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Bioactivity of extracts from formulation of plant parts and part of different growth stages against fungal plant pathogens

Pradip Sarkar, Tushnima Chaudhuri, Susamoy Kundu, Ankita Roy, Jayanta Saha and BN Panja

Abstract
The research was conducted to find out effective plant extract based formulations as safe alternative to synthetic chemicals against eight fungal plant pathogens *viz. Colletotrichum capsici, Colletotrichum musae, Colletotrichum gloeosporoides, Alternaria alternata, Diplodia natalensis, Fuasarium oxysporum, Phomopsis vexans & Rhizoctonia solani*. To achieve the objective, different parts of commonly available plants *viz*. ferns, *Clerodendrum, Polyalthia* and *Glycosmis* were selected. Hexane, chloroform and methanol extract of plant materials of selected plants were formulated with light solvent naphtha and surfactant to get a 20% EC formulation. Results of *in vitro* studies revealed that *Polyalthia* old leaf extract showed highest mean per cent inhibition of radial growth (54.62%) over control irrespective of fungi tested and solvent used. Whereas, standard fungicide carbendazim showed 50.27% mean radial growth inhibition. Among the eight test fungi *Rhizoctonia solani* showed maximum (65.17%) sensitivity towards plant extracts whereas, it was least in case of *Alternaria alternata* (29.07%) in terms of mean per cent radial growth inhibition irrespective of treatments. Effect of the plant extract against three post-harvest pathogens were almost comparable irrespective of solvent used whereas there was a clear increasing trend of bio-activity of plant extract against *Rhizoctonia solani* while using solvent with increasing polarity for extraction. In case of *Diplodia natalensis* where methanol extract of selected plant material showed better mean *per cent* inhibition of radial growth (52.85%) than chloroform extract (46.63%) and hexane extract (39.40%) respectively.

Keywords: Plant extract, *Polyalthia longifolia, Glycosmis pentaphylla, Rhizoctonia solani, Diplodia natalensis*

Introduction
Synthetic fungicides have been used indiscriminately to control fungal diseases that causes a great share of loss to global food production. But several undesirable effects on non-target organisms, environment and human health have been reported which are totally undesirable in the era of sustainable agriculture (Yoon *et al*., 2013) [9]. As an alternative strategy to prevent the spread of diseases, natural compounds of plant origin are being tested for their antimicrobial activities by several researchers. Many locally available plants having potential folklore properties have not been explored in details against fungal plant pathogens. Moreover, in many cases bio-activity of plant extract varies with plants parts and stage of plants. Thus, in the present study, an effort was made to explore bio-activity of extracts from four commonly available plants *viz.* Fern (*Dryopterisfilix-mas* (L.) Schott), Orangeberry (*Glycosmis pentaphylla* (Retz.) DC and Debdaru (*Polyalthia longifolia*)) and their different parts at different stages. These were tested against some fungal plant pathogens, *viz. Rhizoctonia solani, Colletotrichum gloeosporoides, C. capsici, C. musae, Phomopsis vexans, Fusarium oxysporium fsp. ciceri, Diplodia natalensis and Alternaria alternata.*

Materials and Methods
Plant material: Following three plants (Tab-1) were selected for extraction and bio-activity testing from fields at Haringhata, Nadia, West Bengal, India. Selection of plant pathogens
Test fungal pathogens that were isolated and collected from Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya culture collection for testing the bioactivity of the plant extracts are *Rhizoctonia solani, Colletotrichum gloeosporoides, C. capsici, C. musae, Phomopsis vexans, Fusarium oxysporium fsp. ciceri, Diplodia natalensis and Alternaria alternata.*

Table 1: List of selected plant materials

Sl. No.	Common Name	Scientific Name	Family	Parts Used
i.	Fern	*Dryopteris filix-mas* (L.) Schott	*Marattiaceae*	Whole leaves
ii.	Orangeberry	*Glycosmis pentaphylla* (Retz.) DC	*Rutaceae*	Leaves and fruit
iii.	Debdaru	*Polyalthia longifolia* **(Sonn.) Thwaites**	*Annonaceae*	Leaves of different stages

Preparation of Plant Extracts

After collection of the above mentioned four plants and their parts, these were shade dried and grinded. The extraction from powdered plant material was done following sequential hot extraction with organic solvents (hexane-chloroform-methanol) based on their polarity using Soxhlet apparatus (capacity 250 ml) for 6-8 hours following standard protocol. Then the crude extract was collected, concentrated in a Buchi Rotavapor at 45^0 C, transferred in a pre-weighed conical flask and evaporated.

Preparation of plant extract formulation 20% EC (w/w)

Extracts of each plant were formulated (20 EC) using light solvent naphtha (LSN) and Surfactant mixture of (A) N-Alkaline Sulfonate and (B) K-Alkaline Sulfonate. The entire formulation procedure was developed and standardized at Bio-formulation Laboratory, Department of Agricultural Chemicals, BCKV, Mohanpur.

***In-vitro* evaluation of the extracts against the test fungi**

Testing of *in- vitro* efficacy of the plant extracts formulations against the test pathogen was done following standard protocol of Poisoned food technique with three replications. Concentration of 0.2% (400µg/mL) of each formulation were tested against all the test pathogen and Carbendazim @5µg/mL was set alongside which served as standard fungicide control for comparative studies.

