GREEN PESTICIDE: MANAGEMENT OF TOMATO DISEASE FROM PLANT PATHOGEN

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Abstract:

Fusariumoxysporum f. sp. *lycopersici* is responsible for causing tomato wilt disease worldwide. The use of chemical fungicides has significant negative effects on the environment and poses hazards to non-target organisms. Plant metabolites and plant-based pesticides have emerged as promising alternatives due to their lower environmental impact and reduced risk to consumers compared to synthetic pesticides. In this study, extracts from *Daturastramonium* L. and *Calotropisgigantea* L. were tested in vitro for their antifungal properties against *Fusariumoxysporum* f. sp. *lycopersici*, aiming to develop eco-friendly management strategies. Various solvent systems, including methanol and water, were employed to extract the desired plants. The antifungal activity of the extracts was evaluated using the poison food technique at different concentrations (4%, 6%, 8%, 10%, and 12%), resulting in a favourable outcome represented as the percentage of inhibition in FOL mycelial growth. To explore the chemical composition of the extracts, GC-MS analysis was conducted, focusing on important phytochemicals such as flavonoids, alkaloids, and phenolic compounds. This study suggests that botanical extracts could serve as effective plant-based fungicides for the treatment of *Fusariumoxysporum* f. sp. *lycopersici*in organic farming.

Keywords:*Fusariumoxysporum* f. sp. *lycopersici*, tomato, plant metabolites, *Daturastramonium* L.,*Calotropisgigantea* L. poison food technique, organic farming

Introduction:

Tomatoes are a major dietary source and a rich source of nutrients such as vitamin C and antioxidants. They also reduce the risk of heart disease and cancer [1]. Tomatoes are widely used worldwide as a vegetable in various commercial forms, such as soup, juice, and flavouring agent. India is the second-largest producer of tomatoes. However, tomato plants are susceptible to many diseases, including late blight, early blight, and bacterial spots, which reduce the production rate of tomatoes.

Fusarium wilt, a fungal disease caused by the soil-borne pathogen *Fusariumoxysporum* f. sp. *lycopersici*, is a common problem in tomato plants. The pathogen enters the plant roots and blocks the conduits that circulate water and nutrients upward. The favourable conditions for wilt disease include acidic soil pH, high humidity, high nitrogen levels, and warm weather. The initial symptoms of wilt disease include drooping and wilting. Yellowing of the lower

leaves often begins on only one side of the plant and gradually progresses upward. The disease also stunts plant growth and ultimately leads to plant death [2, 3, 4].

To reduce the risk of wilt disease, general disease management practices should be followed, such as using plant-resistant varieties, implementing crop rotation every 3-5 years, using only healthy transplants, avoiding excessive nitrogen use, and managing soil pH.

Besides the conventional management of Fusarium wilt, which involves the use of chemical pesticides and fungicides available in the market, it's important to consider the potential drawbacks of excessive chemical usage. While these chemicals can help prevent the disease, their overuse can harm the crop and kill beneficial soil microbes, negatively impacting soil quality. Moreover, they can have harmful effects on consumers, including the risk of cancer and skin problems.

To address this issue, it is advisable to explore natural alternatives. In this regard, *Daturastramonium* L. and *Calotropisgigantea* L. have been tested against *Fusariumoxysporum* f. sp. *lycopersici*, the pathogen that causes Fusarium wilt disease. Both *Daturastramonium* L. and *Calotropisgigantea* L. possess antifungal and antibacterial properties, which make them potential candidates for natural disease control.

Methods and Materials [5]:

Plant Material: Fresh leaves of *Daturastramonium* L. and *Calotropisgigantea* L. were collected in October 2022.

Washing: The collected leaves were thoroughly rinsed to remove any dust particles.

Drying: The washed leaves were dried in the shade for 10-15 days to remove moisture content.

Grinding: Grinding was performed to obtain a homogeneous plant sample, which also increased the surface contact of the plant material with the solvent.

Preparation of Plant Crude Extract: The plant extraction was carried out using the maceration process. Aqueous extracts of *Daturastramonium* L. and *Calotropisgigantea* L. leaves were prepared by soaking 30g of plant powder material in 450 ml of distilled water. Methanol extract was prepared by soaking 20g of plant powder material in 300 ml of methanol. The extract solvents were kept at room temperature and shaken at regular intervals for 5 days. Afterward, the extract solvents were filtered, and the solvent was concentrated by evaporation using a water bath at 70°C. The remaining semi-solid extract of plant leaves was stored in an airtight container at 4°C for further analysis.

