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ORIGINAL ARTICLE **OPEN ACCESS**

HPTLC Fingerprint Profile and Antifungal activity of selected botanical extract against *Fusarium oxysporum* for management of cumin wilt disease

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ABSTRACT
Fusarium oxysporumf.sp. cumini is a fungus that causes wilt disease in cumin all over the world. Chemical fungicides have major environmental consequences and are toxic to non-target creatures. Plant metabolites and plant-based pesticides appear to be among the best choices since they are known to have a lower environmental effect and pose less risk to consumers than synthetic pesticides. In order to establish eco-friendly management, plant extracts of Ricinus communis were used in an in- vitro antifungal test against Fusarium oxysporum f. sp. cumini. Different solvent systems were used to extract the selected plant, including methanol, acetone, aqueous methanol, aqueous acetone, dichloromethanol: methanol, and water. Preliminary phytochemical examination of all extracts revealed positive results for major phytoconstitutes such as phenol, flavonoid, alkaloid, tannin, and so on. The poison food technique was used to assess the antifungal activity of extracts at various concentrations (4 %, 6%, 8%, 10%, 12% and 14 %) on mycelial development of against Fusarium oxysporum and obtained a favourable result in the form of %inhibition. HPTLC fingerprinting was done for each extract and found positive result for flavonoid and alkaloid. This work suggests that botanical extracts might be a useful option in producing powerful plant-based fungicides for the treatment of Fusariumoxysporum f. sp. cumini in organic farming.
Keywords: Fusarium oxysporum f. sp. cumini, cumin, Plant metabolites, Ricinus communis, poison food technique, HPTLC fingerprinting, organic farming

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INTRODUCTION

India is the world's greatest producer, user, and exporter of spices. Cumin (*Cuminum cyminum L.*) is a significant seed spice crop farmed in the states of Gujarat and Rajasthan. Fungal diseases such as wilt, blight, and powdery mildew are serious problems in the cumin crop and cause significant yield losses in the majority of cumin growing areas in India; however, wilt occurs by *"Fusarium oxysporumf.sp. cumini"* is one of the destructive diseases, causing severe yield losses [1]. The wilt caused roughly 40% crop losses, according to *Fusarium oxysporum f. sp. cumini* causes wilt disease. The disease causes withering symptoms in seedlings. Brownish browning is visible when the stem is sliced lengthwise. When the soil temperature in unfertilized fields ranges between 12.5 and 140 degrees Celsius, epidemics occur on a regular basis. *F. oxysporum* is a saprophyte that survives on the detritus of diseased plants as a mycelium and chlamydospores as it is both soil and seed borne and spreads short distances by water, rain splash, and wind, as well as intercultural activities. After an infected plant dies, the pathogen invades tissues, sporulates, and spreads to neighbouring plants. The disease's negative consequences produce a considerable loss in cumin yield. Because the illness is soil-borne, chemical management of the disease becomes challenging [2].

Fungal infections of crops are often managed by the use of resistant cultivars, extended rotations, and fumigants, but mostly through the use of fungicides. The use of synthetic fungicides is not an environmentally friendly strategy because many have been related to major health hazards and an increased prevalence of numerous forms of cancer. Alternative approaches for controlling fungal infections have been investigated, with the goal of reducing the usage of synthetic fungicides by employing chemicals obtained from plant sources [3].

Natural solutions, such as botanical amendments or botanical extracts, are being used to address fungal infections in plants as an alternative to artificial fungicides owing to the fewer harmful effects to human & environmental health hazards or consequences. It can be utilised to create better, safer, and more environmentally friendly fungicides. 'Plant kingdoms' are a wealthy supply of organic chemicals, lots of which have medical use. *Ricinus communis* is one of numerous natural crude medicines derived from plants that have the potential to cure a wide range of diseases and problems. "*Ricinus communis*" is a blooming plant in the Euphorbiaceae family. Plant leaves were employed for fungicidal objective [4].
In this study, the antifungal capabilities of *Ricinus communis* extract were tested in vitro against *Fusarium oxysporum f. sp. cumini* utilising aqueous, methanol, aqueous methanol, acetone, aqueous acetone, and methanol: dichloromethane solvents to develop natural fungicides. All extracts were also subjected to phytochemical testing and HPTLC fingerprinting.

MATERIAL AND METHODS

Plant material:

In November 2020, fresh *Ricinus communis* leaves were collected in the Mehsana district. Dr.Sachin A. Punekar, Scientist & Founder President, Biospheres, Pune, Maharashtra, has authenticated the plants gathered.

Processing of the plant:

Washing: To remove the clinging undesirable particles, the gathered healthy leaves were rinsed with water.

Drying of plant material: Because it lowers the moisture content of fresh materials, drying is a crucial step of dried material preparation for subsequent processing. However, drying conditions have been proven to have a considerable impact on sensory quality, bioactive component stability, and activity. The plant material was dried in the shade for 7-15 days.

Grinding: Grinding to get a homogeneous sample and to increase the surface contact of the sample with the solvent solution.

Storage: Plant powders are Stored at lower temperature [5].

Physicochemical Parameters:

The numerous physicochemical characteristics established in accordance with The Unani Pharmacopoeia of India. 'Odor, taste', 'colour', 'moisture content', 'total ash value', 'acid insoluble ash value', 'water soluble ash value', and 'extraction yield' were all included [5].

Determination of Moisture (Loss on drying)

1.5gm powdered leaves were measured into a weighted plane & slim Porcelain dish. It was dehydrated in the oven at temperatures ranging from 100^{o} to 105^{o} Celsius. Cooling in desiccators and observing weight loss is commonly measured as 'moisture'.

Ash values

The 'total ash', 'acid insoluble ash', and 'water-soluble ash values' of air-dried samples were determined.

Total ash value

Weigh about 2 gram of the air dried material in a formerly burned and tarred silica crucible. Spread the material out evenly and progressively raise the temperature to 500 - 600°C until it is white, showing the carbon absence. Let the remains to cool for 30 minutes in a desiccator before weighing with no time interval. Using air-dried material standards, percentages of 'total ash' were calculated.

Total ash value of the sample15 =100(Z-x)/y %

X= 'weight of empty dish'

Y= 'weight of the drug taken'

Z= 'weight of the dish + ash (after complete incineration)'

Acid insoluble ash

Fill the crucible with 25 mL of 2N Hcl, cover with a watch glass, and slowly boil for five minutes. The watch glass was cleansed with 5 mL of hot water, add the liquid to the crucible. Collect ash-free filter paper containing insoluble elements and wash with hot water till the filtrate is neutral. Move the filter paper comprising insoluble particles to the initial crucible, dry on a hot plate, and burn to steady weight. Enables the residue to cool in desiccators for 30 minutes before weighing without a time gap. Compute the acid-insoluble ash content per gram of air-dried material in milligram.

Water soluble ash

Bring 25 ml of water to a boil in the crucible containing the whole ash for 5 minutes. In a sintered-glass crucible, combine the insoluble ingredients. After cleaning with hot water, ignite for 15 minutes in a crucible at a temperature no greater than 450 °C. Reduces the weight of this residue in milligram in relation to the total ash weight. Determine the concentration of water-soluble ash in mg per gram of air-dried material. Using an air-dried material, the proportion of water-soluble ash was calculated.

Preparation of plant crude extracts:

Plant extraction was accomplished by the maceration process. The methanol, Acetone, aqueous methanol, aqueous acetone, dichloromethane: methanole, and aqueous extracts were made by soaking 30gm of powder plant materials in 30ml of every extractant for 6 days at room temperature. The extracts were separated and concentrated using a water bath at 70°C for the aqueous extract and 50°C for methanol, acetone, and dichloromethane to get semisolid products. The dried extract was placed in an airtight container and kept at 4°C until further examination [6].

The % extractive yield was computed using the formula shown below, and the results are shown as Table No: 1.

% 'Extractive yield (w/w) = weight of dried extract /weight of dried leave × 100'

Phytochemical Screening [7,8]

To examine the numerous chemical groups found in extracts, qualitative preliminary phytochemical experiments were performed. Using recognised techniques, the presence of primary metabolites such as proteins, carbohydrates, and fixed oils and fats was determined. Secondary metabolites in *Ricinu scommunis* leaf extracts included alkaloids, flavonoids, saponins, polyphenols, tannins, terpenoids, and glycosides.

