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Antifungal Activity of Plant Extracts against Post-harvest Fungal Pathogens

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A B S T R A C T

Extracts of certain plants have been used to control the plant diseases since several years. Here we report *in-vitro* studies to examine the antifungal activity of aqueous leaf extractsof three plants and neem oil against three post-harvest fungal pathogens viz., *Rhizopus arrhizus*, *Sclerotium rolfsii*, *Fusarium solani*. Among the leaf extracts, *Duranta erecta* showed maximum antifungal activity against the pathogens, followed by *Lasonia inermis*, Neem oil, and *Cocculus hirsutus*. The degree of inhibition increased correspondingly with increasing concentrations of the plant extracts.Percentage of inhibitions was high at 20% concentration than 10% concentration, except *S. rolfsii* treated with 10% neem oil. Highest growth inhibitions was recorded in *S. rolfsii* treated with *D. erecta*.

Introduction

Recently, the exploitation of natural plant products to control decay and prolong storage life of perishables has received more and more attention (Kamlesh Mathur *et al.*, 2007; Archana Singh *et al.*, 2008; Babu *et al.*, 2008; Jeeva Ram and Thakore, 2009; Chandra and Mahesh, 2013). Biologically active natural products have the potential to replace synthetic fungicides. Plant extracts and essential oils are source of antifungal activity against a wide range of fungi (Anuradha Bandopadyay, 2008; Combrinck *et al.*, 2011). A rapid assay to determine antifungal activity in both plant extracts and essential oils has recently been described by Wilson *et al.* (1997b; Ghasolia *et al.*, 2008; Devchand Salam *et al.*, 2009; Combrinck *et al.*, 2011). Use of oils in the control of plant diseases is of recent development. Some of the advantages of use of oils are low cost, excellent spreading and sticking properties to plant surface and no toxicity towards man and are co-friendly with the nature. The use of oils in the control of plant diseases have been reported by Mishra *et al.*, (1995), Jain and Pathak, (1998), Bernand *et al.* (1999), Vandanapandey *et al.* (2002), Sastry (2002), Rattanapitigorn *et al.*, 2006, Achour *et al.*, (2008) and Saban *et al.*, (2009; Combrinck *et al.*, 2011). The efficacy of plant products against post-harvest fungi was found to vary with concentrations used as reported (Madan

Singh and Jain, 2007; Jeeva Ram and Thakore, 2009; Rathod, 2010; Combrinck, S., *et al*., 2011; Pawar, (2011). Chandra and Mahesh, 2013). Some plants extracted in different organic solvents have shown inhibitory action against different storage fungi (Singh *et al.,* 1993; Mohamed *et al.,* 1994; Hiremath *et al.,* 1996; Kapoor, 1997; Radha*et al.,* 1999; Rana *et al.,* 1999; Jeyaseela *et al.,* 2012). However, active principles of some of plants have been isolated phytochemically and have shown strong inhibitory action against post-harvest fungi.In view of these, the attempt was made to screen some leaf extracts against post-harvest pathogenic fungi by food poisoning technique (Ansari, 1995).

Materials and Methods

Three locally available plant extracts viz., *Lawsonia inermis, Duranta erecta* and *Cocculus hirsutus and* neem oil were evaluated against the growth of three fungal pathogens *Rhizopus arrhizus, Sclerotium rolfsii* and *Fusarium solani* following the procedure given by Ansari (1995) with a slight modification. Fresh leaves, from plants were first washed with tap water and then with sterilized water. Each sample was then homogenized in sterile distilled water at the rate of 1 ml per gram of tissues (1:1 v/w) with a pestle and mortar and filtered through fine muslin cloth. The filtrate was centrifuged at 5000 rpm for 20 min and the supernatant was filtered with sterilized sintered funnel (pore size 1-2 microns), which formed the standard plant extract solution (100%).