Phytochemical Screening of Plant Extracts [5, 6]:

Phytochemical analysis was conducted on the plant extracts to determine the presence of various phytochemical constituents.

Test for Alkaloids: Concentrated plant extracts (0.5g) were mixed with 5 mL of 1% HCl acid. The mixture was gently heated for approximately 20 minutes to ensure proper dissolution of the extract. After cooling, the mixture was filtered, and the filtrates were used for the following tests.

Wagner's Test: A few drops of Wagner's reagent were added to 2 mL of filtrate in a test tube. A positive result was indicated by the formation of a reddish-brown precipitate.

Hager's Test: 2 mL of filtrate was treated with Hager's reagent. The presence of yellow precipitates indicated a positive result.

Test for Phenols: A 50 mg portion of the extract was diluted with 5 mL of distilled water. A few drops of neutral 5% ferric chloride solution were added to the aqueous solution. The formation of a dark brown color indicated the presence of phenolic compounds.

Test for Tannins: 0.5g of the extract was dissolved in 4 mL of distilled water and then mixed with 3 mL of 10% lead acetate solution. The detection of white bulky precipitates indicated the presence of tannins.

Test for Flavonoids: The aqueous solution of the extract was treated with a 10% ammonium hydroxide solution. The presence of yellow fluorescence indicated the presence of flavonoids.

Test for Saponins: 0.5g of the extract was mixed with 5 mL of distilled water and continuously stirred for 15 minutes. The formation of foam indicated the presence of saponins.

Test for Oil: A small amount of the extract was pressed between two filter sheets. The presence of oil stains on the paper indicated the presence of oil.

Test for Carbohydrates: Barfoed reagent was added to 1 mL of filtrate and boiled in a water bath for 2 minutes. The formation of red precipitates indicated the presence of carbohydrates.

Test for Proteins: A few drops of ninhydrin reagent were added to the filtrate. The formation of a blue color indicated the presence of proteins.

Test for Phytosterols: 0.5g of the extract was dissolved in 2 mL of acetic anhydride. To this, 2 to 3 drops of concentrated sulfuric acid were added. Color change in the tube indicated the presence of phytosterols.

Antifungal activity of plant extracts by Poison food method:

The fungal species *Fusariumoxysporum* f. sp. *lycopersici*was initially grown on Potato Dextrose Agar (PDA). Stock solutions of each plant extract were mixed into 10 mL of sterilized PDA medium in Petri plates at different concentrations: 4, 6, 8, 10, and 12 mg% of

medium. Once the medium solidified, a 5 mm disc of actively growing *Fusariumoxysporum* f. sp. *lycopersici*was placed at the centre of each Petri plate containing different concentrations of plant extracts. Negative control plates were prepared without any plant extracts. All the plates were then incubated for 6-7 days at 27°C. The experiment was repeated four times for each plant extract. After incubation, the radial growth of each Petri plate was measured [5]. The results were compared with those of the negative control.

GC-MS Analysis:

The phytochemical evaluation of aqueous and methanolic extracts of *Daturastramonium* L. and *Calotropisgigantea* L. was done by Gas chromatography/ Mass spectroscopy analysis [7].The experimental conditions of the GC-MS spectra were as follows [8]: Technology type: Gas chromatography/Mass spectroscopy, Product group: GC/MS column, Phase of GC-MS: Elite-5ms, Film thickness: 0.25mm, Inner diameter: 0.25mm, the injectable amount: 1µl.

Pot experiment:

The seeds for the control pots were soaked in distilled water that was not treated with any plant extract [9]. On the other hand, the seeds for the test pots were treated with selected plant extracts, namely DM, DW, AM, and AW. After the seed treatment, the seeds were sown into the labeled pots.

Result:

Phytochemical testing:

The extracted contents of whole plants, *Daturastramonium* L. and *Calotropisgigantea* L., were subjected to different phytochemical tests using various solvents such as methanol and distilled water. The purpose was to determine the presence or absence of different bioactive compounds. The results of these tests are presented in Table 1. Qualitative tests were conducted for Alkaloids, Phenol, Tannin, Flavonoids, Saponin, Oil, Carbohydrates, Protein, and Phytosterols. The overall outcomes of the phytochemical tests indicated that, in comparison to other bioactive compounds, the plant extracts contained a significant amount of Phytosterols, Oil, Tannin, and Alkaloids.

