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Eco-friendly management of tomato early blight and wilt by using aqueous extract of *Aegle marmelos*

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Abstract
Tomato is the most important vegetable crop in India. Two common and davasting diseases of tomato are early blight and Fusarium wilt caused by *Alternaria solani* and *Fusarium oxysporum* f. sp. *lycopersici* respectively. The effect of *Aegle marmelos* leaf extract has been studied against *Alternaria solani* and *Fusarium oxysporum*, the causal organism of early blight and wilt of Tomato plant (*Solanum esculentum* Mill) respectively. Aqueous leaf extracts of 2.5, 5.0, 7.5 and 10.0% concentration were suppressed mycelial growth of both pathogenic fungi and the degree of suppression gradually increased with increase in concentration. A concentration of 10.0% aqueous leaf extract were found to be most effective. *Alternaria solani* and *Fusarium oxysporum* significantly decreased from 6.1 cm and 8.0 cm in control to 0.4 cm and 0.8 cm at 10.0% concentration.

Keywords: Leaf extracts, seed borne mycoflora, seedling health

1. Introduction
Tomato is the most important vegetable crop in India. Tomato (*Lycopersicon esculentum*) a member of the family Solanaceae have high nutritive and medicinal values and also have diversified values (Bose *et al*., 1986). It is widely grown in almost all countries of the world due to its adaptability to a wide range of soils and climate (Ahmed, 1976) [1]. The yield of the crop is very low due to more susceptibility toward the diseases of tomato. Over 200 diseases have been reported to affect the tomato plants in the world (Watterson, 1986) [34]. Among them the seedborne pathogens plays a vital rule in disease development (Fakir and Khan, 1992) [10]. Seedborne diseases create a great loss to the production of crops. Six seed borne fungal diseases of tomato *viz.* early blight (*Alternaria solani*), germination reduction (*Aspergillus flavus*, *Penicillium* sp.), fusarium wilt [*Fusarium oxysporum* sp., seed discoloration (*A. fumigutus*) and late blight (*Phytophthora infestans*) have been detected commonly (Fakir, 2001) [35].
In order to reduce the loss, farmers used to treat seeds with chemicals. Chemicals are quite effective in reducing seedborne infection, but they create environmental pollution and also are costly. As an alternate means of avoiding these problems, use of organic/plants extracts as control agents is one of the promising ways. *Aegle marmelos* plant has strong potential to treat disease. It shows antibacterial and antifungal properties.
Aegle marmelos belonging to family Rutaceae, is commonly known as bael/bel in indigenous systems of medicine and has been regarded to possess various medicinal properties. Its leaves are trifoliate symbolizing the *Trimurthies*: Bramha, Vishnu, and Shiva with the spear shaped leaflets reassembling Thrisoolam, the weapon of Lord Shiva. *Aegle marmelos* is slow growing, medium sized tree, up to 12-15 m. tall with short trunk, thick, soft, flanking bark. (Sharma *et al*., 2007) [30]. The bael tree has its origin from eastern ghats and central India. It is native to India (Lambole and Gajera, 2010) [36]. In India flowering occurs in April and may soon after the new leaves appear and the fruit rippers in 10 to 11 months from bloom march to June of the following year. (Orwa, 2009; Chopra and Nayar, 1956) [26, 8]. The leaves of *Aegle marmelos* contains alkaloids, tarpenoids, essential oils *viz.* limonene, phellandrene, cineol, citronella, citral, cumin aldehyde which shows anti-microbial activities. Now a days medicinal plant extracts have been accorded a lot of importance for crop protection against diseases.

By considering these facts, the present investigation was carried out to evaluate efficacy of *Aegle marmelos* leaf extracts against pathogens of tomato early blight (*Alternaria solani*) and fusarium wilt (*Fusarium oxysporum* f. sp. *lycopersici*).

