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"BIOCONTROL OF *ALTERNARIA* LEAF SPOT OF GERBERA BY USING PARTHENIUM EXTRACT "

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ABSTRACT

Gerbera (*Gerbera jamesonii*) is an important ornamental plant known for production of cut flowers. In gerbera massive losses occur due to diseases affecting gerbera growth and development. Among all the diseases, fungal diseases have been found to cause enormous losses. There is need for proper management and reduction of fungal pathogens infecting gerbera plant. The causative agents of diseases were isolated and identified by using morphological and molecular charactoristics. Molecular characterization involved isolating DNA from fungal isolate, PCR amplification of ITS region. Invitro antifungal activity of parthenium extract against *Alternaria* was carried out by using PDA cultures. The biocontrol potential of *Parthenium* was evaluated against *Alternaria.* The Parthenium extracts showed significant antifungal activity against isolated strain of fungal pathogens. Experimental result indicated that lower dilution of extracts, the antifungal activity was maximum.

Keywords: Gerbera, Fungal pathogens, Isolation and identification, Antifungal, plant extracts.

INTRODUCTION

Floriculture is the rapid growing sector in India [1]. In India, varieties of economically important flowers are cultivated such as marigold (*Tagetes erecta*), gladiolus (*Gladiolus grandiflorus*), tuberose (*Polyanthus tuberosa*), anthurium (*Anthurium andrenum*) and gerbera (*Gerbera jamesonii*). Gerbera is one of the important cut flowers in global floricultural market [2]. Gerbera is greatest and latest sensation to Indian floriculture sector. Gerbera (*Gerbera jamesonii*) is an important ornamental plant known for production of cut flowers. In gerbera massive losses occur due to diseases affecting gerbera growth and development. Among all the diseases, fungal diseases have been found to cause enormous losses. There is need for proper management and reduction of fungal pathogens infecting gerbera plant. [3]. *Alternaria* leaf blight is one of the major damaging and serious diseases of gerbera. *Alternaria* leaf blight

or leaf spot is an economically important disease, generally present in all gerbera growing areas and is considered as potential threat to gerbera cultivation [4, 5, 6]. One of the traditional ways to control diseases is use of fungicides [6]. Owing to the limitations on use of physical treatments, chemical control agents such as fungicides, application of biocontrol agents, etc. there is need of an alternative management strategy to control diseases. This strategy needs to be harmful against diseases and also should prove harmless in environment, so that do not enter the food chain. Recently, use of plant extracts in controlling diseases is gaining importance due to their antimicrobial properties. Presence of secondary metabolites and bioactive molecules such as steroids, proteins, etc. in plants help to reduce plant diseases. Various plant extracts from neem, garlic, turmeric, etc. have been known to show antifungal activity [8, 9]. So, use of appropriate plants for control of diseases in gerbera is required to be studied. Hence, in this study the research revolves around studying *Alternaria* disease and biocontrol potential of Parthenium against it.

MATERIALS AND METHODS

A purpose based sampling survey was conducted in Ahmednagar and Nashik district to study the occurrence and prevalence of diseases on gerbera. The vast amount of information was collected regarding the diseases on gerbera [7]. During the survey, the diseased parts from infected Gerbera plants were analysed and brought to the laboratory in autoclaved plastic bags. The samples obtained with typical diseased symptoms were identified and used further for isolation of pathogens [7].

Isolation of the pathogens and maintainance of pure culture: All infected Gerbera plants were taken to laminar air flow for careful observation and parts showing fungal growth were separated for isolation purpose. The infected region was cut into small pieces with clean, sterilized blade and inoculated on petri plates containing sterile PDA media supplemented with Ampicillin (100 mg/ml) by surface inoculation method. After 48 hrs of incubation, mixed fungal cultures were obtained on plates. These cultures were further subcultured several times to obtain pure culture by hyphal tip method. The inoculum consisting only particular species of organisms was transferred onto sterile Potato Dextrose Agar media, labelled properly, and kept in the refrigerator at 4°C for one month to maintain pure cultures [10].

Identification of the isolates: The isolates obtained were identified based on cultural, morphological and molecular characteristics. More than one criteria were used for authentification of the results. Molecular characterization involved isolating DNA from fungal isolate, PCR amplification of ITS region. From the above charactoristics the disease causing organism is identified as *Alternaria*. After lab level identification of the isolates, all the isolates in pure form were sent to NFCCI, Agharkar research institute, Pune for their further identification and confirmation from experts.

Preparation of *Parthenium hysterophorus* plant extract: To prepare extract of *Parthenium hysterophorus,* leaves were collected during flowering stage and washed thoroughly to get rid

off dust and contaminants. These leaves were cut into fine pieces and ground in sterile distilled water using sterilized mortar and pestle. 10 g of leaves sample was ground in 50 ml sterile distilled water and kept at root temperature for 48 hours. Aqueous extract was prepared by filtering this mixture thorugh filter paper and stored at 4 ºC till its further use [12].

