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In vitro efficacy of crude extracts of *Spirulina platensis* CCC 477 on *Rhizoctonia solani* and *Magnaporthe grisea*

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ABSTRACT
Acetone, methanol and ethanol extract of Spirulina platensis CCC 477 were tested against two major fungal rice pathogens, Rhizoctonia solani and Magnaporthe grisea by four different conventional in vitro assays namely, disc diffusion test, agar well diffusion test, mycelia growth inhibition test and poisoned food technique. Concurrent solvent controls, control (deionized water) and positive control (Carbendazim) were also tested simultaneously along with extracts for comparison. No inhibition was observed in the solvent controls and in the negative control. Both the fungal pathogens showed significant reduction in mycelial growth after the treatment with Spirulina platensis CCC 477 when compared to the control. Acetone extract of Spirulina was most effective in controlling Magnaporthe grisea (Pyricularia oryzae) where as Ethanolic extract of Spirulina was effective against Rhizoctonia solani. Significant mycelial growth inhibitions of the tested pathogens were observed under laboratory conditions.
Keywords: *Spirulina platensis CCC 477*, Extract, *Magnaporthe grisea*, *Rhizoctonia solani*, Antifungal property, Mycelia growth inhibition test and Poisoned food technique.

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INTRODUCTION

Blue green algae are known for their antibacterial and antifungal property against plant pathogens [2]. Studies have proved that the algal extracts including *Spirulina platensis* help in seed germination and growth of plants. Phytochemicals present in the algal extracts are responsible for their antimicrobial activity [1]. However, only limited data are available about the antifungal effect of *Spirulina* extracts against cereal crop pathogens. Reviews also suggest the evaluation of cyanobacteria and algae for their potential use in the biological control of plant pathogenic bacteria and fungi [9].

In view of this, laboratory *in vitro* studies were conducted to evaluate the antifungal property of *Spirulina platensis* extracted with solvents *viz.,* acetone, ethanol and methanol against rice pathogens, *Magnaporthe grisea* and *Rhizoctonia solani* which cause blast and blight diseases in rice, respectively.

MATERIALS AND METHODS

The experiments were conducted in the Department of Plant Pathology, International Institute of Biotechnology and Toxicology (IIBAT), Padappai, Tamil Nadu, 601301, India during 2018.

1.0 Source

Test system: *Spirulina platensis* CCC 477 was procured from CCUBGA (Blue green algae division), Indian Agriculture Research Institute (IARI), New Delhi and mass cultured in IIBAT using modified Zarrouk medium (pH 9.5 to 10.0). The culture was maintained in growth cabinet at 3000-3200 LUX with photo period of 16/8 h of light/dark cycle. Photo periodic illumination was provided using photo synthetically active white fluorescent light.

Test Pathogens: The fungal pathogen cultures were obtained from Dept. of Plant Pathology, Faculty of Agriculture, Annamalai University, Chidambaram, Tamil Nadu, India and subcultured /maintained in IIBAT. The pathogens *Magnaporthe grisea* and *Rhizoctonia solani* culture were maintained in Potato Dextrose Agar (PDA) medium. A mycelial disc from the actively growing culture was taken using a cork borer, pressed gently on the potato dextrose agar, under aseptic condition and incubated at 28 ± 2° C for 5 days. Five sub culture plates were maintained for each pathogen. The cultures from these plates were used for the experiments.

Preparation of the Extracts

The solvents used for extraction was acetone (100%), ethanol (95%) and methanol (95%). About 25 grams of shade dried powder of *Spirulina platensis* were mixed with 100 mL of respective solvents and kept under dark condition for 24 hours with intermittent manual shaking. After 24 hours, the extracts were filtered using Whatman No.1 filter paper. The crude residue was soaked again in 100 mL of fresh respective solvents for another 24 hours. The filtrate from the first, second soaking was mixed together and the combined filtrates were concentrated using Rotavapour, R 215 (M/s. Buchi Pvt Ltd) until the material becomes gummy. Then it was diluted using 10 mL of respective solvents and used for the experiment [5, 6]. The phytochemical analyses were done for the extracts and solvents and presented in **Table 1** (**3**, **20 & 21**).

