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EVALUATION OF DIFFERENT ESSENTIAL OILS AND BIO CONTROL AGENTS AGAINST *ALTERNARIA ALTERNATA* THE CAUSAL AGENT OF FRUIT ROT OF JUJUBE

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Abstract.

This study was carried out on the evaluation of different essential oils and biocontrol agents against *Alternaria alternata* the causal agent of fruit rot**.** For the pathogenicity test against *A. alternata* was performed through cut and injection inoculation methods. The antifungal potential of different essential oils like turpentine, laung, neem and castus root was carried out at different doses i.e. 5, 10 and 15% by food poisoned method to find out the effective and suitable oil for the growth inhibition of *A. alternata* and evaluate the effect of some biocontrol agents on growth inhibition of *A. alternata*. The findings of this investigation stated that cut method of inoculation showed higher percentage (2.60) of rotting as compared to injection method of inoculation (2.35). Minimum colony growth of *A. alternata* (31.60, 21.25 and 15.16%) was examined under Laung oil at the dosage of 5, 10 and 15% followed by Neem oil (42.60, 31.60 and 21.30%), respectively. Maximum colony growth of *A. alternata* (62.71, 52.40 and 41.75%) was observed under Castus root oil at the dosage of 5, 10 and 15%. Zero growth of target pathogen was examined under Turpentine at 5, 10 and 15%. Under control the *A. alternata* showed (90 mm) colony growth. Minimum linear colony growth of *A. alternata* was observed for *Hypoxylon* Sp1 (50.31%), followed by *Neurospora* spp. (52.97%), *Lasiodiplodia theobromae* (54.7%), *Chactomium subaffine* (57.07%) and *Fusarium* sp. (65.4%). Maximum mycelial colony growth (90%) was recorded in control. Based on present investigation, Similarly, for controlling the linear colony growth of *A. alternata* under *in vitro* conditions Turpentine oil ranked 1st, Laung oil ranked 2nd, Neem oil ranked 3rd, Castus root oil ranked 4th.

Keywords: *Alternaria alternate.* Biocontrol, Essential oils, Fruit rot of Jujube

Introduction

Jujube (*Ziziphus mauritiana* Lamk.) is also known as ber, desert apple or plum. It belongs to family Rhamnaceae. It is a tropical/subtropical fruit native to the northern hemisphere (Lyrene, 2012). The genus *Ziziphus* has 135 to 170 species (Pareek, 2013), of which 17 are native of (Singh *et al.*, 2014). *Z. mauritiana* is cultivable Ber in drier parts of the subcontinent (Sebastian and Bhandari, 2012). Ber is also cultivated on marginal lands in some African countries (Johnston, 2012). Jujube fruit is a drupe, globose to ovoid, up to 6 x 4 cm in size; skin smooth or rough, glossy, thin but tough, yellowish to reddish or blackish; flesh white, crisp, and juicy, subacid to sweet, becoming mealy in fully ripe fruit (Pareek, 2013; Abbas *et al.*, 2010). Irregular furrowed stones are found in tuberculate seed which contains 6 mm long brown kernels of elliptic shape. Ber fruit is generally eaten fresh and is a rich source of ascorbic acid, essential minerals and carbohydrates (Pareek *et al.*, 2011). Color of fruit is changed from green to yellow to chocolate brown with the maturity and ripening. The economic returns for jujube will depend on the price paid per tree and the price obtained per pound. Producers should develop their own cost and return estimates based on budget templates that are available for tree fruit and nut crops, such as apples or pawpaw (Cai *et al.* 2012). Relatively high prices per pound may be required to recoup establishment costs and generate economic profits (Jones *et al.* 2017). The jujube is being more popular for offering a

