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RESEARCH ARTICLE

ECO-FRIENDLY MANAGEMENT OF *SCLEROTIUM ROLFSII* CAUSING ROOT ROT DISEASE IN CHILLI.

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Abstract

Synthetically chemical fungicides have long been used to reduce the incidence of plant diseases. However they are costly, can have negative effects on the environment and may induce pathogens resistance. The application of chemical compounds is consider as the most in expensive and common method in plant disease control. Therefore efficacy of leaves extracts of *Annona squamosa* Linn, *Brassica campestris* Linn. (Sarson) and *Ocimum sanctum* Linn against *Sclerotium rolfsii* was studied. The in vitro antifungal activity of these *Annona squamosa* Linn, *Brassica campestris* Linn. (Sarson) and *Ocimum sanctum* Linn leaves extract was investigated. The medicinal plant leaves extract of 10%, 25%, 50%, 75% and 100% (without treatment serve as control) in aqueous medium were select for made. Inhibited values recorded. In the case of *Brassica campestris* Linn (Sarson) 8.534, 9.302, 11.24 and 10.86 percentage of inhibition recorded at 10, 25, 50 and 75 percent concentration after 72 hours incubation periods. The *Annona squamosa* Linn leaves gave 21.33mm mycelial growth at 25 percent concentration of with 3.045 percent of inhibition 77.33mm mycelial growth at 50 percent aqueous concentration gave 3.337 least percentage of inhibition. The *Ocimum spp.* revealed 32.00 and 30.68 percentage of inhibition with 17mm and 17.33mm mycelial growth at 50 & 75 percentage concentration of *Ocimum spp.* aqueous leaves extract after 24 hours. The maximum inhibition was 74.33mm and 73.33mm at 25 percent and 50 percent of *Ocimum spp.* aqueous leaves extract after 72 hours of incubation period. All used concentration of three medicinal plant leaves extracts effectively suppress the radial mycelial growth of *Sclerotium rolfsii* ranging from 2.197% of inhibition in *Ocimum spp.* at 25% of concentration after 72 hours, to 32.00% of inhibition in same *Ocimum spp.* leaves extract at 50% concentration leaves aqueous leaves extract after 24 hours incubation periods. However 0% gave by *Brassica compestris* leaves extract of 10 & 25 concentration after 24 hours of incubation period. The present study was conducted with an aim of determining antifungal activity of medicinal plant leaves extract of *Annona squamosa*, *Brassica campestris* Linn. (Sarson) and *Ocimum sanctum* Linn.

Introduction:-

Plant extract are eco-friendly display structural diversity and complexity and frequently contain secondary metabolites. These chemicals bear a variety of properties like antifungal and others (Parajuli et.al., 1998). Herbal medicinal are used as therapeutic agents (Kondratyak and Pezzuto, 2004; Mothana and Lindquist, 2005; Zaidi and

Crow Jr, 2005), many of these plants were screened for various biological and pharmacological activities including antifungal, antibacterial, insecticidal activities. ;(Al-Mughrabi, 2003; Ismail et al. (2003), (2007); Hoffman et.al. 2004).

Synthetic fungicides are currently used as primarily means use for to control plant diseases, however, the attractive control methods are needed because of negative public perceptions about the use of synthetic chemicals resistance to fungicides among fungal pathogens and high development cost of new chemicals. There is therefore the need to search for the use of cheaper environmentally available alternatives such as plant extracts for the control of *Sclerotium rolfsii*. The plants as medicine are widespread throughout the world.

Management of the plant diseases incited by soil borne pathogens is not achievable chemically; due to the wide spread host range, abundant growth of pathogen and its capacity of producing excessive sclerotia that may persist in soil for several years. Organic soil amendment and eco-friendly bio fungicides are a beneficial approach for controlling chilli disease causing root rot by soil born pathogen *Sclerotium rolfsii*.

Large number of plant have been reported to possess fungi toxic could be exploited commercially with practicality no residual or toxic effect on ecosystem (Kumar et al, 2008). Protective, curative and antagonistic activity of different plants against variety of diseases has been reported by several workers (Kandasamy et al., 1974; Hala and Mathers, 1977; Rahber-Bhatti, 1986; Kalo and Tanigueshi 1987). The leaves extract of *Annona squamosa* was record inhibition on radial growth of *Sclerotium rolfsii* Sacc. causing stem rot of Groundnut by Darvin G. (2013). Sehhajpal, A & et al., (2009) was noticed that *Brassica compestris* showed least mycelial growth and inhibition of pathogen *Rhizoctonia solani*. Therefore it is necessary to search for control measures that are cheap, ecological sound the environmentally safe to eliminate or reduce the incidence of economic importance pathogens and to increases both seed germination and yield of plant crops.

