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Amsaraj C
MSC Scholor, Department of plant Pathology, School of Agricultural, Uttaranchal University, Dehradun, Uttarakhand, India

JP Mishra
Principal & Professor, Department of Plant Pathology, School of Agricultural, Uttaranchal University, Dehradun, Uttarakhand, India

Rajendra Prasad
Professor, Department of plant Pathology, School of Agricultural, Uttaranchal University, Dehradun, Uttarakhand, India

Corresponding Author:
Amsaraj C
MSC Scholor, Department of plant Pathology, School of Agricultural, Uttaranchal University, Dehradun, Uttarakhand, India

Effect of different solvents on plant extracts for their antifungal activity against *Alternaria solani*, the causual agent of Early blight of Tomato under *In Vitro*

Amsaraj C, JP Mishra and Rajendra Prasad

Abstract
In present study the effective solvent for plant extracts to increase their antifungal activity were analyzed against *A. solani.* the plant samples of Neem, Aloe vera, Tulsi, veld grape and Turmeric extract was extracted by using three solvents of sterile distilled water, absolutre ethanol and absolute methanol. The prepared extracts were screened against *A.solani* by using "poision food technique", with concentration of 15%, 20% and 25% for water extract and 1.5%, 3% and 5% for Alcohol extract respectively. The methanol extracts showed their superiority in all cases, followed by ethanol and water extract, but in Tulsi water extract 25% showed maximum growth inhibition which is higher than the alcoholic extracts. Among all plant extracts methanolic extract of Turmeric rhizome extract showed maximum growth inhibiotion of 73.55% followed by Veld grape extract inhibited 69.31% of mycelia growth. In ethanol and water extract also Turmeric rhizome extract showed maximum growth inhibiton of 68.25% and 67.73% at their higher concentration of 5% and 25% respectively. All plant extracts have their fungicidal potential to be developed as potent fungicides in organic farming and Integrated Disease Management.

Keywords: Tomato early blight, *Alternaria solani*, Plant extracts, different solvents, Antifungal activity

Introduction
Tomato (*Lycopersicon esculentum. Mill.)* is one of the world's most lucrative and commonly grown vegetables and ranks first among the crops being processed. In India annual production of tomato is 20515MT/814 ha. (NHB2019). In Uttarakhand the annual production of tomato is 93.22 tonnes (NHB2016). Tomato is a small annual /short lived perennial herb of the solanaceae family, clearly native of peru-Equador. Tomato fruit is regarded as safe and nutritive food because of its high nutritive value, it is rich in Vitamin A, C, Ribiflavin and Thiamine. The yield and nutritive values of the tomato fruit is reduced by many diseases like Damping off, *Septoria* leaf spot, *Fusarium* wilt, Early blight and Late blight. Among these early blight caused by *Alternaria solani* is one of the important plant disease. Which causing serious economic losses to the farmers 30% (PKBasu1974). Ultimately, the pathogen growth may be exacerbated by an increase in inoculums from alternative host including weeds or other *Solanaceous* plants. The severity of the disease and its occurrence are highest when plants are mature. The most of the management practices are chemical treatments like fungicides and the cost of the fungicides and application are too high. Also need some skills and knowledge to farmers for handling and storage. Frequent application of synthetic chemicals are hazardous to soil, plant and environment. Some chemical residues may remain its hazardous to human and animal health. To reduce the hazardous effect of synthetic chemicals encouraging the use of botanicals in plant disease management is cost effective and eco-friendly management of plant disease. All the higher plants and their products showing their antifungal activity against several pathogens. For effective crop protection plant extracts can be use with other botanicals like essential oils and also with chemicals as cost effective and eco-friendly management in Integrated Disease Management.

Materials and methods
The present investigation was carried out in the department of plant pathology laboratory, Uttaranchal school of Agricultural, Uttaranchal University, Dehradun, India. (2019-2020).