Test	Procedure	Positive result indicated by
Mayer's test for Alkaloid	Take a few ml of filtrate, add few drops of Mayer's reagent	A white or creamy precipitate
Wagner's test for Alkaloid	Take a few ml of filtrate, add few drops of Wagner's reagent by the side of the test tube	A reddish brown precipitate
Dragendorff's test Alkaloid	Take a few ml of filtrate,Add 1 or 2 ml of Dragendorff's reagent	A prominent yellow precipitates
Detection of Saponin	The extract (50mg)is diluted with distilled water and made up to 20ml. The suspension is shaken in graduated cylinder for 15min.	A two cm layer of foam
Detection of Glycosides	Take 2 ml of filtrate, add 3 ml of chloroform and shake well, add 10% ammonia solution.	Pink colour
Detection of total phenolic compound	The extract 50mg is dissolved in 5 ml of distilled water, add few drops of neutral 5%ferric chloride	dark green color
Detection of tannin	The extract 50mg is dissolved in 5 ml of distilled water, add 3 ml of 10% lead acetate solution	bulky white precipitate
Detection of Flavonoid	Take extract, add 10%ammonium hydroxide	Yellow fluorescence
Molish;s test for carbohydrates	Take 2 ml of filtrate, add two drops of alcoholic solution of α-naphthol, shake well, add 1 ml of concentrated sulphuric acid at side of tube.	violet ring
Barfoed'stest for carbohydrates	Take 1 ml of filtrate,add1ml of barfoed's reagent, heated on a boiling water bath for 2 min	Red precipitates
Detection of protein	Take filtrate, add 2 drops of ninhydrin.	purple color
Detection of oil	A small quantity of extract is pressed between two filter paper	Oil stain on the paper
Detection of terpenoid	Take 0.5ml of extract, add 2 ml of chloroform and few drops of concentrated sulphuric acid.	red brown color
Detection of Anthraquinone	Take extract, Add few drops of 10% ammonia solution, shake it vigorously for 30 second.	pink/violet/red color
Detection of steroids	Take extract, add equal volume of chloroform, add few drops of concentrated Hcl.	brown ring

Preparation of extracts concentrations from various extractants

The 'crude extracts' produced by the various solvents, were concentrated using dimethylsulfuroxide (DMSO) to obtain concentrations of 4,6,8,10,12,14,16mg/ml for each extract.

Test organism

Fungi that cause plant disease were considered. The selected fungal species was *Fussarium oxysporum*, which was received from the Indian type culture collection (ITCC) with the ITCC number 1053 in the month of January 2021. On potato dextrose agar, the fungal isolates were kept at 4°C.

Antifungal activity assay of botanical extracts by using poison food technique

For each treatment, the experiment was carried out three times. Plant extracts from each stock solution were applied to 10 ml of sterilised potato dextrose agar in petri plates at varying concentrations of 4%,6%,8%,10%12%,14%,16%. The centre of the Petri plate was filled with a 5 mm diameter actively growing mycelium disc of the pathogen from a 6–7 day old culture. Negative controls were plates with no plant extract. At 27°C, the plates were incubated. After seven days of incubation, radial growth of

mycelium was measured. The outcomes were compared to a negative control. The experiment was done three times, with the mean of the three measurements used for computations. The percentage of fungal inhibition in treatments was estimated using the formula below;

$L = [(C - T)/C] \times 100$

Where, 'L is the percent inhibition'; 'C is the colony radius in control plate' and 'T is the radial growth of the pathogen in the presence of plant extracts' [9].

Statistical analysis

For the zones of inhibition assessed in each example, means and standard errors of the mean were determined [10].

HPTLC Analysis:

HPTLC studies were carried out using the standard method described by Wagner et al [11].10ul of sample were loaded in Silica gel TLC plate. The samples loaded plate was kept in TLC twin trough developing chamber (after being saturated with solvent vapour) with respective mobile phases, namely, toluene-acetone-formic acid (4.5 : 4.5 : 1) for flavonoids, and Ethyl acetate-methanol-water(10:1.35:1) for alkaloid. The plate was developed up to 90mm. The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in photodocumentation chamber and the images were captured under visible light, UV 254 nm, and UV 366 nm. The peak table, peak display, and peak densitogram were noted.

RESULT AND DISCUSSION

The first phase in this research was to gather and treat plants; following that, several physicochemical features of plant powder should be noted. The following table-1 depicts the observed outcome:

Test parameters	*Ricinus communis*
Color	Light green
Odour	Specific
Taste	Bitter
Moisture	3.7%
Total Ash	7.5%
Acid Insoluble Ash	2.55 %
Water Soluble Ash	1.28%

Table:1 Physicochemical properties of *Ricinus communis* leaves

Table 2 displays the extraction yields as well as the physical properties of plant extracts. *Ricinus communis* extraction yields ranged from 7.72 percent to 19.42 percent in various solvent systems. The yields of extracts varied greatly depending on the extraction solvent and plant material utilised. Extraction yields achieved in Methanol, aqueous methanol, acetone, aqueous acetone, methanol: Dichloromethanol and water solvent systems of 15.28 %, 18.06 %, 7.72 %, 15.65 %, 13.45 % and 19.42 %, respectively.

The extraction yield of acetone extract was the lowest, at 7.72 %. Water extract had the highest extraction yield of 19.42 %. Physicochemical properties of Plant extract such as colour and feeling of touch were observed.(Table :2)

The colour of the extract from the solvents methanol, acetone, and dichloromethane: methanol was discovered to be green, and the sense of touch was found to be sticky. While the extract from aqueous methanol, aqueous acetone, and aqueous is brown in colour and sticky to the sense of touch.

Table:2 Physical characteristics and % yield of Extract:*Ricinus communis*

PLANT	SOLVENT	COLOUR OF EXTRACT	SENSE OF TOUCH	AMOUNT OF EXTRACT(gm)	% YIELD
Ricinus communis	100% methanol	Green	Sticky	4.584	15.28
	50% methanol	Brown	Sticky	5.419	18.06
	100% acetone	Green	Sticky	2.317	7.72
	50% acetone	Brown	Sticky	4.696	15.65
	Hexane/dichloromethane (1:1)	Green	Sticky	4.036	13.45
	Water	brown	Sticky	5.826	19.42

Figure 1 depicts a graphical representation of the % yield comparative for all the extracts of *Ricinus communis*.

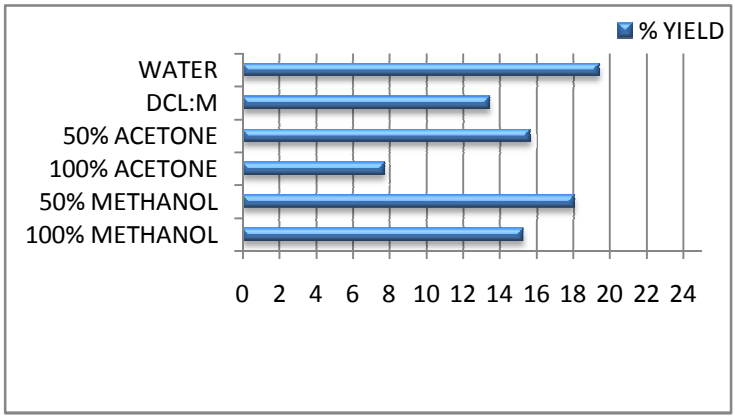


Fig 1: % Yield of extract

The phytochemicals found in plant samples are the focus of natural product biological activity. Phytochemical screening is critical in the study of therapeutic qualities of plant samples. The presence of significant phenolic compounds, saponins, tannin, Flavonoid, and other substances has been identified in the plant's leaves. (Table:3).

TEST	SOLVENT SYSTEM					
	RM	R50M	RA	R50A	RMDM	RW
Alkaloid Mayer's test	+	+	-	+	-	+
Alkaloid Wagner's test	+	+	-	+	+	+
Alkaloid Dragendorff test	-	+	-	+	+	-
Phenol	+	+	+	+	-	+
Tannin	+	+	+	+	-	+
Flavonoids	+	+	+	+	-	+
Antraquinone	-	+	-	+	-	+
Saponnin	+	+	+	+	+	+
Terpenoid	+	-	+	-	+	-
Glycosides	-	+	-	+	-	+
Steroid	+	-	+	-	+	-
oil	+	+	+	+	+	+
Carbohydrate(Mohlis's test)	+	+	+	+	+	+
Carbohydrates (Barfoed test)	+	+	-	+	+	+
Protein (Ninhydrin test)	+	+	+	+	+	+

Table:3 Phytochemical study of plant extract

***In vitro* antifungal activity :**

The in-vitro antifungal activity of leaf extracts of selected plants (*Ricinus communis*) was tested in concentrations of 4,6,8,10,12,14,16 % using different solvents such as methanol, aqueous methanol, acetone, aqueous acetone, Dichloromethane: methanol, and water against *F. oxysporum f. sp. Cumini* with the control. The proportion of *Fusarium oxysporum* mycelial growth suppression was detected using various extracts of selected plants. The results indicated that all tested Bio-agents were efficient at varying percentages for inhibiting mycelial growth of *Fusarium oxysporum f. sp. cumini* under in vitro conditions.

