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Impact of aqueous and organic extracts of *Rhodobryum roseum* on inhibition of fungal and bacterial growth

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Abstract

Mosses have been known for millennia and highly esteemed all over the world as the rich source of bioactive compounds. The research targets on evolution of microbicidal potentialities of *Rhodobryum roseum* (extract) used against selected fungus *(X. oryzae pv oryzae, S. enteric, P. multocida* and *M. plutonius*) and bacteria (*R. solani, S. rolfsii, F. oxysporum and T. indica*) to assay antimicrobial activity. Impact of aqueous and undertaken organic viz., ethanol, acetone, choloform, petroleum ether, methanol extract of *R. roseum,* at varied concentrations and at different time intervals were examined against the growth of bacteria and fungus. All the aqueous extracts were proved to be infective against all the tested pathogens. The antimicrobial potential of six extracts was screened against undertaken bacteria and fungi using micro broth dilution assay. Out of the six (diverse organic and aqueous) extract of *R. roseum* in ethanol and acetone showed maximum inhibitory activity in *S. rolfsii* with the MIC value of 5.00 (µg/ml), along with MFC value of 6.25 (µg/ml) in acetone extract and the value of MBC was recorded utmost in *X. oryzae* with value 3.00 (µg/ml) extracted in ethanol. Over all, the organic extract of *R. roseum* has potent antimicrobial activity and could be possible source of lead molecules considered for the future development of microbicidal agent.

Key Words: ***Rhodobryum roseum, moss, organic and aqueous extract, inhibitory concentration, microbicidal and pathogens***

Introduction

In developing nations like India, microbes are considered as chief causative agents for many serious diseases and are of foremost concern by either private or authorized health care organizations. The financial emergency, low production and high cost of new generation antibiotics, inefficient community access to medicinal and pharmaceutical concern, along with the side effects caused by the synthetic drugs are some of the important aspects. To overcome these issues, research work in many countries is focusing to the vital character of medicinal plants and showing its contribution in wellbeing concern (Kumar *et al.,* 2002). Therefore, in current scenario, rapid growth of medicinally significant multi resistant microbial trains of pathogens attract the researchers in all over world to discover the novel broad-spectrum antimicrobial agents.

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Numerous herbs containing magnificent microbicidal potentialities have been accounted in many previous data (Dewanjee *et al.,* 2007). Various researches have been performed with the extracts of number of plant species for the screening of their antimicrobial activity as well as for understanding the novel antimicrobial complexes, so that, they can be used for human welfare (Sengupta *et al*. 2008)).

Bryophytes (mosses, hornworts, and liverworts) are fascinating group of organisms found in all ecosystems of earth and carry numerous phytochemical constituents, therapeutic agents along with the secondary metabolites i.e. phenols, terpenoids, flavonoids, derivatives of benzoic acids and cinnamic acids, etc. However, due to its small size, inconspicuous place in the ecosystem and lack of commercial value, they are treated as hidden treasure for the world. Thus, the work presented reveals the incredible significance of these small creatures (mosses) and characterize the microbicidal potentialities of the undertaken moss extracts i.e. *invitro* activites of *R. roseum* against economically important crop pathogens.

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Material and Methods

Moss Sampling

Moss was collected from walls, roofs approximately 1.5 meter above ground height and natural rocks which are devoid of no overhanging flora or tree coverings. The estimation was done in different sites (urban, sub-urban and rural) of Kumaon hills (Nainital, Almora, Jageshwar and Mukteshwar) for collecting only mature green plants, while the dead and dried plants (pale in colour) were rejected.

Moss Identification

Bryophytes were collected from different catchment sites of Kumaon hills during 2009-2010. The collected samples were kept in clean plastic bags and were brought to the laboratory for identification with the help of available taxonomical literatures fig.1 (Chopra, 1975; Smith, 1978; Gangulee, 1969; Saxena et al., 2008).