***In vitro* efficacy of *Spirulina platensis* CCC 477**

- **Controls**: Carbendazim 50% W.P served as positive control. Acetone 100%, ethanol 95% and methanol 95% served as solvent controls. Potato Dextrose Medium (Broth/Agar) inoculated with fungal pathogens, without any treatment serves as negative control in mycelia inhibition tests.
- **Incubation condition**: After inoculation, all the agar plates/ erlenmeyer flasks were incubated in an incubator at 28 ± 2 °C for 7 days. For each test pathogen and its control (solvent, positive & negative), three replications were maintained.

Disc Diffusion Test

The 48 hour old actively growing broth culture of test pathogens was used for the experiment. Pathogen cultures were spread on PDA plates using sterile cotton swabs. Sterile filter paper discs (5 mm) saturated with the *Spirulina* extracts were placed carefully on the pathogen swabbed plates using sterile forceps and incubated. The zone of inhibition around the disc was measured on Day 7 and the result was compared with that of positive control.

Agar Well Diffusion Test

Adapting pour plate method, 1mL of inoculums (48 hour**s** old pathogen incubated in PD broth) was added in sterile plates followed by the addition of the PDA medium (20mL). After solidification of the medium, 6 mm well was made on it using a cork borer.100 µl of extracts were added in the wells and the plates were incubated. The zone of inhibition around the well was measured on Day 7 and the result was compared with that of positive control.

Mycelia Growth Inhibition Test by Poisoned Food Technique.

To the sterile petriplates, 500 µl *Spirulina* extract was added followed by the addition of 20 mL PDA. After the solidification of agar, a disc of 6mm diameter from a fully grown pathogen culture was transferred on the centre of the treated PDA plates using a sterile cork borer and incubated. On day 7, the percent inhibition was computed after comparison with the negative control. Fungi toxicity was expressed in terms of percentage of mycelia growth inhibition and calculated as per the formula of Pandey *et al.* [12].

Growth inhibition=(dc-dt) /dc × 100, where, dc= average diameter of fungal colony in control and dt = average diameter of fungal colony in treatment.

Mycelia growth inhibition test by Determination of mycelia dry weight.

500 µl of the extract was added to 100 mL of PD broth taken in a 250 mL Erlenmeyer flask. 6mm agar discs were taken from 5 days old culture plates and inoculated in the treated

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flask. The inoculated flasks were incubated for 10 days and the mycelia were filtered through Whatman filter No.1 paper and washed with deionised water. The mycelia were allowed to dry at 60°C for 6 h and then at 40°C overnight. The filter paper containing mycelia were weighed and the percent inhibition on the basis of dry weight was calculated as

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

Where,
I = Inhibition percent
C = Mycelia dry weight of control (g)
T = Mycelia dry weight of Treatment (g)
Note: Similar experimental procedures were followed for positive, negative and solvent controls for all the above methods.

Statistical analysis

All the statistical analysis was done using ECOSTAT statistical software in SAS environment. One-way ANOVA followed by LSD (least significant difference) for post hoc comparison, was performed to do the comparison of data.

RESULTS

Phytochemical analyses of extracts and solvent

Qualitative phytochemical analyses of extracts and controls were done to understand the nature of the same. All the extracts possess phytochemicals like alkaloids, tannins, flavonoids and saponins which are commonly responsible for the antimicrobial activity. These chemicals were absent in the pure solvents **(Table 1).**

Disc diffusion test

Magnaporthe grisea

The efficacy of the extracts against *M.grisea* based on statistical analysis were Carbendazim (13.0mm) > acetone extract (6.7 mm) and ethanol extract (6.3 mm) > methanol extract (0.0) whereas the solvent controls showed 0% inhibition (acetone (0.0) = ethanol (0.0) = extracts (0.0)) **Figure 1 & 3** and **Table 2**

Rhizoctonia solani

The efficacy of the extracts against *R.solani* based on statistical analysis were Carbendazim (6.0 mm) > ethanol extract (5.3 mm) > acetone extract (0.0 mm) and methanol extract (0.0) where as the solvent controls showed 0% inhibition (acetone (0.0) = ethanol (0.0) = methanol (0.0)) **Figure 1** and **Table 3**

Agar well diffusion test

Magnaporthe grisea

The efficacy of the extracts against *M.grisea* based on statistical analysis are acetone extract (8.3mm) > Carbendazim (7.3 mm) > ethanol extract (5.0 mm) > methanol extract (0.0) where as the solvent controls showed 0% inhibition (acetone (0.0) = ethanol (0.0) = methanol (0.0)) **Figure 1 & Table 2.**

Acetone based *Spirulina* extract showed the maximum inhibition than the positive control. Next to it, ethanol extract showed fungal growth inhibition.

