

**A Case Study on the Isolation, Purification, Identification & maintenance of the Pathogen
Causing Leaf Rot (*Fusarium oxysporum*) in Aloe vera (*Aloe barbadensis* Miller)**

Susheel Kumar¹, Hemendra Kumar Tripathi², Krishna Kumar³, Mohammad Sayeed⁴

¹Associate Professor, Department of Agriculture, Shivalik College of Engineering, Dehradun.

²Professor, Department of Applied Sciences, Shivalik College of Engineering, Dehradun.

³Assistant Professor, School of Agriculture, Galgotias University, Greater Noida, 203201, (UP)

⁴Assistant Professor, Department of Civil Engineering, Shivalik College of Engineering, Dehradun.

Emails: susheel8263@gmail.com, hemendratipathi@gmail.com, krishnakumar@galgotiasuniversity.edu.in

Corresponding Author Email: sayeedraza666@mail.com

ABSTRACT: *Aloe vera* is an oldest medicinal plant grown worldwide. The first written record about the use of *Aloe vera* is found on 6,000 years old clay tablets found in Mesopotamia. The plant was believed to be originated from African continent specifically in Egypt (Daodu, 2000). *Aloe vera* (*Aloe barbadensis*) is a perennial, drought resisting succulent plant belongs to the family Liliaceae. The present investigations were carried out on different aspects like isolation, purification & identification of disease through fungicides and botanicals, disease caused by *Fusarium oxysporum*, leaf rot disease of *Aloe barbadensis*. The *Fusarium oxysporum* isolated from infected *Aloe vera* leaves on PDA medium. The colonies of the fungus white, brown in colour. The pathogen causing leaf rot in *Aloe vera* was isolated on modified Potato Dextrose Agar (PDA) medium after surface sterilization of infected leaf tissue. Following mycelial growth, purification was done using the hyphal tip method, and the culture was maintained on PDA slants. Based on cultural and morphological characteristics, the pathogen was identified as *Fusarium oxysporum*. The colony appeared white and fluffy; microscopic examination revealed septate, hyaline mycelium, with slightly curved, coma-shaped microconidia (0–1 septa), strongly curved macroconidia (1–4 septa), and oval to globose chlamydospores, solitary or in chains. These features matched descriptions by Booth (1971), Singh (2006), Ji et al. (2007), Chavan and Korekar (2011), and Hirooka et al. (2007). The management of *Fusarium oxysporum*, leaf rot disease of *Aloe barbadensis* was done earlier mostly through fungicides to minimizing the yield losses. But fungicides caused many problems in our eco-system. Despite some progress made towards development of Integrated Disease Management. There is a need to exploitation some other alternative tools viz., use of plant extracts and inter-cropping in disease management.

Keywords: *Aloe vera* (*Aloe barbadensis* Miller), Isolation, Purification, Identification & maintenance, Potato Dextrose Agar (PDA).

1. INTRODUCTION

Aloe vera (*Aloe barbadensis* Miller) is a perennial; drought resistant succulent plant belongs to the family Liliaceae. The plant was believed to be originated African continent specifically in Egypt (Daodu, 2000). Distributed to other tropical countries like South Arab,

India, and East Asia. *Aloe vera* is grown largely in South America, Central America, Australia and Africa. There are over 250 species of *Aloe vera* growing around the world however, only three species viz., *Aloe barbadensis*, *Aloe ferox* and *Aloe arborescence* are used as herbal drug *Aloe vera* is cultivated in fairly large area in many in parts of India viz., Uttar Pradesh, Chhattisgarh, Maharashtra, Madhya Pradesh and Gujarat, Tamil Nadu and Andhra Pradesh.