Our goal was to test the application of *Annona squamosa* Linn, *Brassica compestris* Linn and *Ocimum sanctum* Linn medicinal plant leaves extracts, for protecting this plant crop form *S.rolfsii*. The present study was ascertained to investigate the inhibitory effect leaves extracts of *Annona squamosa* Linn, *Brassica compestris* Linn and *Ocimum sanctum* Linn various plants species on the *Sclerotium rolfsii* under in-vitro conditions.

Material and Methods:-

Isolation of *Sclerotium rolfsii*:-

Infected root rot disease of chilli plant caused by *Sclerotium rolfsii* was collected from the field of Aurangabad District. The cut the infected portion into small pieces of about 3-5mm thick and sterilized with 0.1% ($HgCl_2$) 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 in poured petriplates of the medium, mycelial disk 4mm diameter were cut from 4-5 day-old actively growing culture of the *Slcerotium rolfsii* and each was placed in the center of petriplates containing PDA. The effect of plant extracts on inhibition the *Sclerotium rolfsii* was studied using poisoning food technique, (Dhingra and Sinclair, 1985).

Plant species with families selected:-

The effect of plant leaves extracts on inhibition the *Sclerotium rolfsii* was studied. Plant species with species and families were selected for in-vitro evaluation used for this study was presented in the Table 1 below.

Table 1

Sr.No.	Botanical name of medicinal Plants	Families
1	*Annona squamosa* Linn.	Annonaceae
2	*Brassica compestris* Linn.	Brassicaceae / Cruciferaceae
3	*Ocimum sanctum* Linn.	Lamiaceae / Labiaceae

Preparations' of plant extract:-

Fresh leaves of *Annona squamosa* Linn, *Brassica compestris* Linn and *Ocimum sanctum* Linn. were used medicinal plants leaves extract perpetration. These leaves were collected from Phulambari road side's field in Aurangabad District during January, February 2015. These plants leaves, were rinsed in sterile distilled water in two to three

times and dried in shied 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 extract medium for different concentration:-
The plant leaves extract *Annona squamosa* Linn, *Brassica compestris* Linn and *Ocimum sanctum* Linn. of 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 extracts of different concentrations:-
The effect of plant leaves extracts of *Annona squamosa* Linn, *Brassica compestris* Linn and *Ocimum sanctum* Linn on the growth of *Sclerotium rolfsii* were studied by using poisoned food technique. 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 kept as control. All theses poured in to sterilized Petri plates. A mycelial disk of 4mm 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 Petri plates containing the potato dextrose agar medium having different three leaves extracts of *Annona squamosa* Linn, *Brassica compestris* Linn and *Ocimum sanctum* at different 10%, 25%, 50%, 75% concentrations and control under aseptic conditions. The Petri plates 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 *Sclerotium rolfsii* was calculated by using the formula given below.

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

Result and Discussion:-

Antifungal activity of *Annona squamosa* Linn, *Brassica compestris* Linn and *Ocimum sanctum* Linn medicinal plants leaves were assayed by food poisoning method. From table 1, 2 and 3, figure 1 and graph 1-6 revealed that the extract of above three medicinal plants showed reduction in growth of *Sclerotium rolfsii*. Among all three plants extract in aqueous medium *Ocimum sanctum* Linn exhibit maximum antifungal activity & maximum inhibitions percentage 20.8 % with 67.3mm growth at 75 percentage concentration, followed by *Annona squamosa* Linn which at 75 % concentration gave 69mm growth, with 18.8 percent of inhibition. Jalal and Ghaffar (1992) studied antifungal characteristics' of *Ocimum sanctum* Linn. and found that it is leaf extract completely inhibited the *Sclerotium rolfsii* and other fungi. The *Brassica campestris* Linn. aqueous leaves extract resulted maximum growth 77.6 mm and proved as least inhibitor that shows 10.23 % percent of inhibition at 10 percent concentration. The results of fungicidal effects of aqueous extract of all tested three plants showed moderate to minimum activity against *Sclerotium rolfsii* better than no activity of medicinal leaves as fungicides.

