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Original Research Article

In vitro Evaluation of Aqueous and Ethanol Extracts of Botanicals against *R. solani* causing Sheath Blight of Rice

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A B S T R A C T

Aqueous and ethanol extracts of seven botanicals (Aloe vera, Calotropis, Kanel, Neem, Datura, Bhang and Beal) were evaluated against *Rhizoctonia solani* causing sheath blight of rice; by using poison food technique at their concentrations of 20%, 30% and 40%. In case of aqueous extracts Datura has shown maximum growth inhibition per cent as 85.37%, 90.56% and 92.04% at 20%, 30% and 40% of concentrations respectively and it was followed by Bhang and Calotropis. Among ethanol extracts of botanicals at 20% of concentration Calatropis, Datura and Beal have exhibited 100% growth inhibition. Neem at 30% and Kanel at 40% have all so shown 100% of growth inhibition.

Keywords

Sheath bight of rice, Rhizoctonia solani, Botanical extracts and Poison food technique

Introduction

Rice (*Oryza sativa* L.) is the world's most important cereal crop and is a staple food crop for 60% of the world's population. Rice is a major source of calories for more than 70% population in India. Rice outcomes are affected by biotic and abiotic components which cause yield losses up to 20-30%. In the case of biotic constraints fungal diseases are highly predominant in rice-growing areas across the world. To increase the production per unit area and reduce losses, synthetic chemicals have proved their worth for the first time during 1960's and after, this miraculous performance has attracted major concentration towards it. As a result research on plant-derived natural products for the use in agriculture went into decline for a number of years. But this trend has reversed itself because of the adverse affects on human health and the environment, and the increasing incidence of resistance among pathogens towards synthetic chemicals is also a cause for serious concern. So, this has promoted man to produce natural pesticides. In the midst of all this in different parts of the world attention has been paid to exploiting higher-order plant product as novel chemotherapeutants in plant protection. Sheath blight of rice caused by *Rhizoctonia solani* Kuhn (Teleomorph: *Thanetophorus cucumeris* (Frank) Donk) is a potentially devastating fungal disease under suitable conditions (Dath, 1990). In India, this pathogen has become more prevalent in most

of the improved varieties, which are currently growing in India. Sheath blight being an important disease of the rice crop; has been extensively studied and tried to manage by various researchers but not much success has been gained in this direction. Management of sheath blight has become more problematic in organic rice production and lack of resistant germplasm has further aggravated the concern (Bonmann, *et al.,* 1992).

Researchers conducting many experiments on naturally available botanicals for finding their antimicrobial property against *R. solani* causing sheath blight of rice. Sharma *et al.,* (2017) evaluated aqueous extracts of commonly available ten plant species belonging to ten families *in vitro* for their inhibitory effect on the mycelia growth of *Rhizoctonia solani* and found that the lowest mycelia growth (25.33 mm) was recorded in clove extract of garlic (*Allium sativum* L.) which was found significantly superior among all the treatments. Results in terms of percent growth inhibition of *R. solani* revealed that clove extract of garlic produced maximum inhibition (71.85 %) followed by leaf extract of karanj (38.88 %), bulb extract of onion (37.03%), leaf extract of jatropha (30 %).

Patole and Nature (2011) have conducted experimentation with forty four botanical extracts against *R. solani,* concluded that *Allium sativum, Eucalyptus sp.* and *Zingiber officinale* were exhibited 100% growth inhibition of *R. solani.* Aslam *et al.,* (2010) have been tested five local medicinal plants extracts against *R. solani, A. Solani* and *M. Phaseolina;* stated that *Dodonaea viscose* recommended controlling all three pathogens. Sehajpal *et al.,* (2009) have experimented forty four botanical extracts against *R. solani* by using disc diffusion method, noted that among these, extract of *Allium sativum* has shown highest growth inhibition at least concentration of 100ppm. Upmanyu and Gupta (2002) have stated, out of sixteen botanical extracts *Allium cepa, Ocimum sanctum* and *Phyllanthus sp.* were showed 100% mycelial growth inhibition. Amadioha (2001) has reported that leaf extract of *P. guinensis* was best in control of *R. solani* followed by *C. citrates* and *O. sanctum.*

Considering the importance of botanicals in the organic rice production present investigation has been studied to find out the antifungal nature of aqueous and ethanol extracts of botanicals against *R. solani* under *in vitro* condition.

