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# Efficacy of *Sargassum wightii* against Fusarium wilt of tomato incited by *Fusarium solani*

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**ABSTRACT**
*This study aims to assess the effectiveness of Sargassum wightii as a potential bio-agent against Fusarium solani, the main cause of Fusarium wilt in tomatoes. The disease can occur at any stage of the crop, particularly during flowering and fruiting, leading to significant yield losses ranging from 10% to 90%. Sargassum wightii is commonly used to manage soil-borne diseases. The objective of this research is to evaluate the efficacy of S. wightii against Fusarium solani under in vitro. The poison food technique revealed that the acetone seaweed extracts of Sargassum wightii exhibited the highest inhibition of mycelial growth, measuring 10.25 mm, while the ethyl acetate seaweed extracts showed a slightly lower inhibition at 16.27 mm. In the agar well method, Sargassum wightii at a concentration of 30% demonstrated the maximum inhibition zone of 18.9 mm, with carbendazim at 0.1% following closely at 17.8 mm.*
***Keywords:*** *Fusarium solani, Sargassum wightii.*

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## INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) is a popular and widely cultivated vegetable from the Solanaceae family. It is highly valued for its abundant presence of beneficial compounds, including beta-carotene and lycopene [1]. Its cultivation is widespread across the globe due to its nutritional value and versatile culinary uses. Globally, tomatoes are cultivated on a vast scale, covering an impressive area of 45.8 million hectares and yielding a production of 150.5 million tons, with an average productivity rate of 32.8 tons per hectare [2]. In the Indian state of Tamil Nadu, tomato cultivation spans an area of 38.78 lakh hectares and contributes to a production of 841.21 million tons [3]. Tomatoes are highly susceptible to a wide array of diseases caused by various bio agents and physiological disorders [4]. Among these diseases, Fusarium wilt caused by *Fusarium solani* and *Fusarium oxysporum* f.sp. *lycopersici* is a significant concern [5]. Fusarium wilt can result in substantial yield losses ranging from 10% to 90% [6]. The Fusarium pathogens enter the root epidermis, spread through the vascular tissue, and colonize the xylem vessels of the plant. This colonization leads to vessel clogging, severe water stress, and the development of wilt-like symptoms [11]. In recent years, the emergence of greenhouse-grown tomato plants exhibiting vascular wilt and yellowing symptoms has become a notable threat to profitability [7]. Due to concerns regarding the economic viability and environmental safety of chemical options, greater emphasis is being placed on the biological management of plant diseases. This approach involves harnessing the potential of microflora to combat pathogens. The use of chemical pesticides has been associated with hazards to both the environment and human health, necessitating a shift towards more sustainable and eco-friendly alternatives. Seaweeds possess a diverse array of biological activities attributed to their rich content of polysaccharides, phytochemical derivatives, and bioactive substances. These properties contribute to the ability of seaweed to exhibit antibacterial, antifungal, and antiviral effects [8]. Numerous marine algal species have been found to contain bioactive compounds with antimicrobial properties. These compounds encompass a wide range of chemical classes, including sterols, terpenoids, polysaccharides, peptides, proteins, vitamins, acrylic acid, terpenes, chlorophyllides, phenols, heterocyclic compounds, halogenated ketones, alkanes, and cyclic polysulphides [9]. Several seaweeds, such as *Sargassum polyphyllum, Turbinaria ornata, Gelidiopsis* sp.,

*Padina tetrastomatica,* and *Gracilaria corticata*, have been reported to exhibit growth-stimulating effects on plants and display antagonistic activity against various fungal pathogens [10].

## MATERIAL AND METHODS

### Survey and isolation of *Fusarium solani*

From 2021 to 2022, an extensive survey was carried out to evaluate the incidence of wilt disease in various tomato cultivation regions, including Krishnagiri, Dharmapuri, Namakkal, Trichy, and Pudukkottai districts. Within each village, four fields were chosen, and within each field, four random plots were marked, with an average area of one square meter each. During the survey, the pathogen responsible for the wilt disease in tomato plants was isolated from infected plant parts using the tissue segment method as described by Rangaswami in 1958. The diseased plant specimens were first washed with tap water. Then, the infected root parts were cut into small pieces measuring approximately 5 mm. These root pieces were subjected to sterilization using a 0.1% Sodium hypochlorite solution for a duration of two minutes, followed by rinsing with sterilized water for three times. The sterilized root pieces were then placed onto three replicated Petri dishes containing sterilized PDA (potato dextrose agar) at a volume of 15 ml per plate. The Petri dishes were incubated at room temperature, maintained at 28±2°C, for a period of 7 days. The fungal isolates were subsequently purified using the hyphal tip technique, as described by Windels *et al.* [13].

