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Screening of Bio-control agent for the Eco-friendly Management of fungal Diseases of *Aloe Vera*

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Abstract: ***Fungal Disease of Aloe Vera a important medicinal plant clearly indicates that the plant is attacked by many fungi. it was recorded at all the sampling site given in the paper. A total 15 fungi were recorded. Among these Trichoderma viride Imposed maximum inhibition to the growth the growth of the all fungi. Bio-control agent of Trichoderma viride was found significant on in-vitro growth of fungi. So Trichoderma viride was the most effective antagonist against the test fungi.***

Keywords: *Aloe vera Trichoderma viride* ,fungi, Bio-control agent

1. Introduction

Aloe vera is a species of *Aloe*, native to Northern Africa. It is a stem less or very short stemed succulent plant growing to 30-36 inches tall, spreading by offsets and root sprouts. *Aloe vera* has long been a popular houseplant. Often called the 'miracle plant' or the 'natural healer', *Aloe vera* is a plant of many surprises. It flourishes in warm and dry climates, and to many people it looks like a cactus with fleshy thorny leaves. In fact it is a member of the Lily family, staying moist where other plants wither and die by closing its pores to prevent moisture loss. There are over 300 species of *Aloe*, but it is the *Aloe barbadensis* Miller (*Aloe vera* or "true *Aloe*") plant which has been of most use to mankind because of the medicinal properties it displays. Fully grown, the plant stands 30-36 inches high, and a mature leaf is 2.5-3 inches wide at the base, weighing 1.5 to 2 kg. Ancient records of the Egyptians, Arab, African, Asians and Americans have discussed the different uses and pathological cases in which *Aloes* were administered. *Aloe* was cultivated in Egypt thousands of years ago and was used by the people of the Mediterranean at least 400 years before Christ. *Aloe* is also mentioned in the Bible's New Testament. The Arabs had taken *Aloe vera* plants to India and the Indian people called it savari, The Indians also named *Aloe* Ailwa from which the Greek word alon might have been derived. *Aloe* was also mentioned in ancient Chinese transcripts. It was employed medicinally for eczematous skin conditions in China andIndia under the name Luhui in China and Musabbar in India. The Greeks knew *Aloe* through the Indians. The Greek physician Peter Pedanius Dioscoriades wrote about *Aloe* in his medicinal plant collection materna medica. *Aloe* was first illustrated in the Codex Aniciae Julianae which was written around the year 512 A.D. by Dichotomous. *Aloe* was also mentioned in the writings of the Latin writer, Aurelius Celsus, who wrote a book about medicine and called it De Medicina, which appeared for the first time in the year 1378. In America, *Aloe* was mentioned in Columbus' journals.

It also contains Vitamins B1, B2, B3, B5, B6 and B12 along with choline, calcium (teeth and bone formation, muscle contractions and heart health), magnesium(strengthens teeth and bones, maintains healthy muscles and nervous system, activates enzymes), zinc (speeds up wound healing, mental quickness, assists with healthy teeth, bones, skin, immune system and digestive aid), manganese (activates enzymes, builds healthy bones, nerves and tissues), chromium (assists with protein metabolism and balancing of blood sugars), selenium which all influence our brain performance.

The benefits of using *Aloe vera* were discovered long ago in ancient times. Today, people are still using *Aloe vera* for its many restorative and healing properties. The pulp (gel) of the Aloe leaf has been used both externally and internally by many people. But before learning of the many benefits for using an Aloe Vera product, you should first learn about *Aloe vera*.

Therefore it was decided to take a causal survey of places in and around Jabalpur and record the fungi causing destruction to such a economically important plant. The chemical control strategies were found not to relevant and biological control strategies was found to be appropriate for it therefore the object of keeping in view the economic importance of *Aloe vera*

2. Material and Method

2.1 Survey and Collection of Samples

A systematic survey of different areas of Jabalpur was made and infected part of host plant (*Aloe Vera*) were collected in polythene bags and brought to the laboratory for further mycological analysis.

2.2 Preservation of Samples

For proper drying, diseased plant sample collected during survey were kept between two sheets of blotting paper and placed on a flat surface and kept pressed with light weight material paper was changed regularly after every 24 hrs. till complete dryness. Dried samples were kept in envelopes in mycological herbarium of BCRBC (Biodiversity Conservation and Rural Biotechnology Centre).

2.3 Isolation of Fungi

Two methods *viz;* direct isolation and dilution method or pour plate method were employed for isolation of fungi.

(A) Isolation from Infected Plant Parts
Infected parts of the plant were surface sterilized with 0.05% NaOCl for 3 min and cut into small pieces with the help of sterilized blade. These were then transferred in petridishes containing Potato- Dextrose Agar medium supplemented with Chlorophenical @0.75mg/L (Agrawal & Hasija, 1986). These were incubated at 28 $\pm$ 1 ^{0}C in a BOD incubator and observed daily. Colonies appeared on the surface were transferred directly to PDA slants (Martin 1970; Tsao 1970; Agrawal & Hasija 1986).