Materials and Methods

Seven locally available botanicals were collected and evaluated for antifungal property of their leaf extracts against *Rhizoctonia solani* by poison food technique (Table 1). This experiment was conducted under laboratory condition, in the department of plant pathology, Dr. Rajendra Prasad Central Agricultural University, Pusa (Samastipur), Bihar, India.

Preparation of aqueous and ethanol extracts of botanicals

Fresh plant leaves were collected separately from their respective plants and washed thoroughly for several times with tap water, then rinsed with sterile water for 2-3 times to remove the dirt particles and allowed to dry with the help of blotter paper to remove excess moisture.

Cleaned leaves were cut into small pieces under aseptic condition and 100 g of leaves from each plant have blended in a grinder along with 100 ml of sterile distilled water (for aqueous extract of botanicals) or 100 ml of 96% ethanol (for the ethanol extract of botanicals). Crude extract from blended stuff

was strained through muslin cloth then filtered through Whatman no.41 filter paper in to the sterilized conical flasks under aseptic condition. After getting filtrate in to conical flask, flask was sealed with cotton plug, labeled with respective name and date. That final filtrate was considered as 100% (standard stock solution) and was kept at 5°C in refrigerator for further use.

Evaluation method

Aqueous and ethanol extracts of botanicals were tested at 20%, 30% and 40% of concentrations. To get desired concentrations, 20 ml, 30 ml and 40 ml of standard stock solutions were poured in 80 ml, 70 ml and 60 ml of sterilized molten PDA respectively.

After that, they were poured into Petri plates under aseptic condition. Later 9 mm of pathogen (3 to 4 days old culture) containing PDA disc was transferred on to solidified treated media. Culture transferred media which was not treated with aqueous/ethanol extracts of botanicals was used as control.

Each treatment has been done with three replications. Inoculated plates were kept in BOD at 28 ± 1 ℃. The diameter of fungal growth was recorded after 72 hours of incubation. OPSTAT software was used for the statistical data analysis

By using following formula, per cent growth inhibition of *Rhizoctonia solani* was calculated.

$$\text{Inhibition percentage} = \frac{(C-T)\times 100}{C}$$

Where, C = Diameter of fungal growth (mm) in control plate.

T = Diameter of fungal growth (mm) in treated plate.

Results and Discussion

In vitro evaluation of aqueous extracts of botanicals against *R. solani*

Aqueous extracts of all botanicals (Aloe vera, Calotropis, Kanel, Neem, Datura, Bhang and Beal), which have been evaluated at 20%, 30% and 40% of concentrations by using poison food technique against *Rhizoctania solani;* have shown the considerable amount of growth inhibition after 72 hours of incubation. Readings on Colony growth (G in mm); Per cent growth inhibition (I) have been provided in Table 2 and visualized in Figure 1.

Data presented in Table 2 revealed that, significant maximum mycelial growth inhibition was found in Datura at 20% of concentration as 85.37% with 13.17 mm of mycelial growth where it was 90 mm in control. Aqueous extract of Datura was followed by Bhang (70.93%), Beal (62.04%), Calotropis (47.96%), Neem (38.52%), Aloe vera (35.19%) and Kanel (35%). At 30% of concentration maximum growth inhibition was shown by Datura (90.56%) with 8.50 mm of growth followed by Bhang (73.89%), Beal (64.63%), Calotropis (59.44%), Aloe vera (45.74%), Neem (43.89%) and Kanel (40.37%). At 40% of concentration again Datura has shown maximum growth inhibition per cent (92.04%) followed by Bhang (82.41%), Beal (68.70%), Calotropis (70.00%), Neem (53.52%), Aloe vera (50.74%), and kanel (50.74%).