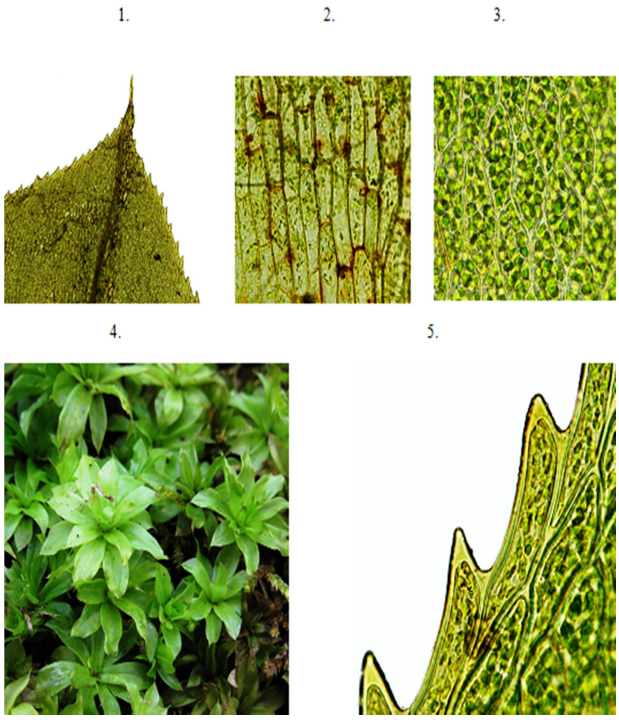

Figure.1 : *Rhodobryum roseum* (Hedw.) Limpr.
1. Leaves, 2. Basal cells, 3. Midleaf cells, 4. Whole plant 5 Margin cells

Moss Extract

The collected moss samples were cleaned carefully to remove the debris and dead material attached and afterword washed in running water and at last with distilled water. The washed samples were dried in shade and then finely powdered (100 g) using grinder. The obtained powder of moss was then extracted by mean of Soxhlet apparatus along with 500 ml of different organic solvents (petroleum ether, chloroform, acetone, ethanol and methanol). The obtained extracts were filtered using muslin cloth and were left at room temperature for complete evaporation. In last step different concentrations (40, 200, 500 and 1000 µg/mL) of crude extract were prepared for experimentation.

Examination of Antimicrobial Activity

Streptomycin and ampicillin antibiotics were used as positive controls to antibacterial activity (*X. oryzae* pv. *oryzae, S. enterica, P. multocida,* and *M. plutonius*). Antimicrobial activity was estimated by using disc diffusion method. The bacterial nutrient agar plates were treated with organic extracts (40 µL/disc) the nutrient and then incubated for 24 hrs at 37 ± 2^0C (Basri and Fan, 2005). Antibacterial activity of the moss extracts was done by measuring the zone of inhibition (ZI) in mm against studied bacterial strains.

To check the fungal activity (*F. oxysporum f. sp. Lycopersci, T. indica, s. rolfsii and R. solanii*) fungicide "Chloramine T" was used as positive control. Plates of PDA (Potato Dextrose Agar) were prepared under aseptic conditions and kept at 28 ± 2^0C for 72 hrs for solidification. Four discs (two treated with moss extract + two controls) were kept in each petri plate with the test fungi.

Calculation of MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration)/MFC (Minimum Fungicidal Concentration)

Janovska and his co workers (2003) carried out micro broth dilution assay to screen out the inhibitory and bacterial/fungicidal concentration of organic extracts. Fresh diluents were prepared for bacteria (nutrient broth) and fungi (potato dextrose agar) and the fresh and revived culture of untaken microorganisms were diluted in broth about 100 folds (100 µL microorganism in 10 mL broth).

CFU (Colony Forming Unit) was measured at optical density (OD) 620 nm (UV-visible spectrophotometer) and was observed 1 x 106 CFU/mL (bacteria) and 1 x109 CFU/mL (fungi). The inoculated tubes of bacteria were incubated for

24 hrs at 37^0C and that of fungi for 72 hrs at 28^0C and later OD was measured at 620 nm via UV visible spectrophotometer.The lowest concentration that inhibited visible growth of tested organisms was recorded as MIC, and that caused no visible microbial growth was considered as MBC.

Statistical Analysis

The experimental data was evaluated statistical by using ANOVA. The study was based on three replicates to examine the effect of treatments separately and were depicted as mean ± SE. Level of significance at $P<0.05$ among undertaken micro-organisms and different extracts was done by using JMP 5.0, SAS Institute, Cary, NC, USA. Furthermore, the mean of treatments were distinguished by using Turkey HSD test.

