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MYCOHERBICIDAL POTENTIAL OF *SCLEROTIUM ROLFSII* SACC. AGAINST *PARTHENIUM*: FACTORS AFFECTING *INVITRO* GROWTH AND SCLEROTIUM FORMATION

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Physiological requirements of two indigenous isolates of *Sclerotium rolfsii* (SRI & SR2) tested as mycoherbicides against *Parthenium*, a noxious weed in India, were investigated and discussed in this paper. Maximum growth and sclerotial formation were recorded on Potato Dextrose Agar medium at 30°C ± 2°C, 6.5 pH. Glucose and potassium nitrate were the best source of carbon and nitrogen respectively.

Keywords : *Sclerotium rolfsii; Parthenium;* Physiochemical Factors.

Introduction

The concept of mycoherbicide is now well recognised in the scientific community and its biological, technological and commercial perspectives are exhaustively reviewed in many publications [1-10]. Realising the potential and need of reliable, safer and low cost weed management strategy in India, a large number of indigenous fungal pathogens including two isolates of *Sclerotium rolfsii* Sacc. (SR_1 & SR_2) have been screened for their herbicidal potential against *Parthenium (P. hysterophorus L)* noxious weed in India [11-20]. Studies conducted earlier reveals that both the isolates of the pathogen incite severe collar rot infection and have significant potential to reduce the population of the weed during rainy season[12-14,19]. Successful exploitation of these pathogens as mycoherbicides needs sufficient amout of inoculum. Therefore, the present investigation was carried out to determine the suitable conditions for maximum *invitro* growth and sclerotial formation. This will also help in the formulation of the inoculum for field applications.

Materials and Methods

a) *Fungal Cultures*: Cultures of two isolated of *S. rolfsii* were obtained from the Mycological Cultrue Collection of Biological Science Department, Jabalpur, isolated earlier from diseased seedlings of *Parthenium*. These were transferred to Potato Dextrose Agar medium and stored at 4°C ± 1°C temperature for further study.

b) *Culture Media : In vitro* growth and sclerotial formation was determined on fourteen nutrient media viz Potato Dexrose Agar, Corn meal agar, Oat meal agar, Malt extract Agar, Nutrient Agar, Asthana and Hawker's medium, Richards medium, Sabourad dextrose agar, Wheat germ glucose agar, Soil extract agar, Hutchinson agar, Host extract agar and Paper extract agar medium. The composition of the meida was as suggested by Agarwal and Hasija.[21] Asthana and Hawkers medium was used as basal medium.

c) *Temperature*: The inoculated plates were kept at 15, 20, 25, 30, 40 and 45°C ± 2°C temperatures in BOD incubators.

d) *Hydrogen-lon concentration*: The fungal isolates were grown in basal medium of different pH values ranges from 5-12 at 28°C±2°C. pH of the medium was adjusted by addition of 0.1 HCl or NaOH before autoclaving.

e) *Carbon sources*: Fourteen carbon sources listed in the table 4 were used. Glucose of the basal medium was replaced singly by various

carbon compounds. The quantity of different compounds was so adjusted so as to contain an amount of carbon equivalent to that of present in the 5 gms of glucose except for strach and dextrin as their empirical formula are not known. In these cases the quantity was similar to that of glucose (5 gms).

f) *Nltrogen Sources*: Similarly for studying the effect of nitrogenous compounds, potassium nitrate of the basal medium was substituted by different nitrogen compounds. The quantity of different compounds was so adjusted so as to contain an amount of nitrogen equivalent to that present in 5 gms of KNO_3.

The quality and quantity of the inoculum are the major factors, influencing the nutritional experiments with fungi[22]. Hence, the inoculation was done by equal size of the circular disc from a seven days old culture grown on Potato Dextrose Agar medium. The observations were recorded after seven and fifteen days for growth (colony diameter) and sclerotial formation respectively. For data analysis method of Abbott[23] was followed.