2. Materials and Methods

2.1 Isolation, purification and identification of pathogen from infected Tomato plant

Plant parts showing wilt and blight characteristics have been collected from the Tomato fields. The samples have been taken from Udgir, District Latur, Maharashtra in the year 2018. The plant parts were examined under microscope to confirm the presence of respective pathogen. First, infected plant parts are cut into pieces (2-3 mm), then it is surface sterilized with 0.1% mercuric chloride solution for 1 Min. The plant parts are washed three times with sterilized distilled water and transferred aseptically on Potato Dextrose Agar (PDA) media. The inoculated plates have been incubated at room temperature (27±2°C) and observations are made daily for emergence of culture. After the development of the fungal colonies stock cultures have been prepared using PDA in test tubes and stored in refrigerator at 4°C. Wilt (*Fusarium oxysporum* f. sp. *lycopersici*) and blight (*Alternaria solani*) pathogens were isolated from infected pigeon pea plants and identified as per the monograph and standard procedures.

Spores of Wilt (*Fusarium oxysporum* f. sp. *lycopersici*) and blight (*Alternaria solani*) were taken from the pure culture and mounted on the clear glass slide. Spores were mixed thoroughly with Lactophenol in order to obtain a uniform spread over on which a cover slip was placed. The spores and hyphae of the fungus were observed by using camera Lucida attached to compound microscope.

2.2 Bio-control of disease causing pathogen by using plant extracts

Leaf extracts of various concentrations (2.5%, 5.0%, 7.5% and 10.0%) of *Aegle marmelos* Corr. was examined against isolated pathogens.

2.2.1 Preparation of aqueous extracts

Green leaf samples (100gm) were collected and washed very carefully with distilled water. Then plant parts were ground with conventional grinder called '*Mortar and pastel*' which is available and popular in every Indian farmer's house. Then grounded material were dipped in to 100 ml distilled water for 48 hours for complete extraction of the active ingredient from the extracted samples (Ahmed *et al*., 2013). After that the water and ground material were filtered with the help of muslin cloth. This extract filtered with the help of Whatman's grade filter paper no. 1. Then crude extracts were preserved in glass bottles and kept in refrigerator at 4 +/- 2^0C for further use.

2.2.2 Mycelial growth inhibition by poison food technique (Nene and Thapliyal, 1993) [24]

Efficacy of leaf extracts with different concentrations were examined by Food poison technique. (Nene and Thapliyal, 1993) [24]. The linear mycelial growth of fungi has been taken after seventh day of incubation.

The required concentrations of plant extracts were obtained by taking 2.5, 5.0, 7.5, and 10.0 mL of extracts in 100 mL of warm agar PDA /GNA media.

The different concentrations of plant extracts prepared in agar media were 2.5, 5.0, 7.5, and 10.0%. The media were poured in sterilized petriplates and allowed to solidify. The control plates were maintained where media was not treated with plant extracts. These plates were inoculated by 4mm disc of *Fusarium oxysporum* f. sp. *Lycopersici* and *Alternaria solani* in the center aseptically. These plates were incubated at 28 $\pm$ 1^0C. The observations were recorded in the form of linear growth of fungal pathogen in centimetre after seven days of incubation period.

Linear mycelial growth inhibition was calculated by following formula:

$$\text{Percentage of fungal growth inhibition} = \frac{gC+gT}{gC} \text{ X } 100$$

Where,
gC= Mycelial growth of fungus in control plate (cm)
gT= Mycelial growth of fungus in treated plates (cm)

2.3 Data Analysis

Data was analysed by Analysis of Variance (ANNOVA) and LSD was calculated at P=0.05 for significance.