Results and Discussion

Effect of extracts of *R. roseum* were examined against the pathogenic plant fungus

Fusarium oxysporum results depict that, moss extract with chloramine T (positive control), at 1000 and 500 µg ml^{-1} conc. was showing significant higher values except that in methanol extract. However, out of the all undertaken extracts (aqueous and organic) of *R. roseum*, percent inhibition was maximum at 24 hrs (38.37) in acetone extract at 1000 µg ml^{-1} conc. and minimum at 72 hrs (8.29) in methanol extract at 40 µg ml^{-1} conc. (Table 1). Interestingly, aqueous extract of *R. roseum* had no activity against *F. oxysporum* at all conc. and time intervals. In addition, undertaken extracts with chloramine T (positive control) shows significantly higher values at all time interval with 100, 500 and 200 µg ml^{-1} conc. in *Tilletia indica* out of the 6 extracts (aqueous and organic) of *R. roseum*, percent inhibition was maximum at 24 hrs (34.27) with chloroform extract at 1000 µg ml^{-1} conc. and minimum at 72 hrs (9.34) in acetone extract at 40 µg ml^{-1} conc. (Table 2). Interestingly, aqueous extract of *R. roseum* had no activity against *T. indica* at all conc. and time intervals.
Furthermore, results depict significantly higher inhibition growth in petroleum ether, methanol and acetone in 1000, 500 and 200 µg ml^{-1} conc. over to chloramine T. However, in case of *Sclerotium rolfsii* out of the all extracts (aqueous and organic) of *R. roseum*, the maximum percent inhibition was observed in methanol extract (32.23) at 72 hrs in 1000 µg ml^{-1} conc., while, the minimum percent inhibition was observed in petroleum ether extract (8.19) at 72 hrs in 40 µg ml^{-1} conc. (Table 3). Here also, aqueous extract of *R. roseum* had no activity against *S. rolfsii* at all conc. and time intervals.
Nevertheless, for *Rhizoctonia solani* out of the 6 extracts (aqueous and organic) of *R. roseum*, the maximum percent inhibition was observed in acetone extract (24.21) at 72 hrs in 1000 µg ml^{-1} conc., whereas, the minimum percent inhibition was observed in chloroform extract (3.09) at 24 hrs in 200 µg ml^{-1} conc. (Table 4). Interestingly, aqueous, extract of *R. roseum* had no activity against *R. solani* at all conc. and time intervals. On doing comparison between all extracts and chloramine T (positive control), results indicate, significantly higher values in methanol at 24 and 48 hrs in 1000 µg ml^{-1} conc.
According to Mekuria *et al.,* (1999) and Mewari *et al.,* (2007) bryophyte as a new source of antifungal substance in crop protection. Eighteen bryophyte species were screened for antifungal nature of their ethanolic extracts. *B. trilobata, D. albicans, S. quinquefarium, D. denudatum* and *H. spledens* caused the greatest inhibition of mycelial growth of *B. cinerea* and *A. solani.* Also, antimicrobial activities ethanol and petroleum ether extracts of mosses*, R. vagans Jaeg* and *E. plicatus C. Muell* against two fungal pathogen, *B. sorokiniana* and *F. solani* and found both the extracts of mosses were effective for the tested fungi. Mekuria (2005) also reported the antifungal activity of 20 other bryophytes both under *in vitro* and *in vivo* conditions. The bryophytes showed the great antifungal capacity against phytopathogens, various researches revealed that the aqueous, methanolic extract of *R. gangetica* and mosses *C. fontinaloides, A. viticulosus, T. tamarsicinum, E. striatum*, *I. alopecuroides* and *P. formosum* and liverworts *P. platyphylla* and *S. anemorea* had antifungal activity against plant pathogenic fungi (Viz. *B. dothidea, B. cinerea, P. viticola, C. acutatum, M. laxa* and *Calosphaeria s*p.). The fungus toxicity of the aqueous extract was precised by inhibition of percent spore germination and hyphal length using

Table 1: Percent inhibition in the growth of *Fusarium oxysporum* f.sp. *lycopersici* with different extract of *R. roseum*.