Effect of methanol extract:

Result presented in Fig.2 demonstrate the inhibitory effect of methanolic plant extract against F. *oxysporum* at various concentrations after 8 days of incubation. The antifungal activity of methanolic extract is determined with six different concentrations (4,6,8,10,12,14%). Mycelium growth is measured for each concentration after 8 days and their respective radius are compared with the radius of control plate to obtain % inhibition of plant extract against *F. oxysporum*. It is observed that % inhibition for six different concentrations (4,6,8,10,12,14%) are found to be 54.54%,62.5%, 77.27%,79.54%, 97.72%,100% respectively. The growth of *F. oxysporum* is highly inhibited at 14% concentration of methanolic extract compared with the control.

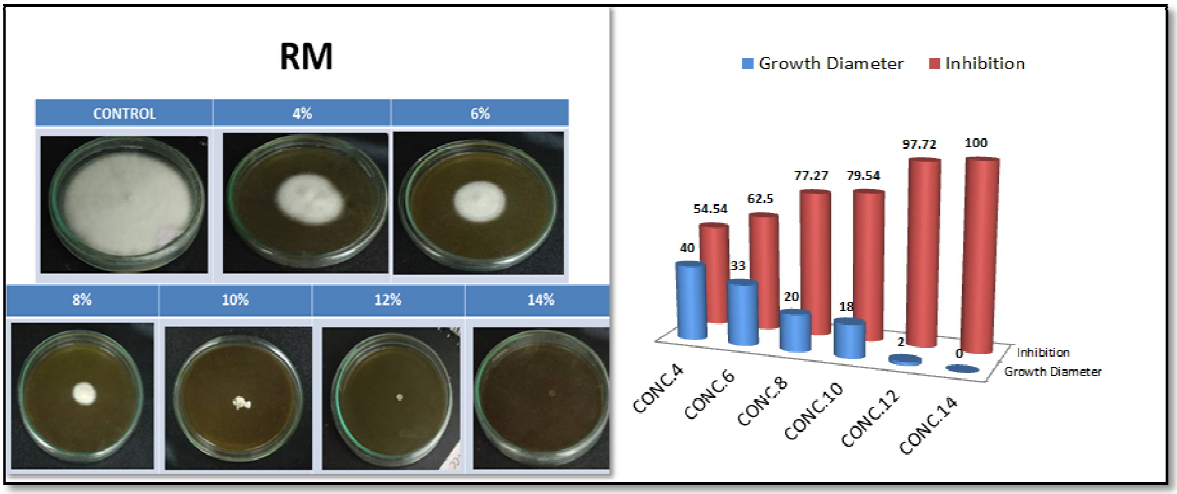


Fig:2 Growth inhibition of methanolic extract against *F.oxysporum*

After 8 days of incubation, the results shown in Fig.3 show that Acetone extract has an inhibitory impact on *F. oxysporum* at varied concentrations. Acetone extract's antifungal activity is tested at six different concentrations (4,6,8,10,12,14%). The percent inhibition of plant extract against *F.oxysporum* is determined by measuring mycelium growth. It is discovered that the percent inhibition for different concentrations (4,6,8,10,12,14%) is 48.31 %, 69.66 %, 73.03 %, 86.51 %, 88.76 %, and 100 %. *F. oxysporum* is completely inhibited (i.e. killed) at a 14% concentration of plant extract.

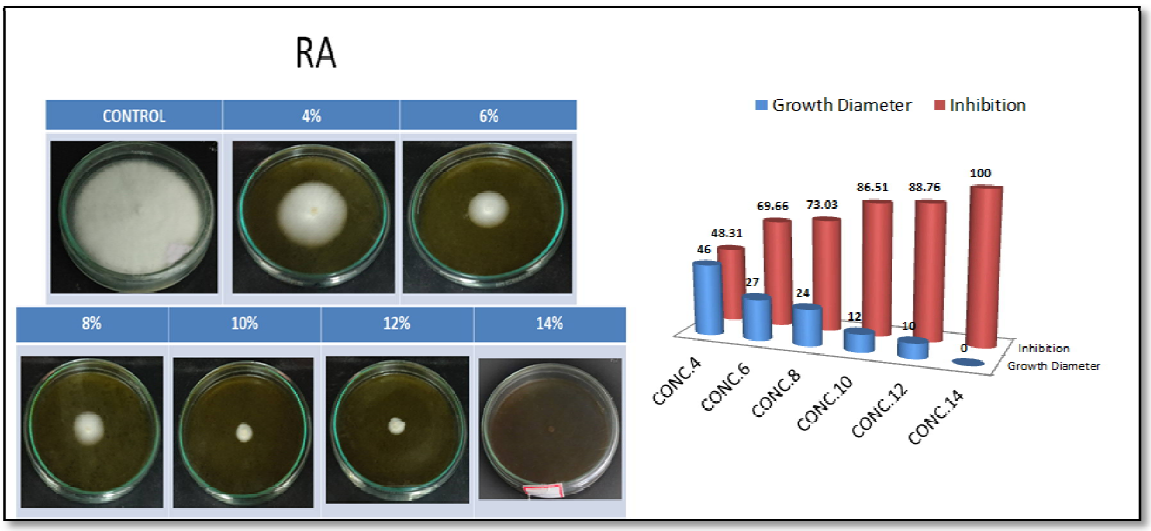


Fig:3 Growth inhibition of Acetone extract against *F.oxysporum*

After 8 days of incubation, the results shown in Fig.4 show that Aqueous methanol extract has an inhibitory effect against *F.oxysporum* at varying concentration. Six different concentrations of Aqueous methanol extract were used to test its antifungal activity (4,6,8,10,12,14%). After 8 days, mycelium development is measured for each concentration, and the radius of each concentration is compared to the radius of the control plate to calculate the percentage inhibition of plant extract against *F.oxysporum*. The percent inhibition for six distinct concentrations (4,6,8,10,12,14 %) is determined to be 54.54 %, 64.77 %, 78.4 %, 92.04 %, 97.72 %, and 100 %. In comparison to the control, F. oxysporum growth is significantly inhibited at a 14% concentration of Aqueous methanol extract.