Isolation
The diseased tomato leaf was identified by visible symptoms. The collected diseased part were excised about 1 cm along with some uninfected region using sterile scalpel and sterilized in a

Petri plate with 0.1% $HgCl_2$ for 2-3 minutes and washed 3-4 times with sterile distilled water, After blot dried on sterile filter paper and transferred into pre-sterilized PDA plates and gentle pressure was given them on to the agar. Incubated at 25±2°C for 3-5 days. After the growth of the pathogen sub cultured by transferring 5 mm mycelial disc by sterilized cork borer and incubated at 25±2 °C for 5-7 days to get pure culture.

Identification of pathogen

The fungus was identified by the morphological characters of greyish coloured mycelia in initial stage and blackish colour in matured stage was observed on culture media. Brown coloured, septate and branched conidophore were observed under Trinocular microscope. After maturation of culture brown coloured muriform shaped conidia with 1 to 6 transverse and 1 to 2 longitudinal septa and the beak at the end was observed under Trinocular microscope.

Collection of plant samples

The listed (table 1) plant sample were collected in Tamil Nadu and Dehradun at farmers field and identified in laboratory.

Table 1: list of the plants and part used for extract preparation

S.no	Plants	Scientific name	Plant parts
1	Neem	*Azadirecta indica*	Leaves
2	Tulsi	*Ocimum sanctum*	Leaves and flowers
3	Aloe	*Aloe vera*	Leaves
4	Veld grape	*Cissus quadrangularis*	Leaves
5	Turmeric	*Curcuma longa*	Rhizome

Preparation of plant extractions

Water extract

After collection of suitable parts of plants were washed under tap water thoroughly for removing dust particles. The washed plant parts were sterilized in 0.1% $HgCl_2$ for 2-3 minutes. After that, shade dried under room temperature for 15-20 days. After drying grinded as fine powder by pestle & mortar. Soaked the powders in sterilized distilled water and shaked vigorously and kepted without disturbance for 24 hrs. After stirring the soaked materials were filtered through double layered muslin cloth, followed by whatman no1 filter paper. The filtrates were stored in 4°C in pre-sterilized flasks/test tubes.

Alcohol extract

For ethanol and methanol extract, the powders were soaked in a solvents at 1:10 ratio and shaked vigorously and kepted without disturbance for 24 hrs. The contents were filtered through Whatman No-1 filter paper and evaporated the filtrates to dryness under water bath. The dried extracts were powdered and dissolved in distilled water.

For standardization of concentration of these effective form the water extracts were used at 15%, 20% and 25% concentrations, while the ethanol and methanol extracts were used at 1.5%, 3% and 5% concentrations, with four replications.

Screening of plant extracts for their antifungal activity against *Alternaria solani In vitro.*

In this method the extracts obtained by different solvents were analyzed against the test fungi. The plant extracts were supplemented to sterilized PDA based on the concentrations and mixed thoroughly. The poisoned media were poured into the sterilized 70 mm petri plates under aseptic condition. The mycelial disc of 5 mm diameter of 7-10 days old culture was cutted by corkborer and transferred into the poisoned medium aseptically. Each concentrations were replicated four times. The petri plates poured with the empty media were considered as control. The petri plates were incubated into the BOD incubator at 25°C until the control plate has full growth. Observations were recorded an alternate days, up to 9 DAI. Percent inhibition of the mycelial growth was calculated by the formulae as follows (Mc Kinney, 1923).

$$I = \left[\frac{C - T}{C}\right] * 100$$

Where

I = Percentage Inhibition

C = Colony diameter in control (mm)

T = Colony diameter in treatment (mm)

Results and Discussion

Screening of plant extracts against *Alternaria solani* for their antifungal activity under *In Vitro* condition.