Discussion:-

The fungicidal efficacy of three plant extracts were tested against *Sclerotium rolfsii* the minimum growth and maximum inhibitory activity found in *Ocimum sanctum* Linn. Three plants extracts showed fungi toxic potentiality out of which *O.sanctum* belongs 11.76 % to 20 % were maximum against the test pathogen, it indicated it broad range of activity as compared to other two *Annona squamosa* Linn, *Brassica compestris* Linn. However, *Brassica compestris* Linn showed least exhibition of test pathogen. These antifungal effects of different concentration of *Brassica compestris* Linn plant leaves extracts on the pathogen *Rhizoctonia solani* was found in Anil Sehajpal, Arora and Parminder Kaur (2009). *O.santum* aqueous extract gave reduction in the work of Awauah (1989) against *Rhizopus* species. Okigbo and Ogbonnanya (2006) reported that *O. gratissum* ethanol extracts inhibited the mycelial growth and spore germinations of many rot causing microorganisms'.

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 extracts	*S.rolfsii* measured 24 hours	given in mm
			24 hr	48 hr	72 hr
1	*Brassica compestris* Linn.	10 %	21	51	78.66
		25 %	20.00	47.33	78
		50 %	19.33	47	76.33
		75 %	20	47.33	76.66
		Control	21	56	86
2	*Annona squamosa* Linn.	10 %	19.66	47.66	73.00
		25 %	21.33	47.33	75.66
		50 %	18.66	49.66	77.33
		75 %	18.66	42.66	70.33
		Control			
3	*Ocimum sanctum* Linn.	10 %	20	48	73
		25 %	18.66	46.83	74.33
		50 %	17	47	73.33
		75 %	17.33	44	68.33
		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 Control (100%) are aqueous medium leaves extract.

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

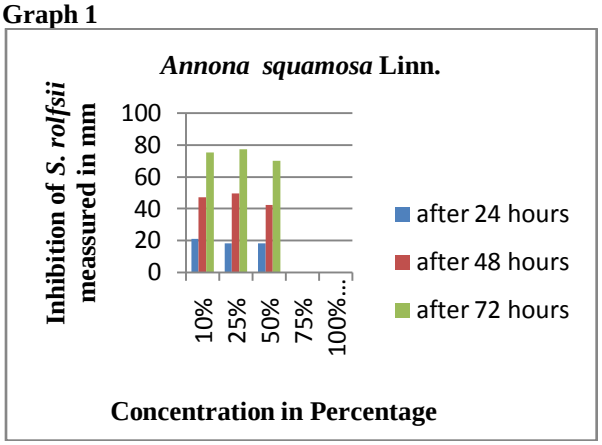
Serial No.	Medicinal plants	Concentrations (%)	Percentage given	of plant	Inhibition (I) extract	of in	*S.rolfsii* following	at concentration
			24 hr	% of I	48 hr	% of I	72 hr	% of I
1	*Brassica compestris* Linn.	10 %	21	0	51	8.928	78.66	8.534
		25 %	21	0	47.33	15.48	78	9.302
		50 %	19.33	6.380	47	16.07	76.33	11.24
		75 %	20	4.761	47.33	15.48	76.66	10.86
		Control	21		56		86	
2	*Annona squamosa* Linn.	10 %	19.66	10.63	47.66	13.34	73.00	8.750
		25 %	21.33	3.045	47.33	13.94	75.66	5.425
		50 %	18.66	15.18	49.66	9.709	77.33	3.337
		75 %	18.66	15.18	42.66	22.43	70.33	12.08
		Control						
3	*Ocimum sanctum* Linn.	10 %	20	20	48	12.72	73	3.947
		25 %	18.66	25.36	46.83	14.85	74.33	2.197
		50 %	17	32	47	14.54	73.33	3.513
		75 %	17.33	30.68	44	20	68.33	10.09
		Control						

* Values are average of triplicate.
* Values measured after deducting or reducing 4mm mycelium disk.

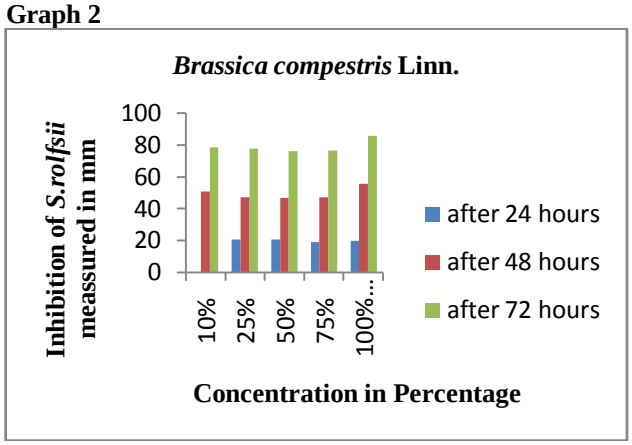
* (I) is denoted for inhibition.
* Note 1- 10%, 25%, 50%, 75% and Control (100%) are aqueous medium leaves extract.