### COLLECTION OF SEAWEEDS

Seaweed species, namely *Sargassum wightii, Ulva lactuca, Gracilaria gracilis, Caulerpa scalpelliformis,* and *Turbinaria conoides*, were collected from various coastal areas in Tamil Nadu. To minimize contamination of the raw biomass, algae floating freely in the coastal zone were collected from the water. The collected algal material underwent a purification process, which involved rinsing with water to remove salt and sand. The larger impurities were then separated, and the biomass was dried until it reached a moisture content of 15%. Afterward, the seaweed was chopped into small pieces and subjected to sun drying for four days, followed by oven-drying at 60°C for 24 hours, as described by Wilk *et al.* [14].

### PREPARATION OF CRUDE EXTRACTS

#### Preparation of hot water extract

The dried seaweeds were individually ground thoroughly using a mixer grinder and then sieved using a nylon sieve to eliminate seaweed fiber. To prepare the hot water extract of the seaweed, the method outlined by Ambika and Sujatha [15] was followed. A total of 20 g of the ground seaweed was mixed with 400 ml of distilled water for each sample, and the resulting suspension was autoclaved at 100°C and 15 lbs pressure for 15 minutes. After autoclaving, the suspension was subjected to centrifugation at 10,000 rpm for 10 minutes. The resulting supernatant was then filtered and stored in a deep freezer for further analysis and study.

#### Preparation of solvent extract

The ground algae were placed into a Soxhlet extractor, consisting of a 500 ml round-bottomed flask and a condenser. The extraction process took place on a water bath for a duration of 12 hours, using 300 ml of 99% acetone and ethyl acetate solvents. Each powdered sample (30 g) was subjected to separate extraction. Subsequently, the crude extracts were filtered through Whatman filter paper No.1, and the resulting filtrates were concentrated under reduced pressure using a rotary evaporator until complete dryness was achieved. The dried crude extracts were then stored at -40°C in a deep freezer for the purpose of testing their antimicrobial activities and conducting phytochemical characterization, following the methods outlined by Abdel-Raouf *et al.* [16].

### POISONED FOOD TECHNIQUE [17]

The extracts of seaweeds, at various concentrations (10%, 20%, and 30%), and the fungicide carbendazim at a concentration of 0.1%, were individually added to autoclaved and melted potato dextrose agar media. The poisoned agar medium was poured into sterilized plates and allowed to solidify. Subsequently, the plates were inoculated with a uniform 9 mm diameter disc taken from a 7-day-old culture grown on potato dextrose agar medium. After inoculation, the diameter of the fungal colony was measured after 7 days. Control plates without fungicide or seaweed extracts were also prepared, and the percentage inhibition over the control was calculated. The experiment was conducted with three replications for each treatment under laboratory conditions at a temperature of 28±1°C. The percentage inhibition of the test fungi was determined using the formula developed by Vincent [18].

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

Where,

I = Percent inhibition of fungal growth

C = Control
T = Growth in treatment

**AGAR WELL METHOD [19]**

A spore suspension of *Fusarium solani* was prepared using sterile distilled water from a 7-day-old culture. Fifteen milliliters of potato dextrose agar (PDA) medium were seeded with 3 ml of the spore suspension, ensuring an adequate spore concentration of 1 × 106 spores/ml. The seeded PDA medium was poured into each Petri plate and allowed to solidify. Equidistant wells were then created using a sterile cork borer. Seaweed extracts at desired concentrations (10%, 20%, and 30%) were prepared, and 0.1 ml of each test extract was pipetted into separate wells. For comparison, carbendazim at a concentration of 0.1% was used, and a suitable control was maintained. Three replications were maintained for each treatment. The plates were incubated at a temperature of 28±2°C for 48 hours, after which the mycelial growth was measured and recorded.