Preparation of fungal cultures: The fungal cultures of *Alternaria* were isolated and identified based on cultural, morphological and molecular characters. These fungal cultures were used for antifungal activity. The isolated fungal cultures were maintained on PDA medium by routine subculturing and stored at 4°C till its further use for performing antifungal activity.

Antifungal activity of parthenium extracts against *Alternaria*: 100 µl of above prepared fungal suspension including spores were spread uniformly on sterile PDA medium plates for each fungi. On these plates, four wells (7 mm) were made using sterile tips. Medicinal plant extracts were prepared as mentioned above and different dilutions of each extract viz. 1:2, 1:4, 1:6 and 1:8 were prepared in sterile water.

EXPERIMENTAL RESULTS AND DISCUSSION

Isolation of the pathogens: The isolation of pathogens considered for the study was carried out from respective disease samples in the laboratory. Plates 1 shows the plant samples infected with different pathogens used for isolation of each pathogen. PDA (Potato Dextrose Agar) media plates and slants were used throughout the isolation procedure. PDA is considered the best and most standard media used for the isolation of the fungal pathogens. Initially, the mixed cultures were obtained for selected strain. Plates 2 displays the plates showing mixed culture isolated from specific diseased plant. These mixed cultures were then subcultured many times to obtain pure cultures. Plates 3 shows plates of pure culture obtained after continuous subculturing.

Maintenance of pure cultures: Selected fungal culture in pure form was maintained on PDA media plates and slants and stored in refrigerator at 4^0C. These cultures were labeled as "Master cultures", which were subcultured after every one month on fresh and sterilized PDA media to maintain activeness and viability of the cultures [10].

Identification: For the identification of the cultures, morphological, cultural and molecular characteristics were studied for selected *Alternaria* isolate. These characters helped in appropriate and exact identification of the isolates. Similar morphological characteristics of *Alternaria alternata* were also reported by Nagrale *et al* 2013 [5].

Cultural characters: The cultural characters of all five isolates of 7 to 9 days old culture on PDA media were studied and all the results were summarized in Table1 and fig number 2 .

Table 1: Cultural characters of *Alternaria alternata*

Cultural characters	Colony characters	Colonies: slow growing Colony color: Dark brown Colony diameter: 22 cm Surface texture: Velvety Colony elevation: None Growth pattern: Flowery

Lactophenol cotton blue staining:

Lactophenol cotton blue staining was done to observe the spores, hyphae and fruiting structures. After staining, these structures were observed as blue and the background was stained light blue in color as shown in figure 3. This staining aided in observing wall of hyphae against light blue background. The same results for fungal staining were also reported by Aneja 2003 [10] and Leck 1999 [11].

Molecular characterization:

For identifying the fungal isolate that infected Gerbera plants, morphological and cultural characters were studied from petri plates containing mycelial growth of each isolate. Further DNA was isolated from each isolate by CTAB method and resolved on 1% agarose gel as shown in Plate 11. This DNA was further used for amplification of specific region using PCR technique. The ITS region of fungal isolates was amplified using ITS1 and ITS4 primers. Based on the similarity and percent match, the isolates were identified.

Development of *Alternaria* leaf blight disease in *Alternaria alternata* infected Gerbera plants: Disease symptoms of *Alternaria* leaf blight was mostly observed on plant parts such as leaves and peduncles. The symptoms of disease caused by *Alternaria alternata*. Lesions were observed on leaves and peduncles. Initially small brown spots along with concentric rings were observed on leaves that coalesced later and covered maximum leaf area. Further, lesions increased with chlorophyll decomposition in leaves that led to change in leaf color from green to yellow. With progress in days after infection, there was progress seen in disease. *Alternaria* leaf blight had led to death of the gerbera plant. Also, dark spots were observed on dead leaves that indicated severity of *Alternaria* leaf blight disease [5].

Effect of *parthenium hysterophorus* extracts: Effect of *Parthenium hysterophorus* extracts on growth of fungal pathogens was studied by conducting antifungal activity. Antifungal activity was estimated by well diffusion method. Different volumes of each extract (1:2, 1:4, 1:6 and 1:8) were prepared in sterile distilled water and used for the study. Antifungal activity was performed against fungal pathogens infecting Gerbera plants as mentioned above. In this experiment, positive and negative control were used, where fluconazole was used as positive control whereas negative control consisted of inoculating only single fungal pathogen on PDA

medium separately. Results of antifungal activity are summarized in table **number 2** and fig number 4 and 5.

Table 2: Antifungal activity of *Parthenium* extracts on fungal pathogens infecting Gerbera plant

	Concentration							
	1:2		1:4		1:6		1:8	
N.	IC	Mean ± SD	IC	Mean ± SD	IC	Mean ± SD	IC	Mean ± SD
1	11.4	11.4 ± 0.5	11.2	11.2 ± 0.7	11.1	11.1 ± 0.5	10.9	10.9 ± 0.5
	10.9		10.5		10.5		10.3	
	12.1		12.2		11.7		11.5	

*MIC- minimum inhibition, SD- Standerd Deeviation

Effect of *Parthenium hysterophorus* plant extract on fungal pathogens:

Table 4 displays the effect of four dilutions (1:2, 1:4, 1:6 and 1:8) of *Parthenium hysterophorus* plant extract on *Alternaria alternate.* Using *Parthenium hysterophorus* plant extract against different fungi, highest antifungal activity of pathenium was observed at 1:2 dilutions.