Rhizoctonia solani

The efficacy of the extracts against *R.solani* based on statistical analysis were methanol extract (14.0 mm) > acetone extract (9.0 mm) > carbendazim (5.7 mm) > ethanol extract (0.0 mm) and whereas the solvent controls showed 0% inhibition (acetone (0.0) = ethanol (0.0) = methanol (0.0) **Figure 1& 4**. Methanol based *Spirulina* extract showed the maximum inhibition followed by acetone extract. **Table 3**

Note: The figures in parentheses are the zone of inhibition observed in mm.

Poisoned Food Technique

Magnaporthe grisea

The sequence of treatments based on percentage mycelia growth inhibition is mentioned as follows: carbendazim (100%) >acetone extract (54.6%) > ethanol extract (45.5%) > methanol extract (28.6%) >ethanol (2.8%) = methanol (2.2%) = acetone (1.9%) Figure 2 & 5 and Table 2

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Acetone based *Spirulina* extract is having most mycelia growth inhibitory effect on *Magnaporthe grisea* followed by ethanol and methanol extracts. The pure solvent treatments shows negligible amount of inhibitions (1.9 - 2.8%) compared to the extracts. Table 2

Rhizoctonia solani

The effects of the extracts in terms of percentage mycelia growth inhibition was analyzed statistically and the results are mentioned as follows. carbendazim (100%) > ethanol extract (2.2%) > acetone extract (1.1%) > methanol extract (0.0%), ethanol (0.0%) > acetone (-0.4%), methanol (-0.7%) **Figure 2 & Table 3.**

Ethanol based *Spirulina* extract showed the maximum fungal growth inhibition compared to other *Spirulina* extracts.

Mycelia dry weight determination

Magnaporthe grisea

The sequence of treatment based on percentage growth inhibition is carbendazim (37.5%) = acetone extract (34.4%) > ethanol extract (22.3%), methanol extract (22.3%) > methanol (9.9%) > ethanol (9.3%) > acetone (7.1%) **Figure 2&6**

Acetone based *Spirulina* extract is having most inhibitory effect on *Magnaporthe grisea* followed by ethanol extract and methanol extract. The pure solvent treatments shows negligible amount of inhibitions (7.1 - 9.9%) compared to the extracts. **Table 2*Rhizoctonia solani***

The efficacy of the extracts in terms of percentage inhibition was analyzed statistically and the results are mentioned as follows; ethanol extract (60.3%) > acetone extract (20.2%) > carbendazim (11.9%) > methanol extract (10.7%) > acetone (5.6%) > methanol (4.8%) & ethanol (2.4%) **Figure 2**

Ethanol based *Spirulina* extract showed the maximum fungal growth inhibition followed by acetone extracts. The solvents show 2.4 to 5.6% of inhibition, which was negligible, compared to the extracts. **Table 3.**

Note: The figures in parentheses are the percentage mycelia inhibition, compared to the negative control.

Table 1: Phytochemical Analysis of Extracts And Solvents

Extracts and solvents	Alkaloids (Mayer's Test)	Tannins (Ferric Chloride Test)	Flavonoids (Sodium Hydroxide Test)	Terpenoids/ steroids (Salkowski Reaction)	Saponins (Foam Test)
Acetone extract	Present	Present	Present	Present	Present
Ethanol extract	Present	Present	Present	Present	Present
Methanol extract	Present	Present	Present	Present	Present
Acetone	Absent	Absent	Absent	Absent	Absent
Ethanol	Absent	Absent	Absent	Absent	Absent
Methanol	Absent	Absent	Absent	Absent	Absent

Mean of three replications

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Table 2 Efficacy of *Spirulina platensis* Extracts Against *Magnaporthe grisea*