Evaluation of ethanol extracts of botanicals against *R. solani*

Ethanol extract of seven botanicals (Aloe vera, Calotropis, Kanel, Neem, Datura, Bhang and Beal) have been shown maximum growth inhibition of *Rhizoctania solani* as compared to aqueous extracts at 20%, 30%

and 40% of concentrations. Readings on Colony growth (G in mm); Per cent growth inhibition (I) have been provided in Table 3 and visualized in Figure 2.

Ethanol extracts of all botanicals showed significant amount of growth inhibition. At 20% of concentration Calotropis, Datura and Beal showed 100% of *R. solani* growth inhibition followed by Neem, Kanel, Bhang and Aloe vera as 85.00%, 83.70%, 70.74% and 40.56% of growth inhibition respectively. At 30% of concentration Calotropis, Datura, Beal and Neem showed 100% of growth inhibition followed by Kanel, Bhang and Aloe vera as 87.96%, 86.11% and 53.52% respectively. At 40% of concentration all showed 100% of growth inhibition except Bhang and Aloe vera which were showed 88.52% and 72.52% respectively.

At 20%, among aqueous extracts, Aloe vera has given excellent production of sclerotia; Calotropis, Kanel and Neem have given moderate amount, and Bhang and Beal produced few whereas no sclerotia production was observed in Datura (Table 4). At 30% all botanicals have shown few amount of sclerotia production except Kanel and Datura where it is moderate and no production. At 40% no sclerotial production observed in Calotropis, Neem, Datura and Bhang. Few sclerotia produced in Aloe vere, Kanel and Beal. There is no sclerotia production was observed in Ethanol extracts of botanicals except in Aloe vera at 20%. Various studies on successful control of mycelial growth of *R. solani* employing botanical extracts are available. Results of the present investigation in accordance with that of the study conducted by Mohanty *et al.,* (2020), they have reported that aqueous and methanol extract of Datura was found 53.10% and 31.47% of growth inhibition respectively in *R. solani* at 10% concentration, but in present study it was 85.37% at 20% of aqueous extract of Datura. According to Kagale *et al.,* (2004) aqueous and methanol leaf extracts of Datura effectively reduced the growth of *R. solani* (RS7, Anastomosis group AG1) and methanol extract was more effective than aqueous extract. Aqueous extract of Calotropis at 20% of concentration produced 47.96% of growth inhibition and showed wide variation from the experimental results reported by Srinivas *et al.,* (2013), where it was found 84.75% of growth inhibition at 10% of concentration. This variation may be supposed due to difference in antimicrobial activity of locally available botanicals or due variation in pathogen isolates.

Atiq *et al.,* (2014) have been evaluated 5 botanical extracts against *R. solani* and found that Neem has shown 71% of growth inhibition followed by *Allium sativum, Eucalyptus sp.* and *Allium cepa.* Bokhari *et al.,* (2014) evaluated different forms of extracts of *Azadirachta indica* against *R. solani,* reported that leaf methanol extract has shown maximum inhibition of pathogen growth as 83%. Aye and Matsumoto (2011) have tested 16 botanical extracts against sheath blight pathogen; results revealed that Neem extract showed highest inhibition as 87.5% and it was followed by Rosemary extract. de Rodriguez *et al.,* (2007) have stated that ethanol extracts of Neem leaves and seeds were effective against *R. solani.*

From the experimental results it could be say that, aqueous extracts of seven botanicals were reported significant antagonism against *Rhizoctonia solani* as compared to the control. It might be because of the biologically active compounds present in botanical extracts, which act as antagonistic to a soil-borne fungal pathogen. Among the seven botanicals at least concentration (20%), aqueous and ethanol extracts of Datura have produced 85.37% and 100% of mycelial growth inhibition.