**ANALYSIS OF ANTIFUNGAL COMPOUND THROUGH GAS CHROMATOGRAPHY MASS SPECTROSCOPY (GCMS)**

To identify the chemical components of the selected seaweed extract, GC-MS analysis was conducted using a Perkin Elmer Gas Chromatography system (Clarus 500) equipped with a mass detector (Turbo Mass Gold). The analysis utilized an Elite-1 column (100% dimethyl polysiloxane) with dimensions of 30m × 0.25mm ID. The carrier gas employed was helium, flowing at a rate of 1 ml/min. The oven temperature program involved a range from 110°C (held for 2 minutes) to 280°C (held for 9 minutes). The injector temperature was set at 250°C, and the total GC analysis duration was 36 minutes.

For the analysis, 2.0 ml aliquots of the water extract were injected into the chromatograph. The major constituents within the extract were identified using a computer-driven algorithm and cross-referencing the mass spectrum of the analysis with a library (NIST Version 2.0, year 2005) to confirm the identification. The Turbo Mass-5.1 software was employed for the gas chromatography mass spectroscopy (GC-MS) analysis.

**RESULT AND DISCUSSION**

**Evaluation of seaweed extract against Fusarium solani (Agar well method)**

The antifungal activity of seaweed extracts against $Fs_4$ was investigated and the findings are presented in table 2. The zone of inhibition observed varied from 10.9 to 18.9 mm. The most potent antifungal activity was observed in Sargassum wightii extract at a concentration of 30%, resulting in an impressive inhibition zone of 18.9 mm. Following closely, Carbendazim at a concentration of 0.1% exhibited a significant mycelial inhibition zone of 17.8 mm. Conversely, Turbinaria conoides displayed the lowest effectiveness, with a recorded inhibition zone of 10.9 mm. Previous studies conducted by Ambika and Sujatha [20] demonstrated that seaweed extracts, such as Sargassum myricocystum and Gracilaria edulis, exhibited inhibitory effects against the growth of Colletotrichum falcatum. The beneficial effects of seaweed extracts in disease reduction were attributed to the induction of defense enzymes and the presence of laminarin, a carbohydrate with structural similarities to fungal cell wall components. This presence of laminarin may stimulate fungal antagonists and elicit a plant defense response [21]. Krishnamoorthi and Sivakumar [22] reported the antifungal activity of Ulva lactuca seaweed against pathogenic fungi including Alternaria solani, *Aspergillus clavatus, Aspergillus niger, Aspergillus flavus*, and *Fusarium oxysporum* using the disk diffusion method. El-Sheekh et al. [23]confirmed the inhibitory effects of marine algal extracts against the plant pathogenic fungus F. oxysporum using the agar diffusion assay technique. These previous studies provide further support and alignment with the findings of the present study.

**Evaluation of Acetone seaweed extract against Fusarium solani (Poison food technique)**

In the poison food technique experiment (Table 4), various acetone seaweed extracts were evaluated for their effectiveness. The results indicated that Sargassum wightii extract at a concentration of 30% exhibited the highest inhibition of mycelial growth, with a measurement of 8.62 mm. This was followed by Carbendazim at a concentration of 0.1%, which recorded a mycelial growth inhibition of 12.63 mm. Conversely, Turbinaria conoides extract at a concentration of 30% showed the least inhibition of mycelial growth, measuring 42.12 mm. Phenolic acids and phytoalexins were identified in the acetone extract of the brown alga Sargassum wightii, and this extract displayed antifungal activity against Rhizoctonia solani, the pathogen responsible for rice sheath blight [24]. Similarly, the acetone extract of another brown alga, Sargassum vulgare, was found to contain phenolic acids and flavonoids that exhibited antifungal effects against *Pythium aphanidermatum*, the causal agent of Pythium leak disease in potatoes [25]. These findings align with the results of the present study, providing further support for the observed antifungal properties of the seaweed extracts.