Graph 1, 2:- Inhibition of *Sclerotium rolfsii* in *Annona squamosa* Linn and *Brassica compestris* Linn. leaves extract as following.

Graph 1

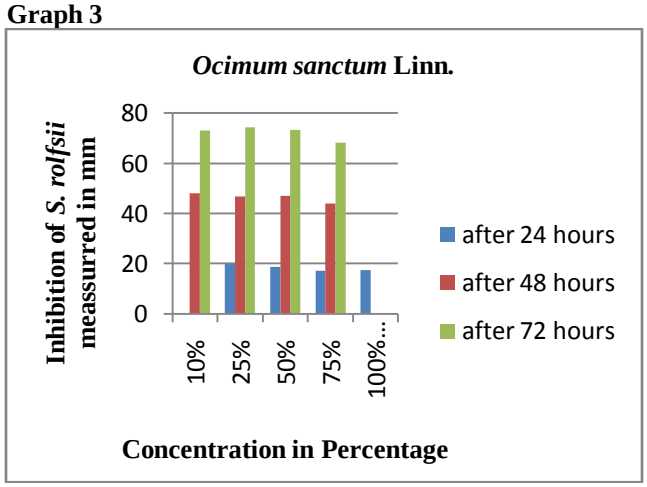


Graph 2



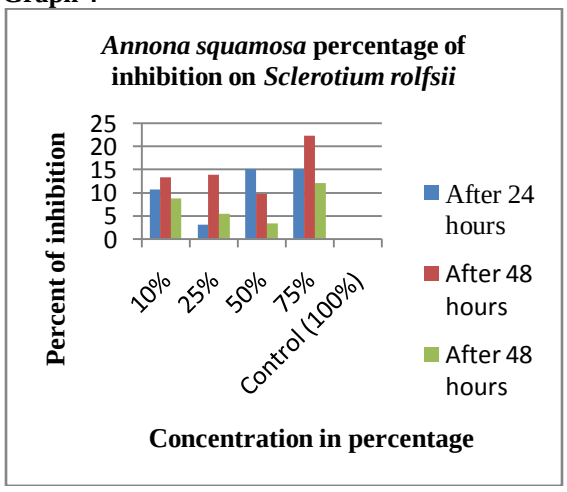
Graph 3:- Inhibition of Sclerotium rolfsii in *Ocimum sanctum* Linn leaves extract.

Graph 3

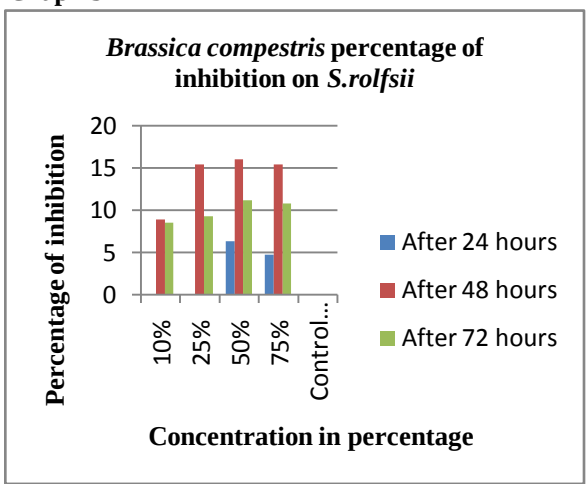


Graph 4, 5:- The percentage of inhibition of *Sclerotium rolfsii* in *Anonna squamosa Linn.*and *Brassica compestris.*

Graph 4



Graph 5



Graph 6:- The percentage of inhibition of *Sclerotium rolfsii* in *Ocimum sanctum.*