The extracts were individually incorporated into PDA medium at 10% and 20% concentrations in 250 ml conical flasks and sterilized at 1.1 kg / cm^2 for 15 min. These were poured in 90 mm sterilized petridishes with three replications for each extract. Control was maintained without extracts. All the Petri dishes were inoculated with 7 mm disc of mycelium of the pathogen and incubated at 25º ± 2^0C. After five days the radial growth of mycelium was recorded. The percent growth inhibition over control was calculated out using the formula of Bliss (1934)

$$I = C\text{-}T/C \times 100$$

Where, I is inhibition percent, C is colony diameter in control (mm) and T is colony diameter in treatment (mm).

The data obtained from the present experiments was statistically analyzed using Analysis of Variance (ANOVA). Duncan's multiple range test (DMRT) at 5% level of significance was used to separate group means when ANOVA was significant.

Results and Discussion

The antifungal activity of three leaf extracts and neem oil against three post-harvest fungal pathogenic fungi is presented in Table 1a and Table 1-4 as inhibition percentage (I%). It was observed from table 1 that all leaf extracts and neem oil showed antifungal activity on post-harvest fungi. Among the plant extracts and neem oil evaluated, the maximum inhibition of mycelial growth was observed in extract of *D. erecta*, the next best were *Lawsonia inermis,* neem oil and *Cocculus hirsutus.* Plant extracts of *Lawsonia inermis, Cocculus hirsutus* and Neem oil had no effect on the growth of *R. arrhizus* at both concentrations, but they could inhibit sporangial formation. However*R. arrhizus* was significantly inhibited by *Duranta erecta* at 20% concentration (Plate -1). Percentage of inhibition increased correspondingly with increasing concentrations of the plant extracts. All plant extracts exhibits their antifungal activity significantly at 20% concentration than 10% concentration, except *S. rolfsii* treated with 10% neem oil (Table -4).

In the case of 10% concentration, *F. solani* could show both highest and lowest growth inhibition when treated with *Lawsonia* and *Cocculus*, respectively. At 20% concentration, maximum inhibition was noticed in *S. rolfsii* treated with *D. erecta* whereas lowest inhibition was found in *F. solani* treated with *Cocculus hirsutus.* Plant extract of *D. erecta* and *Lawsonia inermis* significantly inhibited the growth of *S. rolfsii* (94.89%) and *F. solani* (77%) at 20% concentration, respectively.

Table.1 Effect of plant extract of *Lawsonia inermis*, Duranta erecta, *Cocculus hirsutus* and neem oil on the growth of three post-harvest fungi at different concentrations

