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“Study of fungicidal activity of certain medicinal plants against *Sclerotium rolfsii* causing root diseases of vegetables.”

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Abstract: The medicinal plant aqueous leaves extracts of *Ficus racemosa* (Fig), *Ficus religiosa* Linn (Peepul) and *Tagetes erecta* (Zendu / Marigold) were assessed in vitro for antifungal activity against *Sclerotium rolfsii* Sacc. The causal agent of root rots in chilli. The assessment of fungicidal property was carried out by poisoning food technique using five different concentration 10%, 25%, 50%, 75% and 100% (without treatment severed as control). Assessment was carried out in terms of percentage inhibition (% of I) of *S.rolfsii*. The all three plant leaves extract of selected were found to be effective in inhibiting the mycelial growth. The extract of *Ficus racemosa* (Fig) strongly inhibited the *Sclerotium rolfsii* at the concentration of 75, with 72.46% of strong inhibition with 7.16mm inhibited mycelial after 24 hours of incubation period this was followed by *Ficus religiosa* Linn (Peepul) aqueous leaves extract gave 67.95% moderate percentage of inhibition with 8.333mm inhibited mycelial at 25 percent concentration after 24 hours of incubation period. The least percent of inhibition 56.59% with 37.33mm inhibited mycelial was recorded in *Tagetes erecta* (Zendu / Marigold) at 75 percent concentration after 72 hours of incubation period.

Key words- *Ficus racemosa* (Fig), *Ficus religiosa* Linn (Peepul), Medicinal plant, *Sclerotium rolfsii* and *Tagetes erecta* (Zendu / Marigold).

I. Introduction

India has lots of medicinal plants have chemicals properties against plant diseases. In recent years, secondary plant's metabolites (Photochemical) previously with unknown pharmacological activities have been extensively investigated as sources of medicinal agents. These chemicals bear verities of properties viz. antifungal and others [1]. In recent years much attention has been also given to non-chemical systems for protect crop against many plant pathogens, even if their effectiveness has been reported in many diseases of various crops. The biotic control of plant diseases may have minimum adverse effect on physiological process of plants and less environmental hazards [2] as well may create a fever health problems compared to the synthetic alternatives. [3] the blind dependence on synthetics is over and people are returning to the naturals with a hope of safe and security. The use of biological compounds extracted from plants may be an alternative to conventionally used fungicides to control pathogenic fungi, due to their begin bioactive chemicals [4].

Control measures, like host resistance has not yet become a viable measure, no resistant variety has been developed and released against this soil born pathogen causing *Sclerotium rolfsii* of vegetables. In order to overcome these problems, higher concentrations of chemicals had been used, but these increased the risk of high-level toxic residues in the products which are not beneficial for human beings and environment.

Fungicides such as Captan Calixin have been used for seed dressing and other chemicals such as methyl bromide and Chloropicrin are used as soil fumigants to the control of this pathogen. These chemicals are environmentally hazardous, recently banned, not readily available and difficult therefore to adopt in subsistence agriculture in West Africa [5].

An attempt was made to employs plant extract against *Sclerotium rolfsii* of plant pathogen medicinal plants have no doubt remained the major sources of ant fungicides or antifungal agent used in world wide. In previous studies, it was observed that no previous work has been done to examine the antifungal activity of different concentration of leaves extract of *Ficus racemosa* (Fig), *Ficus religiosa* Linn (Peepul). The aim of the study is to assess the antifungal activity and to determine the zone of inhibition of extracts *Ficus racemosa* (Fig), *Ficus religiosa* Linn (Peepul) and *Tagetes erecta* (Zendu / Marigold) medicinal plants against the test *Sclerotium rolfsii* fungus.

II. Materials and Methods

Isolation of *Sclerotium rolfsii*.