The inhibitory effect of the plant extract on fungal growth was determined by measuring the average diameter of the colony at periodic interval till the control plates showed full growth. Efficacy of botanicals was expressed as per cent inhibition of radial growth over the control which was calculated by using the formula-

$$\% \text{ Radial growth inhibition (I)} = \frac{\text{Radial growth in control(C)} - \text{Radial growth in treatment(T)}\ X\ 100}{\text{Radial growth in control(C)}}$$

Statistical analyses

Statistical analyses of all data were done using MSTATC and Microsoft Office Excel 2007 software, Factorial CRD ANOVA for lab experiments were done.

Result and Discussion

Hexane Extract: Among the hexane extracts of selected plant materials, *per cent* inhibition of radial growth over control was recorded highest in case of *Polyalthia longifolia* old leaf extract (48.78%) and lowest in case of fern extract (25.45%) against all test pathogens. Hexane extract of *Polyalthia longifoli* new leaf showed 33.69% mean inhibition of radial growth of the test pathogen. *Glycosmis pentaphylla* leaves and *Glycosmis pentaphylla* whole inflorescence showed similar inhibitory effect (~ 27%). Carbendazim showed a mean of 50.27% radial growth inhibition over control irrespective of tested fungi (Table no.-2, Plate -1 and 2) which was higher than any plant extract tested.

Among the test pathogens, *Rhizoctonia solani* was highly inhibited by the plant extracts (47.92%) but the lowest inhibition was seen in *Alternaria alternata (19.13%)* by the plant extracts and Carbendazim fungicide. Sensitivity of plant pathogenic fungi to plant extracts (as measured by radial growth inhibition), could be arranged in following sequence of highest to lowest as *Rhizoctonia solani* (47.92)>*Colletotrichum musae* (39.44)>*Diplodia natalensis* (39.40) >*Colletotrichum capsici* (39.24) >*Phomopsis vexans* (33.55)>*Fusarium oxysporium* (32.65) >*Colletotrichum gloeosporioides* (32.23) >*Alternaria alternata* (19.13). (Table no.-2, Plate-1 and 2)

Hexane extract of fern effectively/ significantly inhibited the radial growth of *Phomopsis vexans* (46.29%) and *Colletotrichum capsici* (45.18%) but was less effective against other test fungi. Interestingly extract prepared from *Polyalthia longifoli* old leaves showed higher radial growth inhibition of *Rhizoctonia solani* (85.92%), *Colletotrichum capsici* (56.29%), *Colletotrichum musae*(52.22%) and *Colletotrichum gloeosporioides* (51.85%) than that of standard fungicide and also effectively controlled the growth of *Phomopsis vexans* (41.11%). In case of *Polyalthia longifoli* new leaf extract, it was found to inhibit growth of *Colletotrichum musae* (52.59%) and *Colletotrichum capsici* (50.00%) as compared to standard fungicide 41.85% & 6.29% respectively.

Glycosmis *pentaphylla* inflorescence and Glycosmis *pentaphylla* leaf extract showed similar trend in controlling *Colletotrichum capsici* (41.74% and 31.85% respectively) and *Colletotrichum musae* (37.03% and 34.07% respectively).

Table 2: Per cent radial growth inhibition over control of different plant pathogenic fungi by hexane extracts of plants

Treatments (T)	*C. capsici*	*C. musae*	*C. gloeosporoides*	*Alternaria alternata*	*Diplodia natalensis*	*Rhizoctonia solani*	*Fusarium oxysporum*	*Phomopsis vexans*	Mean of% inhibition irrespective of fungi
Fern	45.18	24.07	17.40	17.40	6.66	24.07	21.48	46.29	25.45
Polyalthia old leaf	56.29	52.22	51.85	14.81	40.74	85.92	48.51	41.11	48.78
Polyalthia new leaf	50.00	52.59	25.55	29.25	41.11	24.07	26.66	20.18	33.69
Glycosmis inflorescence	40.74	31.85	20.37	18.51	26.29	31.85	19.25	27.03	27.14
Glycosmis leaf	37.03	34.07	19.62	22.22	22.59	31.48	19.62	32.59	27.34
Carbendazim	6.29	41.85	57.40	12.59	99.00	90.00	60.37	34.07	50.27
Mean of% inhibition irrespective of treatment	39.24	39.44	32.23	19.13	39.40	47.92	32.65	33.55	
	Treatment (T)		Fungus (F)		Interaction (F X T)				
S.Em±	0.67		0.78		1.90				
CD 5%	1.89		2.19		5.34				