Test	DM	DW	AM	AW
Alkaloid test Wagner's test	+++	++	+	++
Alkaloid test Hager's test	+++	++	++	+
Tannin	+	+++	+	+++
Phenol	+	++	+++	++
Flavonoid	+++	+	++	+
Saponin	+	+++	++	++
Carbohydrates	++	+	+	++
Protein	_	+	+	++
Phytosterol	+++	+++	+++	+++

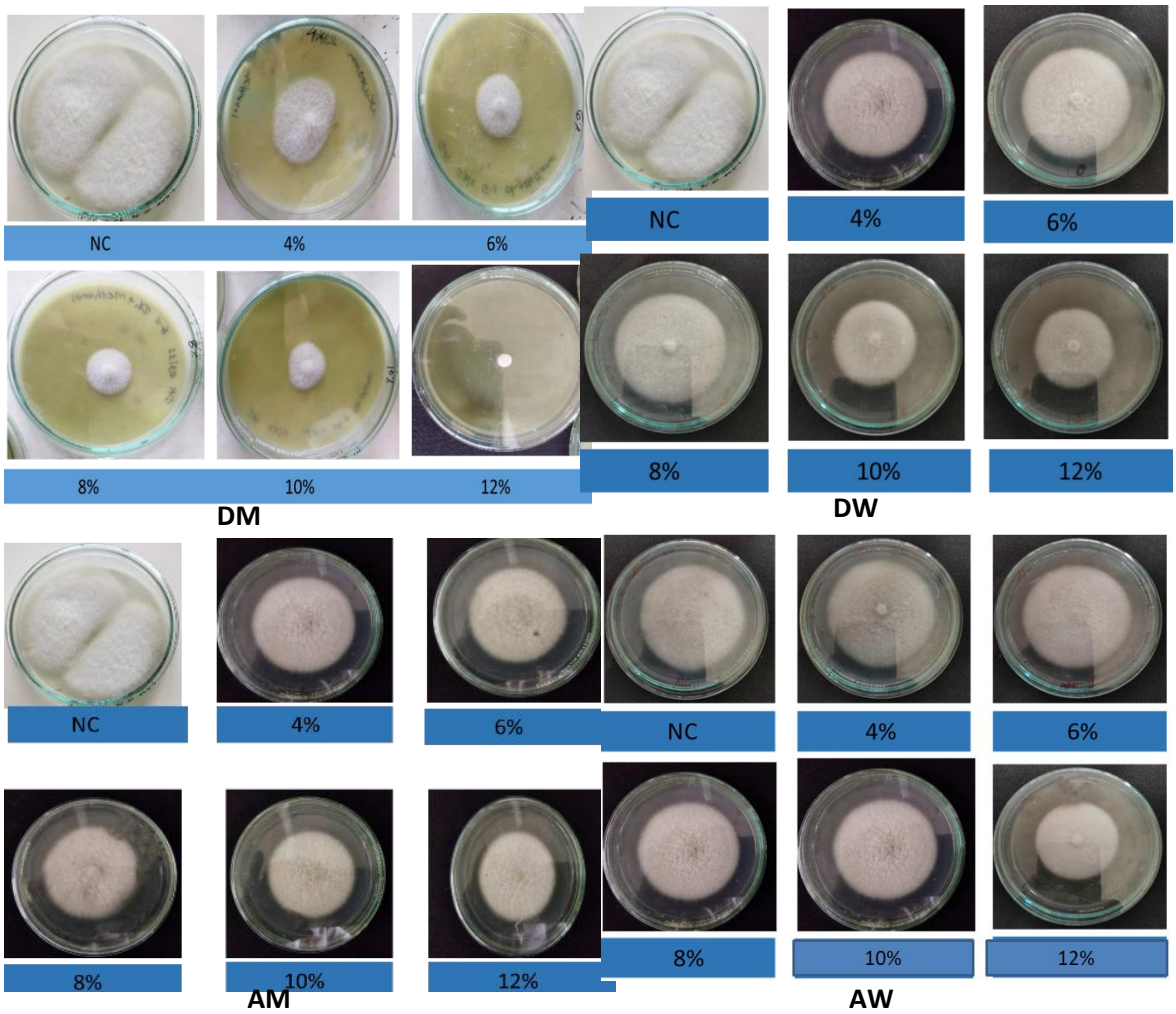
(Table1: Phytochemical screening of plant extract; DM:Methanol extract of *Daturastramonium* L.DW: Water extract of *Daturastramonium* L.,AM:Methanol extract of *Calotropisgigantea* L.,AW:Water extract of *Calotropisgigantea* L.)