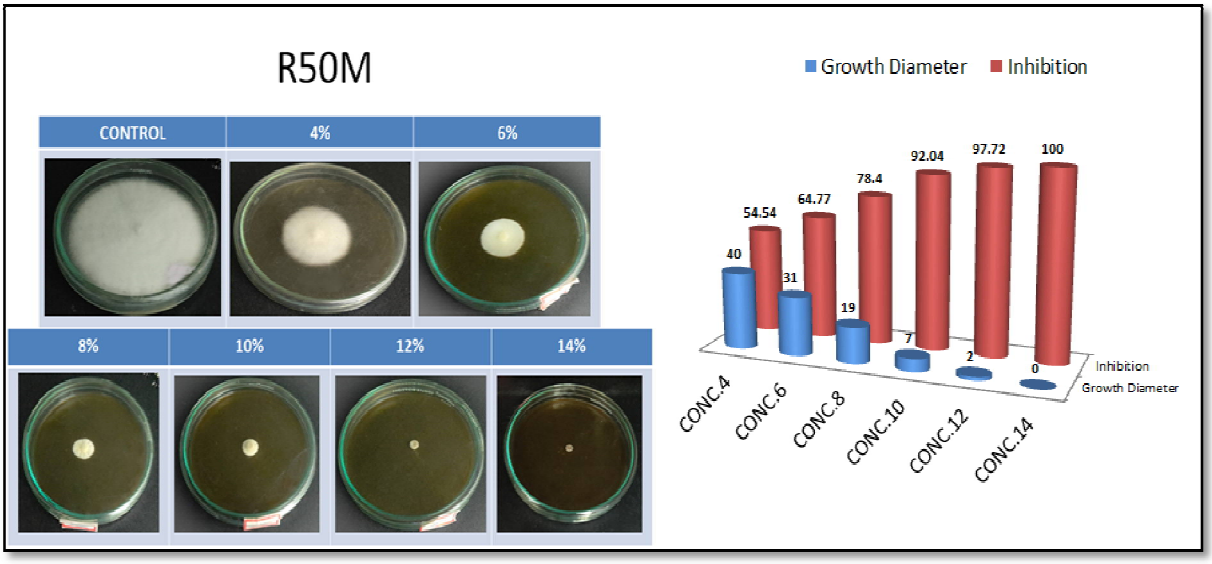


Fig:4 Growth inhibition of Aqueous methanol extract against *F.oxysporum*

The results given in Fig.5 reveal that Aqueous Acetone extract has an inhibiting effect on *F. oxysporum* at various concentrations after 8 days of incubation. The antifungal activity of aqueous acetone extract is investigated at seven different concentrations (4,6,8,10,12,14,16 %). Mycelium growth is used to evaluate the percent inhibition of plant extract against *F. oxysporum*. The percent inhibition for different concentrations (4,6,8,10,12,14,16 %) is determined to be 54.54 %, 67.04 %, 79.54 %, 84.09 %, 93.18 %, 97.72 %, and 100 %. At a 16% concentration of plant extract, *F. oxysporum* is totally inhibited i.e 100%.

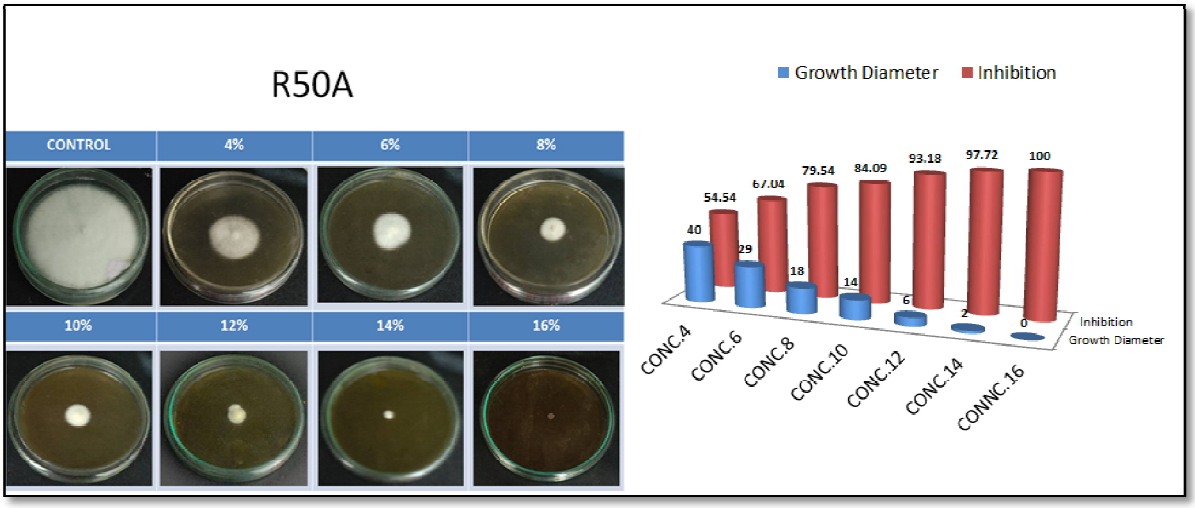


Fig:5 Growth inhibition of Aqueous Acetone extract against *F.oxysporum*

The findings shown in Fig.6 show that after 8 days of incubation, Dichloromethane: methanol extract has an inhibitory impact on *F.oxysporum* at varied concentrations. At five different concentrations (4,6,8,10,12 %), the antifungal activity of dichloromethane: methanol extract is examined. The growth of mycelium is used to determine the percentage of inhibition of a plant extract against *F.oxysporum.* The percent inhibition for various concentrations (4,6,8,10,12%) is determined to be 50%, 62.5 %, 72.72 %, 79.54 %, and 100%. *F.oxysporum* is completely inhibited, i.e. 100%, at a 12% concentration of plant extract.

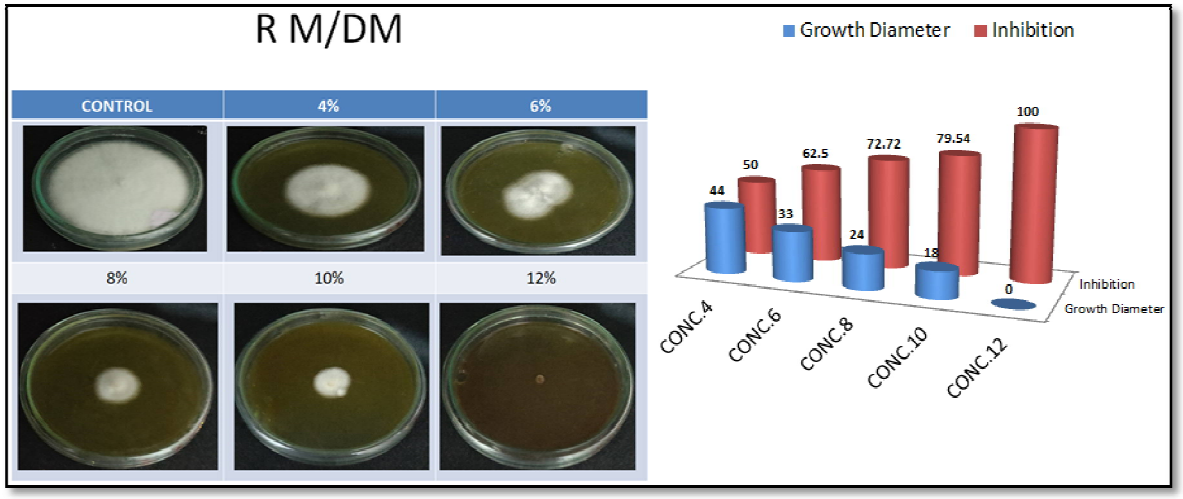


Fig:6 Growth inhibition of Dichloromethane:methanol extract against *F.oxysporum*

The results reported in Fig.7 reveal that after 8 days of incubation, aqueous extract has an inhibitory effect on *F.oxysporum* at various concentrations. The antifungal activity of aqueous extract is tested at six different concentrations (4,6,8,10,12,14 %). Mycelium growth is used to calculate the percentage of inhibition of a plant extract against *F.oxysporum*. The percent inhibition for various concentrations (4,6,8,10,12,14 %) is determined to be 54.54 %, 69.31 %, 82.95 %, 90.9 %, 98.86 %, and 100%. At a 14% concentration of plant extract, *F. oxysporum* is entirely inhibited, i.e. 100% inhibited.

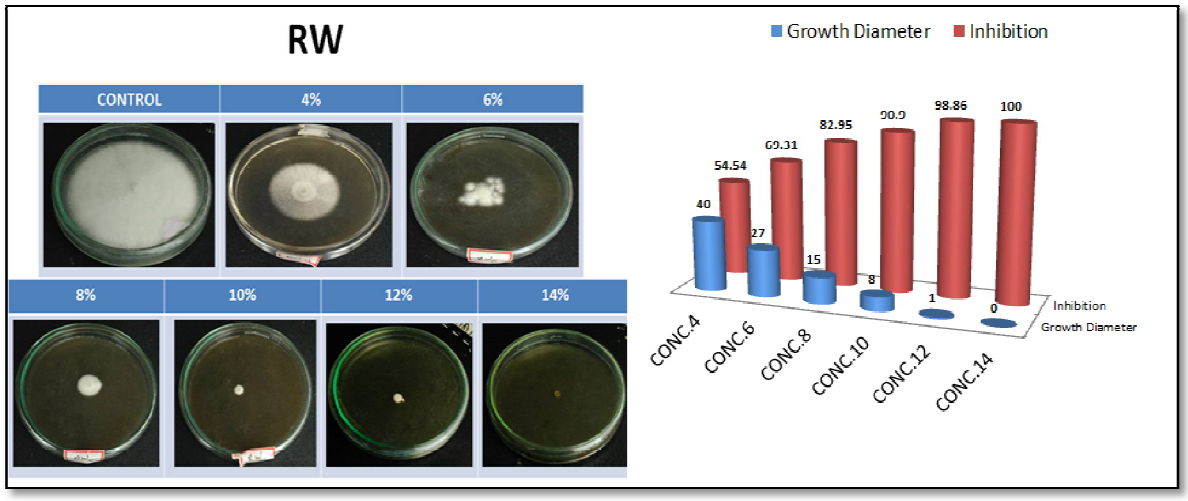


Fig:7 Growth inhibition of aqueous extract against *F.oxysporum*

Table 3 shows the mean SD value of growth inhibition by different solvent systems at different concentrations. According to the results, the dichloromethane: methanol extract inhibits the most at 12 %, the aqueous acetone extract inhibits the most at 16%, and the rest of the extract inhibits the most at 14%.