Nature of extract	Concentration (µg ml^{-1})											
	Time (hrs.)											
	1000			500			200			40		
	24	48	72	24	48	72	24	48	72	24	48	72
Petroleum ether	35.34^{c} ±0.27	33.32^{a} ±0.25	30.35^{a} ±0.27	24.22^{a} ±0.25	27.28^{a} ±0.20	26.35^{a} ±0.24	23.25^{a} ±0.16	19.31^{a} ±0.18	14.20^{a} ±0.23	15.32^{a} ±0.24	17.35^{d} ±0.26	14.27^{b} ±0.17
Methanol	17.28^{d} ±0.16	16.31^{b} ±0.21	15.33^{b} ±0.25	15.29^{b} ±0.04	16.34^{b} ±0.24	13.28^{b} ±0.21	13.24^{b} ±0.29	12.25^{b} ±0.26	10.36^{b} ±0.34	11.38^{b} ±0.25	9.30^{c} ±0.22	8.29^{d} ±0.23
Chloroform	30.30^{b} ±0.22	32.34^{c} ±0.21	33.48^{c} ±0.36	30.25^{c} ±0.16	28.23^{c} ±0.18	25.25^{c} ±0.16	25.33^{c} ±0.28	23.34^{c} ±0.32	20.20^{c} ±0.25	22.20^{c} ±0.22	19.31^{a} ±0.17	15.26^{a} ±0.17
Ethanol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Acetone	38.37^{a} ±0.22	36.28^{d} ±0.09	32.29^{d} ±0.21	32.25^{d} ±0.31	33.21^{d} ±0.25	30.26^{d} ±0.18	25.55^{d} ±0.16	28.38^{d} ±0.25	26.25^{d} ±0.13	18.35^{d} ±0.21	16.35^{b} ±0.21	14.23^{b} ±0.24
Water	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Chloramine T	21.34^{e} ±0.19	25.33^{e} ±0.29	26.24^{e} ±0.15	23.38^{e} ±0.27	21.32^{e} ±0.24	24.39^{f} ±0.32	22.30^{e} ±0.22	20.28^{e} ±0.36	18.42^{e} ±0.19	19.36^{e} ±0.25	16.17^{b} ±0.19	13.34^{c} ±0.22

Table 2: Percent inhibition in the growth of *Tilletia indica* with different extract of *R. roseum*.

Nature of extract	Concentration (µg ml^{-1})											
	Time (hrs.)											
	1000			500			200			40		
	24	48	72	24	48	72	24	48	72	24	48	72
Petroleum ether	26.12^{b} ±0.11	24.29^{e} ±0.29	22.25^{b} ±0.23	23.24^{c} ±0.18	20.46^{d} ±0.12	17.42^{e} ±0.36	20.37^{d} ±0.26	16.42^{e} ±0.46	16.11^{d} ±0.16	15.35^{c} ±0.19	12.18^{d} ±0.30	11.44^{c} ±0.37
Methanol	25.29^{c} ±0.29	23.26^{d} ±0.15	22.26^{b} ±0.17	23.32^{c} ±0.19	22.33^{c} ±0.29	20.31^{d} ±0.21	20.27^{d} ±0.19	18.27^{c} ±0.17	15.25^{e} ±0.15	18.30^{b} ±0.24	15.34^{b} ±0.27	12.30^{b} ±0.20
Chloroform	34.27^{a} ±0.25	30.29^{a} ±0.19	28.52^{a} ±0.28	26.36^{a} ±0.35	25.33^{a} ±0.29	23.37^{b} ±0.34	25.42^{a} ±0.18	23.26^{a} ±0.24	20.51^{a} ±0.39	13.35^{d} ±0.18	10.30^{e} ±0.20	9.36^{d} ±0.26
Ethanol	23.38^{d} ±0.37	25.34^{c} ±0.22	22.32^{b} ±0.19	26.42^{a} ±0.45	23.18^{b} ±0.21	21.35^{c} ±0.31	24.38^{b} ±0.25	19.52^{b} ±0.37	18.29^{c} ±0.21	15.44^{c} ±0.35	12.48^{d} ±0.35	11.39^{c} ±0.34
Acetone	26.44^{b} ±0.28	25.25^{c} ±0.13	23.29^{c} ±0.22	24.28^{b} ±0.18	20.26^{d} ±0.17	17.30^{e} ±0.23	20.37^{d} ±0.26	18.40^{c} ±0.19	15.31^{e} ±0.17	15.35^{c} ±0.18	13.25^{c} ±0.24	9.346^{d} ±0.26
Water	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Chloramine T	25.31^{c} ±0.25	28.26^{b} ±0.18	23.27^{c} ±0.18	22.25^{d} ±0.15	20.27^{d} ±0.18	24.15^{a} ±0.17	21.32^{c} ±0.19	17.48^{d} ±0.39	19.32^{b} ±0.16	20.33^{a} ±0.18	16.53^{a} ±0.28	14.30^{a} ±0.24

***Values are represented as mean ± SD.**

***The treatment means were separated using Tukey HSD at 0.05% probability level.**

***Level not connected by same letter are significantly different in vertical columns.**

Table 3: Percent inhibition in the growth of *Sclerotium rolfsii* with different extracts of *R. roseum*.