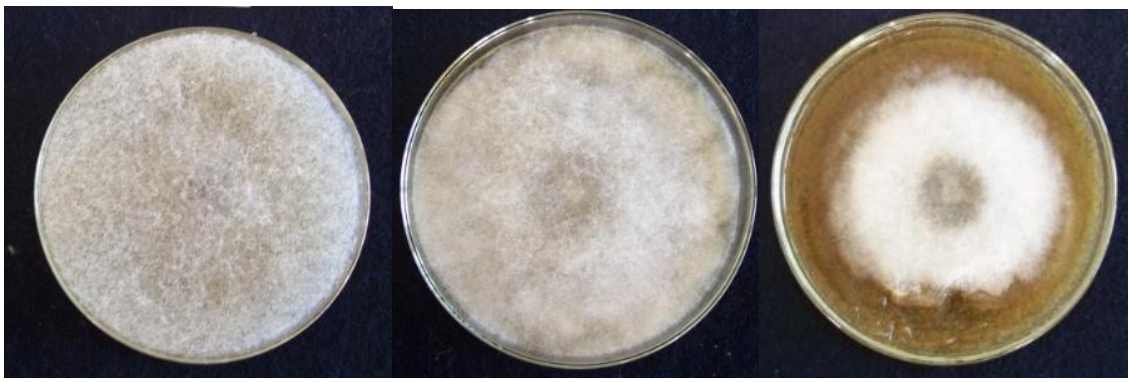
Plant extract	Fungi	Colony diameter (mm) in control	Percentage of inhibition	
			10% concentration	20% concentration
Duranta erecta	*Rhizopus arrhizus*	90.00 ± 0.00	28.14 ± 0.57$^{A, a}$	52.59 ± 2.28$^{A, b}$
	Sclerotium rolfsii	64.67 ± 0.51	45.29 ± 2.39$^{B, a}$	94.82 ± 0.82$^{B, b}$
	Fusarium solani	33.33 ± 0.51	31.61 ± 7.36$^{A, a}$	61.88 ± 2.17$^{A, b}$
Lawsonia inermis	*Rhizopus arrhizus*	90.0 ± 0.00	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}
	Sclerotium rolfsii	71.33 ± 2.05	41.05 ± 0.64$^{B, a}$	42.80 ± 1.75$^{B, a}$
	Fusarium solani	58.0 ± 0.77	54.02 ± 2.21$^{B, a}$	76.87 ± 3.47$^{C, b}$
Cocculus hirsutus	*Rhizopus arrhizus*	90.00 ± 0.00	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}
	Sclerotium rolfsii	71.33 ± 2.05	10.61 ± 1.17$^{A, a}$	10.98 ± 1.90$^{B, a}$
	Fusarium solani	34.00 ± 0.00	9.80 ± 1.51$^{A, a}$	17.65 ± 4.53$^{B, b}$
Neem oil	*Rhizopus arrhizus*	90.00 ± 0.00	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}
	Sclerotium rolfsii	52.67 ± 2.82	15.46 ± 4.31$^{B, a}$	14.18 ± 4.80$^{B, a}$
	Fusarium solani	41.33 ± 3.34	37.47 ± 4.05$^{C, a}$	57.02 ± 3.84$^{C, b}$

The same superscript uppercase letters (CAPITAL) between fungal species within treatment did not differ significantly at 5% level by DMRT; The same superscript lowercase letters (small letters) between treatments did not differ significantly at 5% level by DMRT.

Values are mean ± SEM

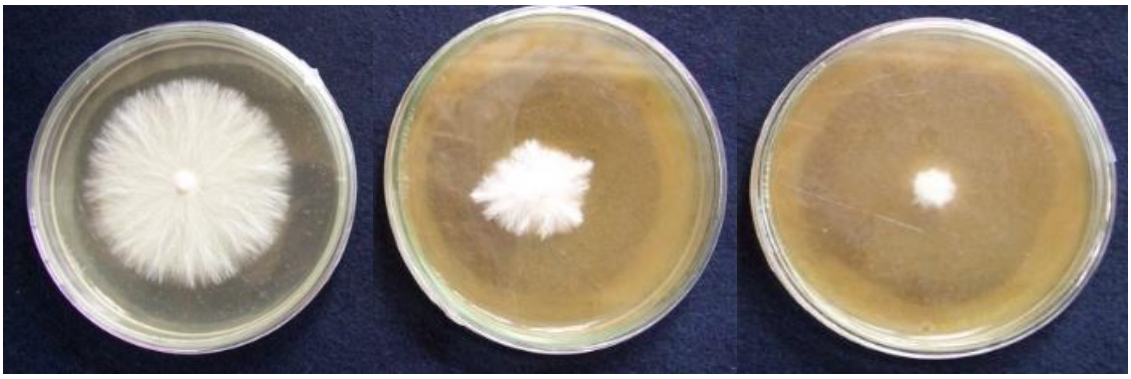
Plate.1 Effect of leaf extract of Durantaerecta on the radial growth (colony diameter) of five post- harvest fungi at 10% and 20% concentration

Rhizopus arrhizus



Control 10% 20%

Sclerotium rolfsii



Control 10% 20%

Fusarium solani.

