *et al.,* 2018).**

They play essential roles in plant biochemistry and physiology, functioning as antioxidants and enzyme inhibitors. Furthermore, they have demonstrated a variety of beneficial effects in animal models, including anti-allergic, anti-inflammatory, anti-viral, anti-proliferative, and anti-carcinogenic properties **(Nijveldt *et al.,* 2001).**

Among the plants rich in flavonoids is lemongrass *Cymbopogon citratus* which has bioactivity as an antioxidant, antimicrobial, antifungal, antibacterial, insecticidal, and insect repellent **(Mirghani *et al.,* 2012; Pan *et al.,* 2022).** On the other hand, they have been recognized as promising biopesticides; however, these revisions did not adequately report the modes and mechanisms of action underlying their insecticidal activity **(Schnarr *et al.*, 2022).** Many researchers have studied the insecticidal effect of flavonoids on insects in general such as moths, beetles, and true bugs **(Riddick, 2021)** and on Lepidoptera particularly (**Jadhav, 2012; Diaz and Palacios, 2015; Su *et al.*, 2018; Sepahvand *et al.,* 2024).** They found that it had an effect of feeding behavior, survival, and development.

Extracts from *C. citrates* are used in the fungicide industry as controlling plant pathogens including *Aspergillus* spp.; *Fusarium* spp.; *Penicillium* spp. and *Alternaria* spp. (**Mori *et al.,* 2017; Israa *et al.*, 2024).**

Flavonoids have shown promise as natural agents against fungal pathogens in plants, effectively inhibiting the germination of spores and causing cellular lysis **(Pusztahelyi *et al.*, 2015)**. Compounds like quercetin and phenolic acids, such as ferulic acid, exhibit significant antifungal activity against various phytopathogens, including *Alternaria alternata*, *Rhizoctonia solani*, *Fusarium oxysporum*, *Botrytis cinerea*, and *Phytophthora infestans*. Their diverse mechanisms make them valuable for sustainable agricultural practices **(Wianowska *et al.*, 2016; Shoaib *et al.*, 2019).**

For the phytosanitary treatment of crops, plant-based formulations are promising substitutes for synthetic chemical pesticides. To make a product that is safe, easy to use, and has a long shelf life, most technical pesticides are made ahead of time by mixing the active ingredients with adjuvants, diluents, preservatives, and inserts (**Ayiare *et al.,* 2023)**.

The present study aims to 1) extract flavonoid compounds from lemongrass herb and determine the phytochemical analysis; chemical composition by using high performance liquid chromatography (HPLC). 2) prepare flavonoid compounds as a soluble liquid (SL) formulations and evaluate them as insecticidal; antifungal agents against *Spodoptera littoralis* and *F. oxysporum, F. solani* and *A. solani*.

2. Material and methods:

2.1. Plant sample collection

Fresh sample of lemongrass herb (*C. citratus*) belongs to the poaceae family was collected from Horticulture Research Institute, Agricultural Research Centre (ARC), Giza, Egypt, and then transferred to the laboratory for washing with tap water and dried at room temperature. The dried herbs were ground separately to powder form and extracted as desirable.

2.2. Tested chemicals

- Standard of rutin (RUE), gallic acid (GAE) and folin-ciocalteau reagent were obtained by Sigma-Aldrich Company.

- Methanol alcohol (CH_3OH 99%); Hydraulic acid (HCl), Sodium carbonate ($NaCO_3$), Sodium Nitrite ($NaNO_2$), Aluminum chloride ($AlCl_3$), lead acetate ($pb(CH_3OO)_2$) and Sodium hydroxide (NaOH), Surface active agents: Tween 20; Tween 40 and Tween 80 all chemicals were supplied by EL-Gomhoria Co., Cairo, Egypt.

2.3. Preparation of crude plant extract

A total of 100 g of the dried leaves powdered of *C. citratus* has been extracted with 500 ml methanol alcohol (99%) by means of soaking for 48 hrs. The extract, then was filtered by filter paper whatman No.1. The plant build up was re-extracted with the expansion of new methanol alcohol for another twice, then filtered and evaporated by rotary evaporator at 40 ˚C and stored at 4 ˚C until used. The percentage yield for crude methanolic extract was calculated as follows:

% yield= weight of extract (g) / weight of plant (g)× 100

The crude methanolic extract was used for insecticidal; antifungal assays and identification of bioactive compounds.

2.4. Phytochemical analysis

2.4.1. Determination of total phenolic content

The colorimetrically method of folin-ciocalteau described by **Jonathan *et al.* (2012)** was used. An aliquot (100 μl) of extract was diluted to 3 ml with double-distilled water and well mixed in a dry test tube, then 0.5 ml of folin-ciocalteau reagent was added. After 3 min, 2 m1 of saturated sodium carbonate solution (20%) were added to the mixture and kept in the dark place for 60 min. The absorbance was measured at λ= 650 nm by UV-VIS spectrophotometer (Milton Roy, Model 601) against a blank sample (100 µl methanol 80% and reagent only). Phenolic contents were calculated from the standard solution prepared from pure gallic acid (10 mg/100 ml double-distilled water). Total phenolic content was performed in triplicate and expressed as mg (GAE)/g dry weight.

2.4.2. Determination of total flavonoid

The colorimetrically method described by **Quettier *et al.* (2000)** was used one ml of extract or standard solutions of rutin (RUE) was added to 4 ml double distillated water, and 0.3 ml (5% $NaNO_2$). After 5 min, 0.3 ml of (10%) aluminum chloride ($AlCl_3$) was added. After 6 min 2 ml of 1 M sodium hydroxide (NaOH) solution was added and the total volume was made up to 10 ml with double-distillated water. Rutin (0.1 g dissolved in 100 ml ethanol 98%) was determined by reading the developed red color at λ= 510 nm by UV-VIS spectrophotometer (Milton Roy, Model 601) against a blank sample (1.0 ml methanol 80% and reagent only). Total flavonoid contents were expressed as mg (RUE) /g dry weight.