Nature of extract	Concentration (µg ml^{-1})											
	Time (hrs.)											
	1000			500			200			40		
	24	48	72	24	48	72	24	48	72	24	48	72
Petroleum ether	21.46^{b} ± 0.37	24.65^{a} ± 0.30	30.29^{a} ± 0.32	14.47^{a} ± 0.15	25.14^{a} ± 0.11	27.28^{a} ± 0.28	21.25^{a} ± 0.31	22.24^{a} ± 0.25	23.107^{a} ± 0.11	8.28^{b} ± 0.46	8.30^{b} ± 0.48	8.19^{b} ± 0.23
Methanol	24.47^{a} ± 0.43	26.35^{b} ± 0.36	32.23^{b} ± 0.37	15.33^{b} ± 0.27	27.26^{b} ± 0.21	28.28^{b} ± 0.30	24.31^{b} ± 0.25	23.34^{b} ± 0.20	24.22^{b} ± 0.30	8.69^{b} ± 0.29	8.32^{b} ±0.46	8.36^{b} ±0.21
Chloroform	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ethanol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Acetone	15.33^{c} ±0.32	18.28^{c} ±0.07	16.21^{c} ±0.28	29.45^{c} ±0.47	31.01^{c} ±0.85	30.32^{c} ±0.43	23.55^{c} ±0.27	21.33^{c} ±0.54	21.11^{c} ±0.16	8.31^{b} ±0.33	8.46^{b} ±0.38	8.26^{b} ±0.34
Water	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Chloramine T	19.32^{d} ±0.06	21.17^{d} ±0.27	18.15^{d} ±0.22	16.26^{d} ±0.25	18.50^{d} ±0.35	15.46^{d} ±0.33	14.15^{d} ±0.23	15.17^{d} ±0.27	13.21^{d} ±0.34	12.21^{a} ±0.46	14.21^{a} ±0.40	11.27^{a} ±0.36

Table 4: Percent inhibition in the growth of *Rhizoctonia solanii* with different extract of *R. roseum*.

Nature of extract	Concentration (µg ml^{-1})											
	Time (hrs.)											
	1000			500			200			40		
	24	48	72	24	48	72	24	48	72	24	48	72
Petroleum ether	7.15^{e} ±0.23	5.63^{c} ±0.48	3.25^{e} ±0.20	6.50^{e} ±0.31	7.24^{f} ±0.19	3.05^{e} ±0.06	15.26^{a} ±0.28	20.28^{a} ±0.39	0.0	9.28^{d} ±0.30	3.23^{d} ±0.29	0.0
Methanol	15.21^{d} ±0.27	15.46^{b} ±0.38	15.12^{d} ±0.17	11.42^{d} ±0.24	14.46^{e} ±0.42	5.66^{d} ±0.23	11.29^{b} ±0.30	6.08^{b} ±0.13	0.0	0.0	0.0	0.0
Chloroform	14.30^{c} ±0.22	12.62^{d} ±0.36	3.34^{e} ±0.29	17.25^{c} ±0.25	23.16^{d} ±0.13	3.16^{e} ±0.13	11.16^{b} ±0.24	3.09^{c} ±0.14	0.0	14.33^{a} ±0.37	3.09^{d} ±0.14	0.0
Ethanol	9.69^{b} ±0.21	15.23^{b} ±0.28	16.48^{c} ±0.41	10.15^{b} ±0.12	18.12^{c} ±0.11	12.16^{c} ±0.24	9.35^{c} ±0.49	14.26^{d} ±0.25	13.31^{a} ±0.43	8.33^{c} ±0.31	6.25^{c} ±0.25	6.18^{b} ±0.21
Acetone	7.57^{e} ±0.33	21.31^{a} ±0.28	24.21^{b} ±0.28	6.44^{e} ±0.12	19.28^{b} ±0.25	13.42^{b} ±0.33	5.31^{d} ±0.24	15.42^{e} ±0.25	10.31^{b} ±0.26	4.39^{b} ±0.31	8.27^{b} ±0.35	9.30^{c} ±0.30
Water	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Chloramine T	12.37^{a} ±0.32	14.24^{e} ±0.30	19.27^{a} ±0.25	16.23^{a} ±.20	17.26^{a} ±0.22	14.21^{a} ±0.26	15.25^{a} ±0.20	13.28^{f} ±0.29	11.22^{c} ±0.36	14.35^{a} ±0.22	12.27^{a} ±0.32	13.32^{a} ±0.28