Water extracts

The maximum growth inhibition was observed in Turmeric rhizome extract with growth inhibition of 67.73% followed by Veld grape extract 61.38% at the concentration of 5%, it was par with Tulsi extract 59.26%. However, Turmeric extratct and Tulsi extract at 3% concentration showed 58.21% and 53.97% growth inhibition, it was par with the 5% concentration of Aloe vera and Neem extract with the growth inhibition of 54.5%. likewise, Aloe vera, Neem and Veld grape extracts shows the growth inhibition of 49.73%, 47.98% and 46.56% at the concentration of 3%, it was par with each other. However, Neem and Aloe vera and Tulsi extratct shows similar growth inhibition of 43.38% at 1.5% concentration. It was par with Turmeric extract at 1.5% concentration with growth inhibition of 42.33%. The least growth inhibition was observed by 1.5% concentration of Veld grape extract with the growth inhibiton of 33.86%. Among all the water extracts Aloe vera extract shows the least growth inhibition of 51.86% at higher concentrations.

Ethanol extracts

Turmeric rhizome extract showed the maximum growth inhibition of 68.25% at 5% concentration and 60.28% at 3% concentration among all the ethanolic extracts. Followed by Neem extract at 5% concentration showed the growth inhibition of 57.67% and 56.61% growth inhibition was shown by Veld grape extract at 5% concentration, it was par with 5% of Aloe vera extract with the growth inhibition of 55.56%. However, 5% of Tulsi and 3% of Neem extracts shows growth inhibition of 52.91% and 50.79%. followed by Veld grape and aloe vera extracts shows the growth inhibition of 49.74% and 48.15% at the concentration of 3%, it was par with 1.5% of Turmeric extract with the growth inhibition of 47.09% and 1.5% of Neem extract with the growth inhibition of 46.56%. However, 3% concentration of Tulsi extratct and 1.5% of Aloe vera extract showed almost similar growth inhibition of 41.8% and 41.27%. The least growth inhibition was observed in 1.5% of Tulsi extract with the growth inhibition of 35.45%, followed by 1.5% concentration of Veld grape extract with the growth inhibition of 38.62%. Among all extracts Tulsi extracts shows the least growth inhibition in higher concentration of 5% with the percentage inhibition 52.91%.

Methanol extract

The Turmeric rhizome extract at 5% concentration proved their superiority among all the plant extracts with the growth inhibition of 73.55%, followed by Veld grape extract shows the gowth inhibition of 69.31% at 5% concentration. However, 3% of Turmeric and Veld grape extract shows the growth inhibition of 65.08% and 64.0%, it was superior than the higher concentration (5%) of other plant extracts, it was par with 5% of Neem extract with the growth inhibition 61.9%. However, 5% of Aloe vera extract shows the similar growth inhibition of 58.2% and 1.5% of Turmeric extract shows the growth inhibition of 56.08%. likewise, 3% of Aloe vera and Neem extract shows the similar growth inhibition of 54.5%, it was par with 1.5% of Aloe vera extract with the growth inhibition of 48.68%. Similarly, Tulsi extract at 5% concentration and 1.5% of Neem extract shows the similar growth inhibition of 46.56%. The least growth inhibition was observed in 1.5% of Tulsi extract with the growth inhibition of 33.33% and followed by 40.21% growth inhibition was observed in 3% of Tulsi extract. Among all the methanol extract the least growth inhibition was observed by Tulsi extract with the growth inhibition of 46.56% at the higher concentration of 5%.