In vitro antifungal activity:

The results presented in Table 2 demonstrate the inhibition of mycelial growth of *Fusariumoxysporum* f. sp. *lycopersici* by the plant extracts of *Daturastramonium*L.and*Calotropisgigantea* L., using both methanolic and aqueous solvents. As per the results, at the highest concentration tested (12%), the plant extracts exhibited significant antifungal activity, as evidenced by the lowest mycelial growth of Fusarium. Specifically, the methanolic extract of *Daturastramonium*L.displayed substantial antifungal activity compared to the other extracts.

Concentration of extracts	**% Inhibition**			
	DM (%)	**DW (%)**	**AM (%)**	**AW (%)**
4%	55.81±0.4	16.28±0.45	41.86±0.18	15.12±0.34
6%	59.30±0.52	18.60±0.42	44.19±0.21	17.44±0.34
8%	62.79±0.34	20.93±0.51	45.35±0.35	19.77±0.44
10%	67.44±0.22	38.37±0.31	46.51±0.33	22.09±0.21
12%	81.39±0.18	39.53±0.20	48.84±0.43	37.21±0.23

[Table 2: Growth inhibition of *Fusariumoxysporum* f. sp. *lycopersici;*Data presented in Mean±SD]



[Figure:1Growth inhibition of*Fusariumoxysporum* f. sp. *lycopersici* by plant extracts; DM:Methanol extract of *Daturastramonium* L.DW: Water extract of *Daturastramonium*L.,AM:Methanol extract of *Calotropisgigantea* L.,AW:Water extract of *Calotropisgigantea* L.]

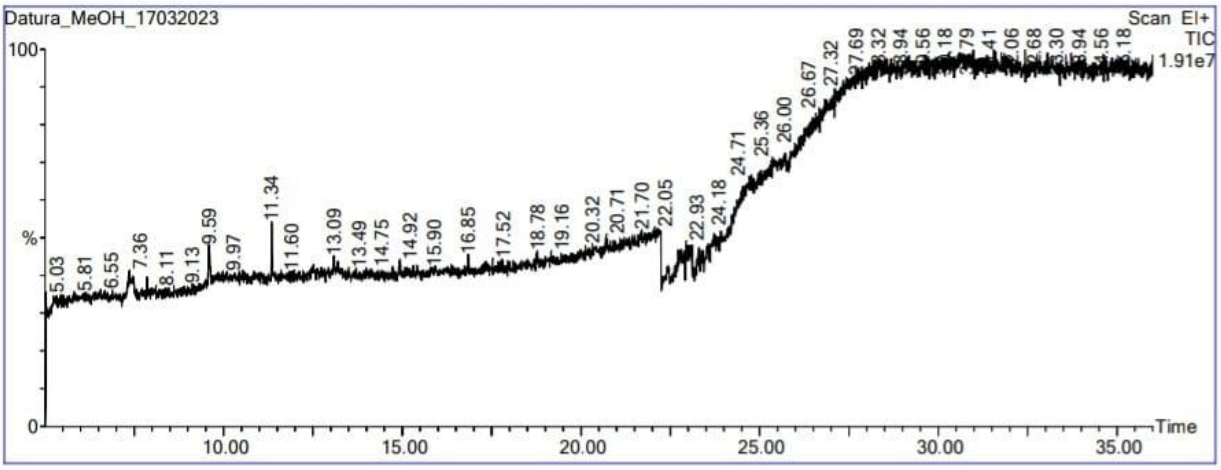
After 7 days of incubation, the results depicted in the above figures illustrate that the mycelial growth of *Fusariumoxysporum* f. sp. *lycopersici*is inversely related to the increasing concentration of the selected plant extracts (of *Daturastramonium* L. and *Calotropisgigantea* L.) in both methanol and aqueous solvents. Notably, Fig 1 highlights the significant antifungal activity of the methanolic extract of of*Daturastramonium*L..