perennial woody species that reduce soil erosion while producing an economic crop following the government policy of converting small grain production to conservation forestry on the Loess Plateau. For more jujube production, there is an urgent need for the specific fertilization of jujube trees. Traditional intensive agriculture aimed at maximum productivity with large amounts of inorganic fertilizers and pesticides is now being blamed for declining soil fertility and negative environmental effects (Schieber *et al.* 2018). Jujube, locally called 'Ber, is an indigenous fruit of China and South Asia. Produced in moderate regions such as China, India, Pakistan, Syria, Malacca, Australia and Malaysia. It is also grown in parts of Afghanistan, Iran and Russia (Liu *et al.* (2018). China is perhaps the most important country for jujube cultivation, where it is known as the "Chinese dates", with hundreds of varieties, some being seedless. In northern China, it is considered one of the principal fruits. In the US this fruit has been introduced but is not grown on a large scale (Azam *et al.* 2016). In Pakistan, it is widely distributed in three provinces i.e. Khyber Pakhtunkhwa (Banu, Karak and Kohat districts), Punjab (Attock, Chakwal and Mianwali districts) and Sindh province (Karachi, Hyderabad and Nawabshah districts). In Pakistan, jujube is cultivated on an area about 5.425 ha with an annual production of 28.000 tones (Qamer *et al*., 2018). *Alternaria* and *Aspergillus* fruit rot are the most commonly occurring fruit rots in Ber. Temperature and relative humidity affect fruit rot development and establishment of pathogen infection. For this disease, the congenial temperature ranges from 20 to 30°C and high relative humidity (>90%). However, the temperature and relative humidity affect the severity and pathogenicity of fruit rots caused by several pathogens that are, *A. alternate*, *A. niger*, *Colletotrichum capsici*, *Drechslera australiensis*, *Fusarium moniliforme* and *F. solani* (Datar, 2015). Jujube is being attacked by several insect pests and diseases. The diseases included *Alternaria* fruit rot**,** *Aspergillus* fruit rot, *Botrytris* fruit rot is the major limiting factors in terms of yield losses both qualitatively and quantitatively (Raji and Raveendran, 2013). Among the above-mentioned diseases, fruit rot of jujube caused by *Aspergillus niger* is one of the major post harvest infection in which it may cause considerable losses in some cases up to 94% to the jujube growers. In Pakistan, this disease invariably appears every year in the jujube orchards causing significant yield and quality losses. The disease is more sever in rainy season and fruit symptoms appeared in two forms; spherical depressed spots occurred in scattered form on the pericarp only and black rot restricted to internal fruit tissues (Yehia, 2013). Ripened fruits are susceptible to attack by a variety of pathogenic fungi and bacteria that can colonies them during the period of development on and off the tree. Ber fruits are susceptible to several postharvest diseases (Eckert & Eaks, 1989). During the period of packaging, storage and transport fruits may be exposed to various decay-causing microflora. Some of the predominant organisms observed on freshly harvested fruits were *Aspergillus niger*, *A. sydowii*, *Rhizopus oryzae*, Penicillium chrysogenum, *Alternaria* tenisima, Phoma spp., *Cuvrularia* spp., of which A. niger and *R. oryzae* caused the greatest spoilage *in-vitro* Kim *et al.* (2014) found that Ulocladium chartarum, Phoma hissarensis and Botryodiplodia theobromae caused decay losses more frequently in the packages. When fruits are weakened by senescence and chilling injury, they are attacked by several pathogens that cause fruit rot (Patil, 2015). About 16 fungi belonging to 12 genera have been reported to cause fruit rot in Ber during harvest, transit and storage. Several other minor pathogens also cause fruit rot in Ber, including *Geotrichum* spp., Phoma herbarium, *Phytophytora micotianae* and *Sclerotium rolfsii* (Pareek *et al.* 2012). The soft rot of jujube starts as light brown lesions at the site of a cut or incision and later changes to dark brown or black with the increase in severity of the symptoms. The outer skin of the spot remains intact but the flesh inside turn dark brown; visibly macerated and water soaked. As the time of incubation advances rotting penetrates inside the tissue up to the seed in the centre and quickly encircles it, emitting a foul smell (Singh *et al.* 2014; Michailides, 2017) reported that plant extracts as biopesticides act as a vital component for the management of this disease. Neem leaf extract gave 58.6% inhibition in radial growth and 56.5% in spore germination at 10% concentration followed by *Ocimum sanctum* which was found effective and gave 54.7% inhibition in radial growth and 50.4% in spore germination over control. Rukhsana *et al.* (2017) determined potential of allelopathic weed aqueous extract

against *P. glomerata*, *A. niger* and *Drechslera tetramera* under the *in-vtiro* condition at various concentrations. According to their result, fungal growth inhibited significantly at 70% concentration of aqueous extract against all causal pathogen followed by 60, 50, 30, 20 and 10% concentration. However, minimum antifungal activity was seen at 0% concentration against *P. glomerata*, *A. niger* and *D. tetramera* under *in-vtiro* condition.

