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STUDIES ON LEAF SPOT OF ALOE (*ALOE VERA*) CAUSED BY *FUSARIUM SOLANI (MART)* SACC.

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Abstract- The present investigation entitled "Studies on leaf spot of Aloe (*Aloe vera*) caused by *Fusarium solani (Mart)* sacc" was done for accessing symptoms of the pathogen including its isolation, purification and pathogenicity test. It also included the study of various factors favoring disease, Biochemical changes in aloe leaf, as influenced by fungal infection. *In vitro* evaluation of chemicals, botanicals and bio agents against *F. solani was also done. Fusarium solani* was observed to be associated with the symptoms on the leaves of *Aloe vera* and the leaf inoculation method was observed to be the best for the confirming the pathogenicity of causal agent. It was found that the fungus grew profusely at 30^{0}C and pH 8.0. Potato dextrose agar medium was observed to be the best for fugal growth. Carbendazim at 250,500 and 1000 ppm concentrations inhibited the fungal mycelium when amended with PDA. Garlic bulb extract was observed to most effective out of six botanicals tested. Similarly, *T. harzianum* inhibited the fungal growth to its maximum. Decreased concentrations of N, P, K, Cd, Ni, Zn, protein and aloin was observed due to infection where as concentration of Fe and Mn was increased. The concentration of Pb remained unchanged.

Keywords- leaf spot, pathogenicity, *in vitro,* inoculation etc.

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Introduction

Aloe vera (*Aloe barbadensis (Mill.*) [Syn. *Aloevera* (L.), commonly known as Ghikanvar or Guar patta, member of family Liliaceae is a native of Canaries (South Africa) naturalized in the Mediterranean and introduced in the warm and dry climate of India. *Aloe vera* is one of the important medicinal plants in India and is known for its various nutritive and medicinal values. This plant is considered as "a new plant resource with the most promising prospects in the world". The mucilaginous pulp of plant is found to be full of 200 components and 74 known nutrients along with 93-96 percent of water, rest is solid which contains active principle like glucose, galactose, mannose and galacturonic acid, unidentified aldopentose, protein (0.013 %) with 18 amino acids, B sitosterol, Vitamins (B_1, B_2, B_6 and C) minerals, cholesterol, steroids and lignin in small proportions. This has the higher quantities of anthraquinones including Aloin, Barbaloin, Aloe emodin and Amodin, which are responsible for the cathatic action.

The crop is attacked by three major foliar diseases *viz*., leaf spot caused by *Alternaria alternata* and *Fusarium solani;* leaf rot by *Erwinia chrysanthemi* and leaf rust caused by *Rare nelia aloii*. Leaf spot, also known as ring spot, caused by *F. solani* becomes an important and limiting factor in its cultivation because the disease reduces the quality and yield of leaves due to which the market value declines. This crop is also vulnerable to certain abiotic, non-pathogenic and physiological disorders such as slime mold and tip dieback. The present studies were proposed to delineate the effects of leaf spot caused by *F. solani* and its management employing chemicals, botanicals and bio-agents.

Materials and Methods

The method involved studying symptoms of leaf spot caused by *Fusarium solani* during the months of November to April. The symptoms were first seen on the upper leaves of 2 to 3 month old aloe plant in the form of water soaked, brownish small circles. In older spots, a dark pinkish brown dead tissue is formed. After that Collection, isolation and purification of pathogen from diseased samples were done from the fields of Botanical garden, Department of Crop and Herbal Physiology, J.N. Krishi VishwaVidyalaya, Jabalpur in order to carry out our present investigation. Isolations were made from the leaves of diseased plants showing characteristic symptoms on PDA media by incubating fungus at 25±1^{0}C for seven days. The various methods involved in doing so involved Leaf inoculation method, Detached leaf technique and Pin-Prick method. Effect of various environmental factors like effect of temperature *viz*., 20, 25, 30 and 35 ^{0}C were selected to study the effect of temperature on growth, sporulation and conidial development of *Fusarium solani,* effect of pH to the levels of 5, 6, 7, 8 and 9 by adding Hydrochloric acid (HCL) and sodium hydroxide (NaOH) of N/10 concentration prior to pouring on solid media like Potato dextrose agar (PDA), Czapek's agar medium (CA), Maize Sand Medium, Richards agar medium and soil extract agar medium were tested for the effective growth of the test fungus and Corn-meal sand medium was used as non synthetic media. A total five fungicides *viz*., Carbendazim, Carboxin, Captan, Mancozeb and Thiram at 250, 500 and 1000 ppm concentration were evaluated on various media mentioned above for study of growth rate and conidial development of *Fusarium solani* under varying condition. Further on evaluation of plant leaf extract of important plants namely Neem (*Azadirachta indica*), Lantana (*Lantana camera*),Ipomoea *(Ipomoea fistula)*, Ashok (*Polyanthus longfolia*) Tulsi (*Ocimum sanctum*) and bulb of garlic (*Allium sativum*) were also done on the growth and sporulation of the fungus. Three bio-control agents which included one bacterium *Basilus subtilis* and two fungal organisms *Trichoderma harzianum* and *T. viride* were tested for their antagonistic efficacy against *Fusarium solani*. The fungal bio-agents were multiplied on PDA and *B. subtilis* was maintained and multiplies on nutrient agar medium. The technique of dual inoculation as suggested by [1] was followed to study the antagonistic effect of the organism against *F. solani*. For the estimation of aloin contents of leaves high Performance thin layer chromatography (HPTLC) method was used.