Control 10% 20%

Plate.2 Effect of leaf extract of *Lawsonia inermis* on the radial growth (colony diameter) of five post -harvest fungi at 10% and 20% concentration

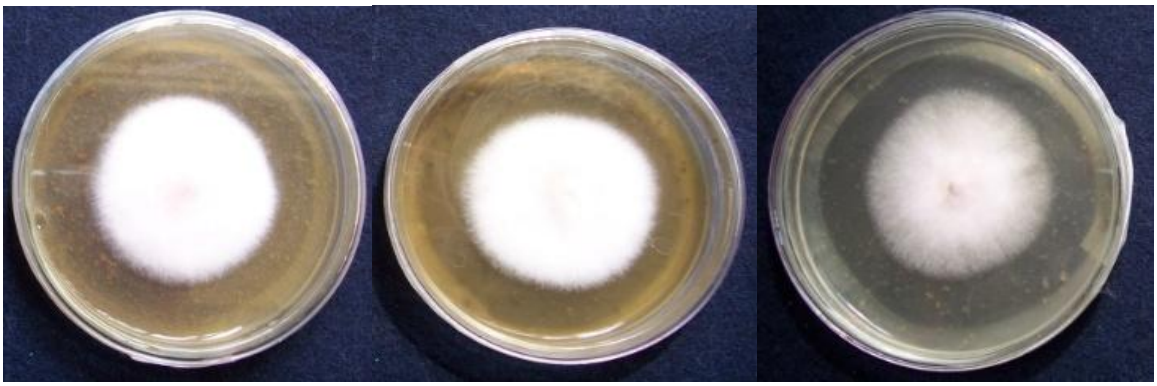
Rhizopus arrhizus

Control 10% 20%

Sclerotium rolfsii

Control 10% 20%

Fusarium solani



Control 10% 20%

Plate.3 Effect of leaf extract of *Cocculus hirsutus* on the radial growth (colony diameter) of five post-harvest fungi at 10% and 20% concentration

Rhizopus arrhizus

Control 10% 20%

Sclerotium rolfsii

Control 10% 20%

Fusarium solani

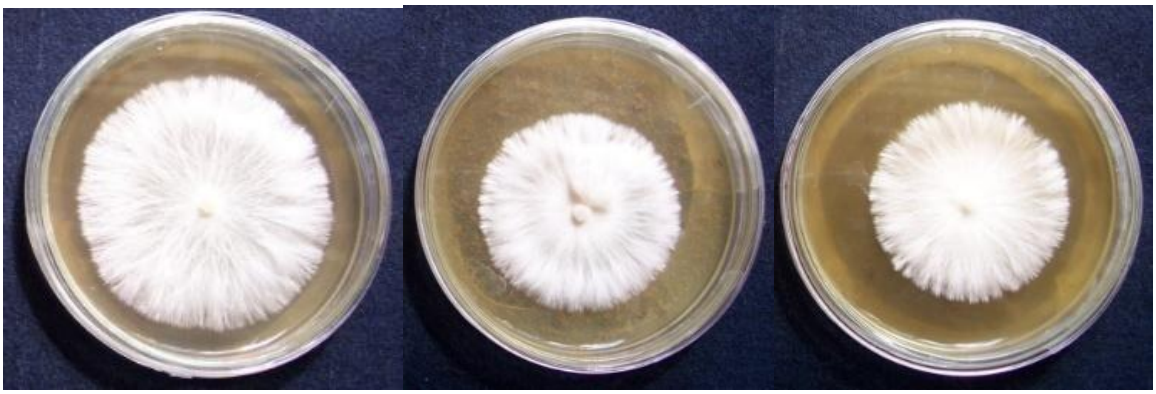
Control 10% 20%

Plate.4 Effect of neem oil (*Azadirachta indica*) on the radial growth (colony diameter) of five post harvest fungi at 10% and 20% concentration