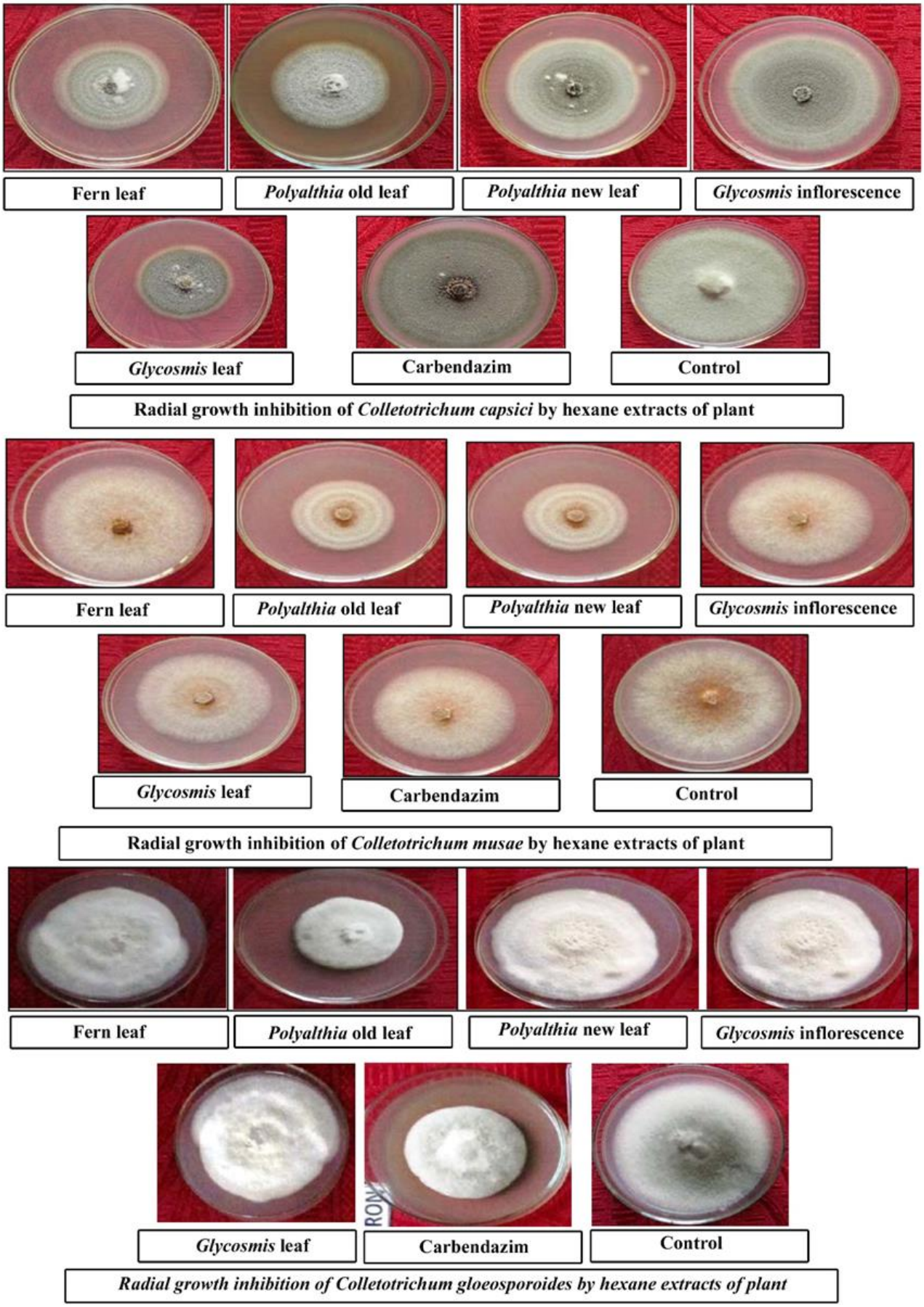

Plate 1: Radial growth inhibition of different fungal pathogen by Hexane extracts of different plants or plant parts