Results and Discussion

Aloe (*Aloe barbadensis)* is attacked by two major foliar diseases *viz*., leaf spot

caused by *Alternaria alternata* and *Fusarium solani* individually and leaf rot in which *Erwinia chrysanthemi* is associated as a pathogen. Out of these the leaf spot ,also known as ring spot caused by *Fusarium solani* ,became an important limiting factor in its cultivation as the disease reduces the quality and yield of leaves due to which the market value declines. Therefore, the present investigation were undertaken to evaluate suitable synthetic media for the mass production of *Fusarium solani*, determination of temperature and pH requirement for its proper growth and sporulation. Experiments were also conducted to evaluate the effect of leaf spot fungus on the nutritional composition of leaf along with the pathogenic behaviour of *F. solani.*

Identification and morphology – *Fusarium solani* was consistently found to be associated with the disease. The pure culture of the organism grows as grayish white and fluffy mycelium with a deep violet-blue pigment developed on Potato Dextrose Agar medium. Hyphae are septate and hyaline. Conidiophores are simple (non-branched) or branched monophialides (phialides with a single opening). Macro conidia are moderately curved, stout, and thick-walled; usually 3-5 septate, measure 4-6 x up to 65 µm long, and are borne on short conidiophores that soon form sporodochia. Microconidia are borne from long monophialides, are one to three-celled, 2-5 x 8-16 µm long, and occur in false heads only (in cluster of conidia at the tip of the phialide). Chlamydoconidia are present (sometime Profuse) and occur both singly and in pairs. The organism was also isolated by [2,3] who identified the organism as *F. solani* on the basis of characters describe above.

Pathogenicity: The pathogenic behavior of *F. solani* was confirmed by adopting three methods namely pin prick, leaf inoculation and detached leaf techniques. Although the pathogenicity was confirmed by following all the methods. The symptoms appearance on 6th days after inoculation in leaf inoculation method where as appearance of lesion was noted on 10th and 12th days in detached leaf and pin prick methods respectively. [4] have also noted the similar results on moth bean infected with *Myrothecium roridum*, and [5] on *Murrya koenigii*.

Influence of various factors on growth rate and conidial development of *Fusarium solani*

Effect of different level of temperature on growth and sporulation of *Fusarium solani*

Temperature is one of the essential requirements for the growth of any living entity existing on this earth. During the course of investigation on the effect of temperature the maximum radial growth (36.2, 70.0, 90.3 mm) was recorded at 30 ^{0}C followed by 25 ^{0}C (26.0, 60.0, 74.4 mm) at 10th, 12th and 15th day after incubation. These results are in conformation with the earlier studies carried out by [6-8].

Effect of pH on growth: The experiments conducted on the effects of various pH ranges revealed that radial growth (26.13 mm) and dry weight (21 mg) of *Fusarium solani* was noted maximum at pH 8, followed by pH 7(17.0 mm) and minimum at pH 9. These finding are in accord with that of [9,10] who also reported that the fungal growth is favored at pH 8.

Effect of media: Testing of various five solid media was done to obtain better mycelium growth of *Fusarium solani*. The finding revealed that the fungus made good mycelial growth and maximum dry mycelium growth on PDA and this medium was noted to be best substrate favoring the growth of *F. solani* [8] also reported that *Fusarium solani* grew best on Potato dextrose agar. [11-13] have also found that PDA is the best medium for the growth of *Fusarium solani.*

In-vitro* evaluation of different chemicals, botanicals and bio-agents against *Fusarium solani:

***In-vitro* evaluation of different chemicals:** Five fungicides namely macozeb, thiram, carboxin, captan and carborandom and maximum radial growth was recorded in mancozeb (74.0 to 78.03 mm) followed by carboxin (34.6 to 56.03 mm). Carbendazim showed inhibition at all concentration followed by captan and thiram. These results are in accord with the findings of [14 and [15] where they observed that carbendazim @ 10 ppm completely inhibited the growth of *F. solani* in PDA amended medium. [13] while working on Soybean also reported that carbendazim inhibited the growth of *F. solani* under *in vitro* conditions. Carbendazim, mancozeb and thiram were found to inhibit the growth of *Fusarium solani.* This finding also confirms that captan, mancozeb and thiram worked well in inhibiting mycelial growth of *F. solani* under laboratory condition when amended with potato dextrose agar. Complete inhibition of *F. solani* by carbendazim (250 ppm) reported by [16].