2.5. Extraction of flavonoids

An aliquot (100 g) of plant powder has been extracted with 500 ml methanol 50% for 24 hrs at ambient conditions by means of shaker and filtered by filter paper whatman No.1 **(Jain *et al.*, 2007)**. The filtrate changed into dealt with 100 ml lead acetate (1%) for 4 hrs for precipitation. The mixture was filtered, and then a mixture of 250 ml acetone and 30 ml of HCl was added to the precipitate, then filtered and evaporated by rotary evaporator at 40 ˚C and stored at 4 ˚C for further analysis. The percentage yield for flavonoid was calculated as follows:

% Yield =weight of precipitate (g)/ weight of plant (g) ×100

2.6. Analysis by High Performance Liquid Chromatography (HPLC)

To identify the flavonoid compounds extracted from lemongrass herb, HPLC analysis was conducted using an Agilent 1260 series system. The separation was performed on a Zorbax Eclipse Plus C8 column (4.6 mm x 250 mm i.d., 5 µm). The mobile phase comprised water (A) and 0.05% trifluoro acetic acid in acetonitrile (B) with a flow rate 0.9 ml/min. The mobile phase gradient was programmed as follows: 0 min (82% A); 0 to1 min (82% A); 1 to 11 min (75% A); 11 to 18 min (60% A); 18 to 22 min (82% A); 22 to 24 min (82% A). The multi-wavelength detector monitored the operation at λ= 280 nm. The injection volume for each of the sample solutions set at 5 µl, and the column temperature was maintained at 40 ˚C. The separated peaks were adjusted, with respect to their certified reference materials (CRMs) at the same retention times (Rts).

2.7. The physico-chemical properties of formulation constituent's

2.7.1. Active ingredient.

- Solubility: The quantities of distilled water, acetone, and xylene were measured in order to determine one gram of the active ingredient's total solubility or miscibility at 20 ˚C **(Nelson and Fiero, 1954)**. The % Solubility was calculated according to the following equation:

% solubility = W/V x 100

[Where; W= active ingredient weight, V= volume of solvent required for complete solubility].

- Free acidity or alkalinity: It was determined according to the method described by **WHO specification (1979).**

2.7.2. Surface active agents

- Hydrophilic-lipophilic balance (HLB): It is believed that a surfactant's water solubility serves as a rough proxy for its HLB **(Lynch and Griffin, 1974).**

- Surface tension: It was determined by using Du-Nouy tensiometer for solutions containing 0.5 % (W/V) surfactant according to **ASTM D-1331 (2001).**

- Critical micelle concentration (CMC): The concentration at which the surface tension of the solution does not decrease when the surfactant concentration increases (CMC) of the tested surfactants was determined according to the method described by **Osipow (1964).**

- Free acidity or alkalinity: It was determined by the same method described before.

2.8. Local prepared soluble liquid formulation.

- Surface tension was ascertained as previously demonstrated.

- Free acidity or alkalinity: It was measured as mentioned before.

2.9. Spray solution at field dilution rate.

- pH: It was determined by using Cole - Parmer pH conductivity meter 1484-44 according to **Dobrat and Martijn (1995).**

- Surface tension: It was determined as mentioned before.

- Electrical Conductivity: It was determined by using Cole-Parmer pH/ Conductivity meter 1484-44, where µmhos is the unit of electrical conductivity measurements according to **Dobrat and Martijn (1995)**.

- Viscosity: It was determined by using Brookfield viscometer Model DVII+Pro, wherecentipoise is the unit of measurement according to **ASTM D-2196 (2005).**

2.10. Biological assays

2.10.1. Insect rearing

Cotton leafworm *S. littoralis* was reared in laboratory for many generations under conditions (26±2 ˚C and 65±5% relative humidity) on castor bean leaves, *Ricinus communis* L. **(Eldefrawi *et al.*, 1964).**

2.10.2. Insecticidal activity assay

The efficacy of the flavonoid extract from *C. citratus* before and after formulation against the neonates of *S. littoralis* was evaluated using leaf-dipping technique **(Ishayaa and Klein, 1990).** Serial concentrations of each compound (1250, 2500, 5000, 10000, 20000 and 30000 ppm) were prepared. Fresh castor bean leaves were dipped in the tested concentrations for 20 seconds and then left to dry. Using water only as the control. Twenty neonate larvae of *S. littoralis* were transferred to Petri dishes (9 cm in diameter). Each treatment was replicated five times. The mortality percentage was recorded after 96 hrs of exposure. The LC_{25} and LC_{50} values were calculated according to the method described by **Finney (1971).**

2.10.3. Residual effect

To study the effect of tested compounds on developmental stages, fifty of neonates of *S. littoralis* larvae were placed in each Petri dish (five replicates for each treatment) and fed on treated castor bean leaves with LC_{25} and LC_{50} concentrations of each concentration for 96 hrs (without changing the treated leaves). The remaining survival larvae were transferred and provided with fresh untreated castor bean leaves to establish the rate of development and growth. Total larval mortality percentages, pupation percentage, adult emergence percentage, were recorded according to these equations:

% of Total larval mortality = (Number of dead larvae/Total number of larvae)×100

% of pupation = (Number of pupae/Total number of larvae) ×100

% of adult emergence = (Number of emerged adults/Total number of larvae) ×100

Also, larval and pupal duration were observed. The percent of hatchability were recorded. The percentage of sterility was calculated according to equation of **Tappozada *et al*. (1968).**

% Hatchability = (No. of hatched eggs / No of deposited eggs) ×100

% sterility = 100 – [(a x b) / (A x B)] ×100

Where: a: no. of eggs laid / female in treatment.

b: % of hatchability in treatment

A: No of eggs laid / female in control.

B: % of hatchability in control.

2.11. Fungal strains used

Old culture *F. oxysporum, F. solani* and *A. solani* was grown in laboratory for 7 days using Petri

dishes with a diameter of 9 cm containing a Potato dextrose agar (PDA) medium and incubated was at 25±2 ˚C.

2.11.1. Antifungal activity assay

Evaluation of antifungal activity of flavonoid extract from *C. citratus* before and after formulation at concentrations (1000, 2000, 4000 and 8000 ppm) against *F. oxysporum, F. solani* and *A. solani* was evaluated by food poisoned technique according to **Mohanty *et al.* (2012).** Added separately concentrations were adjusted at 50 ml of Potato dextrose agar (PDA) medium before solidification, with respect to control PDA medium (without treatment). Then, the samples were inoculated at the center with a mycelial disc (5-mm diameter) taken from the margins of 7 days old *F. oxysporum, F. solani* and *A. solani*. Three replicates were conserved for each treatment and for untreated control. All Petri- dishes were incubated at 25±2 ˚C. The colony diameter was measured until the mycelia were fully covered in the control. The percentage of inhibition mycelial growth was calculated as following equation **(Satya *et al*., 2014).**

$$\mathrm{I}\,\% = \frac{C - T}{C} \times 100$$

Where, C is the average diameter of the mycelia growth in the control, and T is the average diameter of the mycelia growth in the treatment.

