International Journal of Scientific Research in Biological Sciences
Vol.8, Issue.1, pp.60-64, February (2021)
Research Paper
E-ISSN: 2347-7520

Efficacy of *Ocimum Sanctum* against *Alternaria* Causing Diseases on Medicinal Plants

Shruti Ojha[1]*, Mamta Goyal[2], Deepika Chittoriya[3]

[1,2,3]Microbiology Laboratory, Botany Department, S. P. C. Government College, Ajmer, Rajasthan, India

**Corresponding author: ojhashruti@gmail.com, Tel., +919460793300.*

Available online at: www.isroset.org

Received: 23/Dec/2020, Accepted: 20/Jan/2021, Online: 28/Feb/2021

Abstract- *Ocimum sanctum* extracts prepared in methanol were tested for their antifungal activity against *Alternaria alternata* isolated from two important medicinal plants including *Aegle marmelos* and *Emblica officinalis*. Leaf, shoot, and flowers of *O. sanctum* were screened individually for the antifungal activity. Disc diffusion method was conducted against *Alternaria* species for analyzing the antifungal properties of the medicinal plant. Zone of inhibition was measured for evaluating the antifungal activity of the methanolic extracts tested against the test fungal strain. Commercial fungicide at a similar protocol was taken as control. Although all the tested plant parts showed positive results of phytopathogenic growth inhibition, but the leaf extracts showed comparatively better effect than the flower and stem extracts. For both the diseased plants including *A. marmelos* and *E. officinalis*, leaf and flower extracts showed comparatively better results than stem. The consequences of using plant parts of *O. sanctum* as botanical fungicides against the fungal pathogen Alternaria have been discussed. The botanical fungicides could be a potential alternative to the commercial fungicides which cause damage to crop and plant development.

Keywords- Antifungal, bio-fungicides, plant extracts.

I. INTRODUCTION

One of the most important sectors in a country's economy is Agriculture. India is well known for the huge production of crops due to its high requirements and population. Moreover, a majority population of India's infrastructure is engaged in farming [1, 2]. A major global issue is crop production in both quantity and quality. Due to limited resources and enormous requirements, it has become prime important to bring forward some new techniques and protocols to bring the agriculture sector on track and resolve the issues globally. However, farmers use harsh chemicals including pesticides and fungicides to control the growth and attack of the microbes on the crops. These chemicals not only destroy the quality of crops but are equally harmful to human health as well. Among the different microbes, fungal pathogens are well known to cause plant diseases [1, 3]. Fungal diseases can be well treated by the chemical fungicides, but they are also known to have long-lasting toxic effects not only on plant's quality, health, and yields but on living organisms also. This may cause a severe threat to the whole ecosystem with destruction to the entire community [1, 4]. Therefore, it becomes primarily important to bring forward some new organic methodology to not only improve the crop yield but control the spread and growth of the pathogenic fungus on the plants.

Investigation was conducted to study the importance of plants for the growth retardation of microorganisms. Botanical extracts have been studied as an alternative to harmful chemicals as fungicides. The two diseased medicinal plants mainly *Aegle marmelos* and *Emblica officinalis* have been considered for fungal pathogens study. *Alternaria alternata* were isolated and identified from these two infected medicinal plants. *Ocimum sanctum* (family, Lamiaceae) is an Indian medicinal plant well known as the queen of herbs. This Indian plant is considered holy and most cherished herb in the country. The herbal plant holds importance in religious and spiritual traditions in India. Moreover, it plays important role in Ayurveda as herbal medicine to cure many diseases [5]. *O. sanctum* with a rich source of phytochemical compounds is well known for its antifungal activities. Reference [6] conducted a research to determine the potential of *O. sanctum* against dermatophytic fungi. The leaves of this medicinal plants showed antifungal potential measured by the 38 A NCCLS method [6]. Similarly, Reference [7] also investigated the antifungal potential of *Ocimum sanctum* against pathogens. Several fungal pathogens including *Alternaria* were studied [7]. Antimicrobial studies have been conducted considering several categories of plants including vegetables and spices as well [8]. This study supports our research by working on a similar fungal genus and leaves of the medicinal plant. However, methanol has not been tested as any solvent.

Bio-fungicides could be a great alternative against the eradication of fungal pathogens and their destruction on crop production.

II. MATERIAL AND METHODS

Collection of diseased plants

Aegle marmelos and *Emblica officinalis* in diseased state were aseptically collected from Rajasthan, India. The collection was conducted during May-September 2015. The research was conducted in the Microbiology laboratory of S.P.C. Government College, Ajmer. The two parts mainly stem, and leaf was cleaned by autoclaved water.