Table.1 List of the botanicals used for the preparation of extracts

S. No.	Common name	Scientific name	Family	Plant part used for extraction
1	Aloe vera	*Aloe barbadensis*	Asphodelaceae	Leaf
2	Bael	*Aegle marmelos*	Rutaceae	Leaf
3	Bhang	*Cannabis sp.*	Cannabaceace	Leaf
4	Calotropis	*Calotropis gigantea*	Apocynaceae	Leaf
5	Datura	*Datura stramonium*	Solanaceae	Leaf
6	Kanel	*Nerium oleander*	Apocynaceae	Leaf
7	Neem	*Azadirachta indica*	Meliaceae	Leaf

Table.2 Average colony growth (in diameter) and per cent growth inhibition of *R. solani* at different concentrations of aqueous extracts of botanicals after 72 hours of incubation 28 ± 1 ℃

Aqueous extracts	20%		30%		40%	
	G	I	G	I	G	I
Aloe vera (*Aloe barbadensis*)	58.33	35.19	48.83	45.74	44.33	50.74
Calotropis (*Calotropis gigantean*)	46.83	47.96	36.50	59.44	27.00	70.00
Kanel (*Nerium oleander*)	58.50	35.00	53.67	40.37	44.33	50.74
Neem (*Azadirachta indica*)	55.33	38.52	50.50	43.89	41.83	53.52
Datura (*Datura stramonium*)	13.17	85.37	08.50	90.56	07.17	92.04
Bhang (*Cannabis sp.*)	26.17	70.93	23.50	73.89	15.83	82.41
Beal (*Aegle marmelos*)	34.17	62.04	31.83	64.63	28.17	68.70
Control	90.00	0.00	90.00	0.00	90.00	0.00
Factors	**CD at 5%**			**SEm±**		
Botanicals(B)	3.308			1.160		
Concentrations(A)	2.026			0.710		
Interaction(A X B)	5.729			2.009		

G – Average colony diameter (mm); I – Average growth inhibition per cent

Table.3 Average colony growth (in diameter) and per cent growth inhibition of *R. solani* at different concentrations of ethanol extract of botanicals after 72 hours of incubation 28 ± 1 ℃

Ethanol extracts	20 %		30 %		40 %	
	G	I	G	G	I	G
Aloevera (*Aloe barbadensis*)	53.50	40.56	41.83	53.52	24.67	72.59
Calotropis (*Calotropis gigantean*)	0.00	100.00	0.00	100.00	0.00	100.00
Kanel (*Nerium oleander*)	14.67	83.70	10.83	87.96	0.00	100.00
Neem (*Azadirachta indica*)	13.50	85.00	0.00	100.00	0.00	100.00
Datura (*Datura stramonium*)	0.00	100.00	0.00	100.00	0.00	100.00
Bhang (*Cannabis sp.*)	26.33	70.74	12.50	86.11	10.33	88.52
Beal (*Aegle marmelos*)	0.00	100.00	0.00	100.00	0.00	100.00
Control	90.00	0.00	90.00	0.00	90.00	0.00
Factors	**CD at 5%**			**SEm±**		
Botanicals(B)	0.412			0.144		
Concentrations(A)	0.252			0.088		
Interaction(A X B)	0.713			0.250		