2.12. Statistical analysis

The probit analysis of a computer programme (Lpd line) was used to calculate the lethal and sublethal concentrations LC_{25} and LC_{50} values for insect and the median effective EC_{50} and nightly effective EC_{90} concentrations values for fungi according to **Finney (1971)**. One-Way ANOVA with SPSS software version 16.0 (SPSS Inc., Chicago, IL) was done for analysis the mortality percentage in insects and inhibition percentage in fungi. Post-hoc analyses were done by the Tukey HDS test for significant differences ($P<0.05$).

3. RESULTS AND DISCUSSION

In recent years, use of natural products as biological control is a critical issue. The plant extracts are highly concentrated bioactive compounds. In this study, we aimed to focus on lemonegrass (*C. citratus*) methanolic extract which contains phenolic compounds and flavonoid. The compounds are important, because they the benefits of formulation promising biopesticides against insects and pathogens.

3.1. The yield of extract, the total phenolic and total flavonoids content

The results in Table (1) show that the percentage yield of lemongrass (*C. citratus*) crude methanolic extract was recorded 5.8% of dry w. The total phenolic content of methanolic extract showed the content values of 128.5 mg gallic acid (GAE)/g dry w, but the total flavonoids content of methanolic extract showed the content values of 37.6 mg rutin (RUE)/g dry w, compared with gallic acid and rutin (standards). **Falode *et al.* (2022)** reported that the phenolic content of the crude, bound, and free phenol extracts were 516.33, 609, and 780.33 mg/g GAE, respectively, while flavonoid contents were 33.25, 37.77, and 58.13 mg/g QE, However, **Hanaa *et al.* (2021)** found that the *C. citrates* extract had phenolic and flavonoid content as 130.33 mg GAE/g and 193.63 μg QE/g, respectively. **Priadi *et al.* (2022)** showed that the lemongrass leaves had higher flavonoid content ranging from 0.94 to 10.66 μg QE/g extract. **Remy *et al.* (2022)** mentioned that methanol is a good solvent for extraction of large amount of flavonoids better than ethyl acetate, the *C. giganteus* extract had flavonoid content in case of ethyl acetate 134 μg QE/mg extract and in case of methanol 270 μg QE/mg extract.

Table (1). The yield, Total Phenolic and Total Flavonoid contents of crude extract.

Plant	Used part	Color of extract	% Yield	Total Phenolic content (mg GAE/g)	Total Flavonoid content (mg RUE/g)
Lemongrass	leaves	Dark brown	5.8	128.5	37.6

3.2. The yield of total flavonoids

The precipitated total flavonoid from *C. citratus* herb was an amorphous, dark brown in colour. The yield of total flavonoids precipitated was recorded 15.2% of dry plant. There are no guides to be in preceding literature about extraction of *C. citrates* flavonoids.

3.3. Identification of Polyphenols and flavonoid Compounds by HPLC

Use of certified reference materials (CRMs) are helpful to detect the polyphenols and flavonoid compounds in the crude extracts from *C. citratus* by HPLC as shown in Fig. (1A). The results showed that 15 compounds with different retention time (Rts). The results in Table (2) and Fig. (1B) and Fig. (2) indicate that, the polyphenolic and flavonoid compounds of extract contained gallic acid, chlorogenic acid, methyl gallate, coffeic acid, syringic acid, rutin, ellagic acid, coumaric acid, vanillin, ferulic acid, naringenin, rosmarinic acid, cinnamic acid, kaempferol and hesperetin with different concentrations compared with their certified reference materials (CRMs). However, chlorogenic acid (52.62 μg/ml) was the main component of polyphenols and flavonoid in *C. citrates* the extract, followed by methy gallate (22.78 μg/ml), gallic acid (21.32 μg/ml), rutin (11.10 μg/ml), ellagic acid (7.43 μg/ml) and coumaric (6.89 μg/ml) and the other components ranged between 0.34 and 3.34 μg/ml, while catechin, daidzein and querectin were absent in the extract. The results analogous were reported by **Falode, *et al.* (2022)** who reported that, phenolic acids: caffeic, chlorogenic, ferulic, among others and flavonoids: quercetin, kaempferol among others) were presented in *C. citrates* methanolic extract. Lemongrass is rich in bioactive compounds such as flavonoids and phenolic compounds, which consist of quercetin, luteolin, apiginin, isoorientin 2-O-rhamnoside and kaempferol **(Muala *et al.*, 2021; Sorabh *et al.*, 2023).**

3.4.Formulation part

Products with dissolved active components that are typically water-based are known as soluble liquids. This sort of formulation contains dissolved molecules rather than suspended particles. After mixing, agitation is not necessary to keep the constituent parts from settling; for the most part, solutions are visible. Flavonoid extracted from lemongrass tends to have lower viscosity them it mix easily in water and require minimal agitation after dilution.

The physico-chemical tests for the ingredient (i) and additives, soluble liquid (SL) could be made based on the physico-chemical characteristics of the ingredient (i).

Table (2). Polyphenols and flavonoid compounds determined by HPLC in extract of *C. citatus.*

Peak No.	compounds	Rt (min)	Area(mAU*s)	Area (%)	Conc.(µg/ml)
1	Gallic acid	3.462	29.12	7.07	21.32
2	Chlorogenic acid	4.034	37.80	9.17	52.67
3	Catechin	4.408	ND	ND	ND
4	Methyl gallate	5.286	40.71	9.88	22.78
5	Coffeic acid	5.808	6.51	1.58	3.34
6	Syringic acid	6.299	2.32	0.56	1.36
7	Rutin	6.688	7.42	1.80	11.10
8	Ellagic acid	7.207	7.31	1.77	7.43
9	Coumaric acid	8.514	19.18	4.65	6.89
10	Vanillin	9.235	1.13	0.27	0.41
11	Ferulic acid	9.561	6.37	1.54	3.70
12	Naringenin	10.276	4.27	1.03	3.94
13	Rosmarinic acid	11.879	2.76	0.67	2.68
14	Daidzein	15.856	ND	ND	ND
15	Querectin	17.185	ND	ND	ND
16	Cinnamic acid	18.896	11.23	2.72	2.18
17	Kaempferol	19.702	1.34	0.32	0.34
18	Hesperetin	20.983	5.60	1.36	2.62

ND = Not Detected

3.4.1 Physico-chemical properties of flavonoid extracted from lemongrass as active ingredient

Table (3) shows that, the lemongrass extract was totally insoluble in xylene, it was fully soluble in acetone and water. Its free acidity (6.08) indicated that it exhibited acidic properties. Considering these findings, it might be made as SL formulation and requires active agents with an acidic surface to fully interact with ingredient (i).