In vitro methods	Parameters Observed	Negative control	Positive control	Acetone extract	Ethanol extract	Methanol extract	Acetone	Ethanol	Methanol	LSD
Disc Diffusion	*Zone of Inhibition (mm)*[1]	-	13.0[a] (1.0)	6.7[b] (2.1)	6.3[b] (1.5)	0.0[c] (0.0)	0.0[c] (0.0)	0.0[c] (0.0)	0.0[c] (0.0)	1.8
Agar Well Diffusion		-	7.3[a] (0.00)	8.3[a] (1.5)	5.0[b] (1.0)	0.0[c] (0.0)	0.0[c] (0.0)	0.0[c] (0.0)	0.0[c] (0.0)	1.6
Poisoned food technique	*Mycelia growth(cm)*	7.7 (0.2)	0.0[a] (0.0)	3.5[b] (0.5)	4.2[c] (0.1)	5.5[d] (0.2)	7.6[e] (0.2)	7.5[e] (0.1)	7.5[e] (0.3)	0.4
	Growth Inhibition (%)[2]	-	100.0	54.6	45.5	28.6	1.9	2.8	2.2	-
Mycelia dry weight determination	*Mycelia weight(g)*	1.1 (0.0)	0.7[a] (0.1)	0.7[a] (0.1)	0.8[b] (0.1)	0.8[b] (0.1)	1.0[c] (0.0)	1.0[c] (0.0)	1.0[c] (0.1)	0.1
	Growth Inhibition (%)[3]	-	37.5	34.4	22.3	22.3	7.1	9.3	9.9	-

Figures in parentheses are standard deviation: [1]mean of three replications; Means followed by similar letter are not statistically different (One way ANOVA with Least Significant Difference); [2]% Growth inhibition= (Mean mycelia growth of negative control in cm - Mean mycelia growth of extract in cm) × 100 [3]% Growth inhibition = (Mean mycelia growth of negative control in g - Mean mycelia growth of extract in g)/ mean mycelia growth of negative control (g) ×100; LSD- Least significant difference.

Table 3 Efficacy of *Spirulina platensis* Extracts Against *Rhizoctonia solani*

Invitro methods	Parameters Observed	Negative control	Positive control	Acetone extract	Ethanol extract	Methanol extract	Acetone	Ethanol	Methanol	LSD
Disc Diffusion	*Zone of Inhibition (mm)*	-	6.0[a] (0.0)	0.0[c] (0.0)	5.3[b] (0.6)	0.0[c] (0.0)	0.0[c] (0.0)	0.0[c] (0.0)	0.0[c] (0.0)	0.4
Agar Well Diffusion		-	5.7[c] (0.6)	9.0[b] (1.0)	0.0[d] (0.0)	14.0[a] (2.0)	0.0[d] (0.0)	0.0[d] (0.0)	0.0[d] (0.0)	1.5
Poisoned food technique	*Mycelia growth(cm)*	9.0 (0.0)	0.0[a] (0.0)	8.9[ab] (0.2)	8.8[b] (0.3)	9.0[ab] (0.0)	9.0[c] (0.1)	9.0[ab] (0.1)	9.1[c] (0.1)	0.2
	Growth Inhibition (%)[2]	-	100.0	1.1	2.2	0.0	-0.4	0.0	-0.7	-
Mycelia dry weight determination	*Mycelia weight(g)*	0.8 (0.1)	0.7[ab] (0.0)	0.7[b] (0.0)	0.3[a] (0.2)	0.8[ab] (0.0)	0.8[ab] (0.0)	0.8[c] (0.1)	0.8[c] (0.1)	0.1
	Growth Inhibition (%)[3]	-	11.9	20.2	60.3	10.7	5.6	2.4	4.8	-

Figures in parentheses are standard deviation: [1]mean of three replications; Means followed by similar letter are not statistically different (One way ANOVA with Least Significant Difference); [2]% Growth inhibition= (Mean mycelia growth of negative control in cm - Mean mycelia growth of extract in cm)×100 [3]% Growth inhibition = (Mean mycelia growth of negative control in g – Mean mycelia growth of extract in g)/ mean mycelia growth of negative control (g) ×100; LSD- Least significant difference.