***Values are represented as mean ± SD.**

***The treatment means were separated using Tukey HSD at 0.05% probability level.**

***Level not connected by same letter are significantly different in vertical columns.**

Table 5 . Activity Index of the growth of *Xanthomonas oryzae* pv. *oryzae* with different extract of *R. roseum*

Nature of extract	Concentration (µg ml^{-1})			
	1000	**500**	**200**	**40**
Petroleum ether	0.0	0.0	0.0	0.0
Methanol	11.21^{c} ±0.32	12.19^{c} ±0.29	11.27^{c} ±0.24	10.22^{c} ±0.26
Chloroform	0.0	0.0	0.0	0.0
Ethanol	11.26^{c} ±0.34	12.31^{c} ±0.28	11.27^{c} ±0.27	10.36^{c} ±0.32
Acetone	0.0	0.0	0.0	0.0
Water	0.0	0.0	0.0	0.0
Ampicillin	18.19^{b} ±0.22	15.24^{b} ±0.22	13.28^{b} ±0.28	11.21^{b} ±0.20
Streptomycin	20.29^{a} ±0.36	18.26^{a} ±0.34	16.20^{a} ±0.25	13.26^{a} ±0.28

Table 6: Activity Index of the growth of *Melissococcus plutonius* with different extract of *R. roseum*

Nature of extract	Concentration (µg ml^{-1})			
	1000	**500**	**200**	**40**
Petroleum ether	0.0	0.0	0.0	0.0
Methanol	12.19^{c} ±0.23	13.27^{a} ±0.28	11.21^{a} ±0.33	6.37^{d} ±0.27
Chloroform	0.0	0.0	0.0	0.0
Ethanol	13.23^{b} ±0.29	12.30^{b} ±0.24	11.23^{a} ±0.28	10.15^{a} ±0.14
Acetone	15.26^{a} ±0.25	11.24^{c} ±0.29	11.23^{a} ±0.28	10.18^{a} ±0.15
Water	0.0	0.0	0.0	0.0
Ampicillin	15.24^{a} ±0.28	13.22^{a} ±0.29	11.22^{a} ±0.26	9.22^{b} ±0.27
Streptomycin	13.20^{b} ±0.23	11.31^{c} ±0.32	10.27^{b} ±0.32	8.33^{c} ±0.26

***Values are represented as mean ± SD.**
***The treatment means were separated using Tukey HSD at 0.05% probability level.**
***Level not connected by same letter are significantly different in vertical columns.**

Table 7: Activity Index of the growth of *Pasteurella multocida* with different extract of *R. roseum*.

Nature of extract	Concentration (µg ml^{-1})			
	1000	**500**	**200**	**40**
Petroleum ether	0.0	0.0	0.0	0.0
Methanol	0.0	0.0	0.0	0.0
Chloroform	12.26^{d} ±0.27	13.22^{b} ±0.26	11.23^{c} ±0.27	10.23^{a} ±0.28
Ethanol	20.24^{a} ±0.30	18.23^{a} ±0.28	15.48^{a} ±0.15	13.22^{b} ±0.27
Acetone	16.24^{b} ±0.25	13.26^{b} ±0.27	14.25^{b} ±0.32	12.20^{c} ±0.31
Water	0.0	0.0	0.0	0.0
Ampicillin	15.27^{c} ±0.26	13.30^{b} ±0.23	11.24^{c} ±0.28	9.20^{d} ±.23
Streptomycin	15.26^{c} ±0.30	12.26^{c} ±0.32	10.23^{d} ±0.29	8.29^{e} ±0.21

Table 8: Activity Index of the growth of *Salmonella enterica* with different extract of *R. roseum*.

Nature of extract	Concentration (µg ml^{-1})			
	1000	**500**	**200**	**40**
Petroleum ether	0.0	0.0	0.0	0.0
Methanol	0.0	0.0	0.0	0.0
Chloroform	0.0	0.0	0.0	0.0
Ethanol	11.28^{a} ±0.18	15.21^{a} ±0.25	11.22^{a} ±0.23	13.22^{a} ±0.26
Acetone	13.19^{b} ±0.16	16.26^{b} ±0.19	13.25^{b} ±0.21	11.19^{b} ±0.22
Water	0.0	0.0	0.0	0.0
Ampicillin	20.38^{c} ±0.24	16.31^{b} ±0.27	13.28^{b} ±0.28	10.21^{c} ±0.24
Streptomycin	18.30^{d} ±0.31	16.29^{b} ±0.17	13.28^{b} ±0.28	11.30^{b} ±0.33

***Values are represented as mean ± SD.**

***The treatment means were separated using Tukey HSD at 0.05% probability level.**

***Level not connected by same letter are significantly different in vertical columns.**

Table 9: Minimum Inhibitory Concentrations (MIC), Minimum Fungicidal Concentrations (MFC) and Minimum Bactericidal Concentrations (MBC) of different extract of *R. roseum* against different pathogens (µg/ml).