Table (3). Physico-chemical properties of Flavonoid extracted from lemongrass as active ingredient

Solubility			Free acidity
Water	Acetone	Xylene	
100	100	NS	6.08

NS: not soluble

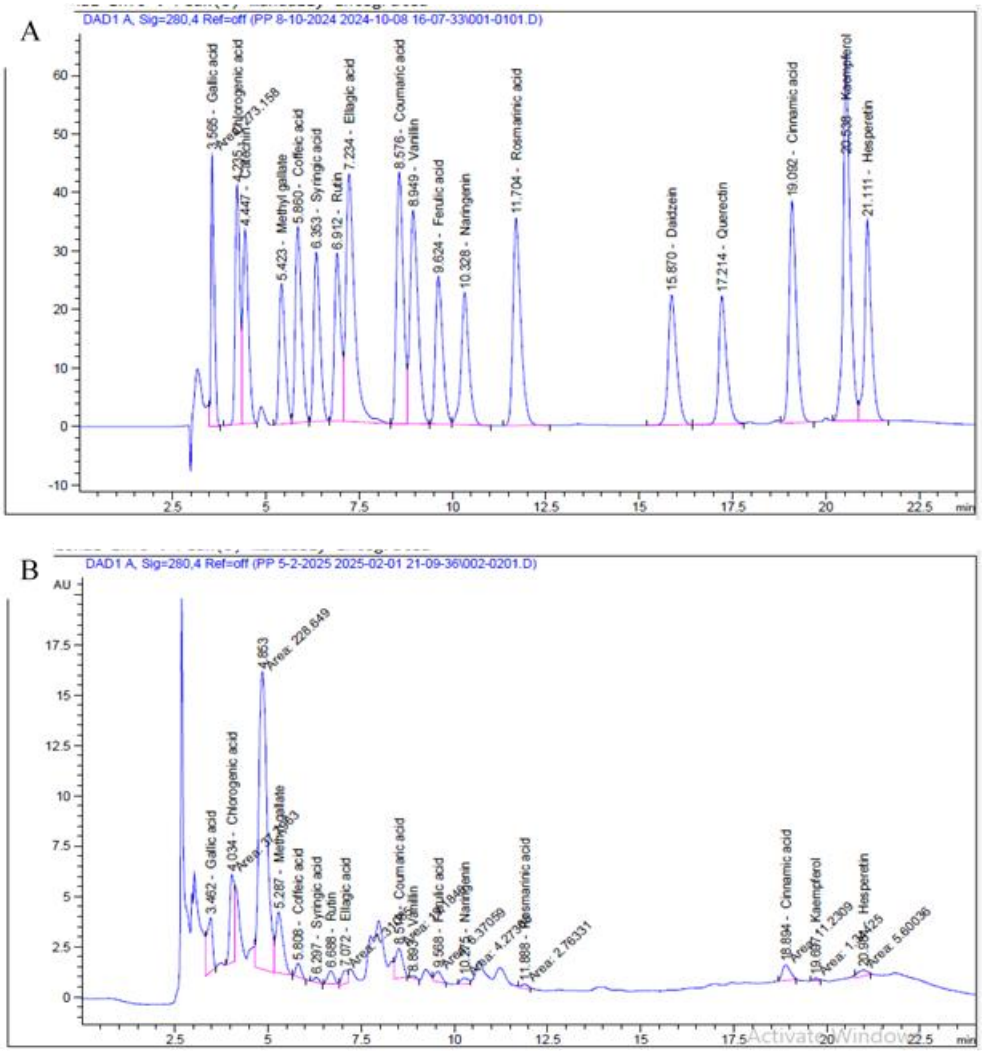


Fig. (1) (A). Chromatogram of reference standards, (B). Chromatogram profile of polyphenols and flavonoid compounds in extract of *C. citratus* by HPLC

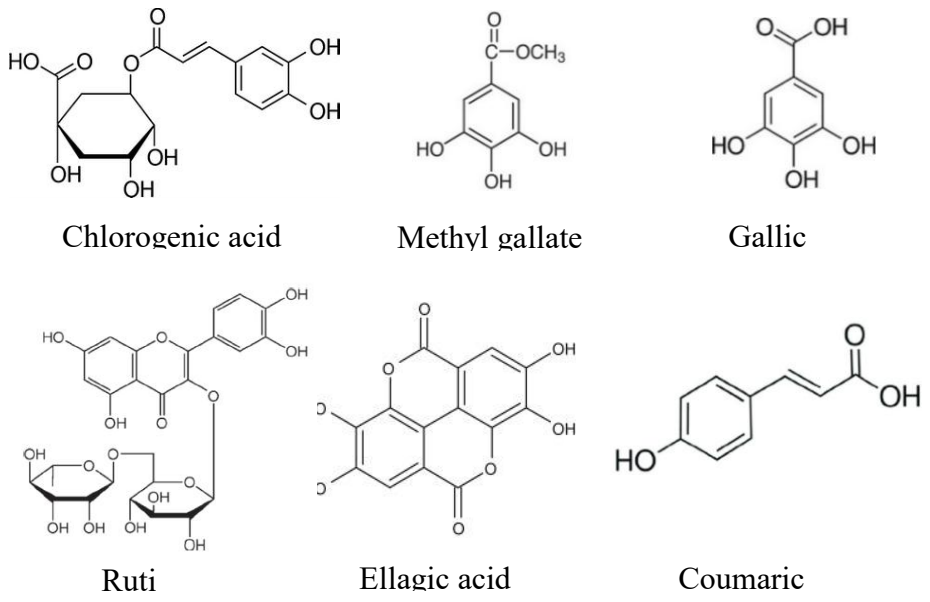


Fig. (2) Chemical structures of the main components of polyphenols and flavonoid in *C. citratus* extrac

3.5.Physico-chemical properties of adjuvants

Data in Table (4) show physico-chemical properties of Tweens 20, 40 and 80 as surface active agents. All of them showed very close surface tension values, their values were 36, 37.02 and 39.2 dyne/cm for Tween 20, 40 and 80, respectively. Also all of them showed an HLB value, greater than 13 meaning that they are considered dispersing agents, but there were clear differences between them in CMC values, where they showed 0.2, 0.4 and 0.5%, respectively. All of them demonstrated that, they are acidic in terms of free acidity or alkalinity, with Tween 80 having the highest value (0.49), followed that with Tween 40 (0.13) and Tween 20 (0.0196). This active ingredient (a.i) can be formulated as a SL using any of the three surface active agents, depending on their free acidity values.

3.6. Physico-chemical properties of local 20% soluble liquid formulation before and after accelerated

Table (5) shows physico-chemical properties of the 20% SL formulation before and after accelerated storage. The formulation's physico-chemical characteristics did not exhibit any significant changes; it was entirely soluble, transparent, and sediment-free in both situations, and it had an inverse connection with temperature. It also demonstrated an acidic property that was essentially unchanged before and after storage. The same results were reported by **Osipow, (1964)**. Generally, there were no effective changes before and after accelerated storage.