Figure 1 Effect of *Spirulina* Extracts on *Magnaporthe grisea* (*Pyricularia oryzae*) & *Rhizoctonia solani* Under *Invitro* Test (Zone of Inhibition)

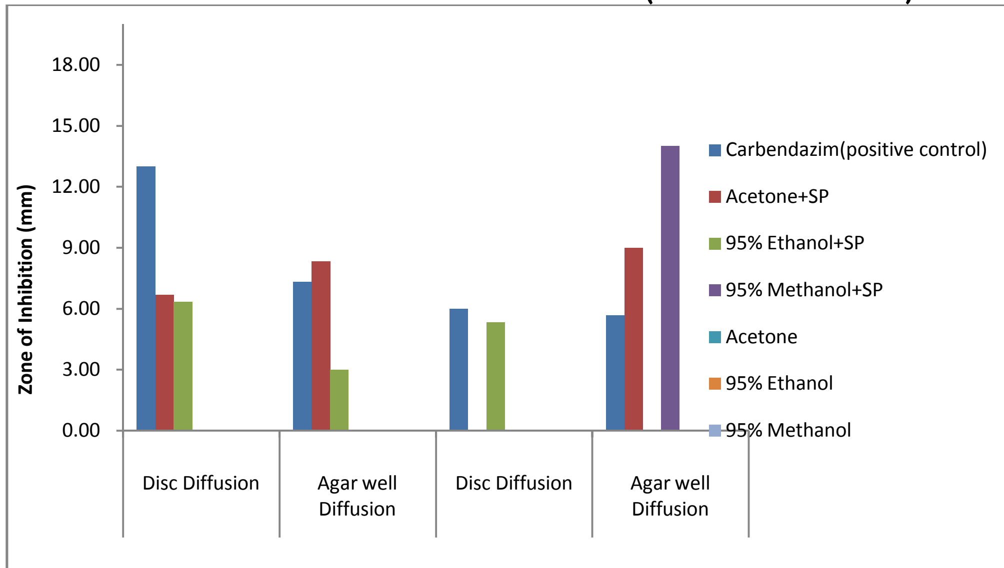


Figure 2 Effect of *Spirulina* Extracts on *Magnaporthe grisea* (*Pyricularia oryzae*) & *Rhizoctonia solani* Under *Invitro* Test (Mycelia Growth Inhibition Assay)

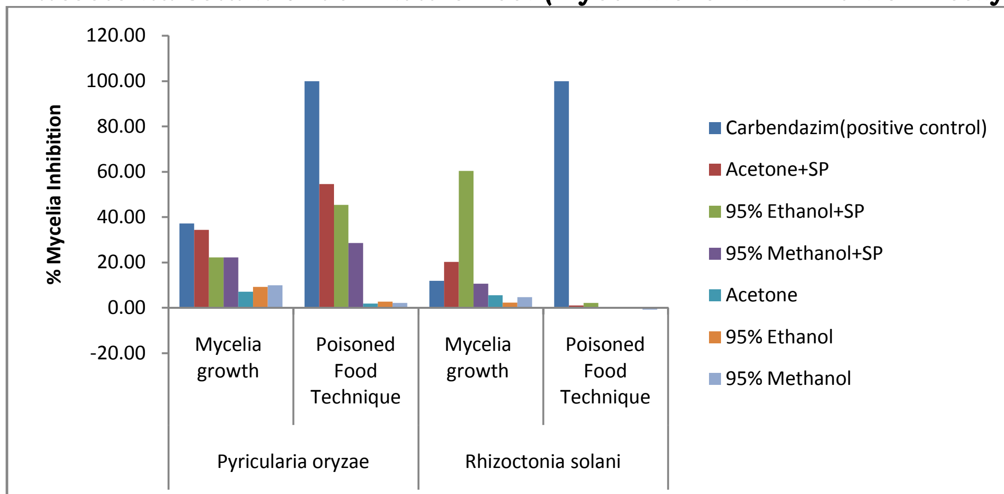
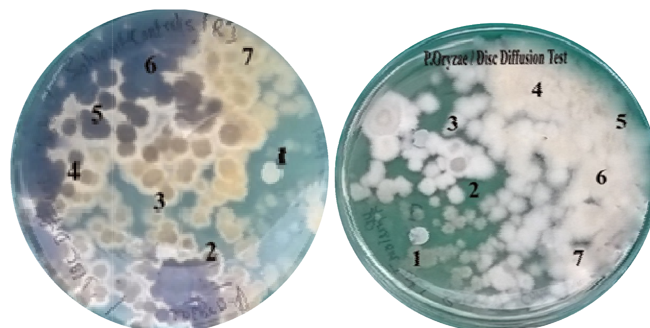


Figure 3 Disc Diffusion Results of Magnaporthe grisea (Zone of Inhibition)