Isolation and identification of pathogenic fungi

The infected parts of both the plants were processed for isolation of pathogenic fungal isolates. Potato dextrose agar medium (PDA) was used for the isolation. Approx. 15ml PDA was aseptically poured into the Petri plates for the isolation of fungal pathogens. Separate 6ml PDA media was poured into autoclaved test tubes for purification of the isolated pathogens by sub-culturing them. The isolated fungal isolates were subjected to the identification.

Culture

The isolated fungi from both the diseased plants including *A. marmelos* and *E. officinalis were Alternaria alternata*. This isolated fungus was isolated and purified in a Potato dextrose agar medium for the antifungal bioassay.

Fungal broth

Potato dextrose broth (medium without agar) was aseptically poured into autoclaved test tubes. The medium without agar does not solidify the media and hence make it suitable for the growth of the fungal mycelium for bioassay. A small piece of the pathogenic fungus was cut from the petri dish and dipped in the broth. The test tubes were incubated for 6-7 days before the experiment.

Botanical extracts

The fresh plant parts including stem, leaves, and flowers of *O. sanctum* were collected from Ajmer, Rajasthan. They were washed to avoid any contamination and debris. The washed parts were dried and crushed in a mortar pestle. Methanol was added individually to each plant part and centrifuged for 15 minutes (10000 rpm). The supernatant obtained was made up to the final concentration by adding methanol and stored for the bioassay protocol.

Antifungal testing

Day one

Sterile Czapek dox agar media (CDA) was poured in the sterile Petri dishes a day before the bioassay and allowed to be solidified. Around 5 mm sterile discs of Whatman's filter paper were prepared and autoclaved. These sterile discs were soaked completely in the botanical extracts already prepared. For controls, the disc was soaked in the fungicide fluconazole. The commercial fungicide was used as the control to analyze the results. Disc diffusion method introduced by Reference [9] was conducted for the bioassay [9].

Day two

The soaked disc if changes color and gets fully impregnated in the extracts, considered suitable for the antifungal bioassay. The CDA poured Petri dishes will be marked with the exact center position of the impregnated disc. A sterile autoclaved cotton bud will be dipped completely in the fungal broth of the pathogenic fungus and allowed to spread uniformly on the media. This process allows the uniform spread of the pathogenic fungus all over the media. The impregnated disc will be carefully placed in the center. A similar procedure will be followed for the controls. The Petri plates were sealed properly and incubated for 5 to 7 days at 25±2°C. zone of inhibition in millimeters was measured of both the treatments and the controls.

Statistical analysis

For the antifungal bioassay, colony diameter in millimeters were measured for evaluations. Average measurements were studied for each treatment. One way analysis of variance (ANOVA) was conducted to study the significant difference among the individual treatment and controls. Tukey's HSD test was done to study the significant differences among the groups. The measurements in the graphs and tables are Mean ± SI.

III. RESULTS

The efficacy of methanol extracts of *O. sanctum* showed positive effects on the growth retardation of *Alternaria alternata* (Fig. 2). Disc diffusion method was used for the bioassay. Both the diseased plants were treated with the botanical extracts of *O. sanctum.* The results are reported in Table 1 which shows a huge impact of the botanical extracts against the pathogenic fungus *Alternaria*. For both the diseased plants, leaf methanolic extracts showed better results compared to stem and flowers (Table 1). For *A. marmelos*, a maximum of 23±2 mm inhibition zone was measured whereas, for *E. officinalis*, 25±2 mm was observed for the leaf extracts prepared in methanol solvent. Whereas controls showed 13±1mm and 13+2 mm for *A. marmelos* and *E. officinalis*, respectively. On the contrary, the shoot extracts for *A. marmelos* showed a zone of inhibition of 22±3 mm. Similarly, the flower extract in *E. officinalis* showed 24±2 mm. These results indicate the close comparisons and the phenomenal impact of the botanical extracts of *O. sanctum* prepared in the methanol solvent. Figure 1 shows the graphical representation of the results which indicate the significant difference (P<0.05) of all the treatments in comparison to the controls which were the commercial fungicide.

Table 1. Effect of methanolic extracts prepared with three plant parts of *O. sanctum* against *A. alternata* isolated from *A. marmelos* and *E. officinalis*. Average measurements of colony diameter (mm) were studied for evaluations.