3.7. Physico-chemical properties of spray solution at field dilution rate

Table (6) demonstrates the spray solution's physico-chemical characteristics. A pesticide's biological activity against the target pest species is reatly influenced by its physical and chemical properties. Particularly,a pesticide's physical characteristics dictate its mode of action, dose, application method, and the ensuing environmental chemo dynamics **(Zacharia, 2011)**.The chemical make-up and formulation of pesticides have a significant impact on their physical characteristics. Surface tension and pH are decreased in the spray solution, whereas electrical conductivity and viscosity are increased. When the spray solution's surface tension decreases, its wettability and spreading on the treated surface improve, which increases the pesticide's deposit and activity **(Osipow, 1964)**. Pesticide efficacy may rise as a result of the pH value decreasing when electrical conductivity rises according to **Tawfik *et al.* (1987)** who stated that, when pH of the pesticide spray solution decrease and its conductivity increases, so did its retention and efficacy. According to **Richardson (1974)**, increasing the viscosity of the spray solution reduced drift and increased the spray solution's retention and sticking on the plant's surface, which could explain the relationship between increasing viscosity and increasing pesticidal efficiency.

Table (4). Physico-chemical properties of adjuvants

Adjuvants	Surface tension (dyne/cm)	HLB	CMC (%)	Free acidity as % H_2SO_4
Tween 20	36	>13	0.2	0.0196
Tween 40	37.02	>13	0.4	0.13
Tween 80	39.2	>13	0.5	0.49

Table (5). Physico-chemical properties of Flavonoid extracted from lemongrass 20% soluble concentrate local formulation before and after accelerated storage

Before storage						After storage					
Surface tension (dyne/cm)	**Free acidity**	**Solubility**	**pH**	**Sed.**	**App.**	**Surface tension (dyne/cm)**	**Free acidity**	**Solubility**	**pH**	**Sed.**	**App.**
32.800	0.534	soluble	7.88	Nil	clear	32.776	0.514	soluble	2.676	Nil	clear

Sed.: Sedimentation, App.: Appearance

Table (6). Physico-chemical properties of spray solution at field dilution rate

Viscosity centipoise	Electrical conductivity (µmhos)	pH	TDS	Salinity	Surface tension (dyne/cm)
1.61	317	2.89	152	0.1	35.4

3.8. Biological assay

3.8.1.Toxicity responses of the neonates of *S. litttoralis*

Table (7) Illustrates the toxicity responses of the neonates of *S.litttoralis* after 96 hrs of feeding on treated castor bean leaves with the tested compounds.

Data revealed that both compounds had an adverse impact on neonates at the tested concentrations that ranged from 1250 to 30000 ppm. Formulation of flavonoid extract (SL) demonstrated more efficacy than flavonoid extract as a.i, where the mortality rate was 88% after treated with the highest concentration (30000 ppm) of SL while it was 80% in case of flavonoid extract (a.i) alone. The six doses tested of both compounds provoked a significant mortality compared to the control. Also, the percentage of larval mortality was increased with increasing the concentrations. The F-value indicated that formulation (SL) is more effective than flavonoid extract (a.i), where the F-value of formulation (SL) was 204.4 compared to 178.21 for flavonoid extract (a.i). The LC_{25} and LC_{50} values for formulation (SL) were 905.16 and 3074 ppm, respectively, While they were less effective in case of flavonoid extract (a.i) (16003 and 5689.3 ppm, respectively). The benefits of formulation for Essential oil based biopesticides have been shown in recent studies. These benefits include improved stability, increased dispersion, and a more persistent release, and reduce degradation under environmental conditions **(Campolo *et al.*, 2020).**

Table (7). Responses of active ingredient (a.i) and its formulation against neonate of *S. littoralis* after feeding on treated castor bean leaves for 96 hrs.

Conc. (ppm)	% Mortality	
	Active ingredient (a.i)	Formulations (SL)
Control	5 ± 1.58	5 ± 1.58
1250	19 ± 1.87	28 ± 1.22
2500	40 ± 2.24	51 ± 1.87
5000	55 ± 1.58	68 ± 2.55
10000	67 ± 2.55	79 ± 3.67
20000	73 ± 2.55	82 ± 2.00
30000	80 ± 2.24	88 ± 1.22
F- Value	178.21	204.04
LC_{25} (95%CL)	1600.3 (1078.6 - 2141.3)	905.16 (567.67 - 1265.07)
LC_{50} (95%CL)	5689.3 (4602.8 - 6958.1)	3074.2 (2406.1 - 3763.6)
Slope (±SE)	1.224 ± 0.117	1.270 ± 0.121

Mean of % M, Standard error ±SE, 95% CL: Lower and upper 95% Confidence Limit.

The development of insects was studied after treatment of neonate of *S. littoralis* with LC_{25} and LC_{50}. Table (8) shows that the tested compounds were significantly LC_{25} and LC_{50} affected the development of *S. littoralis*. The percentage of larval mortality was increased with increases of the concentration and the time intervals (4, 7, 14, and 21 days). It was noted that, the significant effect of the compounds appeared 96 hrs after treatment. After that, there was slight increase in the mortality. At the end of larval stage, the mortality percentage for LC_{25} and LC_{50} were 44 and 71.2% for flavonoid extract (a.i), 57.6 and 75.6% for formulation (SL) compared with control which was 6.5%. The components of flavonoid extract may contribute to its insecticidal activity, leading to substantial larval mortality rates, which can be due to their ability to disrupt various physiological processes in insects or attributed to its interference with the insect's metabolic processes and gut enzymes, leading to mortality or reduced fitness **(Rattan, 2010; Isman, 2020). Su *et al.* (2018)** studied the effect of various flavonoids on larval survival of *S. litura*, they noticed that larval growth and development were significantly reduced.