Discussion

Gurjar M S *et al*. (2012) [3]. Extracted different plant species including Neem, Aloe vera and Turmeric by using different solvents including Water, Ethanol and Methanol against list of pathogens including *A.solani*. Similarly Rabia N and Asghani B(2012) analysed the antimicrobial potential of *Ricinus communis* leaf by water, ethanol and methanol extracts against bacterial and fungal pathogens and proved that methanol extracts was inhibited both fungal and bacterial pathogens than ethanol and water extract. Similarly, Paola D D.(2011) [6] assayed three solvents(water, buffer and acid extracts) on different species of plant tissues like S*alvia spp* and *Aloe vera* against *A. solani* and isolated the potential source of antimicrobial compounds and he proved that acid extracts showing more potential on S*alvia spp extract.*

Rex B *et al*, (2019) [8] tested 10 plant extracts by crude extract method against *A. solani* among these Turmeric rhizome extract shows the more growth inhibition of 89.44% at higher concentration. Nivedha M *et al*,.(2019) [5] Analysed the 25 plant extracts by crude extract method against *A. Solani* at 10% concentration among those plant extracts Tulsi, Veld grape and Neem were used in present study and among these Neem (59.88%) extract shows highest growth inhibition then Tulsi(57%) and Veld grape(41.1%).

Ahiladevi P *et al*,.(2013).Analysed antifungal activity of 20 plant extracts by crude extract method. In present study Neem, aloe vera and Veld grape extracts were used. Among these Neem and aloe vera shows more inhibition of 74.19% and 74.06%, Veld grape extract shows the poor inhibition activity of 7.71% at 10% concentration. In present study, In case of powdered water extract Veld grape extract showing more inhibition (61.3%) than other plant extracts at higher concentration of 25%.

Muthomi J W *et al.*, (2017). Tested ethanol extract of 10 plant species against *A. solani* including Turmeric, Neem and Aloe vera. Among these Turmeric extract shows more growth inhibition of 72.9%. Similarly, Aqsa A *et al.* (2010) [1] tested ethanol extracts of 5 plant species, among these *Dodonaea viscose* shows more growth inhibition of 56.96% and *Azadirecta indica* shows 42.9% of growth inhibition at the concentration of 200g/l. Suleiman M N (2010). Analysed the Methanol extract of Neem and pawpaw against *A solani*. And he proved that crude extract of Paw paw shows more growth inhibition than the Neem extract at different concentrations.

Table 2: Antifungal activity of solvent extracts of different plants on mycelial growth of *A.solani.* on PDA. (Mycelial growth (mm*).

Concentration of plant extracts (%)	**Mycelial growth (mm*) of *A. solani* on PDA containing water extract of plants**				
	Neem	**Aloe vera**	**Tulsi**	**Veld grape**	**Turmeric**
15	35.67	35.67	35.67	41.67	36.33
20	32.67	31.67	29.00	33.67	26.33
25	28.67	28.67	25.67	24.33	20.33
Control	63.00	63.00	63.00	63.00	63.00
SE(m) ±	0.58	0.67	0.793	0.577	1.15
CD at 5%	2.04	2.35	2.80	2.037	4.07
Concentration of plant extracts (%)	**Mycelial growth (mm*) of *A. solani* on PDA containing ethanol extract of plants**				
	Neem	**Aloe vera**	**Tulsi**	**Veld grape**	**Turmeric**
1.5	33.67	37.00	40.67	38.67	33.33
3	31.00	32.67	36.67	31.67	25.00
5	26.67	28.33	29.67	27.33	20.00
Control	63.00	63.00	63.00	63.00	63.00
SE(m) ±	1.36	0.43	0.88	1.86	0.694
CD at 5%	4.80	1.52	3.11	6.55	2.45
Concentration of plant extracts (%)	**Mycelial growth (mm*) of *A. solani* on PDA containing methanol extract of plants**				
	Neem	**Aloe vera**	**Tulsi**	**Veld grape**	**Turmeric**
1.5	33.67	32.33	42.00	26.33	27.67
3	28.67	28.67	37.67	22.67	22.00
5	24.00	26.33	33.67	19.33	16.67
Control	63.00	63.00	63.00	63.00	63.00
SE(m) ±	0.98	0.58	0.544	0.58	0.43
CD at 5%	3.46	2.04	1.92	2.08	1.52

* mean of all four replications

Table 3: Antifungal activity of solvent extracts of different plants on mycelial growth of *A.solani.* on PDA. (Percent growth Inhibition (%).