Results and Discussion

a) *Culture Medium*: It is evident from the table 1 that both the isolates of *S. rolfsii* showed maximum growth and sclerotial formation on Potato Dextrose agar medium. It was followed by Asthana and Hawker's Medium and Host extract medium. They failed to produce sclerotia on Oat meal, Corn meal and Malt extract, Richerds agar, Nutrient agar and Sabourauds media except few sclerotial initial formed on medium Oat meal by SR2. Asthana and Hawker's Medium was selected as basal medium for subsequent studies due to its synthetic nature.

b) *Temperature*: The data presented in the table 2 clearly indicates that both the isolates could grow within a temperature range of 15-45° C ± 2°C. With gradual increase in temperature from 15°C there was a grdual increase in colony diameter, being maximum at 30°C ± 2°C and then reduced drastically beyond 35°C ± 2°C. Only a trace of hyphal extension were observed at 40°C and 45°C ± 2°C temperatures. Similar trends were also recorded in sclerotial formation in both the isolates. Maximum number of sclerotia was recorded at 30°C followed by 35°C and 25°C± 2°C temperatures. Lower and higher temperatures did not favour sclerotial formation. This indicate that slightly higher temperature is essential for the formation of sclerotia. However, contrary to this several workers recorded best growth and sclerotial formation in some isolates of *S. rolfsii* in between 20-25°C temperature, while below or above these did not support the same[25-27]. The size and maturation of sclerotia were also found to be highly variable at different temperatures. At lower temperature sclerotia required longer period for maturation. Largest size and higher rate of maturation of sclerotia were recorded at 30°C ± 2°C. A general opinion about these findings is that the fungus have some distinct races amongst different isolates.

c) *Hydrogen-lon concentrations*: It is evident form the table 3 that both the isolates of *S. rolfsii* attained their maximum growth at slightly acidic medium i. e. 6.5 pH. pH 7.0 and 5.0 were also found to support significant growth while dicrease or increase in pH beyond these considerabley reduced the growth and only few mycelial strands around the incoulum were observed at pH 10.0 and 11.0. Maximum number and larger size of sclerotia were observed at 5.0 pH. It was followed by 9.0 pH which did not support the

Table 1. Effect of different nutrient media on radial growth and intensity of sclerotium formation in *Parthenium* isolates of *Sclerotium rolfsii.*

Nutrient media		SR_1[a]		SR_2[a]	
		Colony diameter* (cm)	Intensity of Sclerotium formation**	Colony diameter* (cm)	Intensity of Sclerotium formation**
1.	Potato Dextrose Agar	6.21 ± 1.108	40.55 ± 1.024	5.69 ± 1.212	27.05 ± 1.115
2.	Hutchinson Agar	1.97 ± 1.162	2.98 ± 1.237	1.69 ± 1.021	2.08 ± 1.120
3.	Wheat Germ	3.13 ± 1.062	2.45 ± 1.072	1.50 ± 1.001	1.68 ± 1.041
4.	Wheat Agar	3.14 ± 1.032	3.31 ± 1.310	2.81 ± 1.342	1.45 ± 1.167
5.	Oatmeal Agar	0.64 ± 1.011	0.00	1.37 ± 1.011	0.20 ± 0.003
6.	Corn meal Agar	1.59 ± 1.031	0.00	1.98 ± 1.311	0.00
7.	Malt extract	2.69 ± 1.231	0.00	2.13 ± 1.021	0.00
8.	Host extract agar	4.13 ± 1.212	9.05 ± 1.332	3.80 ± 1.325	7.55 ± 1.305
9.	Asthana Hawker	4.17 ± 1.312	17.55 ± 1.767	3.09 ± 1.31	14.05 ± 1.654
10.	Richards	1.64 ± 1.301	0.00	1.94 ± 1.051	0.00
11.	Nutrient agar	0.03 ± 0.012	0.00	0.44 ± 0.012	0.00
12.	Saubroudes dextrose agar	3.10 ± 1.011	0.00	2.70 ± 0.001	0.00
13.	Paper extract agar	0.27 ± 0.001	6.75 ± 1.208	0.38 ± 0.012	1.45 ± 1.167
14.	Soil extract agar	0.16 ± 0.002	2.55 ± 1.151	0.13 ± 0.005	1.65 ± 1.020

* = after 7 days; * * = after 15 days; a = an average of 4 replicates CD at 5% = 44 (SR_1); 2.25 (SR_2); Corrected by Abbott's Formulae

Table 2. Effect of different Temperature on *in vitro* radial growth and intensity of sclerotium formation in *Parthenium* isolates of *Sclerotium rolfsii.*