GC-MS Profile:

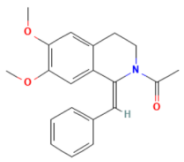
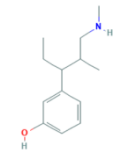
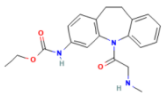
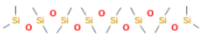
Methanol extract of *Daturastramonium* L.:

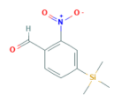
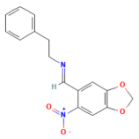
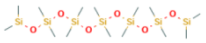
The chromatogram of the GC-MS spectra analysis for the methanolic extract of *Daturastramonium* L. (DM) is presented in Fig 2. The peaks correspond to several bioactive compounds identified in the chromatogram. Table 3 provides a summary of eight bioactive

compounds present in the methanol fraction of *Daturastramonium* L, including their retention time (RT), compound name, molecular weight, molecular formula, peak area (PA), and structures [10]. The identified bioactive compounds in the methanolic extract of *Daturastramonium* L. presented in table 2.



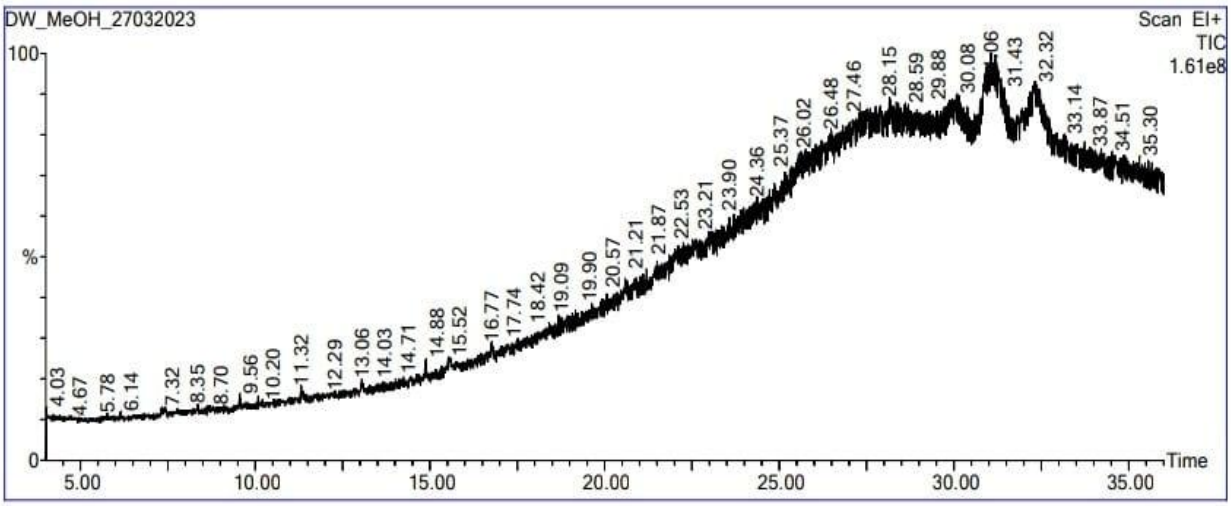
(Figure 2: GC-MS chromatogram of methanol fraction of *Daturastramonium* L. leaves)

SR NO.	RT	NAME OF COMPOND	MOLECULAR FORMULA	MW	PA(%)	STRUCTURE
1	12.518	1,2,3,4-Tetrahydroisoquinolin, 2-acetyl-6,7- dimethoxy-1-phenmethylene-	$C_{20}H_{21}NO_3$	323.4	1.328	
2	16.845	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	$C_{14}H_{42}O_6Si_7$	503.7	1.781	
3	18.505	N-Desmethyltapentadol	$C_{13}H_{21}NO$	207.31	1.371	
4	20.394	Carbamic acid, N-[10,11-dihydro-5-(2-methylamino-1-oxoethyl)-3-5H-dibenzo[b,f]azepinyl]-, ethyl ester	$C_{20}H_{23}N_3O_3$	353.4	1.564	
5	20.705	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	$C_{16}H_{48}O_7Si_8$	577.2	1.485	

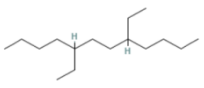
6	21.210	Benzaldehyde, 2-nitro-4-trimethylsilyl-	$C_{10}H_{13}NO_3Si$	223.3	1.800	
7	21.705	4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene)tyramine	$C_{16}H_{14}N_2O_4$	298.29	2.237	
8	22.053	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	$C_{14}H_{42}O_6Si_7$	503.07	2.244	