Pathogen	Petroleum ether		Methanol		Chloroform		Etahnol		Acetone		STANDARDS
Fungi	**MIC**	**MBC/ MFC**	**MIC**	**MBC/ MFC**	**MIC**	**MBC/ MFC**	**MIC**	**MBC/ MFC**	**MIC**	**MBC/ MFC**	**MIC (MBC/MFC)**
S. rolfsii	2.50	5.00	2.50	4.50	2.50	5.00	5.00	6.00	5.00	6.25	0.50 (0.80)
R. solani	2.50	4.50	0.65	0.65	2.50	5.00	0.65	0.65	0.65	1.25	1.00 (1.25)
T. indica	0.65	0.85	0.65	0.75	0.65	1.25	0.65	0.75	0.65	0.65	0.50 (0.60)
F. oxysporium	1.25	1.25	0.65	0.75	0.65	0.65	1.25	1.75	1.25	1.75	0.50 (0.60)
Bacteria											
P. multocida	-	-	-	-	1.25	1.25	0.65	0.80	1.25	1.65	0.50 (0.70)
S. enterica	-	-	-	-	-	-	1.25	1.25	2.50	2.50	0.25 (0.25)
M. plutonius	-	-	2.50	3.00	-	-	1.50	1.50	1.25	2.50	0.65 (0.80)
X. oryzae	-	-	2.50	2.75	-	-	2.50	3.00	-	-	0.65 (0.75)

Chloramines T and Ampicillin are used as standards for fungi and bacteria respectively

hanging drop method and comprising species to species interaction, type and amount of extract was attained. The highest antifungal activity was traced in 100 percent methanolic extract (Deora and Suhalka , 2017; Latinović *et al.,* 2018). Another research revealed by Latinović *et al.* (2019) showed that, the extracts of three bryophyte species are confirmed to have suppressive effects on grey mould disease (*B. cinerea*). Methanol extracts of one leafy liverwort (*P. platyphylla*) and two mosses, one aquatic (*C. fontinaloides*) and one terrestrial (*A. viticulosus*), were applied *in vitro* to *B. cinerea*, after which experiment confirmed inhibition of fungal growth was observed. Antifungal activities of methanol extract of the moss species *Fontinalis antipyretica* Hedw. var. *antipyretica*, *Hypnum cupressiforme* Hedw., and *Ctenidium molluscum* (Hedw.) Mitt., were also analyzed by Veljić *et al.,* in 2009.

Effect of extracts of *R. roseum* were examined against the pathogenic plant bacteria

On comparing all undertaken extracts with positive control (ampicillin and streptomycin) significantly lower values were observed for *Xanthomonas oryzae.* Out of the six extracts (aqueous and organic) of *R. roseum*, the activity was maximum 12.31 with ethanol extract and minimum 10.22 with methanol extract (Table 5). No extract shows better activity when compared to standard antibiotics.

However, in case of *Melissococcus plutonius* out of the six extracts (aqueous and organic) of *R. roseum*, the activity was maximum 15.26 with acetone extract and minimum 6.37 with methanol extract (Table 6). As compared to standard antibiotics acetone extract shows good activity. In ethanol and acetone extract highly significant values were observed at 40 µg ml^{-1} conc. over both the positive control.

In addition, *Pasteurella multocida* out of the six extracts (aqueous and organic) of *R. roseum*, the activity was maximum 20.24 with ethanol extract and minimum 12.20 in acetone extract (Table 7). On comparing all undertaken extracts with positive control (ampicillin and streptomycin) significantly higher values were obtained in ethanol and acetone at all conc. except, at 500 µg ml^{-1}conc.