3. Experimental Results

3.1 Effect of *Aegle marmelos* Corr. on growth of *Alternaria solani*

As shown in Table 1 & 2 and Fig. 1, the average diameter of colonies of test fungi in poisoned food plates (Plate I) were significantly lesser than that of colony diameter in control plates, which is indicative of antifungal potential of extracts. The inhibition was concentration dependent. Among the extract used, the maximum inhibition of the mycelial growth 0.4 cm. obtained with 10.0% concentration which accounted for 93.44% reduction of mycelial growth over control. This was followed by 7.5% concentration with 1.8 cm. growth and 70.49% reduction; whereas, 5.0% concentration showed 3.3 cm. growth which accounted for nearly half (45.9%) reduction of mycelial growth over the control. Control plate showed 6.1 cm. growth.

The values of F indicated that there was significant variation due to various concentrations of aqueous extracts, while the variation was statistically non-significant. The mycelial growth significantly and gradually decreased from 6.1 cm. in control 0.4 cm. at the concentration of 10.0%.

Table 1: Effect of *Aegle marmelos* Corr. on growth of *Alternaria solani*

Sr. No.	Aq. Extract concentration (%)	Linear mycelial growth in cm.				Growth inhibition %
		R1	R2	R3	Mean	
1	0	6.2	6	6.1	6.1	0
2	2.5	5.2	5.3	5.1	5.2	14.75
3	5	3.4	3.3	3.2	3.3	45.9
4	7.5	1.9	1.8	1.7	1.8	70.49
5	10	0.7	0	0.5	0.4	93.44

SE					0.1378	
CD 5%					0.3184	
CD 1%					0.4631	

Table 2: Analysis of variance (ANOVA)

Source	df	SS	MSS	F	S/NS
Concentration	4	66.276	16.569	581.3684211	S
Replications	2	0.112	0.056	1.964912281	NS
Error	8	0.228	0.0285	-	-
Total	14	66.616	-	-	-