Materials and Methods

A sampling of jujube infected fruits: The collected specimens were brought to a mycological laboratory at Faculty of Crop Protection, Department of Plant Pathology, Sindh Agriculture University Tandojam, for isolation and identification of the causal agent.

Isolation and identification of the causal fungus: Diseased specimens showing the symptoms of fruit rot was brought to mycology laboratory at Faculty of Crop Protection Department of Plant Pathology, Sindh Agriculture University Tandojam. The infected portion including fruits was cut into small pieces of 3 to 4 mm length and was surface sterilized with 5% commercial bleach (sodium hypochlorite) for 2 minutes. The sterilized pieces were washed two times with sterilized water and shifted sterilized filter paper for drying and then sterilized portions were kept on petri plates containing fresh potato Dextrose Agar (PDA) medium. Usually, five pieces of infected samples were kept in every plate. All petri plates were kept in an incubator at 25°C±2 temperature for 7 days to observe sporulation of the fungi. Meanwhile, diverse fungal colony appeared which was purified using the single spore isolation technique and hyphal tip method. The colony growth of fungus was recognized based on their morphological characteristic.

Pure culture of the causal fungus: After identification of target fungus the pure culture of fungus was made and maintained for future use.

To perform pathogenicity test: The pure cultures of isolated fungus were maintained on PDA in culture tubes which were stored in the refrigerator at 24°C and used frequently. These were multiplied on 2% PDA for two-three weeks. The inoculum potential of isolated fungus was prepared by taking 1gm culture in 20 ml distilled water. Pathogenicity tests were carried out by injection and disc method.

To evaluate the efficacy of different essential oils on the mycelial colony growth against the causal agent under *in-vitro* conditions: The efficacy of different essential oils such as laung, neem, tarpin, castor, chamomile and essential oil was examined under *in-vitro* conditions against *alternaria alternata* the causal agents of fruit rot of jujube. The essential oils were used, with three different doses (5%, 10% and 15%) with 3 replications. Pathogen was cut from 8-10 days old culture plate by using sterile cork borer (5mm) and placed in the centre of the PDA plate and incubated at 28ºC. Petri dishes without essential oil were treated as control. The radial colony growth of *alternaria alternata* was recorded by drawing two mutually perpendicular lines on the back of the Petri plates crossed each other at the centre of the plate. The data on colony growth was recorded along with these lines in millimeter after every 24 hours until the plates were completely fill in any treatment.

To test the different biocontrol agents on the mycelial colony growth of the causal fungus under *in-vitro* conditions: Biocontrol agents like *Trichoderma viridae* and other biocontrol agents were evaluated against *A. alternata* the causal agent of fruit rot of Jujube. For this purpose, dual culture technique was used.

Results

This study was carried out on the evaluation of different essential oils and biocontrol agents against *A. alternata* the causal agent of fruit rot**.** For this, the pathogenicity test against *A alternata* was performed through cut and injection inoculation methods. The antifungal potential of different essential oils like turpentine, laung, neem and castus root was carried out at different doses i.e. 5%, 10% and 15% by food poisoned method to find out the effective and suitable oil for the growth inhibition of *Alternaria alternata* and evaluate the effect of some biocontrol agents on growth inhibition of *Alternaria alternata*.

Rotting (%) through cut and injection inoculation methods: Results regarding the rotting (%) through cut and injection inoculation methods are presented in Figure-(1A & B). The data shows that rotting of 2.60 cm and 2.35 cm of *Alternaria alternata* in jujube was observed by cut and injection method of inoculation. On the basis

of percentage, it was observed that cut method of inoculation showed higher percentage of rotting as compared to injection method of inoculation.

Linear colony growth of *Alternaria alternata* under different essential oils: The results regarding linear colony growth of *Alternaria alternata* under different essential oils is presented in (Figure-2 A B,C,D & E). The data clarified that minimum colony growth of *Alternaria alternata* (31.60, 21.25 and 15.16mm) was examined under Laung oil at the dosage of 5%, 10% and 15% followed by Neem oil (42.60, 31.60 and 21.30mm), respectively. Maximum colony growth of *Alternaria alternata* (62.71, 52.40 and 41.75mm) was observed under Castus root oil at the dosage of 5%, 10% and 15%. Zero growth of target pathogen was examined under Turpentine at 5%, 10% and 15%. Under control the *Alternaria alternata* showed (90 mm) colony growth. On the basis of means, Turpentine oil ranked 1st, Laung oil ranked 2nd, Neem oil ranked 3rd, Castus root oil ranked 4th for controlling colony growth of *Alternaria alternata* under *in-vitro* conditions. Statistical analysis of the obtained data reveals that there was a significant difference in linear colony growth of *Alternaria alternata* among the essential oils at different dosages.