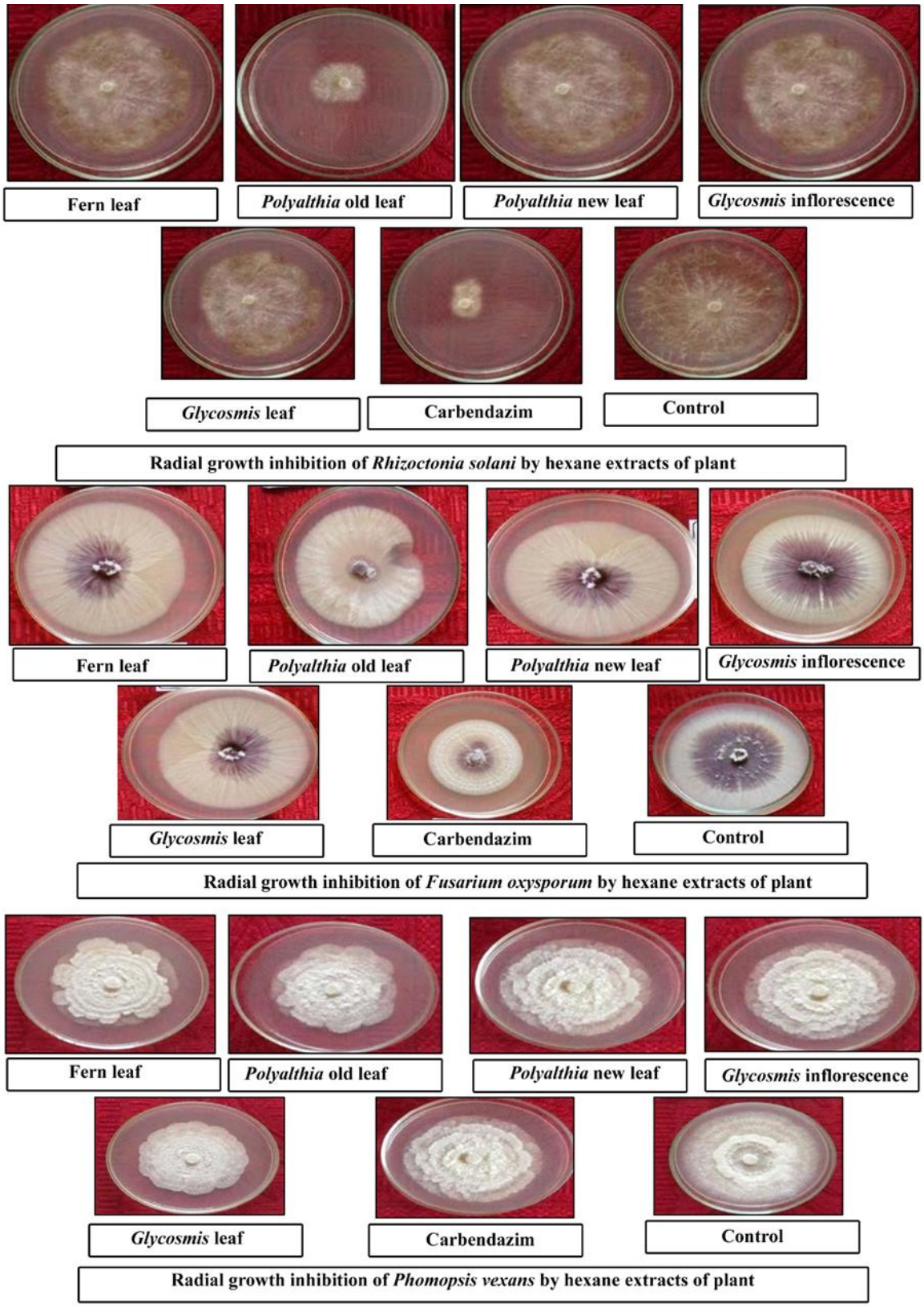

Plate 2: Radial growth inhibition of different fungal pathogen by Hexane extracts of different plants or plant parts

Chloroform Extract

Chloroform extract of selected plant materials along with standard carbendazim fungicide showed the 34.45% to 56.86% mean inhibition of radial growth over control irrespective of test fungi. Among the five plant extracts highest mean inhibitory effect was showed by *Polyalthia longifoli* old leaf extract (56.86%) over control followed by Fern extract (46.34%) against the test fungi. Chloroform extract of *Polyalthia longifoli* new leaves and *Glycosmis pentaphylla* whole inflorescence showed similar mean inhibitory effect (~37%) and *Glycosmis pentaphylla* leaves extract exhibited least mean inhibitory effect (34.45%) as compared to other extracts. Carbendazim, that was used as standard treatment showed a mean radial growth inhibition of 50.27% over control (Table-3, Plate-2) irrespective of fungi tested.

Among the different plant pathogenic fungi *R. solani* was highly inhibited (67.28% mean inhibition) and *P. vexans* was minimally inhibited (34.79% mean inhibition) by all the plant extracts. Themean radial growth inhibition of test fungi by chloroform extract of plant materials followed the sequence of highest to lowest as *R. solani* (67.28%) >*Diplodiana talensis* (46.63%) > *C. capsici* (43.69%) > *C. gloeosporioides* (43.43%) >*C. musae* (39.99%)>*Fusariumoxysporium* (39.26%) >*Alternaria altermata* (35.30%) >*P. vexans* (34.81%) (Table-3,Plate-2).

Fern extract significantly checked the growth of *Colletotrichum capsici* (64.07%), *Colletotrichum musae* (55.40%) and *Phomopsis vexans* (49.62%). Radial growth of *R. solani* (77.03%), *Colletotrichum gloeosporioides* (60.70%) and *Alternaria alternata* (56.29%) inhibited by *Polyalthia* old leaf extract. *Polyalthia* old leaf extract also showed satisfactory inhibition of*Colletotrichum capsici* (54.44%), *Diplodia natalensis* (50.00%) and *Fusarium oxysporum* (45.55%). *Glycosmis* inflorescence extract showed highest *per cent* inhibition of radial growth over control of *Rhizoctonia solani* (85.18%) among all the five plant extracts. *Glycosmis* leaf extracts showed maximum radial growth inhibition of *P. vexans* (41.11%) followed by *C.capsici*(40.74%).