Fig 1 *Alternaria* leaf spot (Infected plant)

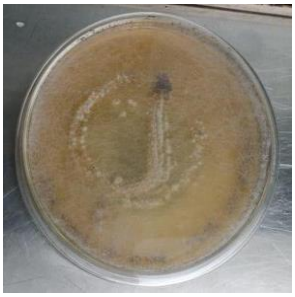
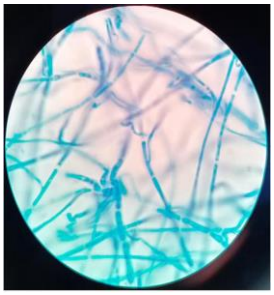


Fig 2 *Alternaria* cultural charctoristics



ig 3 *Alternaria* morphological charactoristics

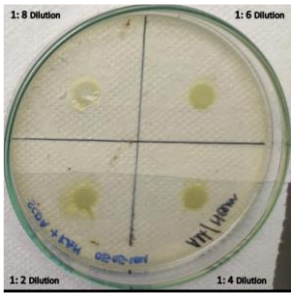


Fig 4 *Alternaria – Parthenium hysterophorus* Extract - Before

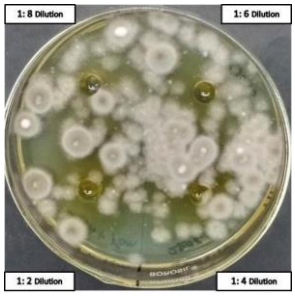


Fig 5 *Alternaria - Parthenium hysterophorus* Extract – After

REFERENCES

[1]Saikia, B., Mazumder, N. and Dutta, P. 2020. Studies on foliar fungal diseases of Gerbera (*Gerbera jamesonii*) in high humid condition of Assam. Bulletin of Environment, Pharmacology and Life Sciences. 9(2): 111-115.

[2]Praveen, N.M., Vijayaraghavan, R., Beena, S. and Krishnan, S. 2018. Occurrence of powdery mildew disease of gerbera in Kerala. J. Horti. Sci. 13(2): 217-220.

[3]Deshmukh, G., Jhade, R. K. and Alawa, S. L. 2019. Economic feasibility of gerbera (*Gerbera jamesonii* L.) under protected cultivation with special reference to Chhindwara district of Madhya Pradesh International Journal of Chemical Studies. 7(2): 1765-1768.

[4]Bhat, H. A., Ahmad, K., Ahanger, R. A. Qazi, N. A. Dar, N. A. and Ganie, S. A. 2013. Satus and symptomatology of *Alternaria* leaf blight (*Alternaria alternata*) of Gerbera (*Gerbera jamesonii*) in Kashmir valley. African journal of agricultural research. 8(9): 819-823.

[5]Nagrale, D. T., Gaikwad, A. P. and Sharma, L. 2013. Morphological and cultural characterization of *Alternaria alternata* (Fr.) Keissler causing blight of gerbera (*Gerbera jamesonii* H. Bolus ex J. D. Hook). Journal of applied and natural science. 5 (1): 171-178.

[6]Bhat, H. A., Ahmad, K., Ahanger, R. A., Wani, S.h. Bhat, A. H. and Dar, S. A. 2017. Fungicidal management of leaf blight of gerbera (*Gerbera jamesonii* Hook) in Kashmir valley. Journal of Pharmacognosy and Phytochemistry. 6 (4): 384-387.

[7]Praveen, N. M., Vijayaraghavan, R., Kumar, K. A., James, D 2017. An investigation on the etiology and characterization of fungal diseases of Gerbera. International journal of current microbiology and applied sciences. 6 (6): 2038-2043.

[8]Waghmare, M. B. 2012. Efficiency of mycotoxins of some plant extracts against *Alternaria alternata* (Fries) Keissler causing leaf spot of Gerbera. Current Biotica. 6(2): 240- 245.

[9]Shukla, A. and Dwivedi, S. K. 2012. Bioefficacy of plant extracts against Fusarium species causing wilt in pulses. IOSR journal of Engineering. 2(1): 136-144.

[10] Aneja, K. R. 2003. Experiments in Microbiology, Plant Pathology and Biotechnology. New age international publications limited. Fourth edition.

[11] Leck, A. 1999. Preparation of Lactophenol cotton blue slide mounts. Community eye health journal. 12 (30): 24.

[12] Parthasarathi, T., Suganya, V. and Sivakumar, R. 2012. Allelopathic Effect of Aqueous Leaf Extract of *Parthenium hysterophorus* L. on Seed Germination and Seedling Growth in Greengram, Blackgram and Groundnut. Madras Agric. J., 99 (7-9): 514-517.