B) Pour Plate Method or Dilution Method
10 grams of each sample was oven dried and grinded in vortex miser. Theses samples were dissolved in 100 ml of sterile distilled water and thoroughly shaken to obtained 1:10(10-1) dilution and then serial dilutions were prepared by adding sterile distilled water. 1.0 ml of each sample was pipeted aseptically in pre-sterilized petridishes and PDA medium was poured. These were incubated in a BOD incubator at 28 $\pm$ 1 ^{0}C, and as soon as colonies appeared were transferred to PDA slants (Agarwal & Hasija 1986).

2.4 Microscopic Studies and Identification of Fungi

Identification of fungi was done after studying the morphological and cultural characteristic with the help of manuals monographs and papers of various workers. Identification of isolated of fungi was made on the basis of the morphological characteristics with the help of available literature (Subramaniam 1971; Van Arx 1981; Barnet and Hunter 1972; Clement and Shear 1931; Ellis 1971, 76; Ellis and Ellis 1985' Thomas and Raper 1945; Sutton 1980).

2.5 Determination of Frequency

The frequency of different fungi was determined by using following formula.

$$= \frac{\text{Total no. of colonies of Individual Fungus in a plate}}{\text{Total no. of different fungi in a plate}} \times 100$$

$$\text{Percentage (\%) Frequency} = \frac{T_1}{T_2} \times 100$$

Where
T_1 = Total no. of colonies of Individual Fungus in a plate
T_2 = Total no of different fungi in a plate

2.6 Screening of Antagonist

Screening of *Trichoderma viride* against isolated pathogen (Singh *et al*., 2014) was carried out by following methods:

***in vitro* Colony interaction:-**

The pure culture of the test isolate was grown in petri plates containing autoclaved solidified PDA medium and were inoculated with the culture of two micro organisms (target and antagonist). Antagonist (*Trichoderma viride*) strains and test pathogen containing young mycelial growth were placed adjacent to each other, 3-5 cms apart. All experiments were carried out induplicates. The placement of inoculum was in such manner so as to give equal opportunities to the two test fungi for growth (Johnson and Curl, 1972). A separate set containing only test pathogen was kept as control. The plates were incubated in BOD incubator for 7 days at 28 $\pm$ 2 ^{0}C.

Percent inhibition in growth (colony diameter) was calculated by the formula suggested by Vincent (1974).

$$\text{Percent (\%) inhibition} = \frac{r_1 - r_2}{r_1} \times 100$$

Where
r_1 = diameter of pathogen (in control).
r_2 = diameter of pathogen from point of inoculation toward interacting site of antagonists.

All the experiments were done in duplicates and average of two was taken.

3. Result

The main objective of present investigation was to assess the ability of various fungi which cause fungal diseases on *Aloe Vera.* A total number of fifteen fungi were recovered from the different infected parts of plant from different areas of Jabalpur.

Table 1: Percentage frequency of various fungi associated with infected *Aloe Vera* plant

S. No.	Name of Fungi	AVFC. No.	Frequency Percentage
1	*Aspegillus niger*	AV # 04	46.15%
2	*Aspergillus fumigatus*	AV # 03	50%
3	*Alternaria alternata*	AV # 08	30%
4	*Alternaria dianthi*	AV # 11	33%
5	*Curvularia lunata*	AV # 09	73.3%
6	*Fuasrium roseum*	AV # 15	50%
7	*Fusarium oxysporium*	AV # 20	90%
8	*Colletotrichum dematium*	AV # 18	42.85%
9	*Nigrospora oryza*	AV # 24	50%
10	*Rhizopus sp.*	AV # 26	52.3%
11	*Phoma multirostrata*	AV # 19	42.2%
12	*Epicoccum sp*	AV # 32	26.13%
13	*Torulla sp*	AV # 25	21.12%
14	*Mucor sp*	AV # 22	69.28%
15	*Trichoderma viride*	AV # 14	66.6%

4. Distribution and Frequency

During the course of present investigation isolation of fungi from the different infected parts of plant were done. Pour plate method and direct isolation method were mainly used. PDA media was found quite satisfactory for isolation of fungi. Observation made during entire period of investigation reveal that maximum fungi were encountered in pour plate or soil dilution method, when PDA medium was used. A total no. of fifteen fungi were isolated (Table-1). Fusarium was the most prevalent fungus represented by two spp. Amongst the various *Fusarium species*. Fusarium oxysporium was the most dominant spp. (90%). It was followed by *Drechslera australiensis* (73.3%) & *Trichoderma viride* (66.6%), *Alternaria dianthi* was encountered in few samplings (33.3%) *Alternaria alternate*, & *Aspergillus Fumigatus* also showed maximum frequency in comparision to others Many fungi *viz; Fusarium oxysporium* are known to incite severe infections in various plant spp. (Bilgrami *et al* ., 1979,81,91; Sarabhoy *et al.*,1986) these were also encountered in the infected part at considerable frequencies (Bilgrami *et al* ., 1979,81,91). The antifungal properties of many saprophytic fungi were also recorded abundantly in almost all the samples during the present investigation. *Trichoderma spp*. were selected for further investigation.