Table 3: Per cent radial growth inhibition over control of different plant pathogenic fungi by chloroform extracts of plants

Treatments (T)	***C.capsici***	***C.musae***	***C.gloeosporoides***	***Alternaria alternata***	***Diplodianatalensis***	***Rhizoctoniasolani***	***Fusariumoxysporum***	***Phomopsisvexans***	**Mean of% inhibition irrespective of fungi**
Fern	64.07	55.40	46.66	46.66	46.29	62.22	44.81	49.62	46.34
Polyalthia old leaf	54.44	34.48	60.74	56.29	50.00	77.03	45.55	32.22	56.86
Polyalthia new leaf	52.59	42.33	32.22	34.81	27.40	54.81	32.22	20.74	37.18
Glycosmis inflorescence	44.07	27.59	31.85	26.29	28.88	85.18	27.77	30.37	37.69
Glycosmis leaf	40.74	38.29	32.96	35.18	28.14	34.44	24.81	41.11	34.45
Carbendazim	6.29	41.85	57.40	12.59	99.00	90.00	60.37	34.07	50.27
Mean of% inhibition irrespective of treatment	43.69	39.99	43.43	35.30	46.63	67.28	39.26	34.79	
	Treatment (T)		Fungus (F)		Interaction (F X T)				
SEm±	0.45		0.52		1.27				
CD 5%	1.26		1.46		3.56				

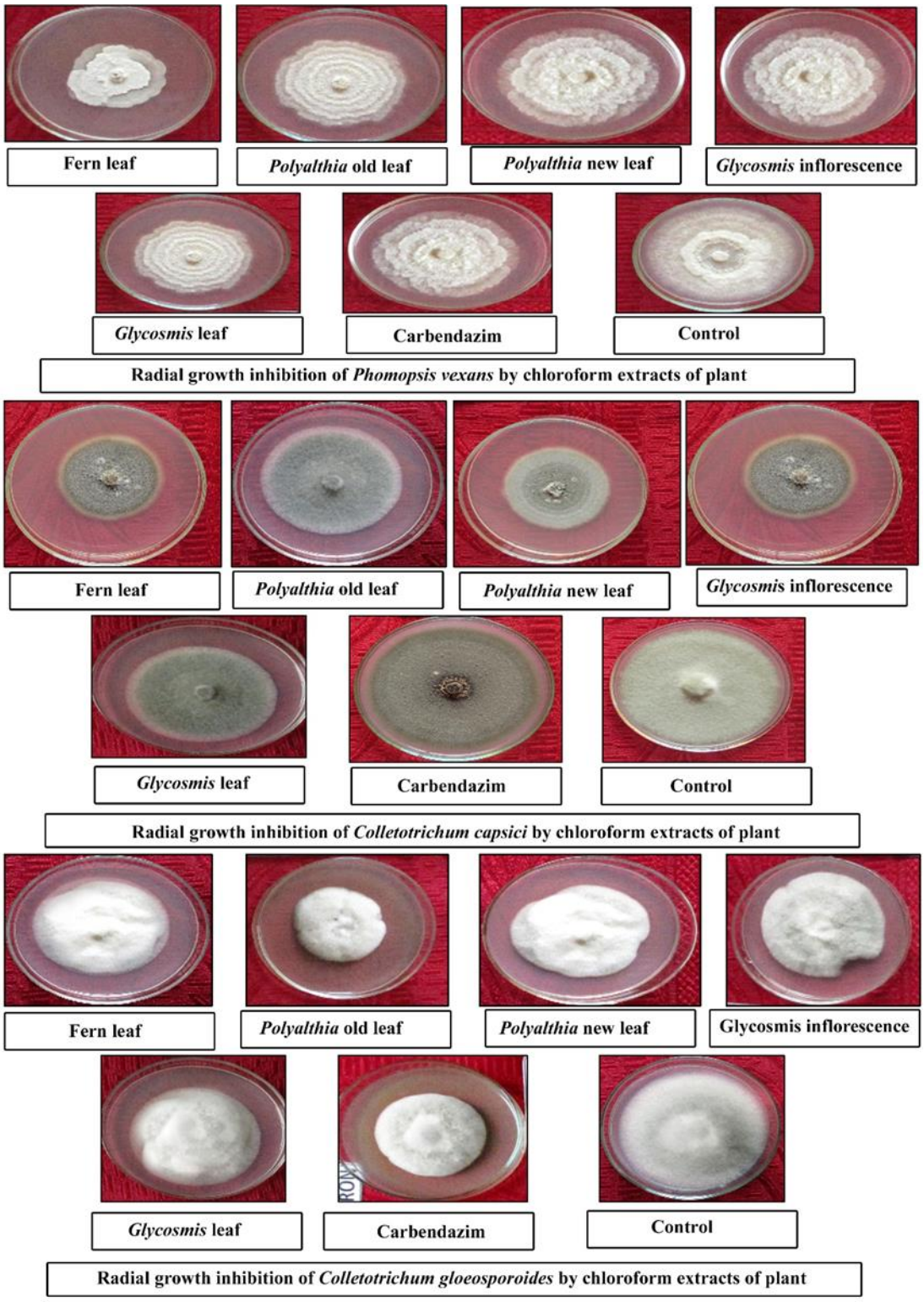

Plate 3: Radial growth inhibition of different fungal pathogen by Chloroform extracts of different plants or plant parts