Infected root rot disease of chilli plant caused by *Sclerotium rolfsii* was collected from the field of Aurangabad. The cut the infected portion into small pieces of about 3-5mm thick and sterilized with 0.1% (HgCl₂) mercuric

chloride solution for few seconds and rinsed thrice in sterilized distilled water, and then placed on filter paper at room temperature. The tissue sections were then placed on potato dextrose agar and incubated at room temperature for seven days. Ultimately the pure culture of the pathogen was isolated subsequently maintained on the potato dextrose agar medium. Potato dextrose agar medium was prepared and after room temperature poured petriplates, mycelial disk 4mm diameter were cut from 4-5 day-old actively growing culture of *Sclerotium rolfsii* and each was placed in the center of petriplates containing PDA. The effect of plant extracts on inhibition of mycelial on the growth of *Sclerotium rolfsii* was studied using poisoning food technique, [6].

Plant species with families selected.

The plant species with families were selected for in-vitro evaluations used for this study were presented in the Table 1 below.

Table 1 Plant species and families selected for this study.

Sr.No.	Botanical name of medicinal Plants	Families
1	<i>Ficus racemosa</i> (Fig)	Moraceae
2	<i>Ficus religiosa</i> Linn (Peepul)	Moraceae
3	<i>Tagetes erecta</i> (Zendu / Marigold)	Asteraceae

Preparations' of plant extract:

Fresh leaves of *Ficus racemosa* (Fig), *Ficus religiosa* Linn (Peepul) and *Tagetes erecta* (Zendu / Marigold) were used for leaves extract preparation. The *Tagetes erecta* (Zendu / Marigold) leaves were collected from near phulambari road, but *Ficus racemosa* (Fig) and *Ficus religiosa* Linn (Peepul) are locally available plants. These leaves were collected during January, February 2015. These plants leaves, were rinsed in sterile distilled water in two to three times and dried in shade at room temperature, after which they were milled motor and pestle and electric blender to make powder. The powders were packed in to bottles and in air tight plastic pouches.

Preparation plant leaves extract medium for different concentration:

The plant leaves extract were made with at the rate of one ml/one gm or one gm / one ml of sterilized distilled water, autoclaved, cooled and then strained through muslin cloth. This formed a standard plant extract were made in aqueous medium of 10%, 25%, 50%, 75% and control (a without plant leaves extracts) concentrations.

Studies effect of plant leaves extracts of different concentrations:

The effect of plant leaves extracts of *Ficus racemosa* (Fig), *Ficus religiosa* Linn (Peepul) and *Tagetes erecta* (Zendu / Marigold) on inhibition of the *Sclerotium rolfsii* was studied using poisoning food technique, (Dhingra and Sinclair, 1985)[6]. From standard stock solutions of plants leaves 10, 25, 50, 75 percentage concentrations was prepared separately by adding the required quantity of plants extract to the molten potato dextrose agar medium. One set is made without plant extract and keep it as controlled. All these poured in to sterilized petri plates. A mycelial disk cut from the periphery to 3-4 days old colony of *Sclerotium rolfsii* grown on potato dextrose agar medium were centrally placed in each of the petriplates containing the potato dextrose agar medium having medicinal plant leaves of *Ficus racemosa* (Fig), *Ficus religiosa* Linn (Peepul) and *Tagetes erecta* (Zendu / Marigold) at different 10%, 25%, 50%, 75% concentrations and control under aseptic conditions. The petriplates contains the PDA medium inoculated with the pathogen alone served as control. All these petriplates were incubated at room temperature. There are three replication were maintained for each treatment. The diameter of the colony was measured in two directions and average was recorded. The inhibition the growth of *Sclerotium rolfsii* was calculated by using the formula given below.

$$\text{Percentage of inhibition} = \frac{[\text{Diameter of colony}] - [\text{Diameter of colony in treatment}]}{\text{Diameter of colony control}} \times 100$$

Table 1- Medicinal plants in aqueous medium of following concentrations impression radial growth of *Sclerotium rolfsii* measured in mm after 24 hours.