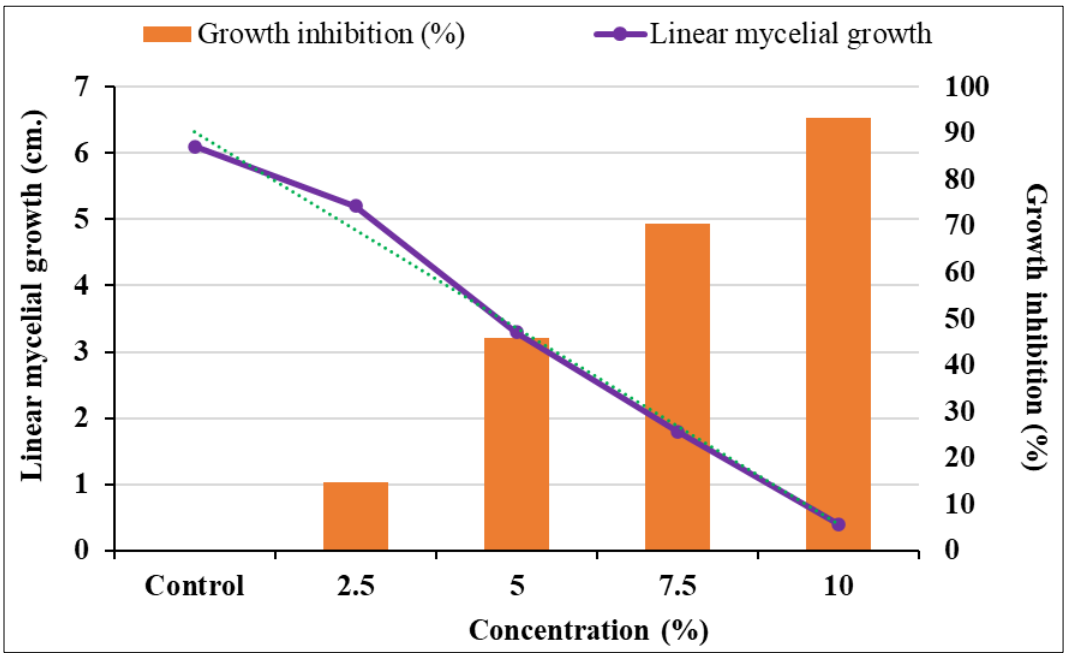


Fig 1: Effect of *Aegle marmelos* Corr. on growth of *Fusarium oxysporum*

3.2) Effect of *Aegle marmelos* Corr. on growth of *Fusarium oxysporum:*

The efficacy of plant extract in reducing mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici* is illustrated in Table 3 & 4, Fig. 2 and Plate II. All the treatments were significantly inhibited the growth of *F. oxysporum*. The maximum inhibition of mycelial growth of *Fusarium oxysporum* was reported in 10.0% concentration, which was 0.8 cm., and 90.0% followed by 7.5% concentration with 1.8 cm. and 77.5% growth inhibition. Whereas, 5.0% concentration recorded 3.1 cm. and 2.5% concentration showed 6.1 cm. mycelial growth and 61.25% and 23.75% growth inhibition respectively. In control plate, the mycelial growth reported was 8 cm. on seventh day of incubation. The mycelial growth significantly and gradually decreased from 8.0 cm. in control and 0.8 cm. at the concentration of 10.0%.

Table 3: Effect of *Aegle marmelos* Corr. on growth of *Fusarium oxysporum*

Sr. No.	Aq. Extract concentration (%)	Linear mycelial growth in cm.				Growth inhibition %
		R1	R2	R3	Mean	
1	0	8.2	8	7.8	8	0
2	2.5	6.1	6	6.2	6.1	23.75
3	5	3.2	3.1	3	3.1	61.25
4	7.5	1.7	1.8	1.9	1.8	77.5
5	10	0.9	0.8	0.7	0.8	90
SE					0.1049	
CD 5%					0.2423	
CD 1%					0.3524	

Table 4: Analysis of variance (ANOVA)

Source	df	SS	MSS	F	S/NS
Concentration	4	108.876	27.219	1649.6364	S
Replications	2	0.028	0.014	0.8485	NS
Error	8	0.132	0.0165	-	-
Total	14	109.036	-	-	-