**Evaluation of different solvent extract against Fusarium solani (Poison food technique)**

Distinct seaweed extracts were prepared using three different solvents and their efficacy was assessed using the poison food technique, as shown in Table 3. The results revealed that the acetone seaweed extract exhibited significant inhibition of F. solani mycelial growth, with an inhibition zone of 10.25 mm at a concentration of 30%. Similarly, the ethyl ester seaweed extract demonstrated notable inhibition, measuring 16.27 mm at a concentration of 30%. The hot water seaweed extract exhibited the highest inhibition, with a remarkable zone of 21.81 mm at a concentration of 30%. De Corato et al. [26] conducted in vitro and in vivo tests using extracts from two brown and three red macroalgae to evaluate their effectiveness against three phytopathogenic fungi. The antifungal activity was attributed to the presence of various compounds such as fatty acids, polysaccharides (laminarans, fucoidans, and alginates), and phlorotannins (phlorethols, fucophloretols, and eckols). Similarly, Ibraheem et al. [27] found that methanolic extracts of the brown algae Sargassum latifolium and Padina gymnospora exhibited antifungal activity against phytopathogenic fungi Fusarium solani and Rhizoctonia solani. Soliman et al. [28] identified an iron-monocarbonyl compound in chloroform extracts of the red algae Gracilaria confervoides, which displayed inhibitory action against soil-borne pathogenic fungi of cucumber including Rhizoctonia solani, Fusarium solani, and *Macrophomina phaseolina*. Additionally, El-Sheekh et al. [29] found that extracts and powders of the green seaweeds Ulva fasciata and *Enteromorpha flexuosa* inhibited the growth and affected the microsclerotia and conidia formation of soil-borne fungi *Macrophomina phaseolina* and Fusarium solani, potentially due to the presence of antifungal compounds such as iron-monocarbonyl and their functional groups.

**GC-MS analysis of Sargassum wightii**

The GC-MS analysis of the acetone extract of Sargassum wightii, as presented in table 5 (Fig. 7), identified several active compounds including 2-Pentanone, 4-hydroxy-4-methyl, n-Hexadecanoic acid, Phytol, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, and 2-Pentadecnone, 6,10,14-trimethyl. These compounds are likely responsible for the growth inhibition of various pathogenic fungi. Rosaline *et al.* [30] reported that the GC-MS analysis of Sargassum wightii extract revealed the presence of the antibacterial compound mono-ethylhexylphthalate. Furthermore, Zawawy *et al.* [31] found various antimicrobial compounds, including n-Hexadecanoic acid, 2-Pentanone-4-hydroxy-4-methyl, 2-methoxy-4-(2-propenyl), and phenol, in the methanolic extract of Sargassum wightii.

**Table1: Collection of seaweeds from various seashores of Tamil Nadu**

| S.NO | Seaweed | Common name | Place of collection |
|---|---|---|---|
| 1 | *Sargassum wightii* | Grown algae | Rameshwaram |
| 2 | *Caulerpa scalpelliformis* | Green algae | Mandapam |
| 3 | *Turbinaria conoides* | Grown algae | Tuticorin |
| 4 | *Ulva lactuca* | Green algae | Mandapam |
| 5 | *Gracilaria gracilis* | Red algae | Rameshwaram |

**Table 2: Evaluation of seaweed extract against *Fusarium solani* (Agar well method)**

| S.NO | Treatments | Mycelial growth (mm) (10DAI) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 10% | Percent inhibition over control | 20% | Percent inhibition over control | 30% | Percent inhibition over control |
| 1 | Sargassum wightii | 16.8 $^{a}$ | 81.33 | 17.8 $^{a}$ | 80.22 | 18.9 $^{a}$ | 79.00 |
| 2 | Caulerpa scalpelliformis | 12.6 $^{de}$ | 86.00 | 12.8 $^{bc}$ | 85.77 | 13.1 $^{bc}$ | 85.44 |
| 3 | Turbinaria conoides | 9.8 $^{e}$ | 89.11 | 10.2 $^{c}$ | 88.67 | 10.9 $^{c}$ | 87.89 |
| 4 | Ulva lactuca | 14.4 $^{cd}$ | 84.00 | 15.1 $^{b}$ | 83.22 | 15.9 $^{b}$ | 82.33 |
| 5 | Gracilaria gracilis | 10.5 $^{ef}$ | 88.33 | 11.2 $^{c}$ | 87.56 | 11.4 $^{c}$ | 87.33 |
| 6 | Carbendazim 0.1% | 15.8 $^{bc}$ | 82.44 | 16.9 $^{ab}$ | 81.22 | 17.8 $^{ab}$ | 80.22 |
| 7 | Control | 90.00 | - | 90.00 | - | 90 | - |