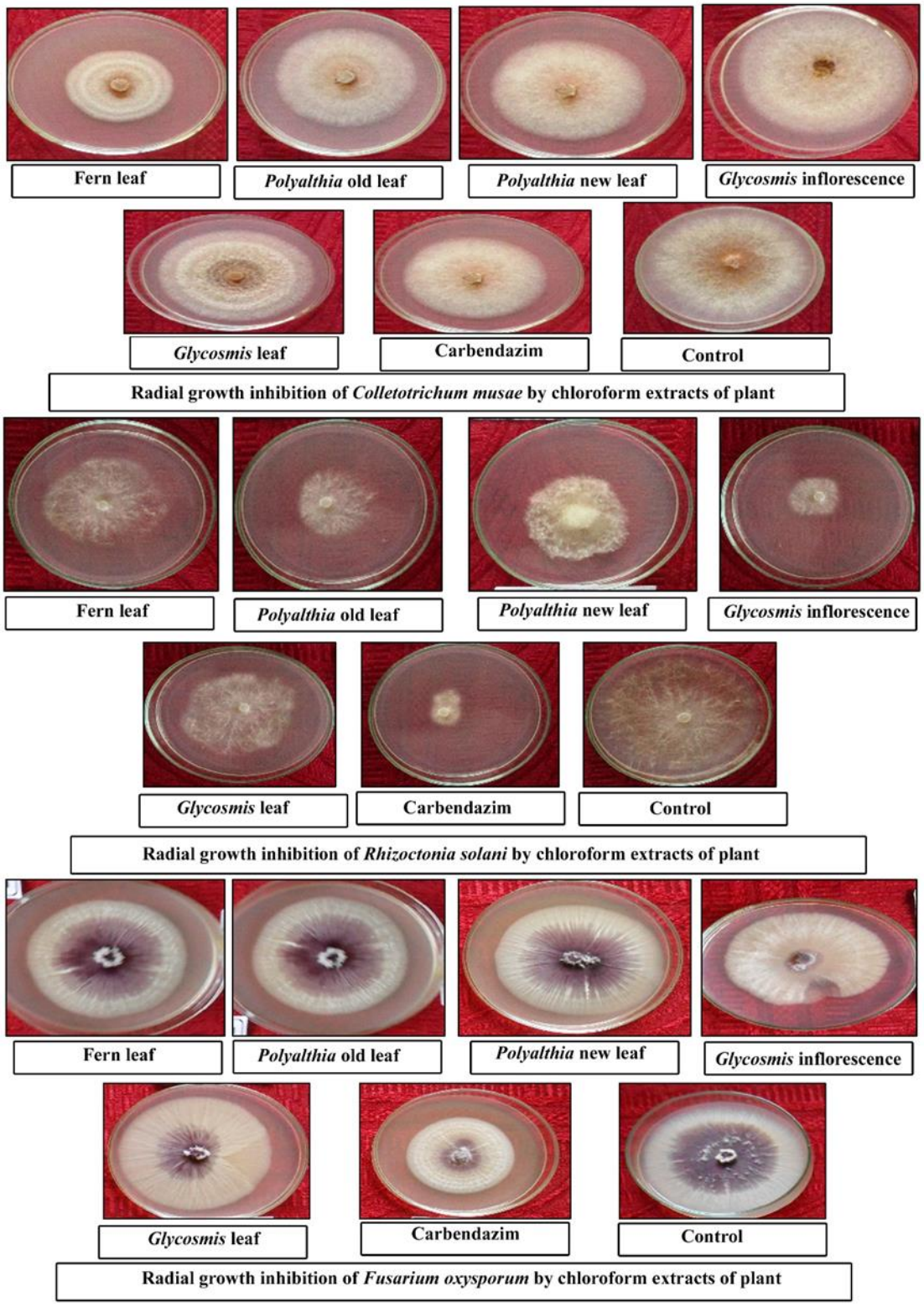

Plate 4: Radial growth inhibition of different fungal pathogen by Chloroform extracts of different plants or plant parts

Methanol Extract

The radial growth of the test fungi was inhibited by 39.32% to 58.22% over control in methanol extracts of selected plant materials. Among the methanol extracts, highest mean inhibitory effect was recorded for *Polyalthia* old leaf extract (58.22%) over control irrespective of fungi followed by fern extracts (51.78%) which was statistically at par to standard fungicide carbendazim. However, *Glycosmis* whole inflorescences and leaf extracts showed relatively poor mean inhibitory effect (~40%) as compared to other plant extracts irrespective of fungi tested against (Table - 4).

Among the different plant pathogenic fungi, highest mean radial growth inhibition was observed against *R. solani* (80.30%), while lowest mean radial growth inhibition was observed against *A. alternate* (32.77%) by the plant extracts (Table-4). Sensitivity of plant pathogenic fungi to plant extracts as measured by radial growth inhibition could be arranged in following sequence of highest to lowest as *R. solani* (80.30%) > *P. vexans* (60.06%) >*Diplodianatalensis* (52.85%) > *C. capsici* (48.94%) >*C. musae* (40.37%)>*C. gloeosporioides* (36.10%)>*F. oxysporum* (34.75%)>*A. alternata* (32.77%) (Table-4.).

Among the methanol extract of all the selected plants, *Polyalthia* old leaf extract showed highest inhibition in radial growth of *P. vexans* (78.51%), *C. capsici* (72.59%) and *C. musae* (67.40%). Fern leaf extract showed significantly inhibitory effect over control against *Alternariaalternata* (55.92%). Whereas methanol extracts of *Polyalthia* old leaf and *Glycosmis* whole inflorescence showed worthy inhibitory effect against *R. solani* (88.14% and 85.18% respectively) which was comparable to carbendazim (90%) (Table-4.).