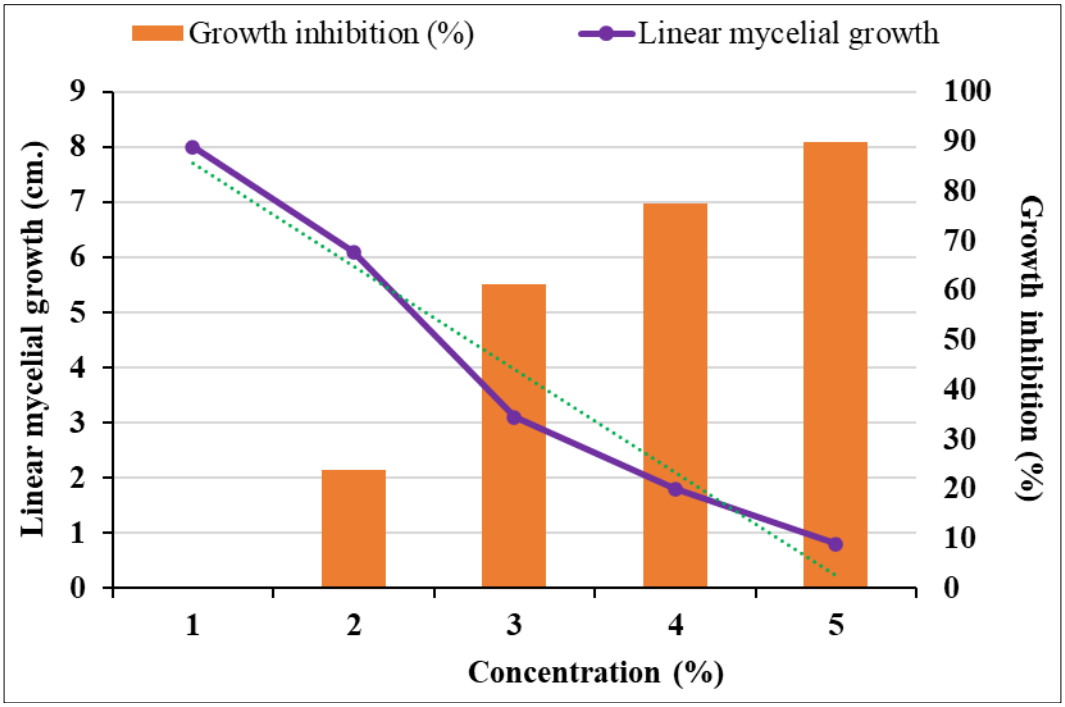
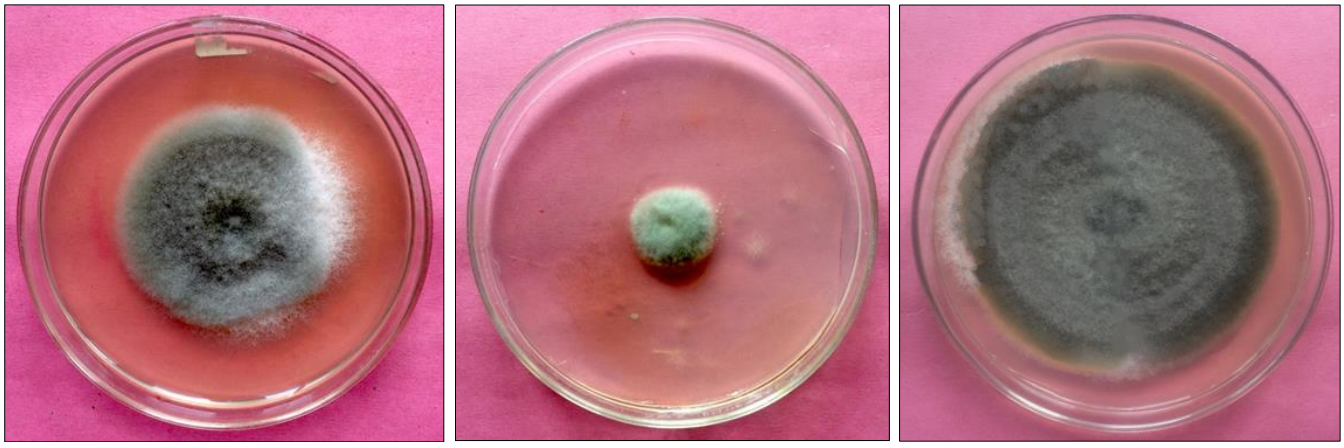
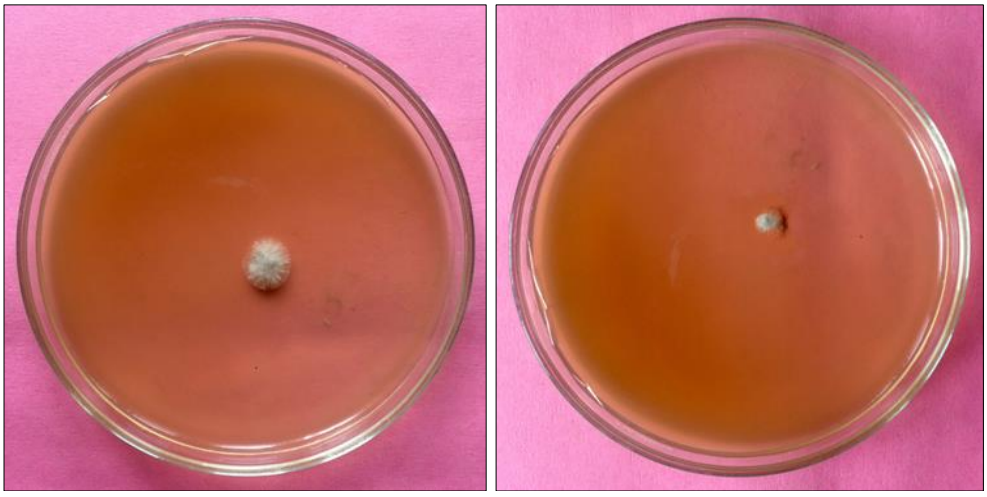