Aloe vera is the oldest medicinal plant grown worldwide. The first written record about the use of *Aloe vera* is found on 6,000 years old clay tablets found in Mesopotamia. It is grown in warm tropical areas and cannot survive freezing temperature. Hot climate suits the growth. *Aloe vera* is a stemless succulent plant growing to 80-100 cm tall, spreading by offsets and root sprouts. The leaves are Lanceolate, thick and fleshy, green to grey-green, with a serrated margin. The flowers are produced on a spike up to 90 cm tall, each flower pendulous, with a yellow tubular corolla 2-3 cm long. The tissue in the center of the aloe leaf contains a gel which known as aloe gel or *Aloe vera* gel. When a leaf is cut an orange yellow sap drips from the open end. A stabilized product is incorporated in a wild variety of preparation, including juice, gel, ointments, cream, lotion and shampoos (Daodu, 2000).

As medicinal herb *Aloe vera* has been used externally to treat various skin conditions such as cuts, burns and eczema. Furthermore, *Aloe vera* gel has been reported to be very effective for the treatment of sore and wounds, skin disease, cold and cough, constipation, pile and fungal infection aloe plants can be used for treatment of asthma, ulcer and diabetes. (Daodu, 2000; Djeraba and Quere, 2000; Olusegun, 2000). It is alleged that sap from *Aloe vera* eases pain and reduces inflammation (Davis and Moro 1989). The essential fatty acids in *Aloe vera* are beneficial, not only nutritional but also act as anti-inflammatory agent. *Aloe vera* is famous for facilitating, digestion, aiding blood circulation, as well as improving kidney, liver and gall bladder function. It has minimum of three anti-inflammatory fatty acids, which helps in smooth functioning of the stomach, small intestines and colon. It has a natural property to alkalize digestive juices which prevents over-acidity. The *Aloe vera* juice concentrates are high in essential enzymes, which stimulate digestion and liver functions. The synergistic effect of *Aloe vera* juice used in combination with a few other herbs does wonders as a liver-cleansing agent. *Aloe vera* supplements also contain a rare natural ingredient called Saponins, which is provided by nature to cleanse and flush out waste products and toxins. (Kumar, *et al.* 2010).

A new leaf rot disease was observed in *Aloe barbadensis* for the first time in India in 2000 (Mandal and Maiti 2005). The disease was very serious and abundant in the area where abundant moisture available. The infection started from the leaves edge and the symptoms appeared on the leaves, and leaves become rotten and dried with dark brown to black color with crescent shape. In order to control the disease, it is necessary to know and identify the causal agent of the disease; however, there is lack of ample information regarding on the aspect of leaf rot disease on aloe plant. Therefore, this study was undertaken in order to determine the incidence of the leaf rot disease on aloe, identification of the causal agent and their managements.

The present investigation entitled “Management of leaf rot disease in *Aloe vera* (*Aloe barbadensis* Miller)”

2. MATERIALS AND METHODS

The present investigation entitled “A Case Study on the Isolation, Purification, and Identification & maintenance of the Pathogen Causing Leaf Rot in *Aloe vera* (*Aloe barbadensis* Miller)” was undertaken during the course of investigation in the discipline of Agriculture, College of Agriculture, Narendra Deva University of Agriculture and Technology, Kumarganj, Faizabad (U.P.). The field experiment was carried out at Main Experiment Station, Horticulture and analytical works undertaken in laboratory, Department of Medicinal and Aromatic Plants during the year of 2014 and 2015.

The experimental site is located at Main Experiment Station, Horticulture, College of Horticulture & Forestry, Narendra Deva University of Agriculture and Technology, Kumarganj, Faizabad (U.P.), on the Faizabad Raibareli road at distance of 42 km from Faizabad which is situated under Indo-gangetic plains at 26.47 N latitude, 82.12 °E longitude and altitude of 113 meters from mean sea level. The experiment was conducted during *Kharif* season 2014 and second 2015, at experimental farm of Medicinal and Aromatic Plants of N.D.U.A.T. Kumarganj, Faizabad. Randomized Blok Design (RBD) was adopted with three replications.