| Treatments | Mycelial growth(mm) (7DAI) | | | | | |
|---|---|---|---|---|---|---|
| | 10% | Percent inhibition over control | 20% | Percent inhibition over control | 30% | Percent inhibition over control |
| *Sargassum wightii* | 18.72 a | 79.2 | 14.32 a | 84.08 | 8.62 a | 90.42 |
| *Caulerpa scalpelliformis* | 32.75 c | 63.61 | 27.65 d | 69.28 | 21.53 d | 76.08 |
| *Turbinaria conoides* | 48.92 e | 45.64 | 45.57 f | 49.36 | 42.12 f | 53.20 |
| *Ulva lactuca* | 29.6 c | 67.1 | 23.74 c | 73.62 | 19.58 c | 78.24 |
| *Gracilaria gracilis* | 38.19 d | 57.56 | 31.28 e | 65.24 | 27.68 e | 69.24 |
| Carbendazim 0.1% | 23.45 b | 73.94 | 19.59 b | 78.26 | 12.63 b | 85.96 |
| Control | 90.00 | - | 90.00 | - | 90.00 | - |

**Table 3: Evaluation of different solvent extract against *Fusarium solani* (Poison food technique)**

**Table 4: Evaluation of Acetone extract against *Fusarium solani* (poison food technique)**

| Treatment | Mycelial growth (mm) (7DAI) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Acetone | | | Ethyl acetate | | | Hot water | | |
| | 10% | 20% | 30% | 10% | 20% | 30% | 10% | 20% | 30% |
| Sargassum wightii | 20.62 a | 18.36 a | 10.25 a | 23.80 a | 21.25 a | 16.27 a | 27.75 a | 25.34 a | 21.81 a |
| Caulerpa scalpelliformis | 30.02 d | 27.67 c | 24.72 d | 35.37 d | 31.58 c | 26.21 c | 39.42 d | 35.27 c | 29.42 c |
| Turbinaria conoides | 41.65 f | 46.63 e | 52.25 e | 47.26 e | 53.62 d | 56.24 e | 48.98 e | 56.28 e | 59.65 de |
| Ulva lactuca | 24.26 c | 20.62 b | 15.36 c | 27.36 c | 25.83 b | 20.38 bc | 31.38 c | 27.69 b | 24.32 b |
| Gracilaria gracilis | 36.56 e | 31.27 d | 27.65 e | 42.63 e | 37.85 cd | 31.58 d | 45.62 e | 51.63 d | 56.82 d |
| Carbendazim 0.1% | 22.31 b | 20.56 ab | 19.36 b | 25.96 b | 21.76 ab | 18.76 b | 29.56 b | 26.32 ab | 22.16 ab |
| Control | 90.00 | | | | | | | | |

**Table 5: Compound identified in acetone extract of *Sargassum wightii***

| Peak No. | RT | Compounds | Formula | % of composition |
|---|---|---|---|---|
| 1 | 5.936 | 2-Pentanone,4-hydroxy-4-methyl | $C_6H_{12}O_6$ | 84.76 |
| 2 | 19.455 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | $C_{20}H_4O_0$ | 0.48 |
| 3 | 19.703 | 2-Pentadecanone,6,10,14-trimethyl | $C_{18}H_{36}O$ | 0.99 |
| 4 | 19.894 | 9-(2-Carboxyethyl)-2,2,4,4-tetramethyl-1,2,3,4-tetrahydro-y-carboline | $C_{18}H_{24}N_2O_2$ | 0.30 |
| 5 | 21.224 | n-Hexadecanoic acid | $C_{16}H_{32}O_2$ | 9.04 |
| 6 | 22.185 | Phytol | $C_{20}H_{40}O$ | 0.69 |
| 7 | 22.464 | Hexadecanoic acid,1-(hydroxymethyl)-1,2-ethanediyl ester | $C_{35}H_{68}O_5$ | 0.16 |
| 8 | 24.367 | 4,8,12,16-Tetramethylheptadecan-4-olide | $C_{21}H_{40}O_2$ | 0.48 |
| 9 | 25.919 | Phthalic acid, di(2propylpentyl) ester | $C_{24}H_8O_4$ | 2.80 |
| 10 | 34.285 | 9-Desoxy-9x-chloroingol 3,7,8,12-tetraacetate | $C_{28}H_{39}ClO_9$ | 0.30 |
| Total | | | | 100 |