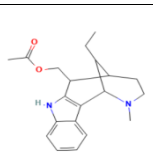
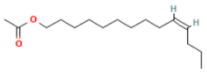
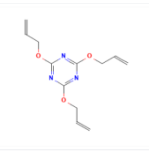
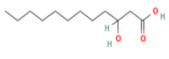
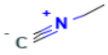
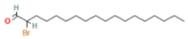
(Table 3: Bioactive compounds identified from the methanolic extract of of*Daturastramonium* L.)

Methanol extract of *Daturastramonium* L.:



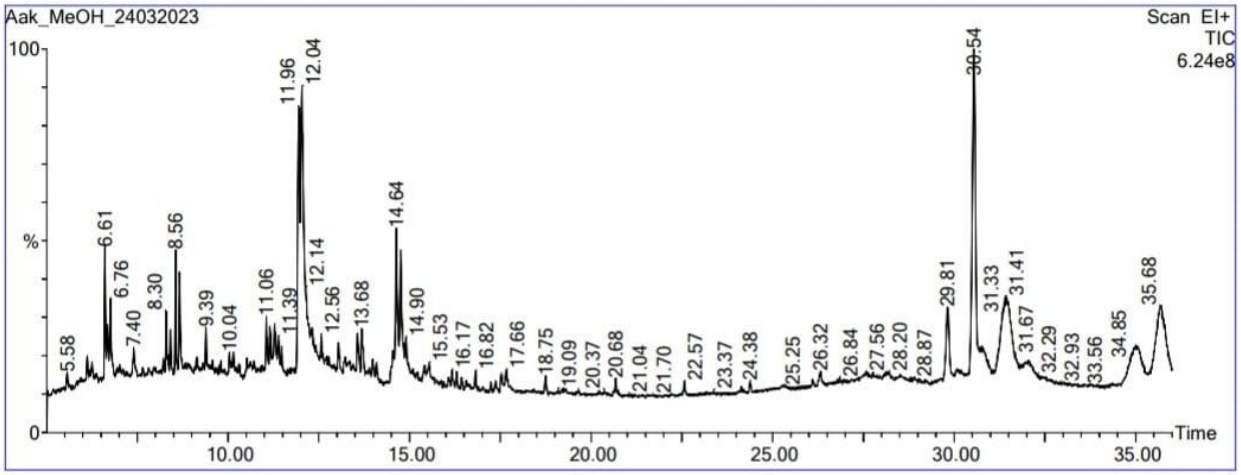
(Figure 3: GC-MS chromatogram of aqueous methanol fraction of *Daturastramonium* leaves)

SR NO.	RT	NAME OF COMPOND	MOLECULAR FORMULA	MW	PA(%)	STRUCTURE
1	27.893	Cis-13-Eicosenoic acid	$C_{20}H_{38}O_2$	310.5	3.396	
2	28.150	Dodecane, 5,8-diethyl-	$C_{16}H_{34}$	226.44	3.545	
3	28.938	Dasycarpidan-1-methanol, acetate	$C_{20}H_{26}N_2O_2$	326.4	3.265	

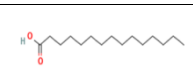
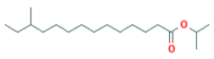
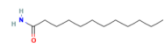
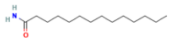
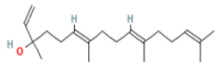
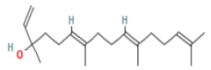
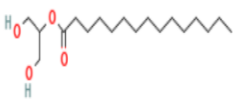
		(ester)				
4	29.947	Z-10-Tetradecen-1-ol acetate	$C_{16}H_{30}O_2$	254.41	2.224	
5	30.075	Triallylcyanurate	$C_{12}H_{15}N_3O_3$	249.27	2.250	
6	30.928	Dodecanoic acid, 3-hydroxy-	$C_{12}H_{24}O_3$	216.32	3.321	
7	31.276	Methyl isocyanide	C_2H_3N	41.05	4.529	
8	32.303	Octadecanal, 2-bromo-	$C_{18}H_{35}BrO$	347.4	5.648	

(Table 4: Bioactive compounds identified from the Water extract of of*Daturastramonium* L.)