4.1 Screening of antagonistic fungi

Indigenous isolates of *Trichoderma viride* were evaluated for their antifungal activity against some plant pathogens as evident from the Table-2 the test fungi, *Trichoderma viride* imposed maximum inhibition in the colony diameter *Aspergillus fumigatus* it was followed by *Colletotrichum dematium* & *Fusarium oxysporium* few of the spp. remain unaffected growth of which was significantly reduced by *Trichoderma viride* beside, inhibition in growth, *T.viride* was also found to be the most effective hyperparasite.

On the basis of experimental evidence, it is apparent from the preliminary data that *Trichoderma viride* are promising sources of antibiotics and hyperparasitism and have considerable potential as biocontrol agent. Hence keeping in view the hazardous impact of agrochemicals on the ecosystem, these organisms must be evaluated extensively and developed as biocontrol agent for the management of soil borne plant diseases.

Table 2: Percentage Inhition of various test pathogen by *Trichoderma viride* strain

S. No.	Name of Fungi	AVFC No.	Percentage Inhition
1	*Aspegillus niger*	AV#04	56.15%
2	*Aspergillus fumigatus*	AV#03	62%
3	*Alternaria alternata*	AV#08	40%
4	*Alternaria dianthi*	AV#11	43%
5	*Curvularia lunata*	AV#09	73.3%
6	*Fusarium roseum*	AV#15	58%
7	*Fusarium oxysporium*	AV#20	80%
8	*Colletotrichum dematium*	AV#18	52.85%
9	*Nigrospora oryza*	AV#24	50%
10	*Rhizopus sp.*	AV#26	48%
11	*Phoma multirostrata*	AV#19	68%
12	*Epicoccum sp*	AV#32	62%
13	*Torulla sp*	AV#25	76%
14	*Mucor sp*	AV#22	81%

5. Summary and Conclusion

Aloe Vera belongs to genus Aloe of the family Asphodelaceae the genus Aloe contain several spp. *Aloe vera* is a common medicinal plant. Various fungi are responsible for the principal disease affecting *Aloe vera*. The most recurrent diseases include aloe rust, sooty mold, basal stem rot and other diseases cause damage to almost all medicinal and field crops. Therefore "Fungi Associated with *Aloe vera* Plant and its Biological Management" is a very important work keeping in mind to the substantial losses and the main objective of this work is to screen out an effective method to combat plant pathogens. Biological control is an important process to reduce incidence of diseases caused by pathogen, infected samples of different infected part were collected from different areas of Jabalpur were brought to laboratory for analysis. Looking to the potential of antagonistic fungi the present work was carried out to find out sustainable antibiotic producing strains of fungi inhabiting *Aloe vera* plant. A total no. of fifteen fungi viz; *Fusrium roseum, Fusarium oxysporium, Alternaria alternate, Alternaria dianthi, Aspegillus niger, Aspergillus fumigatus, Drechslera australiensis, Curvularia senegalensis, Colletotrichum dematium, Nigrospora oryza,* and *Trichoderma viride* were isolated from the leaves sample collected from different areas. Antifungal activities of T.viride were tested against all the fungi.

On the basis of data recorded in the present investigation it can be concluded that *Trichoderma viride* have enormous potentiality to produce antifungal antibiotics can be exploited, for Biological control needs through investigation. It may give promising control to disease caused by above pathogens. Indigenous isolates of *Trichoderma viride* were evaluated for their antifungal activity against some plant pathogens and it is apparent from the preliminary data that the *Trichoderma* act as a promising source of antibiotics and hyperparasitism. The present work aim at establishing the continued need for focused scientific investigation on the subject of "Isolation, Identification & Biological Managment of fungal diseases of *Aloe vera* Plant". It outlines possible directions for future research that could significantly expand

the maximum knowledge in this field. Future work should involve a more extensive research for fungal incidence on *Aloe vera* and the biological control agents biologically sound control management programmes must be built upon general enhancement of antagonistic microbial antagonism to control the fungi. Considerable work on biocontrol of various fungi has already been done and the results are encouraging the advantages of biocontrol are numerous.

Finally in general it can be concluded that the mystery of microbes can be unraveled and their potentials are applied a biocontrol agents. By using microbial technology we will in harmony with nature's checks and balance as a consequence of which sustainable and profitable agriculture can be enjoyed which is beneficial to all ecofriends including human beings.

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