1. Carbendazim; **2** Acetone extract ;3. Ethanol extract ; **4.** Methanol extract; **5.** Acetone; **6.** Ethanol;**7.** Methanol

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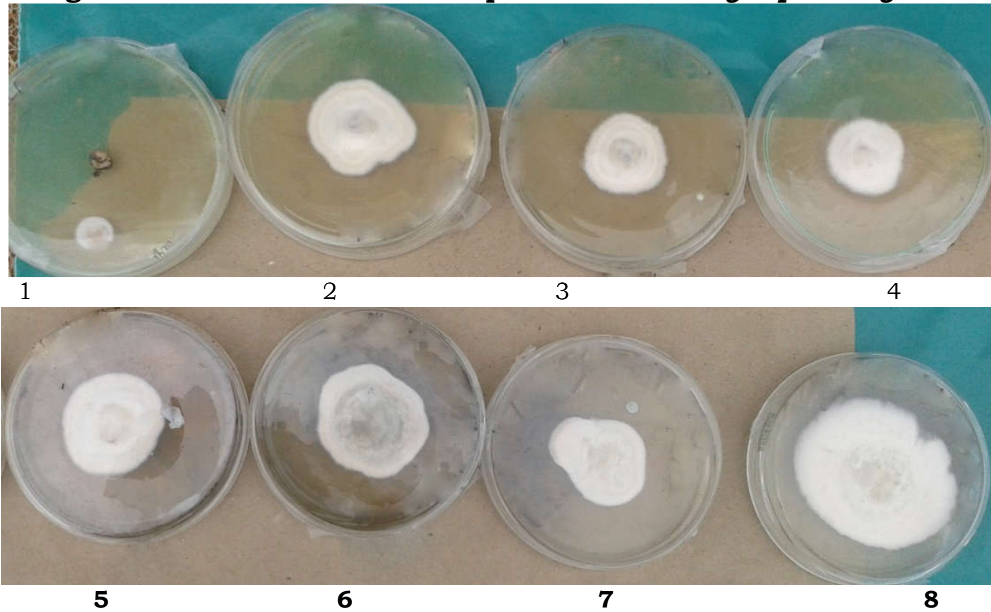
Figure 4: Agar Well Diffusion Results of *Rhizoctonia solani* (Zone of Inhibition)

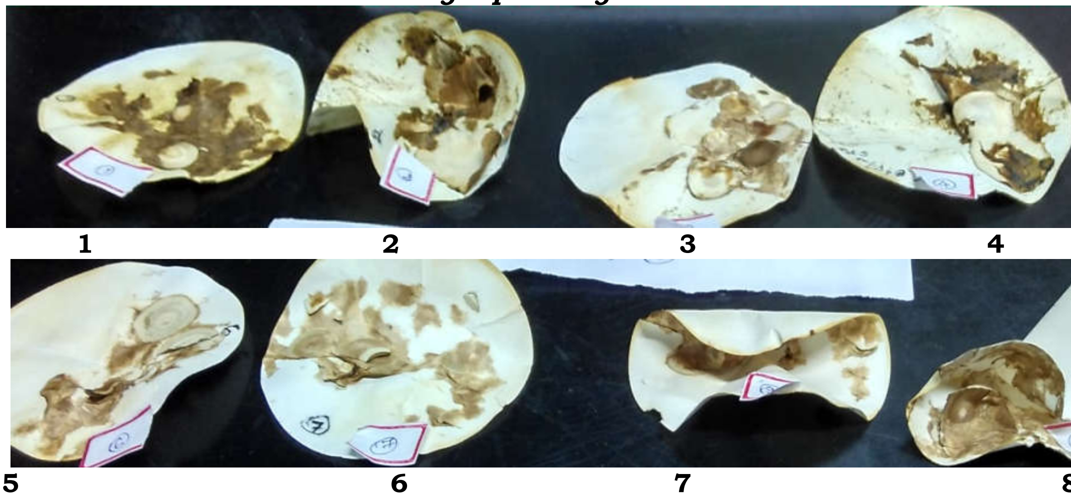
1. Methanol extract; **2.** Acetone extract; **3.**Carbendazim; **4.** Ethanol extract **5.**Acetone; **6.** Ethanol; **7.** Methanol

Figure 5: Poisoned Food Technique Results of *Magnaporthe grisea*



1. Carbendazim; **2**. Methanol extract; **3**. Ethanol extract; **4**. Acetone extract; 5. Methanol **6**. Acetone **7**. Ethanol 8. Negative control

Figure 6 Mycelia Dry Weight Determination (% Growth Inhibition) Results of *Magnaporthe grisea*



1.Ethanol extract: **2**.Carbendazim; **3**. Acetone extract; **4**.Methanol extract; **5**.Ethanol; **6**.Acetone; **7**. Methanol **8**.Negative control.