Temperature	SR_1[a]		SR_2[a]	
	Colony diameter* (cm)	Intensity of Sclerotium formation**	Colony diameter* (cm)	Intensity of Sclerotium formation**
15	1.72 ± 1.022	0.02 ± 0.001	1.68 ± 1.300	0.05 ± 0.001
20	3.43 ± 1.311	1.24 ± 1.069	3.48 ± 1.000	1.28 ± 1.601
25	4.44 ± 1.345	10.75 ± 1.707	3.89 ± 1.305	3.40 ± 1.251
30	5.26 ± 1.227	41.28 ± 1.901	8.62 ± 1.241	48.78 ± 1.035
35	3.35 ± 1.031	36.17 ± 1.667	4.10 ± 1.211	36.28 ± 1.025
40	0.86 ± 0.003	1.20 ± 1.010	1.20 ± 1.010	1.25 ± 1.021
45	0.02 ± 0.010	0.05 ± 0.001	0.08 ± 0.004	0.75 ± 0.012

* = after 7 days; * * = after 15 days; a = an average of 4 replicates CD at 5% = 30.7 (SR_1); 44.84 (SR_2); Corrected by Abbott's Formulae

Table 3. Effect of different Hydrogen ion concentration on radial growth and intensity of sclerotium formation in *Parthenium* isolates of *Sclerotium rolfsii*.

Hydrogen ion Concentration	SR_1[a]		SR_2[a]	
	Colony diameter* (cm)	Intensity of Sclerotium formation**	Colony diameter* (cm)	Intensity of Sclerotium formation**
5.0	3.65 ± 1.301	38.25 ± 1.031	3.27 ± 1.210	32.48 ± 1.092
5.5	3.05 ± 1.250	22.40 ± 1.213	3.00 ± 1.205	20.20 ± 1.132
6.0	3.37 ± 1.350	18.12 ± 1.231	3.38 ± 1.215	0.00
6.5	4.72 ± 1.441	15.16 ± 1.067	4.02 ± 1.313	16.20 ± 1.077
7.0	4.05 ± 1.140	12.26 ± 1.092	3.95 ± 1.125	12.82 ± 1.000
7.5	2.48 ± 1.103	10.21 ± 1.126	2.30 ± 1.103	10.02 ± 1.071
8.0	1.37 ± 1.160	18.58 ± 1.117	2.60 ± 1.019	15.02 ± 1.201
8.5	1.02 ± 1.001	25.75 ± 1.170	1.32 ± 1.107	18.10 ± 1.011
9.0	0.57 ± 0.021	31.78 ± 1.116	1.60 ± 1.110	28.25 ± 1.145
9.5	0.08 ± 0.001	10.50 ± 1.012	0.06 ± 0.003	9.58 ± 1.192
10.0	0.08 ± 0.001	10.50 ± 1.012	0.06 ± 0.003	9.58 ± 1.910
10.5	0.002 ± 0.001	0.00	0.00	0.00
11.0	0.002 ± 0.001	0.00	0.00	0.00

* = after 7 days; * * = after 15 days; a = average of 4 replicates; Temperature 28 ± 2°C; CD at 5% = 18.89 (SR_1); 5.67 (SR_2); Corrected by Abbott's Formulae

Table 4. Effect of different carbon sources on *in vitro* radial growth and intensity of sclerotium formation in *Parthenium* isolates of *Sclerotium rolfsii*.

Carbon Compounds	SR_1[a]		SR_2[a]	
	Colony diameter* (cm)	Intensity of Sclerotium formation**	Colony diameter* (cm)	Intensity of Sclerotium formation**
Glucose	5.83 ± 1.113	46.11 ± 1.024	6.22 ± 1.131	47.34 ± 1.685
Maltose	2.95 ± 1.005	12.52 ± 1.071	3.18 ± 1.114	21.71 ± 1.161
Fructose	2.97 ± 1.011	15.14 ± 1.061	1.91 ± 1.091	12.22 ± 1.117
Mannose	3.01 ± 1.017	24.34 ± 1.134	3.18 ± 1.231	29.48 + 1.231
Xylose	1.97 ± 1.031	5.71 ± 1.310	2.92 ± 1.111	13.71 ± 1.017
Lactose	1.99 ± 1.110	0.79 ± 0.003	1.28 ± 1.007	10.71 ± 1.120
Raffinose	0.07 ± 0.002	10.12 ± 1.015	1.72 ± 1.011	26.21 ± 1.261
Galactose	1.85 1.007	0.70 ± 0.003	4.07 ± 1.001	0.360 ± 0.002
Mannitol	1.78 ± 1.109	0.09 ± 0.002	0.35 ± 0.002	1.08 ± 1.004
Starch	3.72 ± 1.135	11.99 ± 1.081	4.76 ± 1.211	9.12 ± 1.015
Sucrose	4.76 ± 1.210	8.71 ± 1.012	5.18 ± 1.200	3.71 ± 1.110
Dextrin	1.98 ± 1.131	2.52 ± 1.104	1.18 ± 1.191	6.10 ± 1.011
Glycerol	4.62 ± 1.121	0.10 ± 0.001	5.10 ± 1.109	0.36 ± 0.003
Sorbitol	2.76 ± 1.137	0.46 ± 0.031	3.76 ± 1.011	0.94 ± 0.009