Rhizopus arrhizus

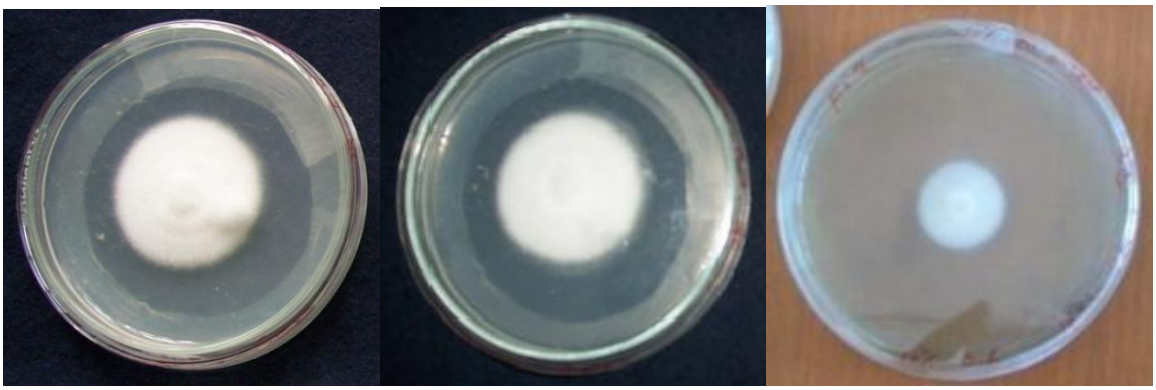
Control 10% 20%

Sclerotium rolfsii



Control 10% 20%

Fusarium solani



Control 10% 20%

Fig.1 Effect of plant extract *Lawsonia inermis* on growth inhibition (%) of fungal pathogens at 10% and 20% concentration

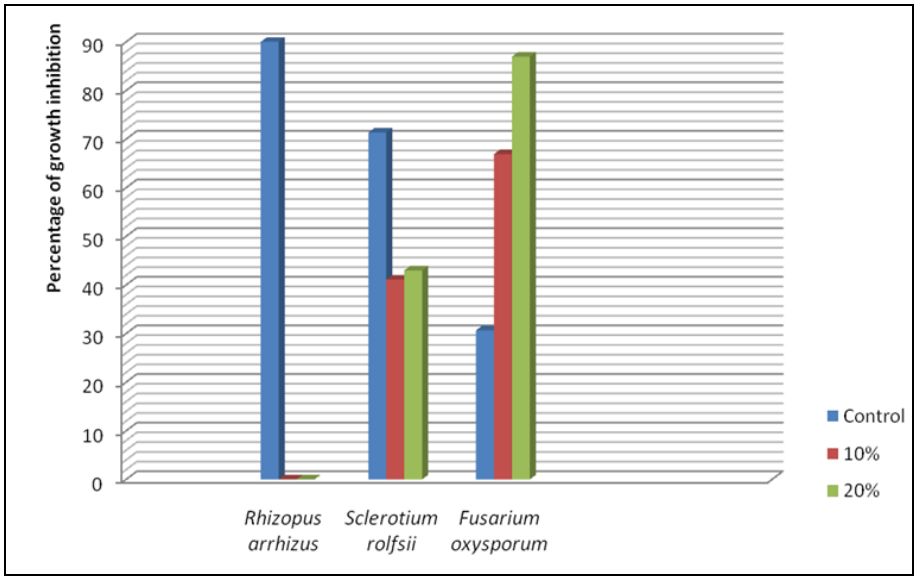


Fig.2 Effect of plant extract of Durantaerecta on growth inhibition (%) of fungal pathogens at 10% and 20% concentration