Further, in case of *Salmonella enterica* out of the six extracts (aqueous and organic) of *R. roseum*, the activity was maximum 16.26 and minimum 11.19 was observed with acetone extract (Table 8). No extract shows better activity when compared to standard antibiotics. Comparison of acetone and ethanol extract with positive both the controls showed significantly lower, but at the same time non significant values were observed at 200 and 500 µg ml^{-1} conc.

Dewanjee *et al.,* (2007) showed that methanol extract of *D. peregrina* fruits (MEDP) and *C. grandis* leaves (MECG) had highest sensitivity against *E coli strains*. MECG showed major activity against *Staphylococcus aureus, E. coli, S. dysenteriae, S. soneii* and *P. aeruginosa*; while resistant to *S. flexneri* and *S. boydii*. In the present study the strain of *E. coli* showed least sensitivity for the ethanol extract. Other results revealed by Abdel-Shafi *et al.,* (2017), the pathogenic bacteria *L. monocytogenes* LMG 10470, *E. coli* LMG 8223, *B. cereus* ATCC 14579 and *P. aeruginosa* LMG 8029 were suppressed by the aqueous methanolic extracts of *Imbibryum* spp., *B. convoluta* and *Trichostomum* spp. The fusion of *Imbibryum* spp. extract and tetracycline has synergistic effects against *P. aeruginosa* while the mixing of *Trichostomum* spp. extract with tetracycline has antagonistic effect against *P. aeruginosa.* Off Petroleum ether, chloroform, benzene, methanolic and aqueous extracts of five ferns *A. caudatum, A. evecta, P. confusa, P. argyraea* and *L. microphyllum* against *Xanthomonas campestris pv. centellae.* The methanolic extracts of all the ferns showed successful antibacterial activity of against the performed bacteria. Phytochemical study of all the extracts showed that antibacterial activity and the MIC and RPI values, *A. evecta* could be used as potential plant for the supervision of pathogenic bacteria, *Xanthomonas campestris pv. centellae* which is known to cause diseases on various crops especially *Centella asiatica* (Gracelin et al., 2012). All of the tested outcomes confirmed that the aqueous methanolic extracts of selected bryophyte species are proven promising antibacterial agents however, Sabovljevic *et al.,* in 2010 also showed that DMSO extracts of three selected bryophyte species, two mosses and a liverwort (*A. undulatum* (Hedw.) P. Beauv., *M. polymorpha* L. sp. *ruderalis* Bischl. and Boisselier, *P. patens* (Hedw.) Bruch and Schimp.) estimated the results by micro-dilution method against eight bacterial species. All examined extracts of bryophytes are confirmed to be active against all bacteria tested.

Out of the six (different organic and aqueous) extracts of *R. roseum* ethanol and acetone extracts showed maximum inhibitory activity in *S.* rolfsii. In addition, maximum MIC value was observed 5.00 (acetone), whereas, MFC value as 6.25 (acetone) and MBC value as 3.00 (ethanol) in *X. oryzae*. Study also shows the greater MIC values with their respective values of MFC/MBC, this may be due to contaminated form of the bio-active compounds. However, in few results these values were found equal to their respective MFC/MBC values, which may be due to presence of a specific group of compounds. Study carried out by Mewari and his coworkers (2008) recorded the higher values of MBC/MFC in methanol extract over their MIC values, while, flavonoid showed the similar results of MIC and MBC/MFC.

The bioactivity data obtained from the present work points toward maximum antifungal activity in methanol, acetone and chloroform extracts of undertaken moss i.e. *Rhodobryum roseum* over other extracts. Furthermore, different extracts of aqueous and organic at different concentrations (1000, 500, 200 and 40 µg ml^{-1}) and at different time intervals (24, 48 and 72 hrs) indicated maximum inhibition on the growth of *F. oxysporum* in acetone extract with 1000 µg ml^{-1} conc. at 24 hrs and *T. indica* in chloroform extract with 1000 µg ml^{-1} conc. at 24 hrs. However, in case of bacteria maximum inhibition was observed on the growth of *P. multocida* in ethanol extract with 1000 µg ml^{-1} conc., which may be due to contaminated form of bioactive compounds (Table 9). Results of few experiments show similar values of MIC and MBC/MFC which may be because of specific group of compound.

Conclusion

Above study concluded that, selected species of bryophyte i.e. *R. roseum* had bioactivity against selected test of bacteria and fungi. Bioactivity varied according to the solvent used, concentrations applied and time intervals. Hence, *R. roseum* products may be considered as bio-control representatives and play noteworthy roles for practical perspectives in a communally, economically and ecologically vigorous crop management structure.

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