* = after 7 days; * * = after 15 days; a = average of 4 replicates; Temperature 28 ± 2°C; CD at 5% = 8.16 (SR_1); 13.11 (SR_2); Corrected by Abbott's Formulae

Table 5. Effect of different organic and inorganic nitrogen sources on *in vitro* radial growth and intensity of sclerotium formation in *Parthenium* isolates of *Sclerotium rolfsii*.

Nitrogen source	SR_1[a]		SR_2[a]	
	Colony diameter* (cm)	Intensity of Sclerotium formation**	Colony diameter* (cm)	Intensity of Sclerotium formation**
Control	trace	0.00	trace	0.00
Serine	0.85 ±	2.23 ± 1.112	1.07±	3.73 ± 1.112
Leucine	0.05 ± 0.020	0.00	1.22 ± 1.001	0.00
Aspargin	1.15 ± 1.100	0.00	0.12 ± 0.001	0.00
Histidine	2.35 ± 1.021	29.73 ± 1.701	5.12 ± 1.211	20.66 ± 1.671
Urea	0.02 ± 0.001	0.00	2.18 ± 1.002	0.00
Peptone	2.71 ± 1.001	12.14 ± 1.237	2.68 ± 1.112	14.26 ± 1.182
Alanine	1.68 ± 1.061	0.77 ± 0.003	1.28 ± 1.002	0.460 ± 0.002
Potassium nitrate	6.08 ± 1.100	46.24 ± 1.667	5.82 ± 1.120	39.00 ± 1.659
Sodium nitrate	0.70 ± 0.004	3.14 ± 1.231	1.51 ± 1.101	1.13 + 1.001
Calcium nitrate	0.95 ± 0.006	0.22 ± 0.001	1.35 ± 1.003	0.22 ± 0.005
Ammonium nitrate	1.55 ± 1.011	0.54 ± 0.002	1.68 ± 1.007	0.00
Ammonium chloride	1.54 ± 1.020	1.13 ± 1.015	1.35 ± 1.201	1.66 ± 1.003
Ammonium sulphate	0.52 ± 0.001	0.13 ± 0.51	0.92 ± 0.051	0.77 ± 0.004
Di-ammonium hydrogen phosphate	3.76 ± 1.121	39.23 ± 1.861	3.64 ± 1.143	37.46 ±1.679

* = after 7 days; * * = after 15 days; a = average of 4 replicates; Temperature 28 ± 2°C; CD at 5% = 10.66 (SR_1); 4.58 (SR_2); Corrected by Abbott's Formulae

growth but favoured the formation of several sclerotia of very smaller size.

d) *Carbon Sources*: Amongst the carbon sources tried glucose was found to be relatively better source of carbon, supported maximum growth and sclerotial formation in both the isolates (Table 4). It was followed by Sucrose and Glycerol for growth and Mannose for sclerotial formation. Sclerotial formation in general was very poor on Lactose, Mannitol, Glycerol and Sorbitol. Both the isolates varied greatly on their Galactose utilization. SR_2 gave moderate growth while SR1 produced very poor growth on it. Similary SR2 showed comparatively more affinity with starch than that of SRI. Further it was observed that in general the carbon compounds which supported good growth were also good source for sclerotial formation except Glycerol and Sucrose where they supported significant growth while failed to induce sclerotial formation in both the isolates.