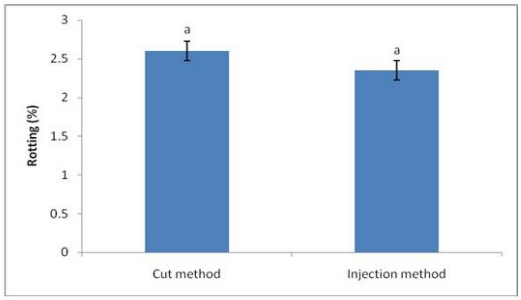

Figure 1A. Rotting (%) through cut and injection inoculation methods

Figure 1B. Pathogenicity through cut and injection inoculation methods

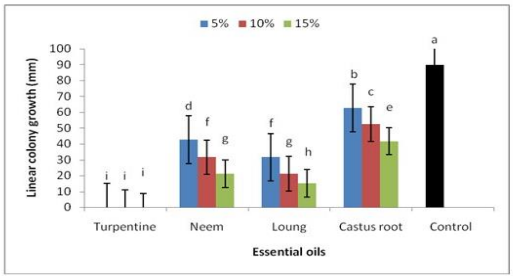

Figure 2.A. Linear colony growth (mm) of *Alternaria alternata* at different concentrations of essential oils in comparison with control

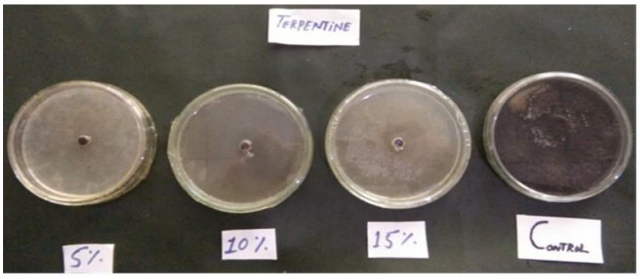

Figure 2.B. Mycelial colony growth (mm) of *Alternaria alternata* at different concentrations of *Turpentine* oil in comparison with control

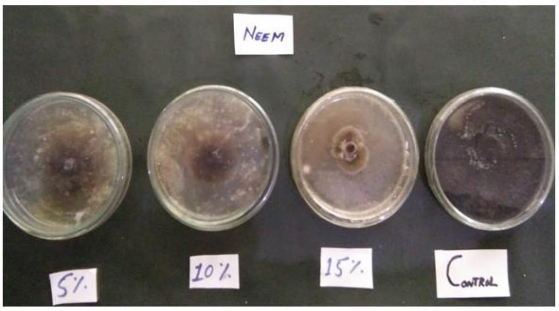

Figure 2 C. Mycelial colony growth (mm) of *Alternaria alternata* at different concentrations of Neem oil in comparison with control

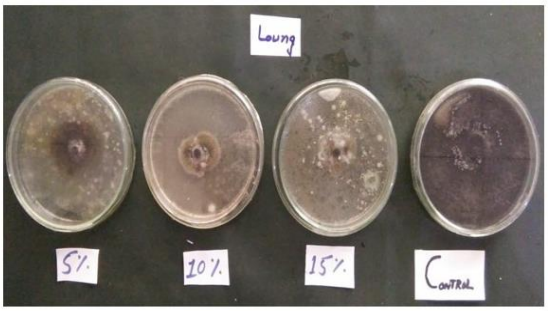

Figure 2D. Mycelial colony growth (mm) of *Alternaria alternata* at different concentrations of Loung oil in comparison with control