Ricinus Communis							
	4%	6%	8%	10%	12%	14%	16%
Methanol	53.54±0.87	61.72±0.75	76.57±0.64	78.64±0.78	97.58±0.52	100±00	-
Aqueous methanol	53.80±0.77	64.55±0.59	78.13±0.25	92.24±0.57	97.64±0.50	100±00	-
Acetone	48.07±0.21	69.47±0.84	73.19±0.61	86.4±0.65	88.25±0.45	99.73±0.46	-
Aqueous Acetone	54.01±0.52	67.71±0.60	79.18±0.32	84.26±0.57	93.16±0.75	97.60±0.55	100±00
Dichloromethane: Methanol	49.7±0.7	62.4±0.55	71.94±0.81	79.61±0.55	100±00	-	-
Water	54.48±0.55	68.83±0.64	82.48±0.85	90.63±0.64	98.68±0.52	100±00	-
Control	00±00	00±00	00±00	00±00	00±00	00±00	00±00

Table: 4 The value means of three replicates ± standard error for % inhibition of plant extract against *F. oxysporum*

The bar chart (fig:8) compares mycelium growth inhibition at various concentrations. The concentration of plant extract is displayed on the x-axis, while the %inhibition is plotted on the y-axis. It can be observed that as the concentration of the plant extract increases, so does the %inhibition. It eventually lowers the radius of mycelium development on the plate. At 14 and 16% concentrations, the percent inhibition increases dramatically. Overall, we can discern a definite rising trend in the percent inhibition with increasing plant extract concentrations.

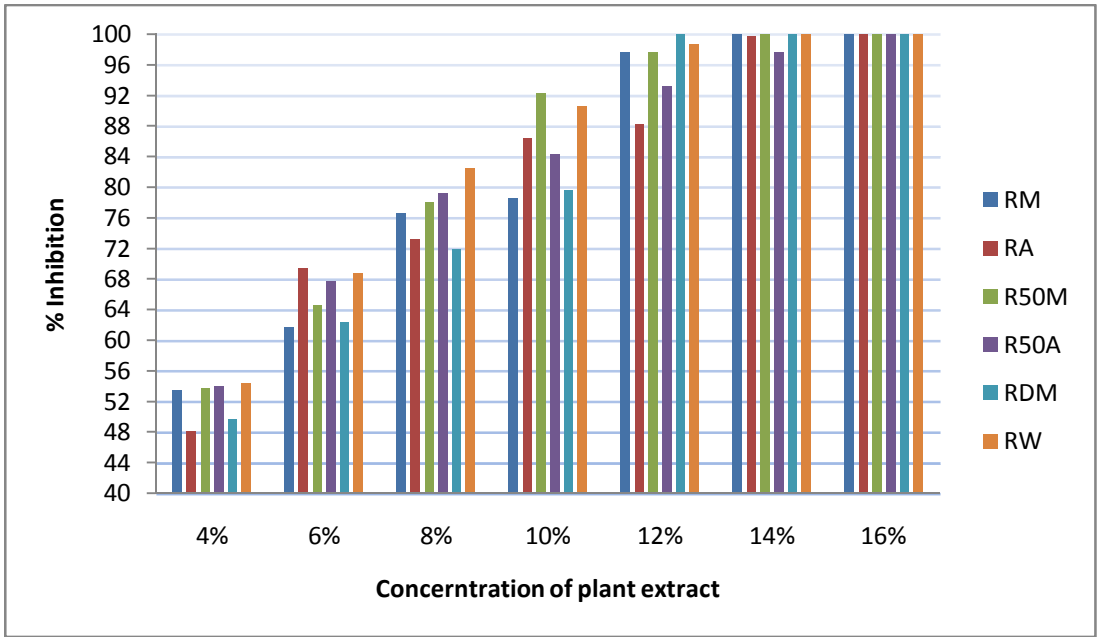
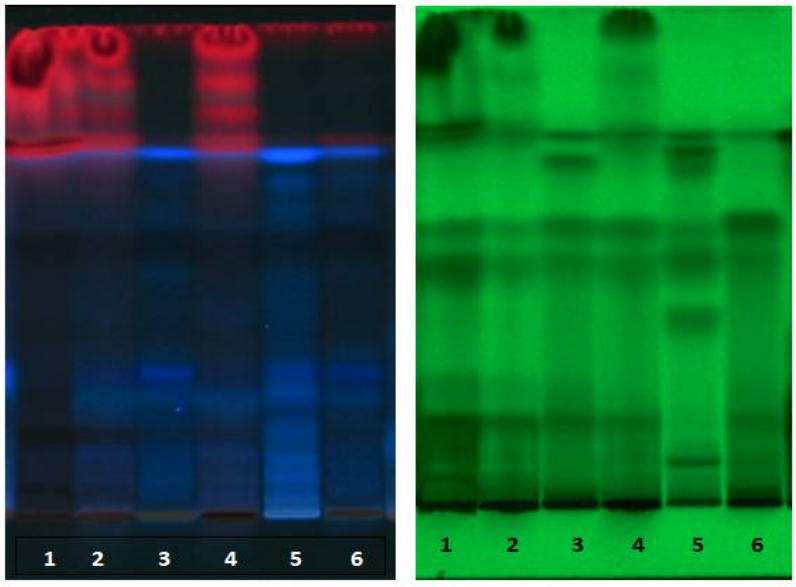


Fig:8 Inhibitory effect of different extract at different concentration

HPTLC fingerprint profile, chromatogram and densitogram for Flavonoid is presented in figure 9 and 10 respectively. A variety of extracts like Acetone extract, Dichloromethane: methanol extract, aqueous methanol extract, Methanol extract, Water extract, aqueous acetone extract of *Ricinus communis* are used for HPTLC Flavonoids profile that represented the presence of 10, 9, 7, 6, 8, 9 respective bands and substantiated 2, 3, 2, 2, 1, 2 types of flavonoids with *Rf* values ranged from 0.06 to 0.96, 0.05 to 0.87, 0.1 to 0.89, 0.1 to 0.87, 0.09 to 0.77, 0.04 to 0.9 respectively.



(A.Fluorescence light) (B.UV light)

[Figure:9 HPTLC (chromatogram). Flavonoids profile of 1.RA(Acetone extract),2. RDM (Dichloromethane: methanol extract),3.R50M(aqueous methanol extract) ,4.RM(Methanol extract),5.RW(Water extract, 6.R50A (aqueous acetone extract)]

Figure 10(a) Peak densitogram of flavonoid for A.RA(Acetone extract),B. RDM(Dichloromethane: methanol extract),C.R50M(aqueous methanol extract) ,D.RM(Methanol extract),E.RW(Water extract,F.R50A(aqueous acetone extract)at 254nm]