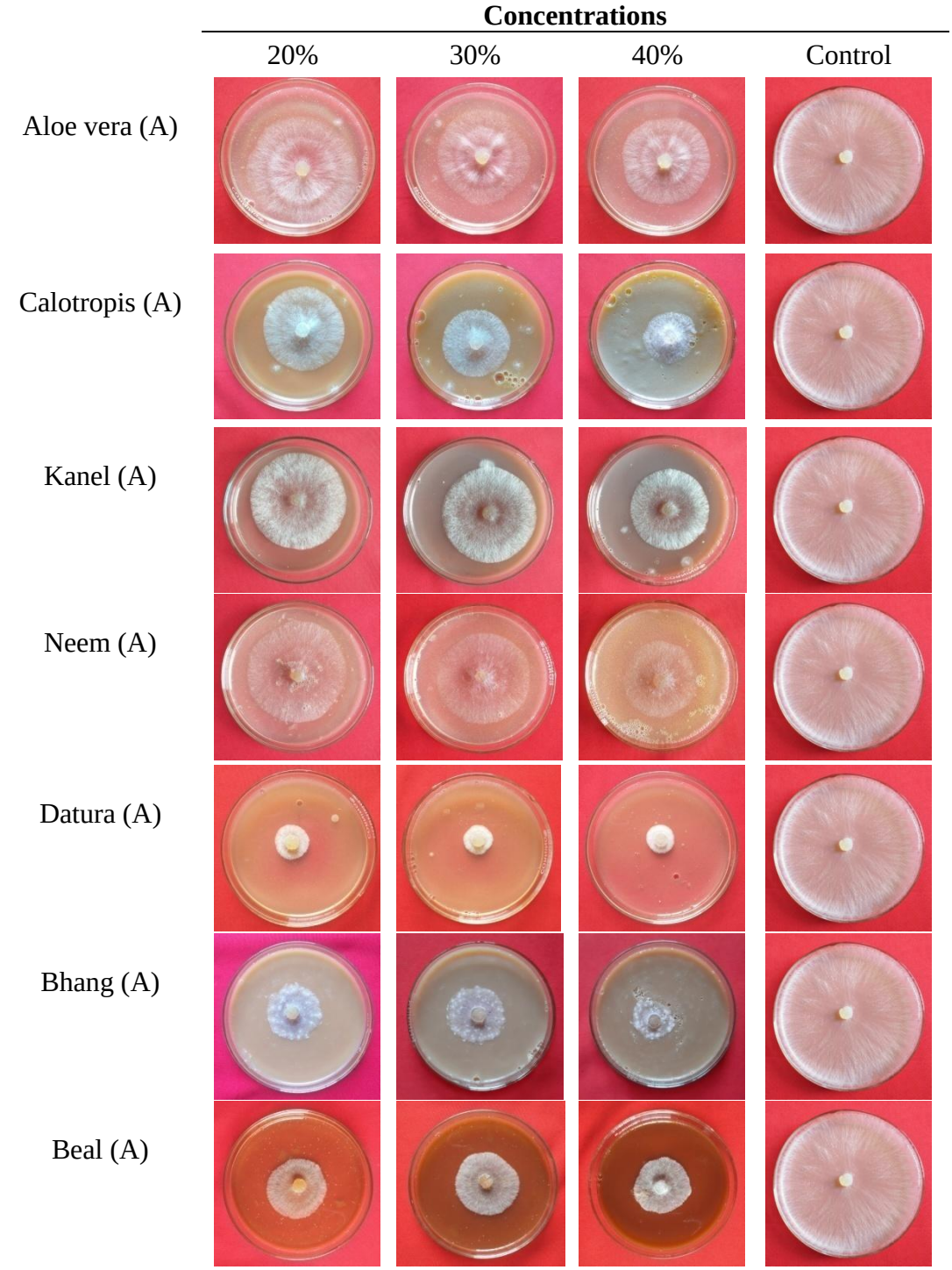
G – Average colony diameter (mm); I – Average growth inhibition per cent

Table.4 Effect of different concentrations of aqueous and ethanol extracts of botanicals on sclerotia production of *Rhizoctonia solani* after 120 hours of incubation at 28+ 1°C

S. No.	Botanicals	Concentrations					
		Aqueous Extracts			Ethanol Extracts		
		20%	30%	40%	20%	30%	40%
1	**Aloe vera**	+++	+	+	+	-	-
2	**Calotropis**	++	+	-	-	-	-
3	**Kanel**	++	++	+	-	-	-
4	**Neem**	++	+	-	-	-	-
5	**Datura**	-	-	-	-	-	-
6	**Bhang**	+	+	-	-	-	-
7	**Beal**	+	+	+	-	-	-
8	Control	+++	+++	+++	+++	+++	+++