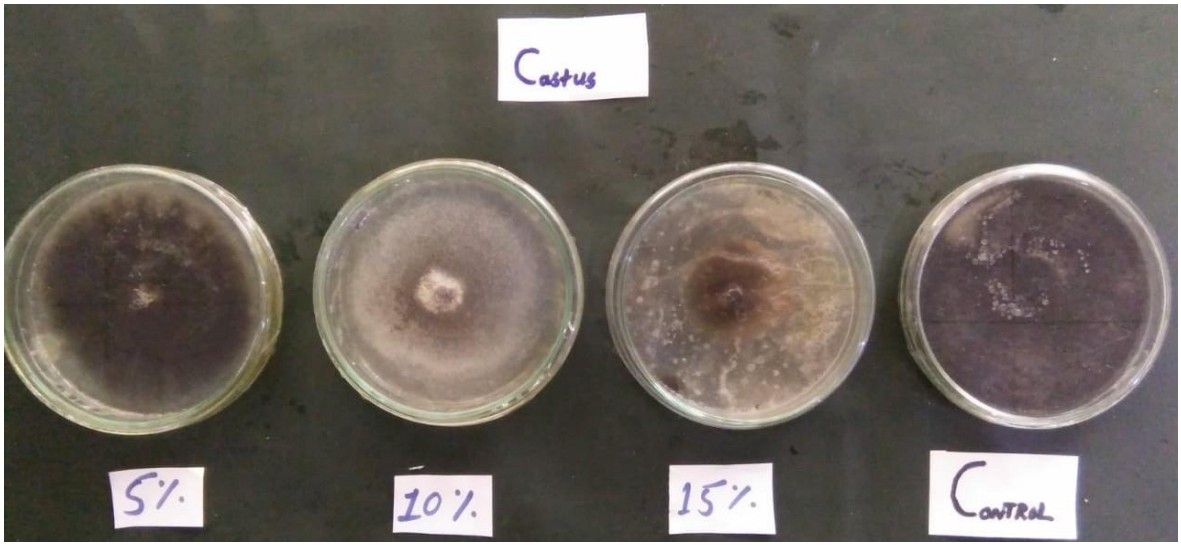

Figure 2E. Mycelial colony growth (mm) of *Alternaria alternata* at different concentrations of Castus root oil in comparison with control

Linear colony growth of *Alternaria alternata* under different biocontrol agents: The results (Figure-3A & B) indicates that minimum linear colony growth of *A. alternata* was observed for *Hypoxylon* Sp1 (50.31mm), followed by *Neurospora* spp. (52.97mm), *Lasiodiplodia theobromae* (54.7mm), *Chactomium subaffine* (57.07mm) and *Fusarium* sp. (65.4mm). Maximum mycelial colony growth (90mm) was recorded in control. Statistically there was significant ($p<0.05$) difference in mycelial colony growth between the biocontrol agents.

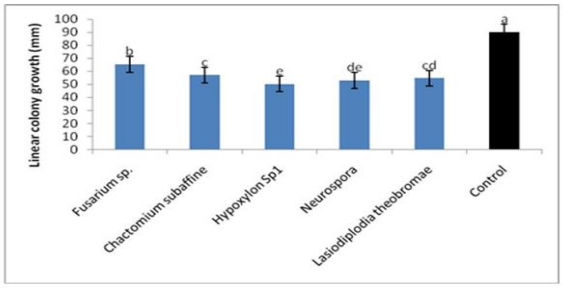

Figure 3A. Linear colony growth (mm) of *Alternaria alternata* under different biocontrol agents in comparison with control

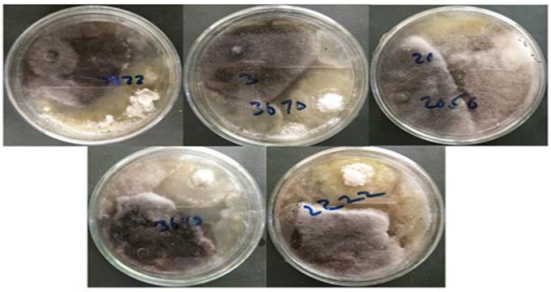

Figure 3B. Mycelial colony growth (mm) of *Alternaria alternata* under different biocontrol agents in comparison with control

Discussions

In our study cut method of inoculation showed higher percentage of rotting as compared to injection method of inoculation. These results are in accordance with the findings of Pawar and Thaker, (2006), they stated that most of the other essential oils were found challenging to combat *A. alternata*, suggesting their use as strong aroma therapeutic agents. For further study of effectiveness of essential oils, the inoculated jujube fruits were immersed in aqueous solution of essential oils. Rukhsana *et al.* (2017) observed minimum rotting (12.53%) for laung oil followed by neem oil (13.30%), tarpin oil (13.42%), rose oil (14.27%), castor oil (15.16%) and chamomile oil (17.25%), respectively. These findings agree with other studies reported by Guynot *et al.* (2003). Several investigations showed that the antifungal activity of the volatile compounds by vapour contact is better than that obtained by broth dilution or by agar diffusion Bouddine *et al.*, (2012). The results obtained in our experimental conditions showed that the antifungal effect of the essential oils and their major components limited the growth of fungus to higher concentration. Even if this method is easy to use and widely utilised, it is principally a qualitative test which gives no more than an idea about the volatile fraction of the essential oils. Among the essential oils, Turpentine oil showed