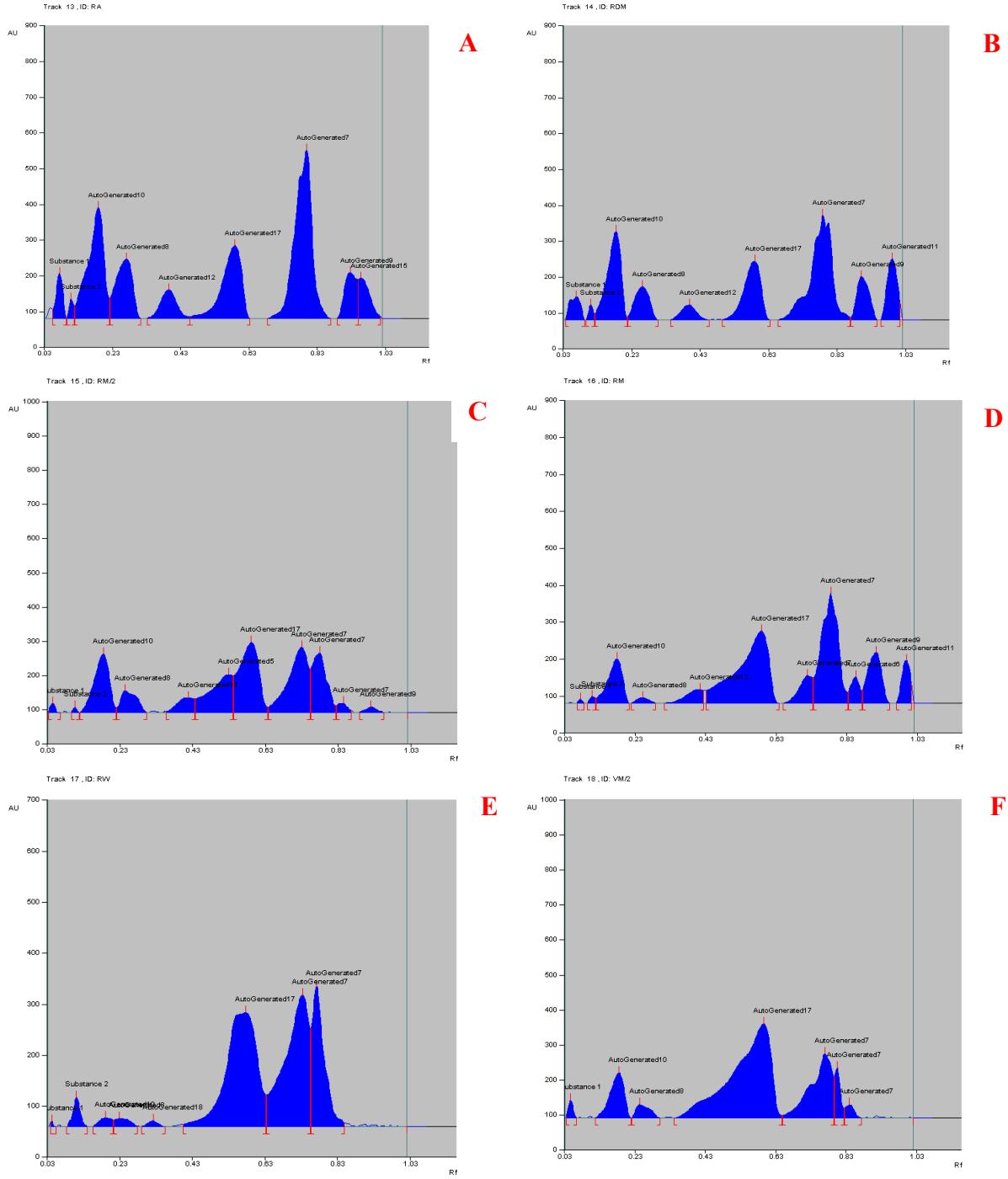
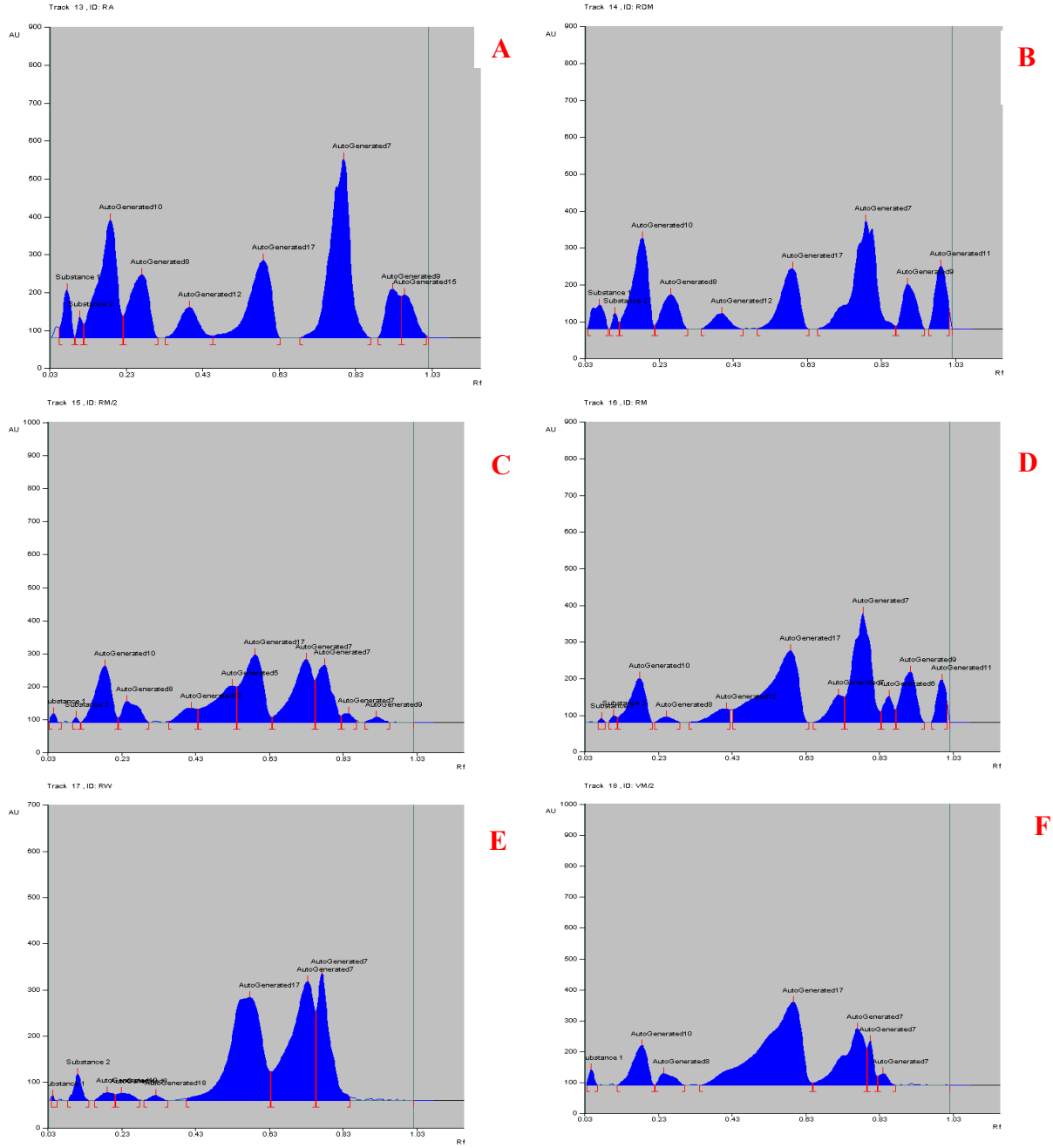


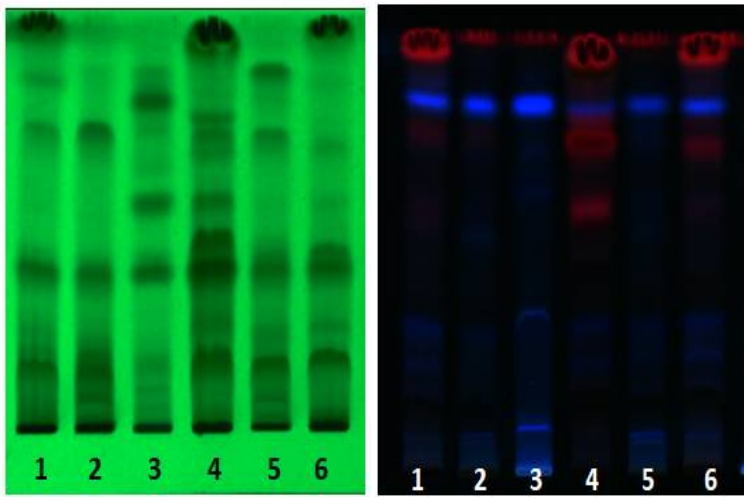
Figure 10(b) Peak densitogram of flavonoid for A.RA(Acetone extract),B. RDM(Dichloromethane:methanol extract),C.R50M(aqueous methanol extract) ,D.RM(Methanol extract),E.RW(Water extract,F.R50A(aqueous acetone extract)at 366nm]