**Fig1: Different seaweeds collected from various seashores in Tamilnadu**

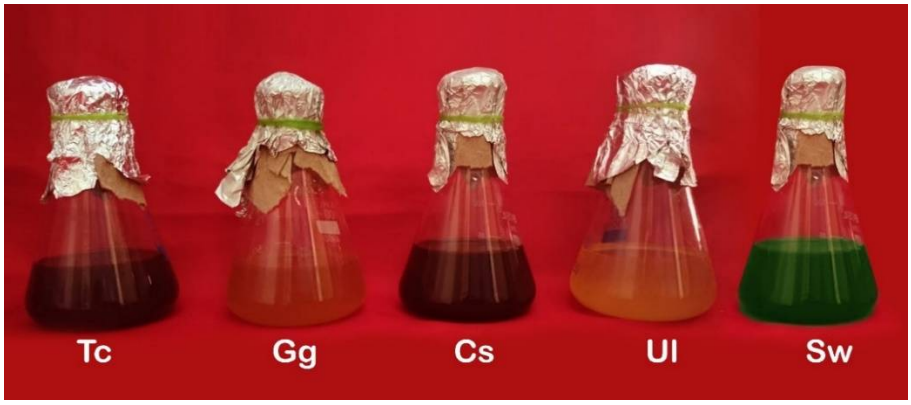


**Fig2: Extracts of different seaweeds**


**Fig3: Different solvent extract of *S. wightii***

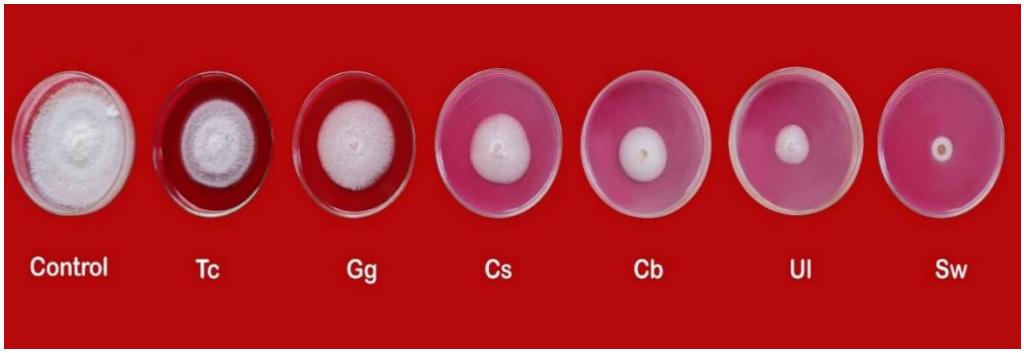


**Fig 4: Evaluation of different solvent extract against *F. solani* (Poison food technique)**

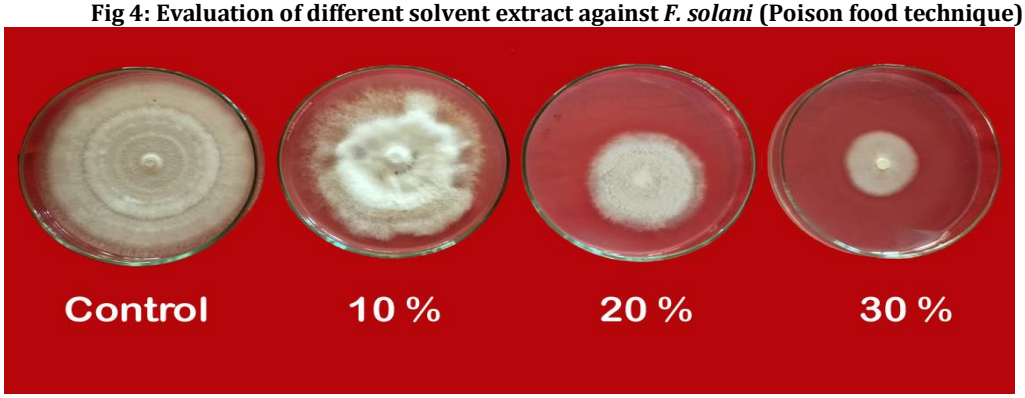