e) *Nitrogen Sources*: The importance of nitrogen in the nutrition of both the isolates of *S. rolfsii* are evident from the fact that the growth of the isolates in the meduim devoid of any nitrogen were only in traces and that too may be due to some nitrogen which might have been introduced into nutrient medium with inoculum (Table 5). This further showed that the isolates were incapable of utilizing atmospheric nitrogen for their growth. They differ slightly in their nitrogen reqirments. They utilized potassium nitrate very profusely for their maximum growth and sclerotial formation. It was followed by Diammonium

hydrogen phosphate and Histidine. Ammonium sulphate, Alanine, Serine, Leucin, Urea, Sodium nitrate, Calcium nitrate and Ammonium chloride were found to be a very poor source of nitrogen for both the isolates. The source of nitrogen also appeared to influence the size of sclerotia. The maximum size was attained on Potassium nitrate while smallest on Ammonium sulphate. The ability of the isolates to utilise nitrates and Ammonium nitrogen beside organic nitrogen places these isolates in the second group of Robbins (1937) classification. There was no strict interrelationship between the growth and sclerotial formation inrespect of nitrogen sources.

The findings of the present investigation clearly indicates that both the isolates slightly differ in their physiochemical requirments. These must be kept in mind during formulation for field application.

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References

1. Auld BA 1995, *Weed Tech. 9* 638
2. Charudattan R 1993, *In: Pestiside interactions in crop Production: Beneficial and deleterious effects*, J Altman (ed.) CRC Press Boca Raton Ann Arbor London PP. 421-432.
3. Freeman T E 1987, *In: Advance in Agriculture Microbiology* NS Subba RAO (ed.) Oxford & IBH Publishing Ltd., New Delhi PP. 419-428
4. Hoagland RE 1996, *In: Microbes and Microbial Products as herbicides.* RE Hoaglland ed. ACS symposium seies 439. American Chemical Society, Washington. PP. 1-52.
5. Jackson MA, Schister DA, Slininger PJ, Boyette CD W, Silman RW and Bothast RJ 1996, *Weed Tech. 10(2)* 1
6. TeBeest DO (ed) 1996, *Microbial control of weeds*, Chapman & Hall New York London P 284.
7. Templeton G E 1992, *In: Colletotrichum: Biology, Pathology and control*, JA Bailey and MJ Jegor (eds.) CAB International Mycological Rescarch Institute, Kew England UK. PP 359-380.
8. Templeton GE and Heiny DK 1989, *In: Biotechnology of fungi for improving plant growth*, JM Whipps and RD Lumsden (eds.) Cambrige University Press Cambridge UK PP. 127-151.
9. Templeton GE, Smith RJ and TeBeest DO 1986, *Rev. Weed Sci. 2* 1
10. Hasija SK, Rajak RC and Pandey AK 1994, *In: Vitas in Seed Biology*, T. Singh & PC Trivedi (eds) Printwell, Jaipur PP. 82-104.
11. Farkya S, Pandey AK and Rajak RC 1994, *Nat. Acad. Sci. Letters 17* 81
12. Mishra J, Pandey AK, Rajak R C and Hasija SK 1994, *Nat. Acad. Sci. Letters 17* 169
13. Mishra J, Pandey AK, and Hasija SK 1995, *Indian Phytopath. 48* 294
14. Mishra J, Pandey AK and Hasija SK 1996, *Indian J. Applied Pure Biol. 11* (2) (In Press)
15. Pandey AK, Luka RS, Hasija SK and Rajak RC 1991, *J. Biol. Control 5* 113
16. Pandey AK, Hasija SK and Rajak RC 1990, *Nat. Acad. Sci. Letters 13* 369
17. Pandey AK, Luka BM, Hasija SK and Rajak RC 1992, *Proc. Nat. Acad. Sci. India 62* 449
18. Pandey AK, Farkya S and Rajak RC 1992, *J. Indian bot. Soc. 71* 103
19. Pandey AK, Mishra J, Rajak RC and Hasija SK 1992, *Nat. Acad. Sci. Letters 15* 111
20. Rajak RC, Farkya S, Hasija SK and Pandey AK 1990, *Proc. Nat. Sci. India 60* 165
21. Aggarwal GP and Hasija SK 1986, *Microorganisms in the Laboratory-A Laboratory guide for Mycology, Microbilogy and Plant Pathology*, Print house (India) Lucknow pp. 155.
22. Ward EWB and Colotelo N 1960, *Can. J. Microl. 6* 545
23. Abbott WS 1925, *J. Econ. Entomol. 18* 265
24. Cormack MW 1948, *Can. J. Res. 26* 71
25. Kheshwal KF 1934, *Indian J. Agric. Sci. 4* 663
26. Lauritzen JJ 1932, *J. Agri, Res. 44* 861