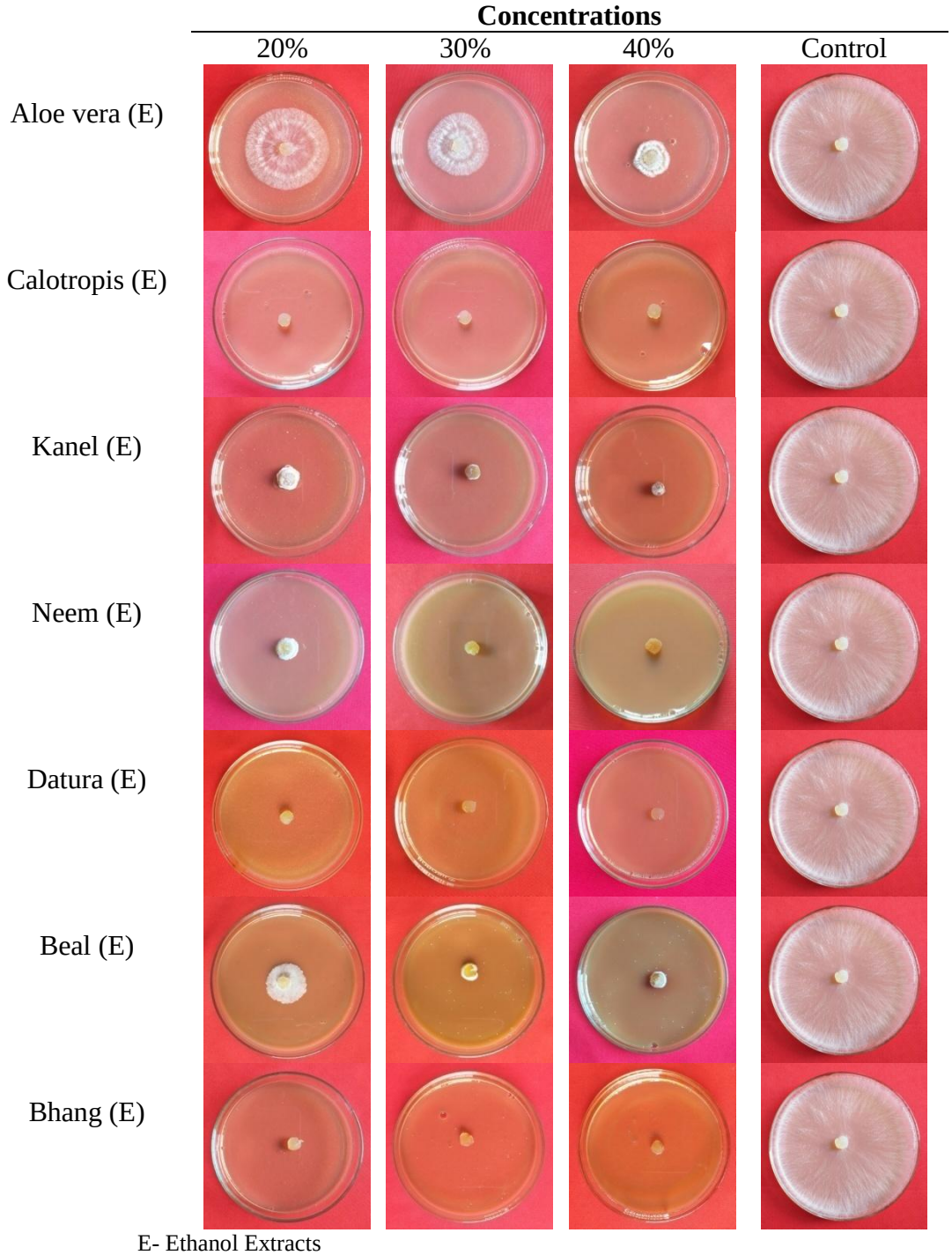
+++ Excellent production, ++ Good production, + Poor production, - No production

Fig.1 Effect of aqueous extracts of botanicals on mycelial growth of *R. solani* at different concentrations after 72 hours of incubation 28 ± 1 ℃



A- Aqueous Extracts

Fig.2 Mycelial growth of *R. solani* at different concentrations of ethanol extracts of botanicals after 72 hours of incubation 28 ± 1 ℃



E- Ethanol Extracts

In case of aqueous extracts after Datura, Bhang and Beal have given highest growth inhibition at 20% of concentration. With increasing concentration, rate of increasing per cent growth inhibition was more in Calotropis and Neem. At lowest concentration of ethanol extracts of botanicals along with Datura, Beal and

Calotropis have also shown 100% growth inhibition. Ethanol extract of botanicals were having more efficacy in inhibiting the mycelial growth of *R. solani.* So, it can be conclude that ethanol enhanced the growth inhibition property of botanical extracts as compared to the aqueous extracts.

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