Serial. No	Medicinal plants	Concentrations (%)	Growth <i>S.rolfsii</i> given extracts measured mm 24 hours		
			24 hr	48 hr	72 hr
16	<i>Ficus racemosa</i> (Fig)	10 %	10	33.66	64
		25 %	11.33	35.33	66
		50 %	10	32.66	59
		75 %	7.16	21	30.66
		Control			
17	<i>Ficus religiosa</i> Linn (Peepul)	10 %	9.333	31	63
		25 %	8.333	19.33	54
		50 %	9.333	31	55.66
		75 %	13.16	29.66	47.66

		Control			
18	<i>Tagetes erecta</i> (Zendu / Marigold)	10 %	15.66	47.33	80
		25 %	13.66	37.66	68.66
		50 %	14.33	27.83	52
		75 %	15	25.33	37.33
		Control			

- * Values are average of triplicate.
- * Values measured after deducting or reducing 4mm mycelium disk.
- * (I) is denoted for inhibition.
- * Note 1-10%, 25%, 50%, 75% and 100% are plant leaves extracts of aqueous medium.

Table 2- The percentage of Inhibition (% of I) of *Sclerotium rolfii* at given plant extract concentration in aqueous medium.

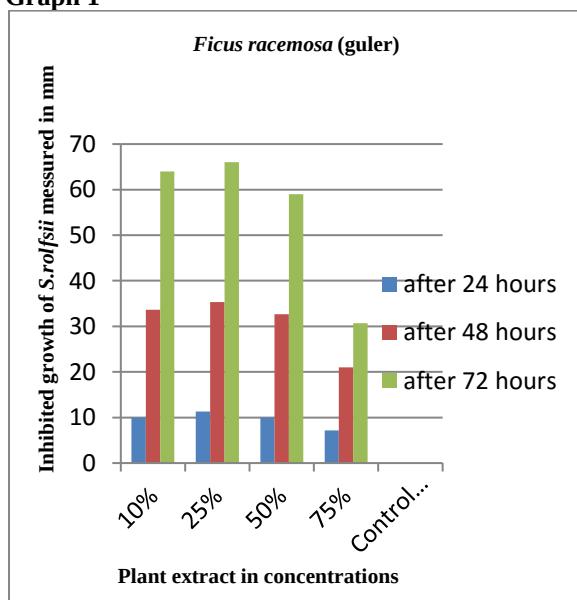
Sr. No	Medicinal plants	Concentrations (%)	Percentage Of Inhibition (I) of <i>S.rolfsii</i> at					
			given	Plant	extract	in	following	concentration
			24 hr	% of I	48 hr	% of I	72 hr	% of I
1	<i>Ficus racemosa</i> (Fig)	10 %	10	61.53	33.66	39.89	64	25.58
		25 %	11.33	56.42	35.33	36.91	66	23.25
		50 %	10	61.53	32.66	41.67	59	31.39
		75 %	7.16	72.46	21	62.5	30.66	64.34
		Control						
2	<i>Ficus religiosa</i> Linn (Peepul)	10 %	9.333	64.10	31	44.64	63	26.74
		25 %	8.333	67.95	29.33	47.62	54	37.20
		50 %	9.333	64.10	31	44.64	55.66	35.27
		75 %	13.16	49.38	29.66	47.03	47.66	44.58
		Control						
3	<i>Tagetes erecta</i> (Zendu / Marigold)	10 %	15.66	39.67	47.33	15.48	80	6.976
		25 %	13.66	47.46	37.66	32.75	68.66	20.16
		50 %	14.33	44.88	27.83	50.30	52	39.53
		75 %	15	42.30	25.33	54.76	37.33	56.59
		Control						

- * Values are average of triplicate.
- * Values measured after deducting or reducing 4mm mycelium disk.
- * (I) is denoted for inhibition.
- * Note 2-10%, 25%, 50%, 75% and 100% are plant leaves extracts of aqueous medium.

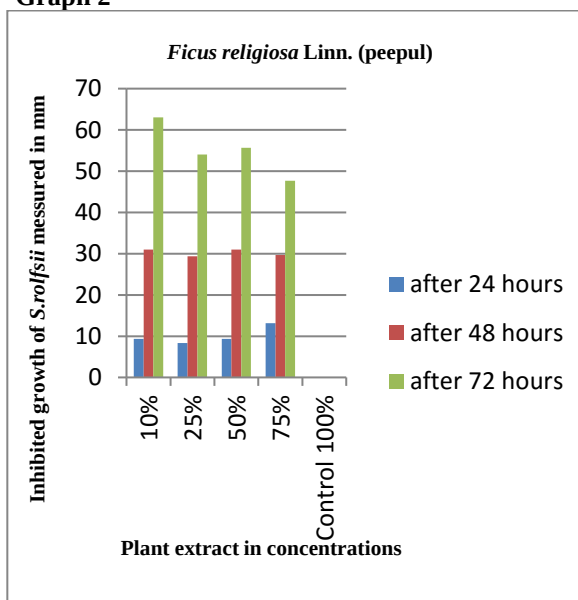
Graph 1- Inhibited growth of *Sclerotium rolfii* in *Ficus racemosa*(Fig) leaves extract.