RA		RDM		R50M		RM		RW		R50A	
Rf value	Assigned substance	Rf value	Assigned substance	Rf value	Assigned substance	Rf value	Assigned substance	Rf value	Assigned substance	Rf value	Assigned substance
0.06	Unknown	0.05	Unknown	0.1	Unknown	0.1	Unknown	0.09	Unknown	0.04	Unknown
0.1	Unknown	0.07	Unknown	0.14	Flavonoid	0.15	Flavonoid	0.16	Unknown	0.09	Unknown
0.23	Flavonoid	0.14	Flavonoid	0.36	Flavonoid	0.31	Unknown	0.3	Flavonoid	0.14	Flavonoid
0.35	Flavonoid	0.23	Flavonoid	0.56	Unknown	0.56	Unknown	0.45	Unknown	0.24	Flavonoid
0.43	Unknown	0.35	Flavonoid	0.64	Unknown	0.67	Flavonoid	0.57	Unknown	0.33	Unknown
0.56	Unknown	0.43	Unknown	0.76	Unknown	0.87	Unknown	0.66	Unknown	0.56	Unknown
0.69	Unknown	0.56	Unknown	0.89	Unknown	-	-	0.73	Unknown	0.66	Unknown
0.8	Unknown	0.66	Unknown	-	-	-	-	0.77	Unknown	0.74	Unknown
0.9	Unknown	0.87	Unknown	-	-	-	-	-	-	0.9	Unknown
0.96	Unknown	-	-	-	-	-	-	-	-	-	-

Table 5: HPTLC: flavonoids profile of studied plants

Chromatogram and densitogram of HPTLC fingerprinting of alkaloid for different plant extract are presented in Figure 11 and 12.

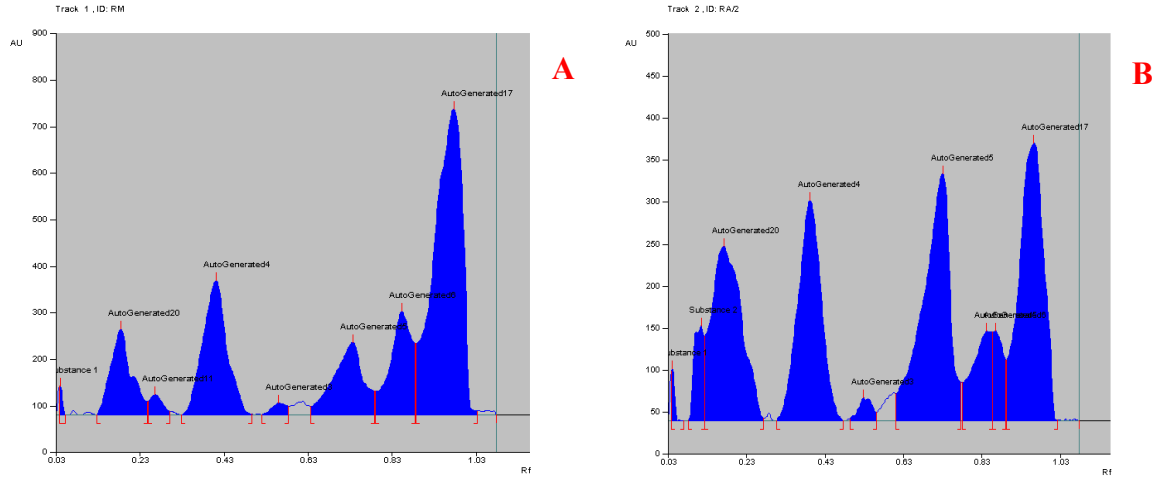


(A.Fluorescence light) (B.UV light)

[Figure:11 HPTLC (chromatogram). Alkaloid profile of 1. RM(Methanol extract),2. R50A(aqueous acetone extract),3. RW(Water extract) ,4. RA(Acetone extract),5. R50M(aqueous methanol extract),6. RDM (Dichloromethane: methanol extract)]

For HPTLC Alkaloid profile, a variety of extracts such as Methanol extract, aqueous acetone extract, Water extract, Acetone extract, aqueous methanol extract, Dichloromethane: methanol extract of *Ricinus communis* were used, which represented the presence of 8, 9, 11, 10, 9, 12 bands with Rf values ranging from 0.04 to 0.89, 0.04 to 0.9, 0.04 to 0.9, 0.04 to 0.92, 0.05 to 0.9, and 0.04 to 0.86 respectively. The band number 4 in aqueous acetone extract, 3 & 6 in Water extract, 2 in aqueous methanol extract, 3 in Dichloromethane: methanol extract are identified as Strychnine, Nicotine, Alkaloid 1, Nicotine & Nicotine respectively.

Figure:12(a) Peak densitogram of Alkaloid for A. RM(Methanol extract),B. R50A(aqueous acetone extract),C. RW(Water extract) ,D. RA(Acetone extract),E. R50M(aqueous methanol extract),F. RDM(Dichloromethane: methanol extract) at 254nm]