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DISCUSSIONS

The current investigation shows that the blue green alga, *Spirulina platensis* has antifungal activity against the pathogens *Magnaporthe grisea* and *Rhizoctonia solani* tested under *in vitro* conditions. Four types of antifungal assays were performed to confirm the results. Disc diffusion test and Agar well diffusion tests are preliminary screening tests used in antimicrobial assay. The compounds responsible for antimicrobial properties may not disperse in the disc or diffuse in agar. In order to ratify the result, the mycelia inhibition assays were also conducted here. The results are comparable in all the antifungal assays. Among the extracts, acetone and ethanol extracts were most efficient against *Magnaporthe grisea* and *Rhizoctonia solani.* The fungicidal activity of *S.platensis* was similar to that of *Nostoc* sp. which was studied against five rice pathogens [8].

The current result is well comparable with the previous investigations [10], [14], [15], [17], [18], [19] and [22]. The literautures state that lipids, tocopherols(13) andC.phycocyanin (11)are some of the chemicals responsible for the antifungal activity of *S. platensis*. Study by Moraes de Souza *et.al.* [19] revealed that the methanolic extract of *Spirulina* has reduced the glucosamine production significantly in the *Aspergillus flavus*. Reed, [16]found out that the phenolic compounds of the blue - green algae contain multi free hydroxyl groups which forms hydrogen bonds with carbohydrates and proteins found in the fungal cell wall .This changes the enzyme nature inside the cell and the complex mixture inhibits the essential metabolism reactions required for reproduction and growth of fungi. Similar mechanisms of inhibition are suspected in this experiment too.

CONCLUSIONS

- *Spirulina* extracts possess antifungal effects against *Magnaporthe grisea* and *Rhizoctonia solani* under *in vitro* experiments.
- Both fungal plant pathogens showed significant reduction in mycelial growth after the treatment with *Spirulina platensis* CCC 477 culture extracts when compared to the control.
- The mycelial growth inhibition effect of ethanol and acetone extracts against *M.grisea* was in the range of 45.5 - 54.6% (Poisoned food technique) and the effect of ethanol extract against *R.solani* (60.3%) (Mycelia Dry Weight Determination test**)** were more than the minimum prescribed (CIB-RC) requirement of 35% reduction in target organism under laboratory conditions tested (**7**).
- Further studies under *in vivo* conditions are required to understand the mechanism involved in the control of the plant pathogens *Magnaporthe grisea* and *Rhizoctonia solani* by *Spirulina platensis* extracts.

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REFERENCES

1. Aly, M. H., Abd El-All, Azza, A. M., Mostafa and Soha, S. M., [2008].Enhancement of sugar beet seed germination, plant growth, performance and biochemical components as contributed by algal extracellular products. J. Agric. Sci. Mansoura Univ., **33 (12):8429-8448**.
2. Archana Tiwari and Akshita Sharma., [2013]. Antifungal activity of *Anabaena variables* against plant pathogens. Int J Pharm Bio Sci., **4(2):(B) 1030-1036**.
3. Bargah, R.K., [2015]. Preliminary test of phytochemical screening of crude ethanolic and aqueous extract of *Moringa pterygosperma Gaertn* . Journal of Pharmacognosy and Phytochemistry; **4(1): 07-09**.
4. ECOSTATS Program version 2012.06.03 SAS version 9.3, SAS Institute Inc., Cary, NC, USA, 2002-2010.
5. El-Baz F.K., El-Senousy, W.M., El-Sayed, A.B. and Kamel, M.M. [2013]. *In vitro* antiviral and antimicrobial activities of *Spirulina platensis* extract. Journal of Applied Pharmaceutical Science., **Vol.3 (12), pp.052-056**.
6. Fayzunnessa, N., Morshed, A., Uddin ,A., Parvin, A. and Saifur, R. [2011]. *In vivo* study on the efficacy of hypoglycemic activity of *Spirulina plantesis* in long evan rats. International Journal of Biomolecules and Biomedicine (IJBB)., **1:27-33**.