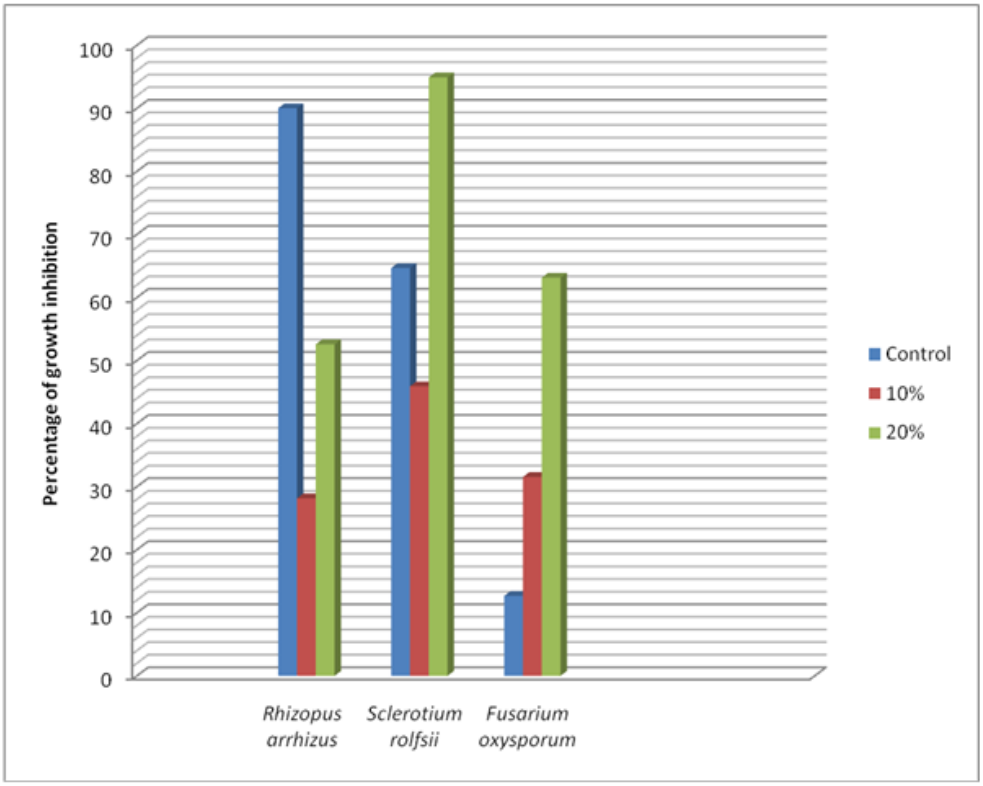


Fig.3 Effect of plant extract of *Cocculus hirsutus* on growth inhibition (%) of fungal pathogens at 10% and 20% concentration