Fig 2: Effect of *Aegle marmelos* Corr. on growth of *Fusarium oxysporum*



2.5% 5.0% Control



7.5% **10.0%**

Plate 1: Effect of *Aegle marmelos* Corr. on growth of *Alternaria solani*

2.5% 5.0% Control

7.5% 10.0%

Plate 2: Effect of *Aegle marmelos* Corr. on growth of *Fusarium oxysporum*

4. Discussion

Tomatoes (*Solanum lycopersicum* L) are the most widely eaten fruit and vegetable crop in the world, with an estimated annual production of 124.4 million tonnes of fresh tomato fruits (Wang *et al*., 2009) [33]. Tomato is the most significant vegetable crop in India, ranking second in terms of output and area globally. China is the world's greatest producer, accounting for 27.8%, followed by India (11.2%) (Kumar *et al*., 2016; Harisha *et al*., 2019; Gupta *et al*., 2021) [21, 14, 13]. It is one of the most extensively grown vegetable crops, with India being the second-largest producer after China. Bacterial, viral, nematode, and fungal diseases may all affect tomato crops. Early blight, caused by *Alternaria solani* and wilt caused by *Fusarium oxysporum*, are most devastating diseases in tomato-growing regions. Tomato production is hampered by the early wilt and blight diseases, which are particularly prevalent in subtropical and tropical areas.

During the present investigation *Aegle marmelos* Corr. leaf extracts of different concentrations showed encouraging effect on pathogenic fungi. Leaf extract was found to be most effective with increasing concentration to control the growth of fungi.

After 7th day of incubation, control plate without leaf extract showed 6.1 cm. linear growth of *Alternaria solani*. Petriplates with 2.5%, 5.0%, 7.5% and 10.0% concentration of aqueous extract showed an average 5.2 cm., 3.3 cm., 1.8 cm. and 0.4 cm. linear growth, whereas, percentage inhibition recorded was 14.75%, 45.90%, 70.49% and 93.44% respectively. While in case of *Fusarium oxysporum*, after 7th day of incubation, control plate without leaf extract showed 8.0 cm. linear growth. Petriplates with 2.5%, 5.0%, 7.5% and 10.0% concentration of aqueous extract showed an average 6.1 cm., 3.1 cm., 1.8 cm. and 0.8 cm. linear growth, whereas, percentage inhibition recorded was 23.75%, 61.25%, 77.50% and 90.0% respectively.

Chemical fungicides have a significant impact on seed-borne fungal infections, but they also have a negative impact on beneficial microorganisms in soils and the environment. Furthermore, indiscriminate fungicide usage is not only harmful to animals and humans, but it also breeds resistance among target pathogens. Because fungicides may harm non-target species (Banerjee *et al*., 2005), researchers are turning to more environmentally benign and cost-effective ways of disease management, such as acid treatments, antagonistic microorganisms, and plant extracts. These are viable options that release plant growth regulators that impact overall crop development and enhance morphological traits.

Plants create a vast range of environmentally benign secondary metabolites and are the richest source of organic compounds (Okigbo and Nmeka, 2005; Jamil *et al.*, 2007; Riaz *et al.*, 2010) [25, 37, 27]. Botanicals, rather than chemical fungicides, are one of the most modern techniques of managing seed-borne and other plant diseases (Howlader,

2003; Islam *et al*., 2006) [15, 17]. Various plant extracts, including garlic clove, neem leaf, allamonda leaf, ginger rhizome, kalijira seed, bel leaf, turmeric rhizome, katamehedi leaf, and onion bulb, were tested for antifungal effectiveness against tomato seed-borne damping-off. Seed treatment with plant extracts had varying degrees of success in terms of tomato percent damping-off. However, neem leaf extract had the greatest seed germination (86.67%) and the lowest incidence of damping-off of tomato, followed by garlic clove and allamonda leaf extract (Islam and Faruq, 2012) [17].