Concentration of plant extracts (%)	Percentage Inhibition (%) of mycelial growth on PDA containing water extract				
	Neem	**Aloe vera**	**Tulsi**	**Veld grape**	**Turmeric**
15	43.39	43.39	43.39	33.86	42.33
20	47.98	49.73	53.97	46.56	58.2
25	54.5	54.5	59.26	61.37	67.73
Control	0	0	0	0	0
SE(m) ±	0.94	1.06	1.26	0.92	1.83
CD at 5%	3.31	3.73	4.44	3.24	6.47
Concentration of plant extracts (%)	**Percentage Inhibition (%) of mycelial growth on PDA containing Ethanol extract**				
	Neem	**Aloe vera**	**Tulsi**	**Veld grape**	**Turmeric**
1.5	46.56	41.27	35.45	38.62	47.09
3	52.80	48.15	41.8	49.74	60.32
5	57.67	55.56	52.91	56.61	68.25
Control	0	0	0	0	0
SE(m) ±	2.16	0.80	1.40	2.95	1.1
CD at 5%	7.62	2.85	4.94	10.4	3.89
Concentration of plant extracts (%)	**Percentage Inhibition (%) of mycelial growth on PDA containing Methanol extract**				
	Neem	**Aloe vera**	**Tulsi**	**Veld grape**	**Turmeric**
1.5	46.56	48.67	33.33	58.2	56.08
3	54.5	54.5	40.21	64.02	65.08
5	61.9	58.2	46.56	69.31	73.55
Control	0	0	0	0	0
SE(m)±	1.56	0.92	0.86	0.92	0.68
CD at 5%	5.5	3.23	3.04	3.23	2.41

SE(m)± - Standard Error mean, CD – Critical Difference.