Graph 2- Inhibited growth of *Sclerotium rolfii* in *Ficus religiosa* Linn (peepul) leaves extract.

Graph 1



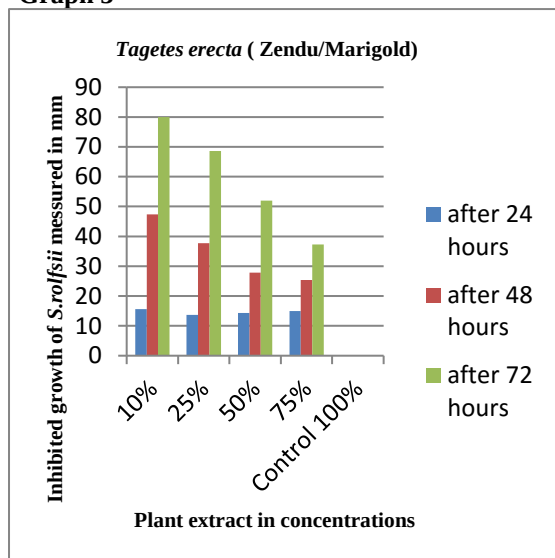
Graph 2



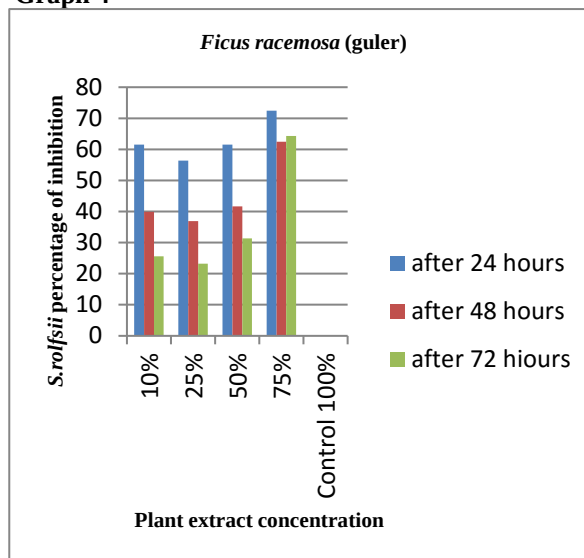
Graph 3- Inhibited growth of *Sclerotium rolfsii* in *Tagetes erecta* (Zendu/ Marigold) leaves extract.

Graph 4- The percentage of inhibition of *Sclerotium rolfsii* of *Ficus racemosa*(Fig).