Table 4: Per cent radial growth inhibition over control of different plant pathogenic fungi by methanolic extracts of plants

Treatments (T)	***C. capsici***	***C. musae***	***C. gloeosporoides***	***Alternari aalternata***	***Diplodianatalensis***	***Rhizoctonia solani***	***Fusarium oxysporum***	***Phomopsis vexans***	**Mean of% inhibition irrespective of fungi**
Fern	64.81	25.55	41.85	55.92	61.85	80.37	33.70	49.62	51.78
Polyalthia old leaf	72.59	67.40	35.18	38.51	44.07	88.14	40.74	78.51	58.22
Polyalthia new leaf	48.51	42.96	39.62	43.70	41.11	77.40	26.29	77.40	49.42
Glycosmis whole inflorescence	45.18	31.85	18.14	18.88	38.51	85.18	21.48	54.44	39.32
Glycosmis leaf	56.29	32.59	23.33	27.03	32.59	60.74	25.92	66.29	40.59
Carbendazim	6.29	41.85	57.40	12.59	99.00	90.00	60.37	34.07	50.27
Mean of% inhibition irrespective of treatment	48.94	40.37	36.10	32.77	52.85	80.30	34.75	60.06	
	Treatment (T)		Fungus (F)		Interaction (F X T)				
S.Em±	1.02		1.17		2.88				
CD 5%	2.86		3.28		8.09				

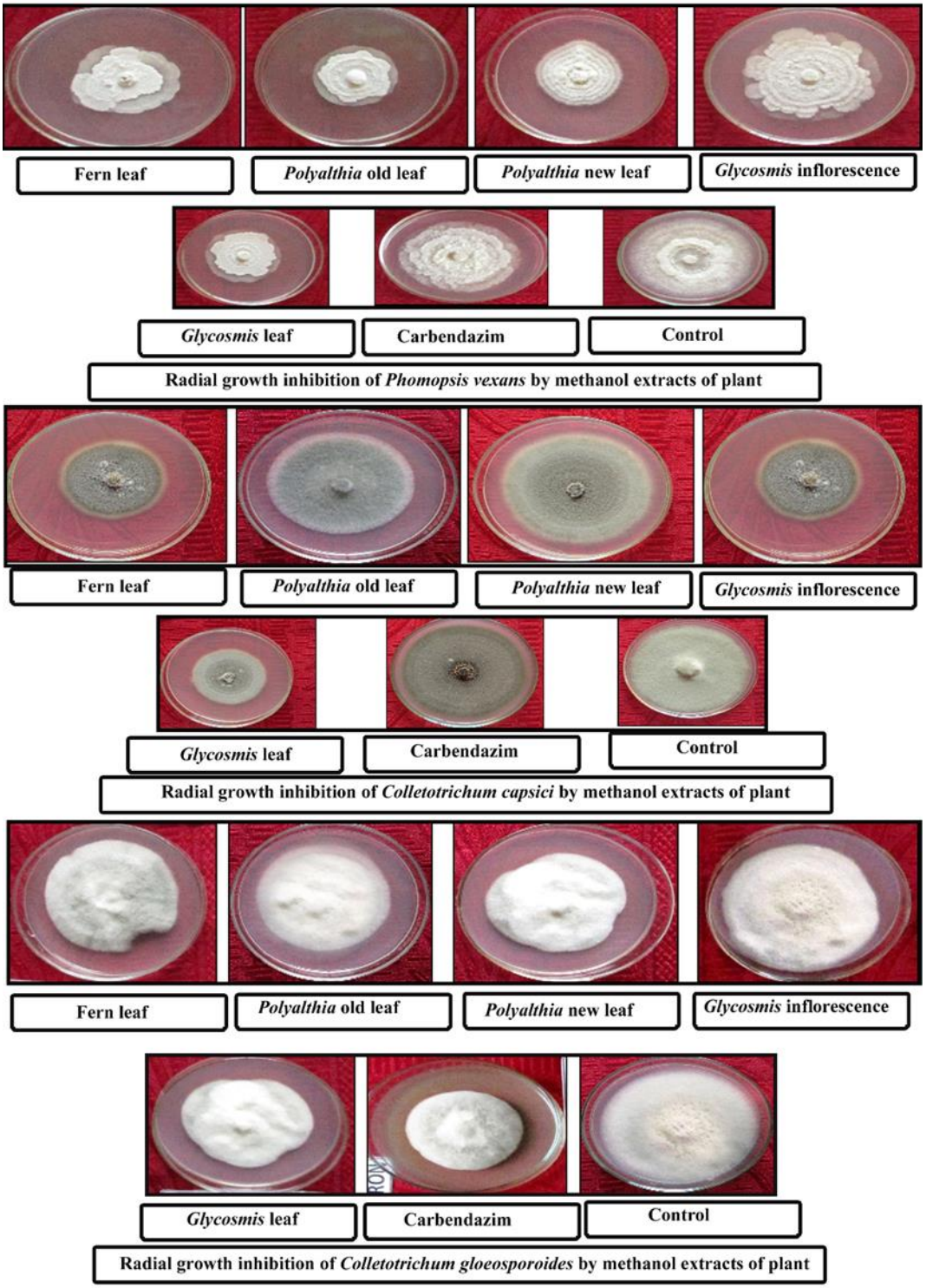

Plate 5: Radial growth inhibition of different fungal pathogen by Methanol extracts of different plants or plant parts