Methanol extract of *Calotropisgigantea* L.:

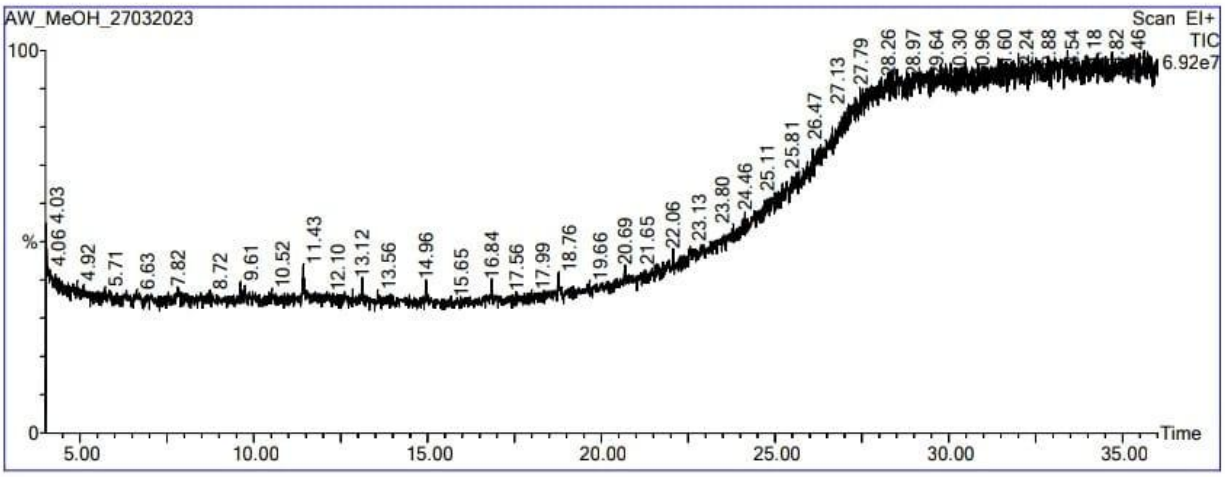


(Figure 4: GC-MS chromatogram of methanol fraction of *Calotropisgigantea* L.leaves)

Sr no.	RT	Name of Compound	Molecular formula	MW	PA(%)	Sructure
1	11.958	Pentadecanoic acid	$C_{15}H_{30}O_2$	242.4	4.938	
2	12.041	i-Propyl 12-methyltetradecanoate	$C_{18}H_{36}O_2$	284.5	9.999	
4	14.636	Dodecanamide	$C_{12}H_{25}NO$	199.33	2.862	
5	14.755	Tetradecanamide	$C_{14}H_{29}NO$	227.39	2.736	
6	29.810	1,6,10,14-Hexadecatetraen-3-ol, 3,7.11.15-tetramethyl-,(E.E)-	$C_{20}H_{34}O$	290.5	2.128	
7	30.543	1,6,10,14-Hexadecatetraen-3-ol, 3,7.11.15-tetramethyl-,(E.E)-	$C_{20}H_{34}O$	290.5	10.087	
8	31.433	Pentadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{18}H_{36}O_4$	316.5	7.837	

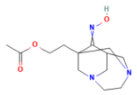
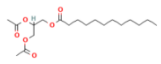
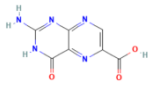
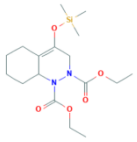
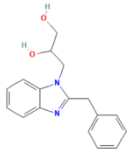
(Table 5: Bioactive compounds identified from the methanol extract of *Calotropisgigantea* L.)

Water extract of *Calotropisgigantea* L.:



(Figure 5: GC-MS chromatogram of aqueous methanol fraction of *Calotropisgigantea*L.leaves)

Sr no.	RT	Name of Compound	Molecular formula	MW	PA(%)	Structure
1	11.426	Cyclononasiloxane,octadecamethyl-	$C_{18}H_{54}O_9Si_9$	667.4	0.560	