7. Guidelines / Data Requirements for Registration of Antagonistic Bacteria under section 9(3B) and 9(3) of the Insecticide Act, 1968 (w.e.f. 1st January 2011).
8. Jaiswal, P., Prasanna, R., Singh, P.K., and Prem Dureja., [2014]. Biocidal spectrum of a rice field cyanobacterium *Nostoc sp.* Egyptian Journal of Biology., **Vol. 16, pp 87-94**.
9. Martin M. Kulik ., [1995]. The potential for using cyanobacteria (blue-green algae) and algae in thebiological control of plant pathogenic bacteria and fungi. European Journal of Plant Pathology., **101: 585-599**.
10. Medina-Jaritz, N.B., Perez-Solis, D.R., Ruiloba de Leon, F., and Olvera-Ramirez, R., [2011]. Antimicrobial activity of aqueous and methanolic extracts from *Arthrospira maxima.* Formatex., **1267-1271**.
11. Murugan,T., Manikantavelu,T., and Saranraj, P., [2012]. Growth and bio-pigment production of three microalgal species in organic and inorganic media and determination of generation time - a comparative study. International Journal of Pharmaceutical & Biological Archives., **3(1):140-145**.
12. Pandey, D. K., Chandra, H., and Tripathi, N. N., [1982]. Volatile fungitoxic activity of some higher plants with special reference to that of *Callistemon lanceolatus* DC., Phytopath.Z., Bd.**105**,Heft **2**.
13. Ramadan, M.F., Asker, M.M., and Ibrahim, Z.K. [2008]. Functional bioactive compounds and biological activities of *Spirulina platensis* lipids. Czech J.Food Sci., **26(3)**, **211-222**.
14. Ramamurthy, V., Raveendran, S., Thirumeni, S., and Krishnaveni, S., [2012] Antimicrobial activity of heterocytic cyanobacteria. Int. J. Adv. Lif. Sci., **1:32-39**.
15. Rania, MAA., and Taha. HM. [2008]. Antibacterial and antifungal activity of cyanobacteria and green microalgae. Evaluation of medium components by placket burman design for antimicrobial activity of *Spirulina platensis*. Global J. Biotechnol. Biochem., **3(1): 22-31**.
16. Reed, J. [1995]. Nutritional toxicology of tannis and related polyphenols in forage legumes. J.Animal Soc., **73:1516-1528**.
17. Santoyo, S., Herrero, M., Javier, F., Cifuentes, A., Ibanez, E., and Jaime, L. [2006]. Functional characterization of pressurized liquid extracts of *Spirulina platensis*. Eur. Food Res. Technol., **224: 75-81**.
18. Sivakumar, J. and Santhanan, P. [2011]. Antipathogenic activity of *Spirulina platensis*. Recent Res. Sci. Technol., **3 (4): 158 -161**.
19. Souza, MM., Prietto, L. T., Denardi de Souza., Ribeiro, AC. and Badiale - Furlong, E .[2011]. Assessment of the antifungal activity of *Spirulina platensis* phenolic extract against *Aspergillus flavus*. Ciênc. Agrotec., **35(6):1050-1058**.
20. Sudha, S.S., Karthic, R., Rengaramanujam, .J. and Athulya. [2011]. Antimicrobial activity of *Spirulina platensis* and *Aphanothece* sp. on selected clinical bacterial isolates and its antioxidant activity. South As. J. Biol. Sci.*,* **1(2)2011:87-98**.
21. Usman, H., Abdulrahman, F.I. and Usman, A. [2009]. Qualitative phytochemical screening and In *vitro* antimicrobial effects of methanol stem bark extract of *Ficusthonningii* (*Moraceae*). Afr. J. Trad. CAM., **6(3):289-295**.
22. Uyisenga, J.P., Nzayino, P.,Seneza, R., Hishamunda, L., Uwantege, K., Gasana,N. and Bajyana. E.S., [2010)]. *In vitro* Study of Antibacterial and Antifungal Activity of *Spirulina platensis*. Int. J. Ecol. and Develop., **16(10): 315-719**.