Sallam and Abo-Elyousr, (2012) [28] reported similar results as tested plant extracts of *Ocimum basilicum*, *Azadirachta indica*, *Eucalyptus chamadulonsis*, *Datura stramonium*, *Nerium oleander*, and *Allium sativum*, caused a significant reduction in the linear growth of *A. solani*. They further reported that reduction was gradually increased by increasing the concentration of extracts in the growth medium. Similar effects of various other plant products effective against *Alternaria* spp. were reported by several other authors (Latha *et al.* 2009; Goussous *et al*. 2010) [23, 38]. The spore germination and mycelial growth of A. solani were suppressed by bulb extracts of *A. sativum*, leaf extracts of *Aegle marmelos*, and flower extracts of *Catharanthus roseus* (Vijayan, 1989) [32]. According to Vijayan (1989) [32], the inhibitory impact of the analysed plant extracts might be owing to their direct toxic action on the pathogen. The active components included in plant extracts may either act on the pathogen directly (Amadioha, 2000) or establish systemic resistance in host plants, resulting in a decrease in disease progression, according to research on the processes of disease suppression by plant products (Kagale *et al*., 2004) [19].

Bowers and Locke (2000) found that 10% aqueous emulsion of pepper, clove and cassia extracts reduced the growth of *Fusarium oxysporum f.sp.chrysanthem*i *(Foc)* in soil by 99.9%, 97.5% and 96.1% after three days of incubation, while 5% aqueous emulsion of pepper extracts reduced the population densities of *Fusarium oxysporum f.sp.chrysanthem*i by 99.9%. Chand and Singh. (2005) explained the inhibitory activity of *Eucalyptus globulus*, *Jatropha multifida*, *Azadirachta indica*, and *Allium sativum* against wilt incidence in chickpea. Uzma *et al*. (2008) [31] investigated the antifungal efficacy of asafetida (*Ferula asafoetida*), black cumin seed (*Nigella sativa*), neem (*Azadirchta indica*) and mustard (*Brassica compestris*) oils against eight fungal species *viz. Aspergillus flavus, Aspergillus niger, Fusarium moniliformae, Fusarium oxysporum, Fusarium nivale, Fusarium semitectum, Alternaria alternata and Drechslera hawiensis.* Sharma and Kumar (2009) [29] reported that the extract of three weed plants, namely, *Capparis decidua*, *Lantana camara* and *Tridax procumbens*, showed antifungal property against *Fusarium oxysporum*. Gaire and Subedi (2013) [11] recorded the antifungal activity Acetone extracts of *Datura stramonium* against several fungi including *Fusarium oxysporum.* Khaleel *et al*. (2014) [20] recorded fungitoxic effect of *Allium sativum* extract, *Azadirachta indica* leaf extract, *Zingiber officinale* Extract, *Calatropis procera* leaf extract, *Moringa oleifera* leaf extract and *Parthenium hysterophorus* L. leaf extract showed against F. *oxysporum* f.sp. *pisi*.

The result of the present study showed identical output with *Aegle marmelos* Corr. leaf extracts as most effective antifungal agent against blight and wilt pathogens of tomato at 10% concentration.

5. Conclusions

In conclusion this study demonstrated that different concentrations of aqueous leaf extract of *Aegle marmelos* Corr. was found considerably strong inhibitory on *Alternaria solani* and *Fusarium oxysporum* and therefore can be used for the biocontrol of early blight and wilt diseases. As a result, this form of management may help to reduce the risks and dangers of harmful fungicides, particularly on vegetables grown for fresh consumption. The active chemicals responsible for these extracts' fungicidal efficacy will be identified via further investigation.

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