high efficacy against linear colony growth of fungus at all concentrations applied. These results are parallel with findings of Jatoi *et al.,* (2020) they stated Neem oil and its leaf extracts significantly ($p<0.05$) reduced severity of fruit rots. Gadhi *et al.* (2018) reported that plant extracts as biopesticdes act as a vital component for the management of this disease. Neem leaf extract gave 58.6% inhibition in radial growth and 56.5% in spore germination at 10% concentration followed by *Ocimum sanctum* which was found effective and gave 54.7% inhibition in radial growth and 50.4% in spore germination over control. Mengal *et al*., (2019) revealed that all plant extracts exhibited significantly different mycelia growth inhibition of pathogen. Among them J. curcas leaf extract showed maximum growth inhibition (62.9%) followed by D. strumarium (55.6%), A. indica (51.9%), M. oleifera (46.9%), C. gigantean (23.45%) and *M. alba* (13.6%), respectively. Mengal *et al*., (2019) revealed that all the concentrations of plant extracts exhibited significantly different in spore germination inhibition of R. stolonifer and A. alternata. Maximum spore germination inhibition was observed on higher concentration compared to lower concentration of marigold, garlic and mint, correspondingly. Jatoi *et al.,* (2020) revealed that all the extracts significantly inhibited the mycelial growth at this concentration wherever *Madhuca longifolia* and *Tagetes patula* showed least mycelial growth. However, the leaf extract of Eucalyptus was found to be most effective as compared to other including control for controlling the disease caused by *A. alternata.* An intense study on these leaf extract may help to use them as an effective biopesticides in commercial scale. Wang *et al.,* (2009) stated that maximum antifungal potential was observed with the extracts of *C. sativa,* which recorded excellent inhibitory activity against *C. lunata* (100%), *A. zinnia* (59.68%), followed by leaf extract of *P. hysterophorus* (50%) against *A. solani*. The efficacy of fungicides it is also grown in summer and rainy seasons. Many fungal diseases have been found to attack cucumber. Leaf spot caused by *Alternaria alternata* (Fr.) Keissler is an important disease reported to be very destructive diseases which affect the growth, yield and quality of cucumber. The use of chemicals for managing the disease is expensive and often leads to environmental pollution, development of fungicide resistant strains of the pathogens and upset of the biological equilibrium in soil (Singh *et al.,* 2014). Among the biocontrol agents, *Hypoxylon* Sp1 showed better efficacy against linear colony growth of fungus. Similar kind of result was reported by Khan *et al.,* (2019) evaluated *Penicillium* species for the control of black rot disease. According to their findings all the penicilum species remarkably control the colony growth inhibition of fungi compared to control. The disease management generally done by the chemical control such as fungicide used commonly under field condition in standing crop, fruit and vegetable plants throughout the year depending upon the intensity of pathogen. Due to over misusage of fungicides causing harmful effects in human health, disturbing the equilibrium of ecosystem and reducing the shelf life of fruits and vegetable, the losses reaches up to 20% in harvested product in the countries (Cappellini and Ceponis, 1984). Whereas, in developing countries the losses rated about to 50% due to poor cultural practices (Eckert & Eaks, 1989). To overcome the problems due to use of fungicide, appropriate cultural and biological control should be practiced during the fruit and vegetable cultivation and avoid the use of fungicide in field conditions.

Conclusions

Based on present investigation, it was concluded that pathogenicity test through cut method is more severe than that of injection method. Similarly, for controlling the linear colony growth of *Alternaria alternata* under *in vitro* conditions Turpentine oil ranked 1st, Laung oil ranked 2nd, Neem oil ranked 3rd, Castus root oil ranked 4th.

Suggestions

As per conclusion it is suggested that *Turpentine* oil should be used for controlling *Alternaria alternate* causing fruit rot of jujube. Further studies should be conducted on the efficacy of *Turpentine* oil against other fungal species on various fruits and vegetables.

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