Graph 3



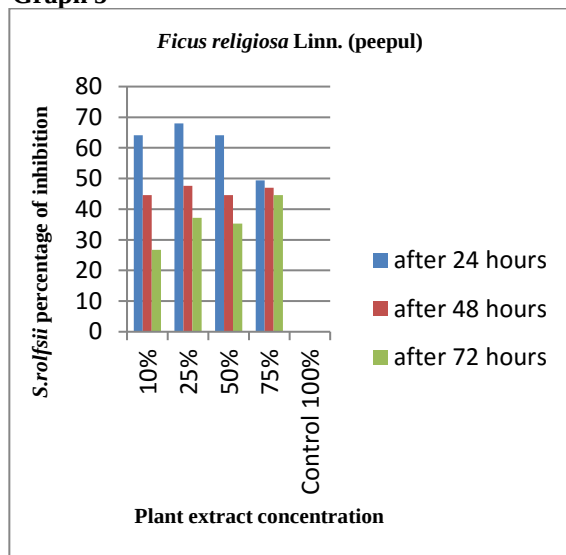
Graph 4



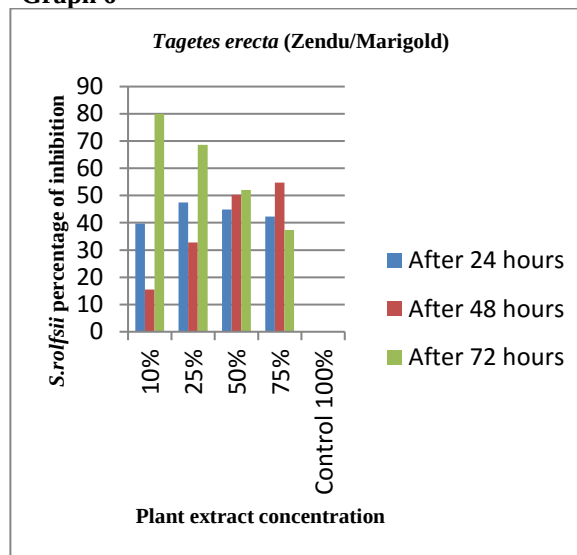
Graph 5- The percentage of inhibition of *Sclerotium rolfsii* of *Ficus religiosa* Linn (peepul).

Graph 6- The percentage of inhibition of *Sclerotium rolfsii* of *Tagetes erecta* (Marigold / Zendu).

Graph 5



Graph 6



III. Result and Discussion:

The effect of *Ficus racemosa* (Fig), *Ficus religiosa* Linn (Peepul) and *Tagetes erecta* (Zendu / Marigold) are in table 2 & 3 at four concentrations. The inhibited the *Sclerotium rolfsii* is shown in table 1, 2, 3, figure 1 and graph 1-6 after 72 hours of incubation, the radial growth of *Sclerotium rolfsii* on plates impregnate with water as aqueous, extraction medium of *Ficus racemosa* (Fig), *Ficus religiosa* Linn (Peepul) and *Tagetes erecta* (Zendu / Marigold) plant extracts concentration. Plate impregnated with aqueous extract of *Tagetes erecta* (Zendu / Marigold) showed highest percentage of inhibitions 56.59percentage with least growth 37.33mm at 75 percentages concentration, while the lowest percentage of inhibition also arise in *Tagetes erecta* (Zendu / Marigold) at 10 percentage concentration, that is about 5.95 least percentage of inhibition with 79mm maximum inhibited mycelial growth. In remains two leaves extract *Ficus racemosa* (Fig), *Ficus religiosa* Linn (Peepul) concentration of aqueous medium moderately suppressed the mycelial growth of *Sclerotium rolfsii*. The result showed that inhibit *Ficus racemosa* (Fig) gave second maximum percentage of inhibition 55.95 percentages with 37mm mycelial growth. To explore the fungicidal efficacy of three plant extracts were tested against *Sclerotium rolfsii*. The inhibitory activities are categorized as strong, moderate and least. Three plant extracts showed fungi toxic potentially against

Sclerotium rolfsii, out of which was strong fungi toxic *Tagetes erecta* (Zendu / Marigold) was observed and *Ficus racemosa* (Fig) plant extract inhibited the pathogen fungus moderately, where as the remaining plant *Ficus religiosa* Linn (Peepul) extracted showed least inhibition with very few difference.

IV. Conclusion

In view of analyzing the table 1, 2, 3, figure 1 and graph 1- 6, we come to conclude that among all three medicinal plant leaves extract of *Ficus racemosa* (Fig), *Ficus religiosa* Linn (Peepul) and *Tagetes erecta* (Zendu / Marigold), the *Ficus racemosa* (Fig) most effectively inhibited mycelial growth of *Sclerotium rolfsii* this was subsequently by *Ficus religiosa* Linn (Peepul) which note down moderate percentage of inhibition. The aqueous leaves extracts of *Tagetes erecta* (Zendu / Marigold) reported least percentage of inhibition (but above 55%). The three selected medicinal plant leaves extract were evident in this studies, they have prove potential to control of plant pathogenic fungi *Sclerotium rofsii* and could be consider for developing new fungicides.

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