The latent effects of *S. littoralis* were significantly decreasing the percentages of pupation, adult emergence, after treatment with the LC_{25} and LC_{50} of the tested compound compared to the control. **Jadhav *et al.* (2012)** tested the effect of three flavonoids on growth and survival of *H. armigera* and *S. littoralis.* They concluded that rutin significantly impacted larval development, increased pupal mortality, and caused malformations in adults, compared to quercetin and chlorogenic acid. The toxicity of these compounds is primarily attributed to their interference with enzymatic activity, inhibition of neurotransmission, and disruption of hormonal regulation, which collectively impairs insect development and reproduction **(Simmonds, 2003).**

The reduction in pupation percentage further indicates its effectiveness in disrupting the insect's normal growth and molting cycles. Moreover, the decline in adult emergence suggests that flavonoids may impede successful metamorphosis, leading to developmental abnormalities **(Aboshi *et al*., 2018).**

On the other hand, the treatments led to a slight significance in elongation of the larval duration but, there was a significance difference in the duration of pupal stage compared to the control. On the contrary, there was a decrease in the lifespan of adults especially when treated at high concentration of tested compounds. **Silva *et al.* (2016)** evaluated the activity of rutin on the biology of the *S. frugiperda*, they found that the rutin flavonoid negatively affected by

prolonging the development time. The prolonged larval and pupal durations suggest that flavonoids may disrupt hormonal regulation, particularly the molting and metamorphosis processes governed by juvenile hormone and ecdysteroids which are critical for insect development **(Koul *et al.*, 2008; Diaz Napal and Palacios, 2015).** Similarly, the shortened adult lifespan in treated groups highlights the long-term physiological stress imposed by flavonoid exposure, reducing reproductive potential and overall population sustainability. **Puri *et al.* (2022)** reported that flavonoids have also been shown to cause malformations, postpone growth and development, and alter the reproductive cycle of adult insects by extending the pre-reproductive period and reducing fecundity, oviposition, and egg hatching. Fecundity was drastically reduced, with females laying fewer eggs compared to controls. Hatchability and fertility rates were significantly reduced for LC_{50} of both treated groups, The decline in fertility and increase in sterility percentages further indicate the potential role of flavonoids as reproductive disruptors, possibly by interfering with gametogenesis or embryonic development reinforcing the hypothesis that these compounds impair reproductive fitness, **(Riddick, 2024).** These results align with previous studies that demonstrated the efficacy of plant flavonoids into population suppression **Goławska *et al.* (2014).**

Table (8). Residual effect of tested compounds at levels: LC_{25} and LC_{50} against neonate of *S. littoralis* after feeding on treated castor bean leaves for 96 hrs and completed development stages.

Parameters		Control	Active ingredient (a.i)		Formulations (SL)	
			LC_{25}	LC_{50}	LC_{25}	LC_{50}
Larvae Mortality (%) ±SE	4 d	2.4 ± 0.40^{c}	23.6 ± 1.17^{b}	49.2 ± 1.36^{a}	28.0 ± 1.79^{b}	51.2 ± 1.35^{a}
	7 d	2.8 ± 0.49^{d}	25.6 ± 1.17^{c}	52.4± 1.94^{a}	33.2 ± 1.74^{b}	54 ± 2.00^{a}
	14 d	3.2 ± 0.49^{c}	30 ± 1.09^{b}	56 ± 4.97^{a}	37.6 ± 2.32^{b}	61.2 ± 5.89^{a}
	21 d	3.6 ± 0.40^{c}	37.6 ± 1.94^{b}	61.2 ± 3.14^{a}	47.6 ± 2.86^{b}	66.8 ± 4.41^{a}
Total larvae Mortality %±SE		6.5 ± 1.50^{d}	44 ± 2.28^{c}	71.2 ± 2.80^{a}	57.6 ± 5.17^{b}	75.6 ± 3.54^{a}
Larval duration (days)		20.2 ± 0.37^{c}	20.4 ± 0.51^{c}	24.6 ± 0.51^{b}	19.0 ± 0.71^{c}	26.8± 0.66^{b}
Pupation% ±SE		93.6 ± 1.17^{a}	56 ± 2.28^{b}	28.8 ± 2.80^{d}	42.4 ± 4.17^{c}	24.4± 3.54^{d}
Pupal duration(days)		11 ± 0.71^{c}	15 ± 0.63^{b}	17.8 ±0.584^{a,b}	15.8 ± 0.74^{a,b}	18.6± 0.68^{a}
Adult emergence% ±SE		88 ± 0.63^{a}	47.2 ± 1.36^{b}	19.6 ± 1.83^{d}	32.4 ± 3.43^{c}	17.2 ± 3.38^{d}
Adult duration(days)		7.2 ± .37^{a}	5.6 ± 0.25^{b}	5.0 ± 0.32^{b}	6.2 ± 0.37^{a,b}	5.0 ± 0.45^{b}
Fecundity % ±SE		1001 ±51.44^{a}	826 ± 35.02^{b}	764.6 ±12.33^{c}	816.4 ±55.82^{b,c}	757 ± 19.87^{c}
Hatchability %±SE		95.55 ± 1.29^{a}	79.46 ± 2.22^{b}	64.46 ± 3.78^{c}	77.23 ± 2.11^{b}	61.18 ± 3.58^{c}
Sterility % ±SE			23.01 ± 2.95^{b}	35.65 ±3.08^{a,b}	25.59 ± 5.68^{b}	40.29 ± 3.48^{a}

Mean values ± standard error followed by different letters in the same raw indicate a significant difference at $P<$ 0.05 according Tukey's HDS comparisons.

Also, **Pereira *et al.* (2024)** confirmed that, flavonoids have the potential to be highly effective insecticides, because of their ability to interfere with digestion, growth, development, and reproduction.

3.9. Evaluation of flavonoid extract as active ingredient (a.i) and (SL) formulation on *F. oxysporium, F. solani* and *A. solani in vitro* conditions

This study, showed Aeffect of flavonoid extract (a.i) alone and formulation (SL) at different concentrations on the linear growth of *F. oxysporium, F. solani* and *A. solani in vitro* conditions. Data in Table (9) and Fig. (3 and 4) illustrated that with flavonoid extract (a.i) or formulation (SL), decreased the straight development of three fungi, its inhibition impact on the linear growth fungal mycelial at all tested concentrations. The flavonoid extract (a.i) displayed the highest mycelial growth inhibition 59.63% for *F. oxysporium,* followed by *A. solani* and *F. solani* 57.78 and 56.67%, respectively, at concentration 8000 ppm. Flavonoid formulation (SL) gave completely inhibition for *F. oxysporium* at concentration of 8000 ppm. Though, the reduction rate was 76.30 and 61.11% for *F. solani* and *A. solani,*

respectively, at the same concentration, The F-value indicated that formulation (SL) is more effective than flavonoid extract (a.i), where the F-value of formulation (SL) were 402.34, 145.89 and 69.4 compared to 183.19, 73.23 and 68.86 for flavonoid extract (a.i) alone against *F. oxysporium, F. solani* and *A. solani,* respectively. The EC_{50} values for formulation (SL) were 1860.90, 1909.98 and 3240.31 ppm, respectively; while they were less effective in case of flavonoid extract (a.i) 5866.38, 4834.23 and 4649.76 ppm respectively. Furthermore, *F. oxysporum* was more sensitive to flavonoid formulation (SL). These results showed clearly that the formulation increased the efficacy of the tested materials against the target pathogen which may be due to the adding of adjuvants to ingredient. This was agreed with **Sobiya *et al.* (2012)** revealed that the methanol extract from *C. citratus* caused maximum inhibition of *Alternaria alternata* and *Alternaria tenuissima* at high concentration of 4% after 5 and 10 days of incubation period. **Yashwanth and Suhas (2021)** showed that the lemongrass oil extract has significant antimicrobial activity against *Botrytis cinerea, Alternaria solani, Rhizoctonia solani, Fusarium oxysporum*, and *Pythium ultimum*. **Mori *et al.* (2017) and Israa *et al.* (2024)**, who showed that the methanolic extract from *C. citratus* used in the fungicide industry as controlling plant pathogens. This effect may be due to polyphenol and flavonoid components in extract.