In *vitro* evaluation of different botanicals: Out of six botanical's tested against *F. solani*, mean radial growth (mm) of the fungus was maximum in Neem (*Azadirachta indica*) and minimum in Garlic (*Allium sativum*). [17] while working with fungitoxic properties of plant extracts have also reported the superior efficacy of *Allium sativum* against *F. solani.* Lantana (*Lantana camera*) however, inferior to garlic but showed its superiority over rest of the botanicals. [18] have also reported the efficacy of *Lantana camera* while working with *F. oxysporum*. [19] the efficacy of plant extracts in inhibiting the growth of *F. solani* (causing wilt in Okra) was investigated *in vitro* using the paper disc method and found that garlic extract produced maximum inhibition. Effective control of *F. solani* by bulb extract of garlic followed by lantana was also reported by [20].

In *vitro* testing for bio-control efficacy of antagonists: The experiment using three bio agents, *viz*., *Trichoderma harzianum, T. viride* and *Bacillus subtilis* against *Fusarium solani* following dual culture method showed that maximum inhibition zone (8.87 %) was formed against *Fusarium solani* by *T. harzianum* and minimum radial growth (8.3 %) was observed agaist *T. viride*. [21] have also noted the superior efficacy of *T. harzianum* against *Fusarium* in Soybean and *Bacillus subtilis* was the second best organism inhibiting the growth of *F. solani* and showed its efficacy over *T. viride*. [22,23] have also reported the similar results against *F. solani* in Soybean. *T. harzianum* is one of the best bio control agents followed by *Bacillus subtilis* against *F. solani* reported by [24].

Effect of *Fusarium solani* on the nutritional composition of aloe leaf: The substance, which are produced or degrade during biochemical changes caused by host parasite interaction may pre-dispose the host or may develop resistance in the host in response to pathogen. A biochemical change in plant caused by pathogen is closely related to the process of pathogenesis. The investigations were carried out to evaluate effect of disease on level of different essential nutrient present in the leaves. The findings revealed that percentage deviation of N, P, K, Cd, Ni, Zn, Protein and Aloin over healthy leaves was 30.1, 33.6, 22.8, 25.6, 50.98, 6.6, 1.4, and 70 while Mn, Fe was increased in the infected leaves and Pb content not affected by infection. Similar finding were also reported by [25] who found initial increase in nitrogen content of sunflower inoculated with *Puccinia halianthi*, [26] found reduced phosphorus content of groundnut infected with *Puccinia arachidis*. Decrease in potassium content in soybean infected by *Xanthomonas campestris pv glycines* was also reported by [27]. The reduction in protein contents may be attributed to utilization of host protein by fungi, which varied in their potential capacity to secrete protease enzyme. The decrease in total nitrogen and protein may be due to rapid respiration as reported by [28]. Similar results have also been reported by [29] in muskmelon and [30] on tomato.

Table-2 *Influence of pH on growth of F. solani*

pH	Mean radial growth(mm)*	Dry weight(mg*)
5.0	9.4	14.6
6.0	12.5	16.0
7.0	15.4	17.0
8.0	26.13	21.0
9.0	8.4	9.6
SE ±	0.73	1.5
CD at 5%	2.31	4.9

*Mean of three replication

Table-1 *Influence of temperature on mean radial growth of F. solani*

Temperature(°C)	Mean radial growth(mm)*after			Number of spores/field	
	10th day	12th day	15th day	Micro	Macro
20	21.2(5.1)**	40.0(6.3)	50.4(7.1)	10	2
25	26.0(6.5)	60.0(7.7)	74.4(8.6)	12	5
30	36.2(6.8)	70.0(8.3)	90.3(9.5)	25	8
35	15.2(4.5)	14.3(3.8)	14.0(3.8)	5	2
SE ±	0.095	0.31	0.08	0.57	0.58
CD at 5%	0.31	1.04	0.28	1.8	1.7

* Mean of three replication

** Figures in parentheses are square root transformed values

Table-3 *Effect of different media on growth and sporulation of F.solani*

Media	Cultural characteristics			
	Colony diameter (mm)	Type of colony	Sporulation	Pigmentation
P.D.A	82.03	Yellowish white cottony fluffy forming zone	Medium	Yellowish brown
Corn-meal sand medium	76.55	Dull white submerged	Poor	White
Czapek's dox agar medium	78.50	white cottony aerial fluffy growth	Medium	White yellow
Richard's agar Medium	42.73	Dull white submerged matted growth	Medium	White yellow
Soil extract agar medium	80.50	white cottony aerial fluffy growth	Medium	White yellow