Management of plant diseases by plant extracts is nowadays considered as an important component of integrated disease management. The plant extract having bioactivity either directly by inhibition of the growth and development of pathogens or by induction of systemic resistance in the host (Akila *et al*., 2011) [2]. Plants are rich source of several secondary metabolites primarily to defend themselves from the herbivore and the natural enemies (Hamburger and Hosettman, 1991) [8]. In general, this plant secondary metabolites have co-evolve to have herbivore. A continuous thrust has been given to search bioactive plant extracts against plant pathogens with an aim to minimizing the ecological side effects (Dubey *et al*., 2010) [7]. In the present studies widely grown plant species were explored to search out potential extracts against some important plant pathogenic fungi and diseases caused by them. Concentration of bioactive secondary metabolites in plant depends on various factors like the growing environment, stress, plant parts, age of the plant-plant parts. As for example in the present laboratory Bhutia, 2013 [4] found that young leaf extracts of *Tectona grandis* had high antifungal activity rather than the well matured senescent leaf extracts.

In the present study three plant species were taken into consideration *viz. Polyalthia longifolia, Glycosmis sp.and* fern for extraction and testing against different plant pathogenic fungi. More over polyalthia new and aged leaves were

extracted separately following sequential extraction method in soxhlet apparatus using hexane, chloroform and methanol as an extracting solvent. Results indicated that plant extracts have significant radial growth inhibitory effect on laboratory culture media at very low concentration 400µg/l. some extracts were better than standard fungicide Carbendazim on the *in-vitro* condition. Among the three different extracts chloroform and methanol extracts were better than hexane extracts. Low polar solvent hexane is used to extract mostly the oils and other low polar plant secondary metabolites. That indicate the plant materials used in the present study had the negligible or very low bioactive oil contents. However, several showed the bioactivity of essential oils of plant origin against several plant pathogenic fungi (Bowers and Locke, 2004; Al-Reza *et al*., 2010) [5, 3].

Methanol extracts were found best among the three solvent extracts followed by chloroform and hexane, indicating majority of the bioactive compounds in the plant material used were polar in nature (Bhutia, 2013) [4]. In the present study it was found that extracts from *Polyalthia* old leaves having better bioactivity than the new leaves extracts irrespective of the fungi tested against and its mean activity was even better than standard fungicide carbendazim. However, mean antifungal activity of *Glycosmis* whole inflorescence and leaf extracts were comparable (Table 6). The variation in the bio-activity may be due to variation in concentration of active secondary metabolites in differential maturity stages in *Polyalthia* (Al-Reza *et al*., 2010) [3].

Table 5: Summary of means of radial growth inhibition of plant pathogenic fungi to plant extracts

Fungi	Hexane	Chloroform	Methanol	Mean
C. capsici	39.24	43.69	48.94	43.96
C. musae	39.44	39.99	40.37	39.93
C. gloeosporoides	32.23	43.43	36.10	37.25
Alternaria alternata	19.13	35.30	32.77	29.07
Diplodia natalensis	39.40	46.63	52.85	46.29
Rhizoctonia solani	47.92	67.28	80.30	65.17
Fusarium oxysporum	32.65	39.26	34.75	35.55
Phomopsis vexans	33.55	34.79	60.06	42.80
Mean	35.45	43.80	48.27	

It was also observed that the test plants pathogenic fungi that include post-harvest pathogens were also differed with respect to their sensitivity towards plant extract formulation. Sensitivity of three different species of *Colletotrichum sp.* to plant extracts were comparable indicating mode of action of the selected plant extracts to *Colletotrichum sp.* are more or less same (Table. 5.). Among the pathogen tested *Rhizoctonia solani* was found to be most sensitive to the plant extracts and interestingly there is an increase in sensitivity with polarity of the extraction solvent used. Similar trend of sensitivity towards more polar solvent extracts was noted in case of *Phomopsis vexans* and *Diplodia natalensis* (Table.5.).

Table 6: Summary of means of activity of plant extracts

Plant Extracts	Hexane	Chloroform	Methanol	Mean
Fern leaf	25.45	46.34	51.78	41.19
Polyalthia old leaf	48.78	56.86	58.22	54.62
Polyalthia new leaf	33.69	37.18	49.42	40.10
Glycosmis whole inflorescence	27.14	37.69	39.32	34.72
Glycosmis leaf	27.34	34.45	40.59	34.13
Carbendazim	50.27	50.27	50.27	50.27
Mean	35.45	43.80	48.27	

There are several reports on management of postharvest pathogens and diseases by plant extracts. Ademe *et al*., 2013 [1] recorded highest inhibition of *C. gloeosporioides* causing papaya anthracnose by the ethyl acetate extract of *Lantana camara.* Similarly, Al-Reza *et al*. (2010) & Camele *et al*. (2012) [3, 6] observed significant growth inhibition of postharvest pathogens by essential oils.

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