Mode of action for flavonoids as antifungal activity was suggested by **Draz *et al.* (2019) and Al Yousef (2013)** who mentioned that the presence of alcoholic agencies inside the structure of flavonoids boom the interest of the plant extract to inhibit pathogenic fungi by increasing the defense effects of infected plants these leads to increasing fungal mortality (fungicidal effect), inhibiting fungal growth (fungitoxic effect) and improving plant growth. So, the flavonoid components are taken into consideration as antiseptic dealers that are converting the cellular protein nature and inducing cell membranes permeability. **Sorabh *et al.* (2023)** reported that the flavonoid mechanism of action may involve disruption of the cell membrane of lipophilic components. The high hydrophobic effect allows them to split up in the fluids of fungal cell. So, it makes structures more permeable in a significant feature and their constituents. **Enyiukwu *et al.* (2016)** installed that flavonoid are enzymes antagonists and may interfere with cell membrane integrity, inhibition of cell division, inhibition of RNA/DNA or protein synthesis and inhibition of cell wall formation. **Taghva *et al.* (2016)** confirmed that flowers boom and improvement, in conjunction with protection against contamination and harm, depend on flavonoids.

Table (9). The efficacy of flavonoid extract as active ingredient (a.i) and formulations (SL) against some plant pathogenic fungi *in vitro*.

Conc. (ppm)	Mycelial growth inhibition (%)					
	F. oxysporum		***F. solani***		***A. solani***	
	active ingredient(a.i)	**Formulation (SL)**	**active ingredient (a.i)**	**Formulation (SL)**	**active ingredient (a.i)**	**Formulation (SL)**
Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
1000	13.25 ± 1.23	34.44 ±1.70	24.81 ± 1.69	40.00 ±1.70	22.59 ± 3.03	33.70 ± 0.98
2000	20.37 ± 2.25	50.00 ±1.92	34.81 ± 0.98	48.89 ±1.11	38.89 ± 1.28	44.4 ± 1.28
4000	35.65 ± 1.28	62.96 ± 0.97	50.00 ± 2.31	61.85 ±1.34	48.15 ± 0.74	53.70 ± 1.61
8000	59.63 ± 0.98	99.90 ± 0.37	56.67 ± 1.11	76.30 ±0.98	57.78 ± 1.28	61.11 ± 1.69
F-Value	183.19	402.34	73.23	145.89	68.86	69.40
EC_{50}	5866.38	1860.90	4834.23	1909.98	4649.76	3240.31
EC_{90}	21784.86	7327.12	95386.59	30252.47	86161.87	144697
Slope	1.249 ± 0.18	1.691 ± 0.22	0.972 ± 0.19	1.0678 ± 0.19	1.011 ± 0.19	0.776 ± 0.19

Control was: (PDA medium without any treatment)

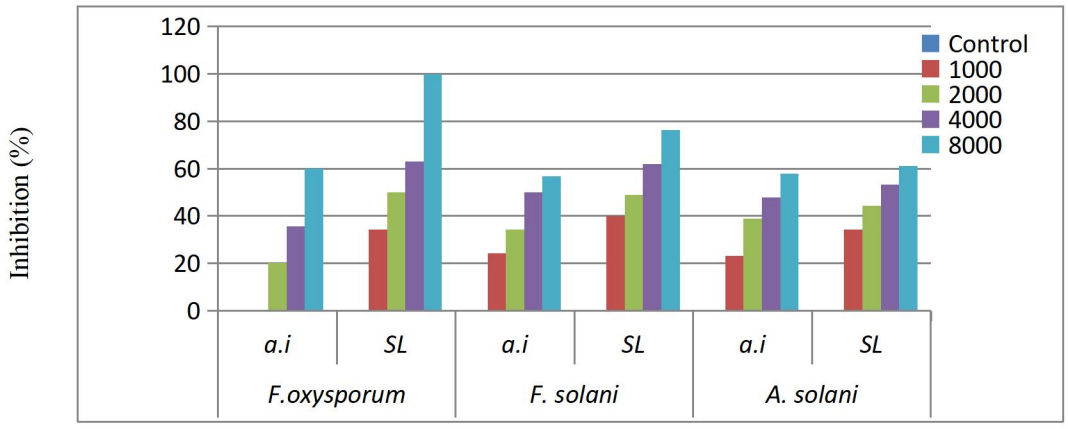


Fig. (3). The mean growth inhibition percent in tested *F. oxysporum, F. solani* and *A. solani* mycelial exposed to concentrations 1000, 2000, 4000, 8000 ppm of flavonoid extract as active ingredient (a.i) and formulations (SL)