Medicinal plant	Concentration (mg/ml)	Leaf	Shoot	Flower	Controls
			Colony diameter (mm)		
Aegle marmelos	100	23±2	22±3	17±2	8+1
	100	21±1	18±2	12±1	10±1
	100	15±1	12±1	13±2	13±1
Emblica officinalis	100	25±2	23±2	24±2	13+2
	100	20±2	19±2	20±2	10±0
	100	20±2	18±1	17±1	13±1

***N=9**

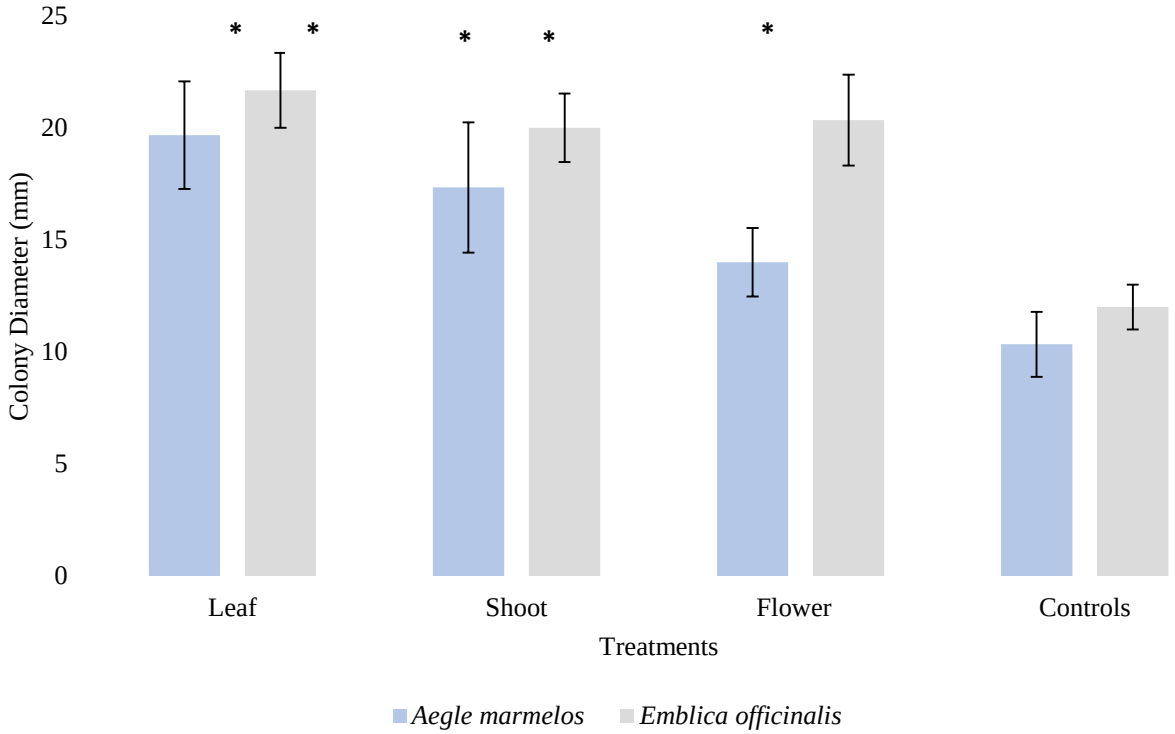

Figure 1. Effect of methanolic extracts prepared by three plant parts of Aegle marmelos and Emblica officinalis on growth inhibition of Alternaria alternata. Leaf, shoot, and flowers were used for preparing botanical fungicides. Commercial fungicide was taken as controls. Growth inhibition was higher in the plant extracts. * indicates $P<0.05$ from ANOVA and Tukey's HSD test.

Figure 2. Antifungal bioassay. In situ experiments showed greater inhibition of fungal pathogen by plant extracts compared to the commercial fungicides. A. infected plant parts of *Aegle marmelos*. B. infected plant parts of *Emblica officinalis*. C. healthy plant parts of medicinal plant *Ocimum sanctum*. D. treatment (plant extract). E. control (commercial fungicide).

IV. DISCUSSION

Ocimum sanctum is rich with vital phytochemicals including phenols, flavonoids, terpenoids, coumarins, steroids, neolignans, phenyl propenoids, essential oils, and many other secondary compounds [10]. The leaves have been reported to be the richest source of all the mentioned phytochemicals. This data supports our results which show the greatest impact of the leaf extracts against the growth retardation of phytopathogen *Alternaria*. A lot of literature is available for the use of *O. sanctum* for the study of several life-threatening diseases including cancer, diabetes, and many more. However, very little work has been conducted for the botanical extracts of *O. sanctum* against antifungal potential. This study has been focused on the growth retardation of isolated fungus *Alternaria* from two disease medicinal plants *A. marmelos* and *E. officinalis*. moreover, this study also aims to improve the production of both the diseased plants which are themselves occupy a great medicinal value. Reference [11] studied the phytochemical compounds of four medicinal plants including *O. sanctum.* Results indicated the presence of alkaloids, phenols, flavonoids, terpenoids, carbohydrates, and reducing sugar in *O. sanctum*. However, a maximum of these compounds was present in the leaves [11]. This again supports the results mentioned in Table 1. The botanical extracts prepared from weed plants have also been studied which have great potential against microbial

growth [12]. The presence of these compounds does not have side effects and is a great substitute for antibiotics [13].