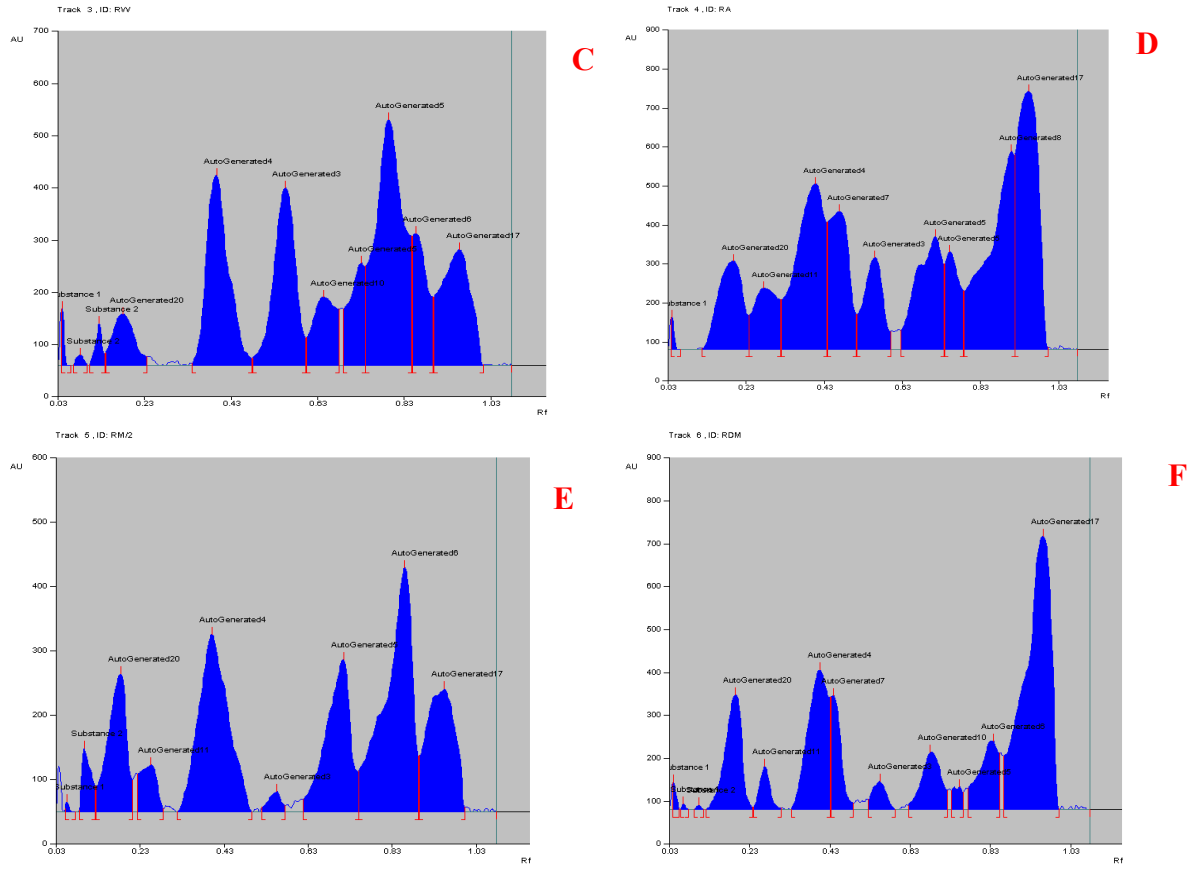
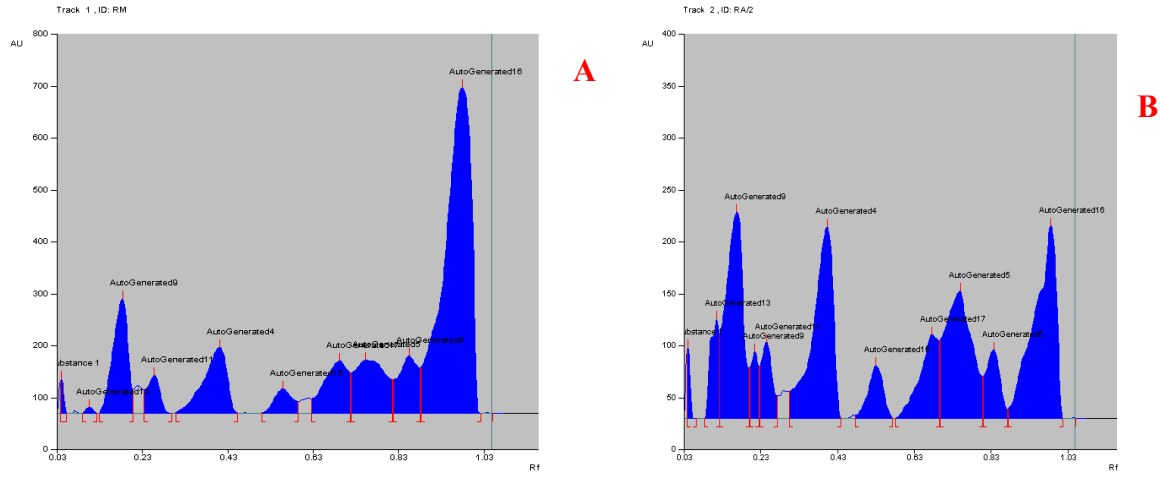
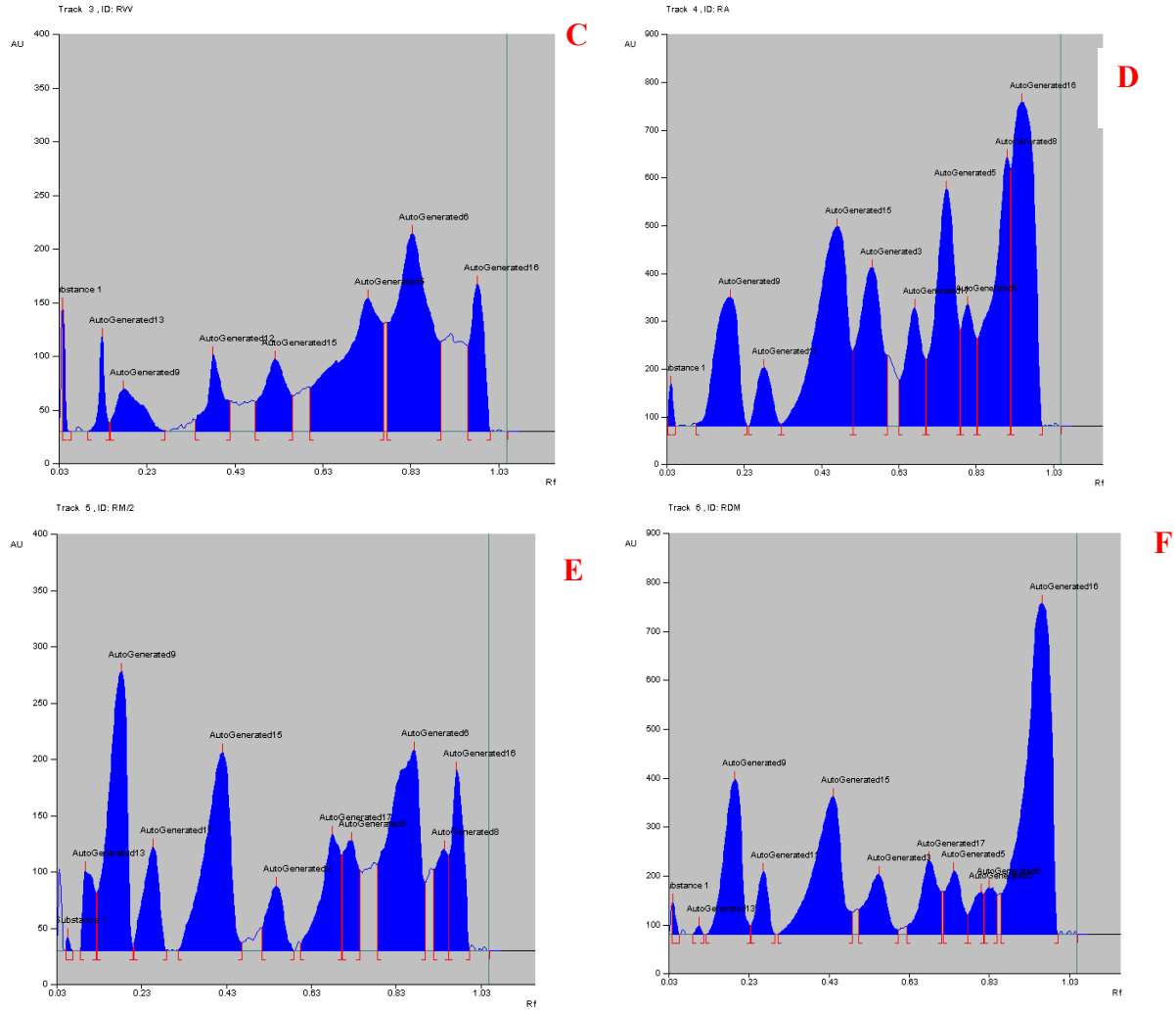


Figure 12(b) Peak densitogram of Alkaloid for A. RM(Methanol extract),B. R50A(aqueous acetone extract),C. RW(Water extract) ,D. RA(Acetone extract),E. R50M(aqueous methanol extract),F. RDM(Dichloromethane: methanol extract) at 366nm]





RM		R50A		RW		RA		R50M		RDM	
Rf value	Assigned substance	Rf value	Assigned substance	Rf value	Assigned substance	Rf value	Assigned substance	Rf value	Assigned substance	Rf value	Assigned substance
0.04	Unknown	0.04	Unknown	0.04	Unknown	0.04	Unknown	0.05	Unknown	0.04	Unknown
0.13	Unknown	0.08	Unknown	0.07	Unknown	0.12	Unknown	0.09	**Nicotine**	0.06	Unknown
0.25	Unknown	0.13	Unknown	0.1	**Nicotine**	0.24	Unknown	0.13	Unknown	0.09	**Nicotine**
0.33	Unknown	0.31	**Strychnine**	0.14	Unknown	0.32	Unknown	0.23	Unknown	0.12	Unknown
0.52	Unknown	0.5	Unknown	0.34	Unknown	0.44	Unknown	0.32	Unknown	0.24	Unknown
0.64	Unknown	0.61	Unknown	0.48	Alkaloid **1**	0.52	Unknown	0.52	Unknown	0.34	Unknown
0.79	Unknown	0.78	Unknown	0.61	Unknown	0.63	Unknown	0.62	Unknown	0.43	Unknown
0.89	Unknown	0.86	Unknown	0.69	Unknown	0.74	Unknown	0.75	Unknown	0.53	Unknown
-		0.9	Unknown	0.74	Unknown	0.79	Unknown	0.9	Unknown	0.63	Unknown
-	-	-	-	0.85	Unknown	0.92	Unknown	-	-	0.73	Unknown
-	-	-	-	0.9	Unknown	-	-	-	-	0.78	Unknown
-	-	-	-	-	-	-	-	-	-	0.86	

Table 6: HPTLC: Alkaloid profile of studied plants

CONCLUSION

Considering the need for alternative biorational fungicides in cumin production management, it was thought interesting to analyze the antifungal effects of locally accessible plant extracts. The results were positive, since one of the plants exhibited total inhibition. This survey suggests that all of the extracts derived from the leaves of *Ricinus communis* include numerous phytochemicals such as phenol, tannin, flavonoid, saponin, and others. Plant extracts of *Ricinus communis* from diverse solvent systems inhibited the growth of *Fusarium oxysporum*. HPTLC fingerprinting confirmed the presence of some flavonoid and alkaloid compound in different extract of selected plants which might be responsible for inhibitory effect against pathogenic fungi. These preliminary in vitro results may be reinforced by other more complete in vivo studies, both under controlled greenhouse circumstances and in the field, to actually evaluate the usage of these extracts in the context of an Integrated Pest Management. Rather than employing chemicals to eradicate this agent, this approach would highlight the use of biological extracts that inhibit numerous elements responsible for biotic stress, such as insects, fungus, weeds, and nematodes.

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