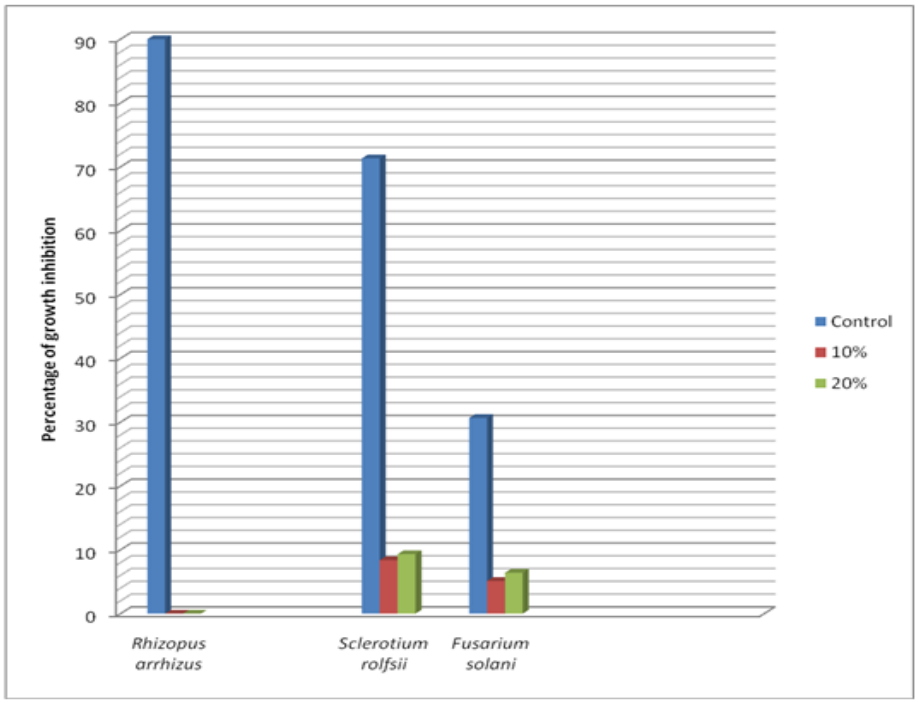
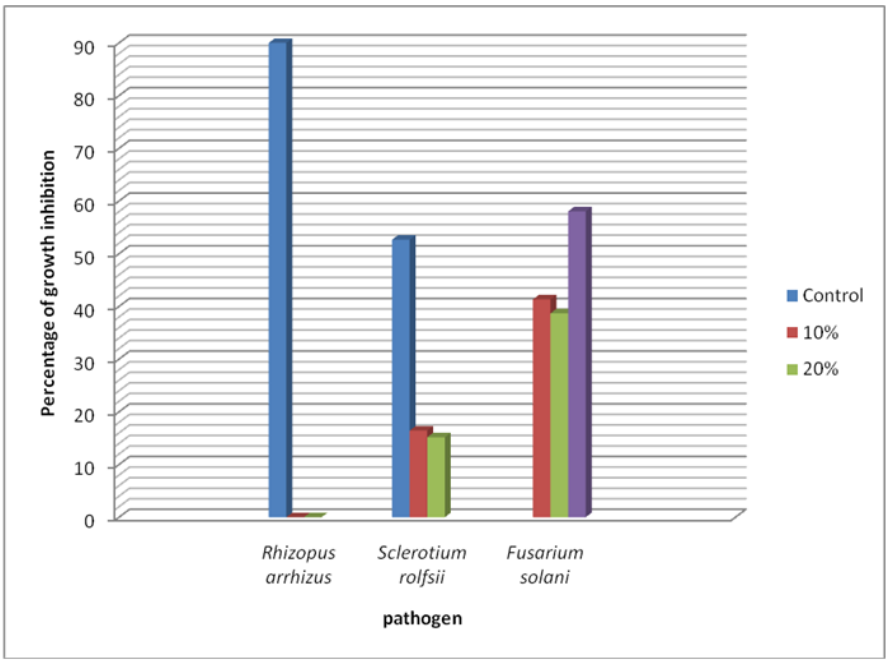


Fig.4 Effect of neem oil on growth inhibition (%) of pathogens at 10% and 20% concentration



In the present study three plant extracts and neem oil tested, which positively checked the growth of pathogens. The present findings emphasis that besides *Lawsonia* leaf extract, *Duranta erecta* was the most effective plant against the pathogens appears a new information. *In-vitro* studies of leaf extract of *L. inermis* showed promising antifungal activity with (77%) inhibition of *F. solani* and 42.97% of *S. rolfsii* was also recorded (Table-1a).The present results similar to that of Beebi Razeena and Rasheed Ahmad (2007). As reported by Purohit and Vyas (2004), principal colouring mater of henna is lawsoniel 12 hydroxy-1, 4 naphthaquinine, which is present in dried leaf at conc. of 1-4% bahemic, arachidic, stearic, palmitic, etc. Which may responsible for antifungal activity of *Lawsonia.*

The results of this *in-vitro* study are in the agreement with the findings of Sheo Raj Singh *et al.*, (2007), who observed Merigold and neem leaf extract reduced the hyphal growth and sclerotial production of *S. rolfsiiin-vitro*. The present results similar to that of Madan Singh and Jain (2007); Jeeva Ram and Thakore (2009), who observed plant extracts against *F. solani.*

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