The botanical fungicides have been seen to be time-consuming, cheaper, and easily available. They do not require many resources and impose no side effects on the plants. The use of medicinal plants in both the diseased and curing provide the best platform to save the environment healthily and most safely. This technique can provide a great opportunity for plant growth and crop protection.

V. CONCLUSION AND FUTURE SCOPE

The study shows an alternative methodology to treat microbial community destroying the plant growth and development. This research brings forward the traditional, organic, cheaper, and absolute harmless way to improve the crops. Medicinal plants play important role in environment and needs to be saved for the future. *Ocimum sanctum* is a medicinal plant which is easily available and holds great potential to treat the fungal diseases in plants. the three plant parts of the plants showed positive impact towards the two phytopathogens tested. Hence, we can conclude from this investigation that medicinal plants particularly, *O. sanctum* can be used as organic fungicides to control the plant diseases.

ACKNOWLEDGMENT

Sincere thanks go to the botany department, S.P.C. Government College, Ajmer, Rajasthan, India for unconditional guidance and facilities provided.

REFERENCES

[1]. J. Singh, S. K. Bhatnagar, and A. Tomar, "Study on fungicidal effect of plant extracts on plant pathogenic fungi and the economy of extract preparation and efficacy in comparison to synthetic/chemical fungicides", *Journal of Applied and Natural Science*, Vol. **11**, Issue **02**, pp. **333- 337**, **2019**.

[2]. GOI, "Economic survey 2010-11, Union Budget". Statistical appendice: Economic survey, **2011**.

[3]. D. Persley, "Diseases of fruit crops", Brisbane: Department of primary industries, **1993**.

[4]. S. A. J. Tarr, "Diseases of sorghum, sudan grass and broom corn", The Commonwealth Mycological Institute, Kew, **1962**.

[4]. P. Pattanayak, P. Behera, D. Das, and S. K. Panda, "*Ocimum sanctum Linn*. A reservoir plant for therapeutic applications: an overview", *Pharmacognosy review*, Vol. **04**, pp. **95–105**, **2010**.

[5]. S. Balakumar, S. Rajan, T. Thirunalasundari, and S. Jeeva, "Antifungal activity of *Ocimum sanctum Linn*. (Lamiaceae) on clinically isolated dermatophytic fungi", *Asian Pacific Journal of Tropical Medicine*, Vol. **04**, Issue **08**, pp. **654-657**, **2011**.

[6]. S. Sharma, R. Singh, and B. Thakre, "Antifungal activity of leaf extracts of *Ocimum sanctum* against fungal pathogens", International *Journal of Current Microbiology and Applied Sciences*, Vol. **08**, Issue **04**, pp. **1210-1214**, **2019**.

[7]. J. Guna Priya, K. Geetha Lakshmi, Niharika Sharma, D.Rajani, "A Comparative Study of Antioxidants and Antimicrobial activity in Vegetables, Leafy Vegetables and Spices," *International Journal of Scientific Research in Biological Sciences*, Vol.**06**, Issue.**01**, pp.**80-85**, **2019**.

[8]. C. Perez, M. Paul, and P. Bazerque, "Antibiotic assay by agar well diffusion method", Acta Biol Med Exp. Vol.**15**, pp. **113-115**, **1990**.

[9]. D. Singh and P. K. Chaudhuri, "A review on phytochemical and pharmacological properties of Holy basil (*Ocimum sanctum L.*)", *Industrial Crops and Products*, Vol. **118**: pp. **367–382**, **2018**.

[10]. S. Ojha, and M. Goyal, "Preliminary phytochemical screening of plant extracts prepared from traditional medicinal plants of Rajasthan", *Asian Journal of Research in Chemistry and Pharmaceutical Sciences*, Vol. **07**, Issue **03**, pp. **819-823**, **2019**.

[11]. D. Chittoriya, M. Goyal, and S. Ojha, "Analysis of phytochemical constituents of selected medicinal weed plants occurring in central region of Rajasthan", Asian *Journal of Research in Chemistry and Pharmaceutical Sciences*, Vol. **08**, Issue **01**, pp. **72-77**, **2020**.

[12]. P. J. Patil and J. S. Ghosh, "Antimicrobial Activity of *Catharanthus roseus* – A Detailed Study", *British Journal of Pharmacology and Toxicology*, Vol. **01**, Issue **01**, pp. **40-44**, **2010**.

AUTHOR'S PROFILE

Shruti Ojha has published over five papers and has attended several national and international conferences related to mycology. Her research work focusses on plant-pathogen interaction, plant pathology, and microbial study related to plants. She has completed her Master of Philosophy as merit holder in the university and aims to finish her doctorate soon. She has more than ten years of research and teaching experience in reputed institutions and laboratories.