**Fig 5: Evaluation of Acetone extract of *S. wightii* against *F. solani* (poison food)**

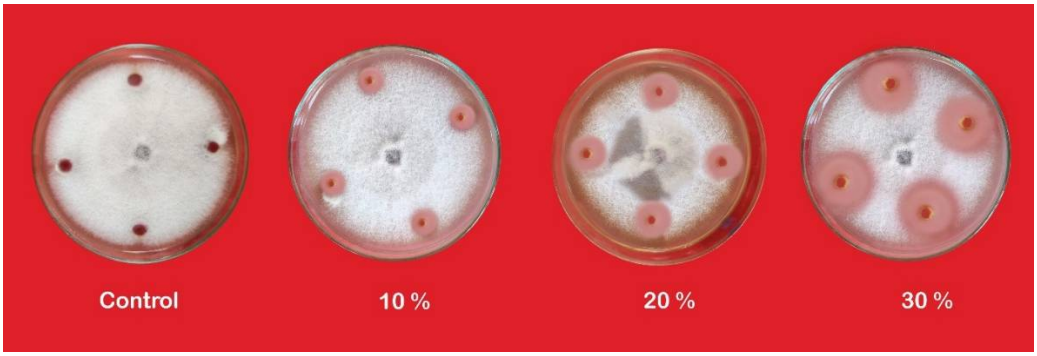


**Fig 6: Evaluation of acetone extract of *S. wightii* against *F. solani* (Agar well method)**

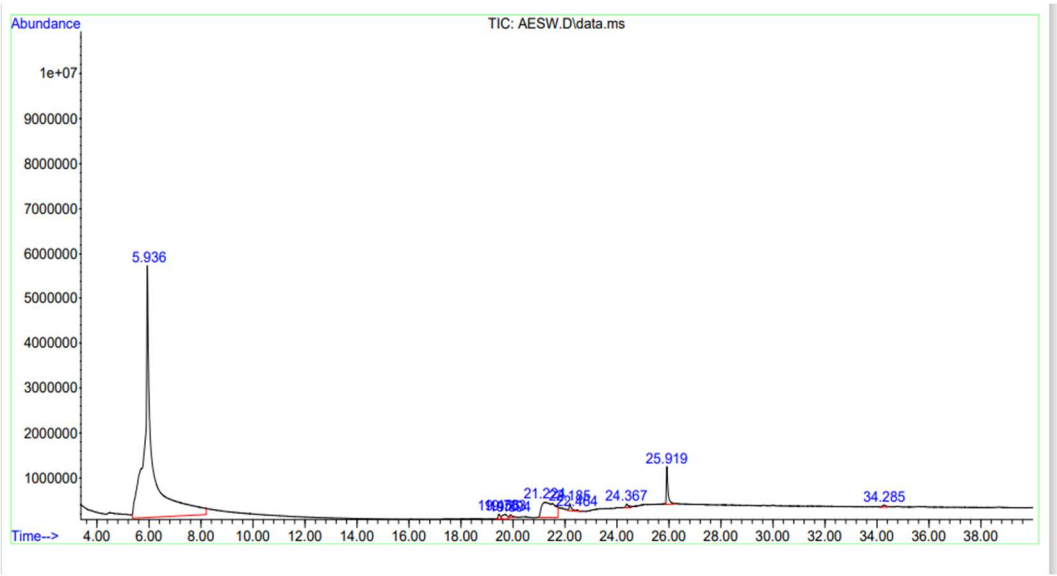


**Fig 7: Compound identified in acetone extract of *Sargassum wightii* by using GCMS**

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