Graph 6

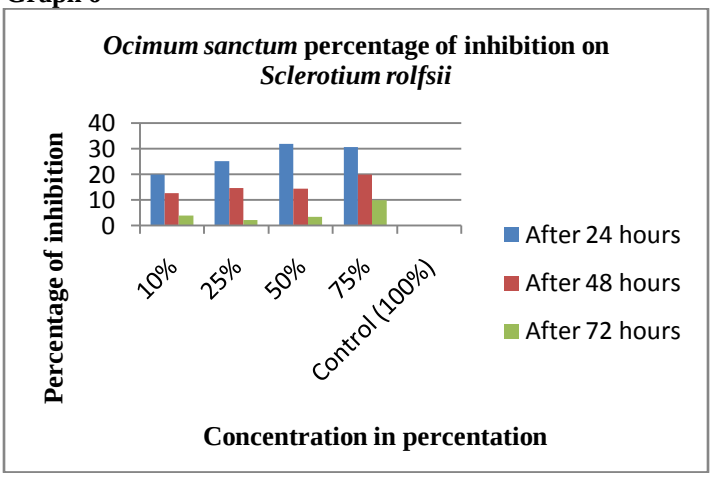
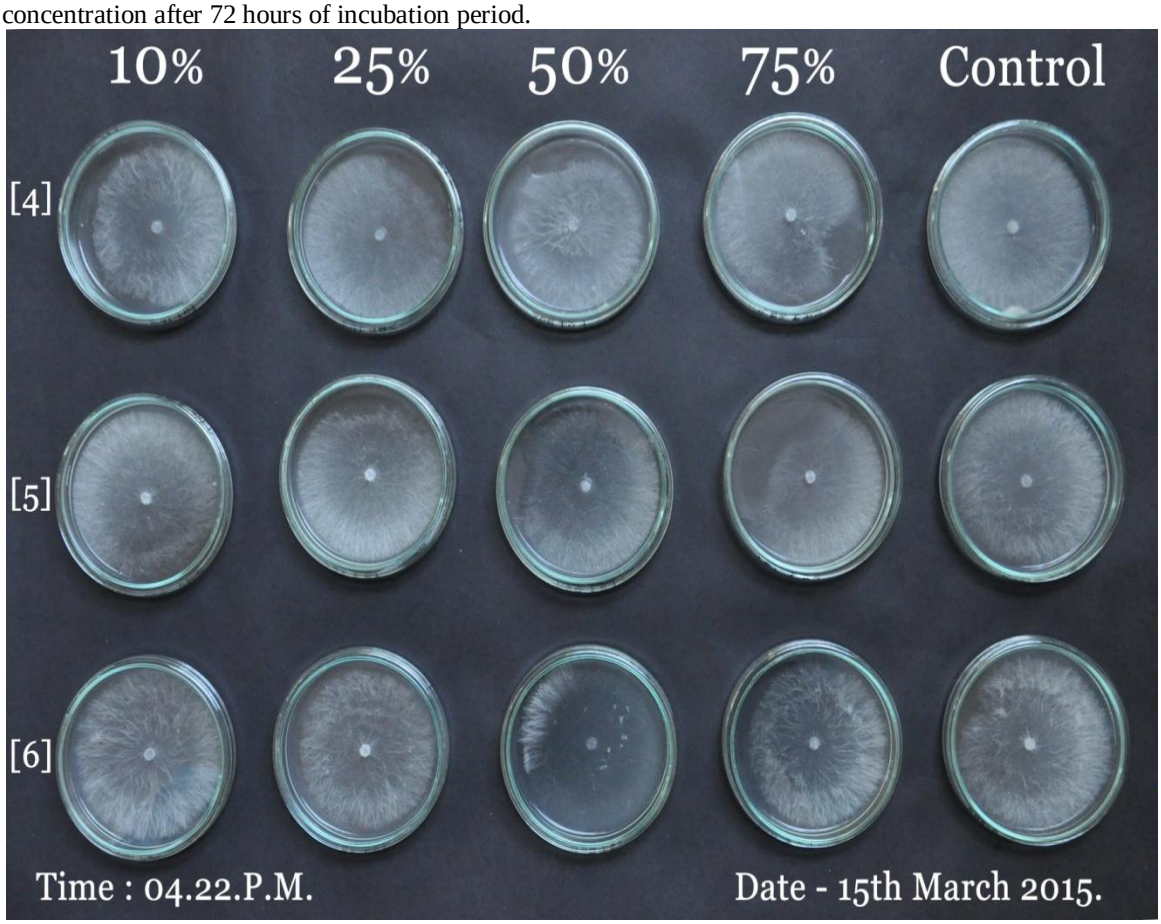


Figure 1: Plant extracts [4] *Annona squamosa*, [5] *Brassica compestris* and [6] *Ocimum sanctum* in following concentration after 72 hours of incubation period.



Conclusion:-

The management of fungal diseases in vegetables with chemicals, under field condition is cost prohibitive, hazardous and cause serious environmental pollution. So efforts are being made these days to shift from the conventional use of chemicals to the use of eco-friendly botanicals for the management. Organic amendments are

not safe to use but also have the capacity to improve soil structure and fertility. Thus, control strategies are now directed towards the use of natural products.

The conclusion of study is *Annona squamosa* Linn, *Brassica campestris* Linn. (Sarson) and *Ocimum sanctum* Linn aqueous extract inhibited the *Sclerotium rolfsii* under in vitro condition.

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