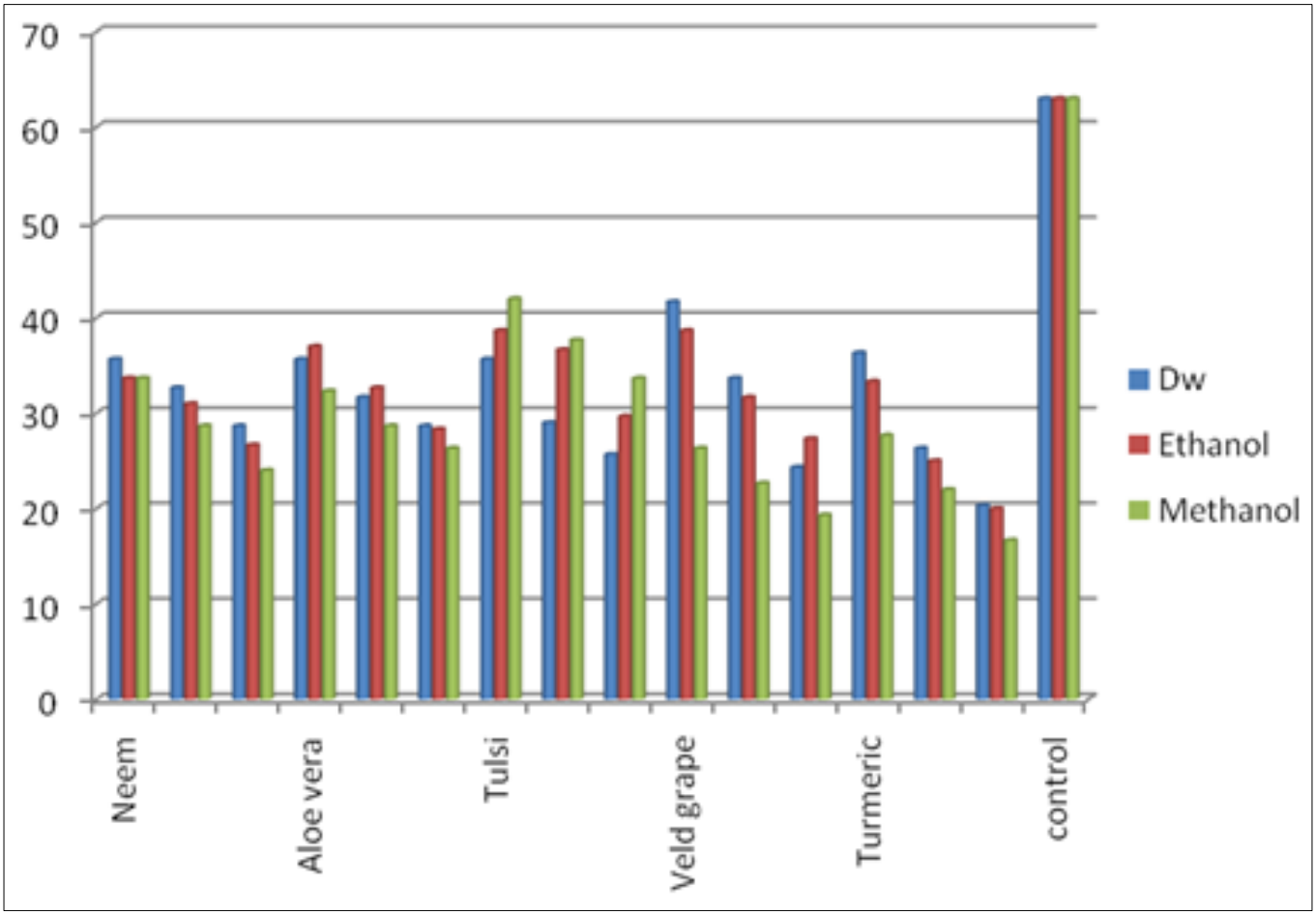
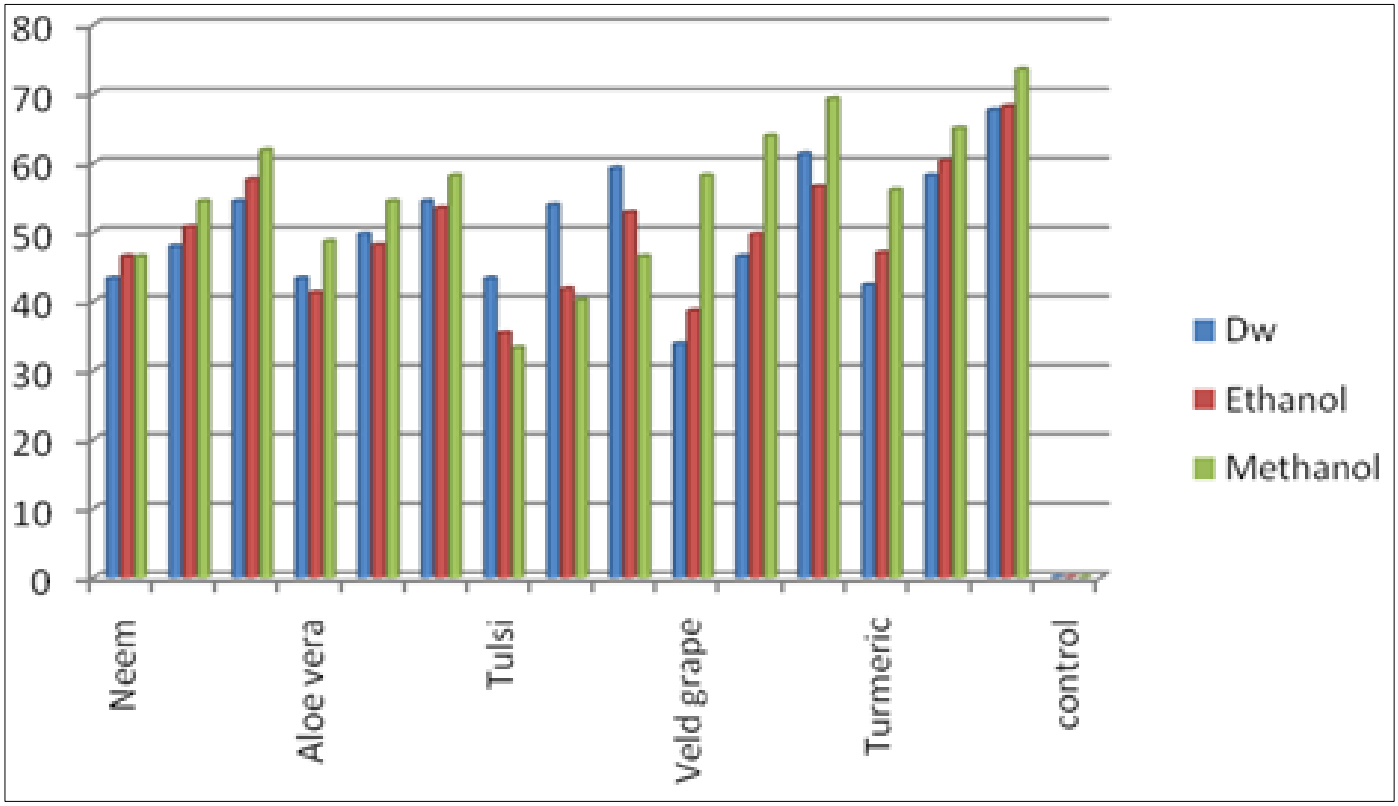
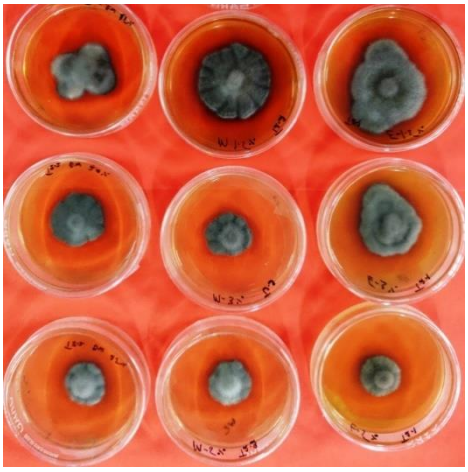