2	13.122	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13, 15, 15-hexadecamethyl-	$C_{16}H_{48}O_7Si_8$	577.2	0.581	
3	16.845	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13 tetradecamethyl-	$C_{14}H_{42}O_6Si_7$	503.07	0.607	
4	18.761	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	$C_{14}H_{42}O_6Si_7$	503.07	0.675	
5	28.645	1-(2-Acetoxyethyl)-3,6-diazahomoadamantan-9-one oxime	$C_{13}H_{21}N_3O_3$	267.32	0.507	
6	29.085	Dodecanoic acid, 2,3-bis(acetyloxy)propyl ester	$C_{19}H_{34}O_6$	358.5	0.494	
7	33.220	Pterin-6-carboxylic acid	$C_7H_5N_5O_3$	207.15	0.662	
8	34.238	1,2-Cinnolinedicarboxylic acid, 1,2,3,5,6,7,8,8a- octahydro-4-trimethylsilyloxy-, diethyl ester	$C_{17}H_{30}N_2O_5Si$	370.5	0.667	
9	35.338	3-(2-Benzyl-benzoimidazol-1-yl)-propane-1,2-diol	$C_{17}H_{18}N_2O_2$	282.34	0.662	

(Table 6: Bioactive compounds identified from the water extract of *Calotropisgigantea* L.)

Pot experiment:

Extracts	Germination rate (%)	Wilt (%)	Survival rate(%)
NC	10±.90	80±1.2	20±1.1
DM	40±0.87	25.00±.90	75.00±0.92
DW	30±0.78	66.67±0.89	33.33±0.90

AM	30±0.90	66.67±1.1	33.33±1.0
AW	20±0.23	50.00±1.4	50.00±1.2

(Table 7: Germination rate, Disease rate and Survival rate in percentage;DM:Methanol extract of *Daturastramonium* L.DW: Water extract of *Daturastramonium* L.,AM:Methanol extract of *Calotropisgigantea* L.,AW:Water extract of *Calotropisgigantea* L.)

The summarized table provides information on the germination rate (%), wilt rate (%), and survival rate (%) for the selected plant extracts. In comparison to the control pots, which had a germination rate of only 10% and a wilt disease rate of 80%, the seeds treated with the selected plant extracts showed a significant improvement in germination rate and a reduction in wilt disease.

Among all the plant extracts, the methanol extract of *Daturastramonium*L.exhibited the highest germination rate and a lower wilt disease rate compared to the others. This indicates that the methanol extract of *Daturastramonium* L.had a positive impact on seed germination and provided some protection against wilt disease. The survival rate was not explicitly mentioned in the information provided.

(Fig 6: Pot assessment of tomato seeds treated with aqueous and methanol extracts of *Daturastramonium* L. and *Calotropisgigantea* L.;NC:Negative control, DM:Methanol extract of *Daturastramonium* L.DW: Water extract of *Daturastramonium* L.,AM:Methanol extract of *Calotropisgigantea* L.,AW:Water extract of *Calotropisgigantea* L.)

The results shown in Figure 6 demonstrate the efficacy of the selected plant extracts (methanol and aqueous extracts of *Daturastramonium*L.and*Calotropisgigantea* L.) in terms of germination rate and disease rate compared to the control plants. It indicates that the plants treated with these extracts exhibited a considerable germination rate and a lower disease rate in comparison to the control plants.This suggests that the plant extracts, particularly the methanol and aqueous extracts of *Daturastramonium*L.and*Calotropisgigantea* L., had a positive effect on seed germination and provided some level of protection against disease.

CONCLUSION:

The results obtained from the study indicate that the plant extracts of *Daturastramonium* L.and *Calotropisgigantea* L.have the potential to be used as biocontrol agents for managing fusarium wilt disease. The extracts exhibited significant antifungal activity against *Fusariumoxysporum* f. sp. *lycopersici*, the causative pathogen of the disease. The presence of various phytochemicals, including phenol, alkaloids, phytosterols, oil, and saponin, in the plant extracts suggests their potential role in inhibiting the growth of the pathogen.

Furthermore, the GC-MS analysis identified several bioactive compounds in the methanol extract of *Daturastramonium* L., which may contribute to its antifungal activity. The pot experiment conducted under controlled conditions demonstrated that treating the plants with the methanol and aqueous extracts *Daturastramonium*L.and*Calotropisgigantea* L.resulted in a significant germination rate and lower disease rate compared to the control plants.

These findings suggest that utilizing natural plant extracts with antifungal properties could serve as an alternative to chemical pesticides and fungicides for managing fusarium wilt disease in tomato plants. This approach not only helps in controlling the disease but also contributes to the maintenance of soil fertility and the reduction of chemical-related risks to the environment and human health.

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