SE ± : 0.94, CD at 5% : 2.90

Table-4 *Evaluation of fungicides against F. solani*

Fungicides concentrations	Radial growth (in mm) after*				%inhibition after 192 hrs
	48 hrs	96 hrs	144 hrs	192 hrs	
Mancozeb					
250 ppm	16.4(4.1)	42.2(6.5)	66.2(8.1)	78.03(8.8)	13.4(3.7)
500 ppm	13.1(3.6)	40.6(6.4)	64.0(8.0)	76.66(8.7)	15.3(3.9)
1000 ppm	12.5(3.6)	38.06(6.2)	63.4(7.9)	74.03(8.6)	17.0(4.1)
Thiram					
250 ppm	18.5(4.3)	23.8(4.9)	34.6(5.9)	42.26(6.5)	54.0(7.3)
500 ppm	0.0(0.7)	16.3(4.1)	25.6(5.1)	38.26(6.2)	57.0(7.5)
1000 ppm	0.0(0.7)	10.06(3.2)	13.9(3.7)	15.63(4.0)	83.0(9.1)
Carboxin					
250 ppm	11.3(3.4)	32.1(5.7)	47.3(6.9)	56.03(7.5)	37.0(6.1)
500 ppm	7.6(2.8)	23.2(4.8)	31.7(5.6)	46.53(6.8)	48.0(6.9)
1000 ppm	0.0(0.7)	12.5(3.6)	26.06(5.1)	34.16(5.9)	61.33(7.8)
Captan					
250 ppm	0.0(0.7)	18.03(4.3)	26.23(5.1)	42.2(6.5)	52.2(7.2)
500 ppm	0.0(0.7)	15.0(3.9)	20.06(4.5)	35.0(5.9)	60.0(7.7)
1000 ppm	0.0(0.7)	8.03(1.9)	11.16(3.4)	16.0(4.0)	81.66(9.0)
Carbendazim					
250 ppm 500 ppm	0.0(0.7) 0.0(0.7)	0.0(0.7) 0.0(0.7)	0.0(0.7) 0.0(0.7)	0.0(0.7) 0.0(0.7)	10.0(3.2) 96.3(10.0)
1000 ppm	0.0(0.7)	0.0(0.7)	0.0(0.7)	0.0(0.7)	100(10.0)
Control	23.0(4.8)**	52.6(7.2)	74.16(8.6)	90.(9.4)	-
SE ±	0.076	0.107	0.07	0.088	0.08
CD at 5%:	0.22	0.309	0.20	0.25	0.24

*Mean of three replication

** Figures in parentheses are square root transformed values

Table-5 *Effect of various botanicals on the growth and development of F. solani*

Name of botanical fungicide	Mean radial growth(mm) at concentration	
	10 %	20 %
Ashok (*Polyanthus longfolia*)	11.3	9.16
Tulsi(*Ocimum sanctum*)	5.2	4.20
Ipomoea(*I. carnea*)	12.00	10.36
Neem(*Azadirachtaindica*)	18.0	17.3
Lantana (*Lantana camera*)	3.74	3.06
Garlic(*Allium sativum*)	3.20	2.80
Control	90	90
SE ±	0.20	0.053
CD at 5%	0.066	0.16

* Mean of three replication

Table-6 *Redial growth and percent inhibition zone of Fusarium solani against three antagonists*

Antagonistic	Colony diameter(mm)*		Inhibition %	Antagonistc index
	Pathogen	Antagonist		
Bacillus subtilis	20.9(4.62)	69.3(8.35)	77(8.80)	Strong
Trichoderma viride	27.1(5.25)	63.4(7.96)	69.13(8.34)	Strong
Trichodermaharzianum	20(4.52)	71.13(8.46)	78.3(8.87)	Strong
Control	90(9.51)**	90(9.51)	0.0(0.7)	Nil
SE ±	0.007	0.22	0.027	
CD at 5%	0.023	0.074	0.088	

* Mean of three replication

** Figures in parentheses are square root transformed values

Table-7 *Percentage of essential nutrients in healthy and Fusarium solani infected leaves of Aloe vera*

Nutrient	Healthy	Disease	Percentage deviation over healthy
Aloin (%)	3.55	1.74	50.98
Protein (%)	1.25	0.93	25.6
Macro-nutrient (ppm)			
N	3.05	2.13	30.1
P	0.98	0.65	33.6
K	2.28	1.60	22.8
Micro-nutreint (ppm)			
Mn	89	111.7	-25.5
Zn	196.5	59.9	70
Fe	292	358.5	-22.7
Pb	27.5	27.5	0
Ni	17.5	17.2	1.4
Cd	7.5	7.0	6.6

Conflict of Interest: None declared

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