Fig 1: Antifungal activity of solvent extracts of different plants on mycelial growth of *A.solani.* on PDA. (Colony diameter (mm)*).

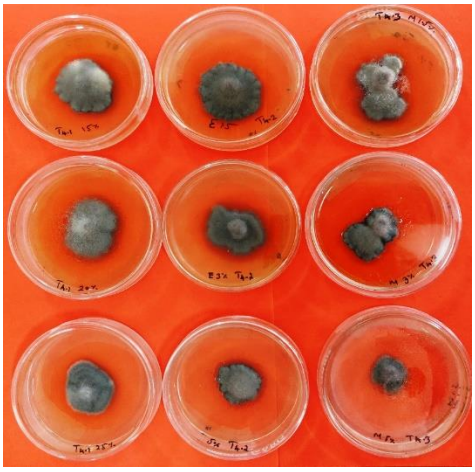


(Each Extracts screened with three concentration; DW-15%, 20% and 25%, Alcoholic Extract-1.5%, 3% and 5%). DW- Distilled water

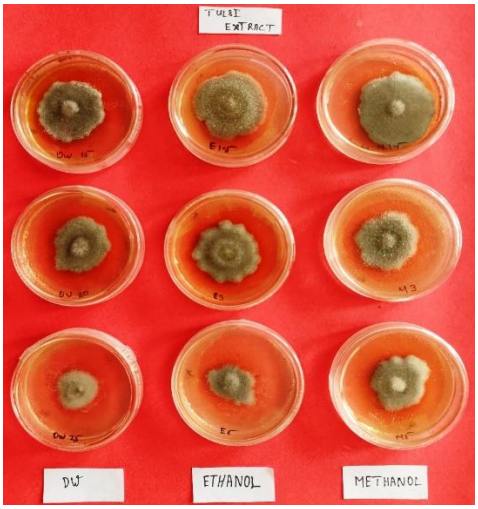
Fig 3: Antifungal activity of solvent extracts of different plants on mycelial growth of *A.solani.* on PDA



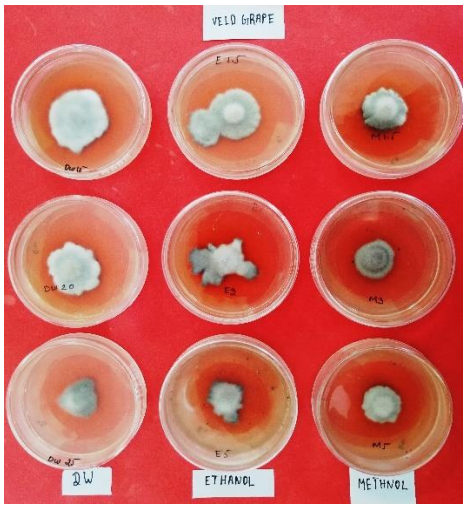
Neem extract



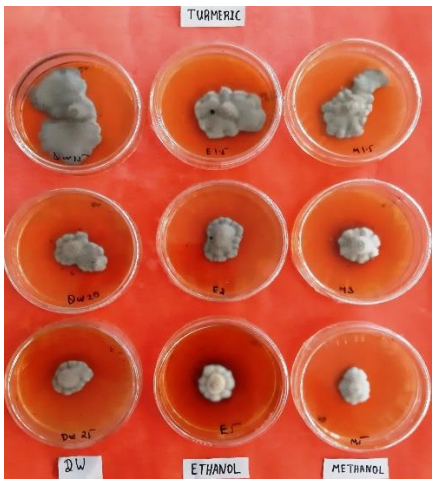
Aloe evra extract



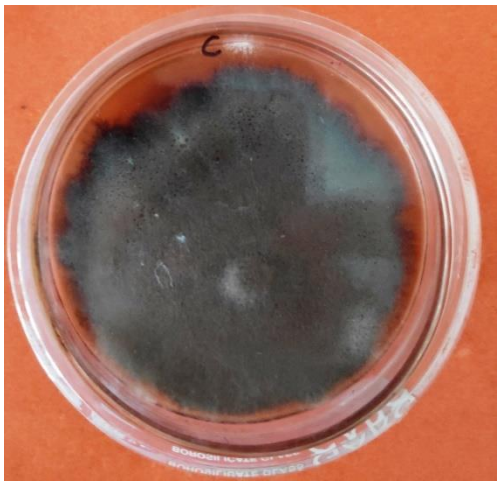
Tulsi extract



Veld grape extract



Turmeric rhizome extract



Control plate

Conclusion

The plant extracts by three solvents (Water, Ethanol and Methanol) were screened against *A. solani*. Under *In vitro* condition. Among these extracts methanol extracts shows more growth inhibition, followed by ethanol and water extracts. Turmeric rhizome extract showed the maximum growth inhibition in all three solvents and also proved that all plants have a fungicidal potent to manage diseases.

Recommendation

In case of field application the plant extracts can be dissolve in water and spray as foliar application. The water extracts can easily prepare by farmers and to increase their efficacy against plant pathogens desired concentration of alcoholic extracts can be mixed. So that the cost of disease management by other chemicals can be reduced.

The botanicals are mostly compatible with each other and also with other chemicals. In integrated disease management the use of all botanicals with chemicals may give effective result in field condition. So we recommending that combination of all botanicals effective in organic farming and Integrated Disease Managemnent.

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