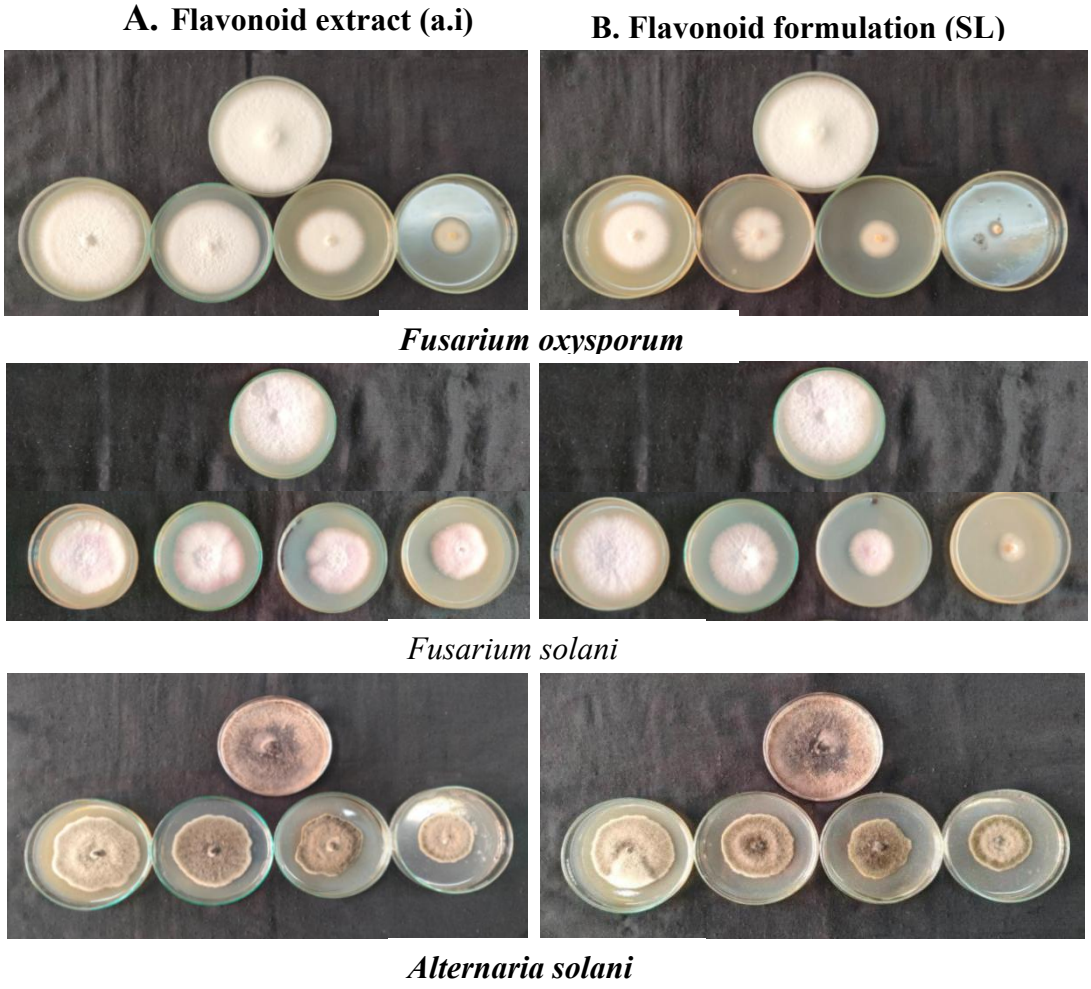


Fig. (4). Antifungal activity of (A) flavonoid extract as active ingredient (a.i) and (B) flavonoid formulation as soluble liquid (SL) with different concentrations (1000, 2000, 4000 and 8000 ppm) of on *F. oxysporum, F. solani* and *A. solani.*

Conclusion:

The findings of this study confirm the potent insecticidal and antifungal properties of flavonoid compounds extracted from *C. citratus* against the neonate of *S. littoralis* pest insects and pathogenic fungi of *F. oxysporum, F. solani* and *A. solani*. The significant reductions in developmental stages of *S. littoralis* larval survival, pupation, adult emergence, fecundity, and fertility coupled with prolonged developmental durations and increased sterility, while inhibit pathogenic fungi, through increasing fungal mortality and inhibit fungal growth development highlighting the potential of flavonoid-based formulations as an effective alternative to synthetic pesticides. Given the increasing concern over pesticide resistance and environmental toxicity, the utilization of plant-derived compounds offers a promising, eco-friendly strategy for pest control. Future research should focus on optimizing formulation techniques to enhance the stability and efficacy of flavonoid-based bio-pesecticides and exploring their field applications for integrated pest management.

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تقييم النشاط الحيوى للفلافونيدات المشتقة من حشيشة الليمون ومستحضراتها ضد دودة ورق القطن وبعض الفطريات المسببة للأمراض النباتية

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الملخص العربى:

النباتات العطرية غنية بالمركبات الفينولية، التي تُعرف بأنها بدائل آمنة للمبيدات الكيميائية. أُجريت هذه الدراسة لتقييم الأنشطة الحشرية والمضادة للفطريات لمركبات الفلافونويد المستخلصة من أوراق حشيشة الليمون (*Cymbopogon citratus*) ومستحضرتها. تم التعرف على 15 مركب من خلال تحليل الكروماتوغرافيا السائلة عالية الأداء (HPLC). كان المركب الرئيسى هو حمض الكلوروجينيك (52.62 ميكروغرام/مل)، وميثيل جلات (22.78 ميكروغرام/مل)، وحمض الجليك (21.32 ميكروغرام/مل)، والروتين (11.10 ميكروغرام/مل)، وحمض الإلجيك (7.43 ميكروغرام/مل)، والكيوماريك (6.89 ميكروغرام/مل)، وذلك وفقًا لمعاييرها. إجتاز المستحضر المجهز جميع الإختبارات المعملية كتركيبة سائلة قابلة للذوبان فى الماء(SL). تم تقييم فعالية تركيزات مختلفة من المستخلص الفلافونيد لحشيشة الليمون. على دودة ورق القطن (حديثة الفقس) قبل وبعد تجهيز المستحضر. أظهرت النتائج أن مستحضر الفلافونويد (SL) له تأثير أكبر من مستخلص الفلافونويد (a.i)، حيث بلغت قيم LC_{50} 3074.2 و5689.3 جزء في المليون على التوالي. بالإضافة إلى ذلك، أظهر كلا المركبين انخفاضًا ملحوظًا في جميع مراحل نمو يرقات دودة ورق القطن، من حيث البقاء على قيد الحياة، والتعذر، وظهور الحشرة البالغة، إلى جانب إطالة فترات النمو، مما يؤثر سلبًا على النشاط التكاثري. من ناحية أخرى، تم تقييم التأثير المضاد للفطريات لنفس المستخلص والمستحضر على فطريات فيوزاريوم أوكسبوروم، وفيوزاريوم سولاني، وألترناريا سولاني معمليا. أكان لأستخدام مستخلص الفلافونويد (a.i) أو المستحضر تأثير مثبط على النمو الخطي للفطريات في جميع التركيزات المختبرة. أظهرت النتائج أن مستحضر (SL) أكثر فعالية من مستخلص الفلافونويد (a.i). حيث أعطى تثبيطًا كاملاً لفطر *F. oxysporium* عند تركيز 8000 جزء في المليون، وبلغت قيمة EC_{50} 1860.90 جزء في المليون. واستنادًا إلى هذه الدراسة والنتائج السابقة، يمكن استخدام تركيبة مستخلص الفلافونويد (SL) كبديل آمن للمبيدات الكيميائية ضد *S. littoralis* و*F. oxysporum* و*F. solani* و*A. solani* بعد استكمال الدراسات اللازمة.