The samples were collected from crop grown at the experimental site. The infected leaves collected from infected plants in sterilized poly bags. Thus, collected typical symptom showing parts were kept in rough dry envelopes and marked clearly mentioning location, infected parts, reaction types, date of collection etc. and brought to the laboratory for isolation of the pathogen. The samples were dried for 24 hours in shade in order to remove excess surface moisture. After drying the samples were kept in B.O.D. incubator in paper envelope marked with date of collection maintained at 6-8 °C for isolation and further studies.

Potato-Dextrose-Agar medium was used as a basal medium for isolation of pathogen. Potato-Dextrose-Agar medium have following composition and prepared by the method are Peeled potato, 200.0 g, Dextrose, 20.0 g, Agar, 20.0 g Distilled water, 1000 ml. The peeled potato was cut into 12 mm cubes. Two hundred grams of potato cubes were rinsed in water and boiled for 20 minutes in 500 ml water. Potato broth was filtered through cheese cloth. At the same time, agar was also melted in 500 ml of water by heating. Potato broth was then poured into the melted agar and dextrose was added in it. The final volume was made up 1000 ml by adding distilled water.

The pH was adjusted to 7.0; the stock solution was then poured in to sterilized 250 ml Erlenmeyer conical flasks up to 1/3rd only. Then it was plugged with non-absorbent cotton. These poured flasks were kept for sterilization in autoclave at 15psi for 20 minutes. After sterilization these sterilized flasks were kept in incubator at 6-8°C for further use. Malt-extract Agar Medium are, Malt Extract, 20 ml, Dextrose, 20 gm, Agar, 20 gm & Water, 1000 ml. Infected leaves were cut with the help of sterilized blade into pieces of 2-3 mm size having half healthy and half diseased tissues. The small pieces were sterilized with HgCl₂ solution (1:1000) for 30 seconds and thoroughly washed in sterilized distilled water thrice. Later on the pieces were placed between two layers of sterilized blotter paper to remove excess of water. Symptomatological studies were carried out in laboratory at different stages and the

characteristics symptoms of the disease were recorded. Cultural characteristics of the fungus were studied on Potato Dextrose Agar medium (PDA), twenty ml sterilized medium was poured into each Petriplate and allowed to solidify. Each Petri plate was centrally inoculated with actively growing mycelial discs (5 mm) and incubated at $24 \pm 1^{\circ}\text{C}$. Observations were recorded for the type of mycelial colony, colour, and its diameter after seven days of inoculation. Morphological characters including size of conidia were measured with the help of ocular and stage micrometer. Color of conidia and septation in conidia were also recorded under microscope. Single spore isolation technique was used for obtaining pure culture of the fungus and subcultures were maintained through hyphal tip method on PDA plates and were preserved at $5 \pm 1^{\circ}\text{C}$ in refrigerator for further studies. The purification culture of fungus was obtained by adopting suspension poured on plain agar petridishes to form a very thin layer on it and spores allowed to settle down on the agar surface. Settled were supported out from each other selected under the microscope and encircled with the help of dummy culture in petridishes. They were lifted along with agar block and transferred to petri dished containing sterilized 2% PDA medium. After proper growth of fungus obtained by single spore culture. Thus, obtained culture was sub cultured at monthly intervals and maintained on potato dextrose agar slants under refrigeration at 6 to 8°C for further studies. The isolated fungus was identified as *Fusarium oxysporum* on the basis of conidial and morphological characters. Slides were prepared in cotton blue and examined under compound microscope (40x) to study the morphological characters of the pathogen. Pathogenicity test was conducted on *Aloe vera* leaves by inoculation and germination technique in petriplates. One leaf each was placed in 30 petriplates, inoculated with spore suspension of *Fusarium oxysporum* and moist chamber is prepared using sterilized filter paper, plates were incubated at $25 \pm 2^{\circ}\text{C}$. The symptoms appeared after 10 days of inoculation. The test pathogen was re-isolated from the infected leaves. The procedure was repeated twice to confirm the pathogenicity. Colour and growth of fungus were recorded on PDA medium after 4-7 days of incubation. Colour and type of mycelium were observed with the help of microscope. Colour, septation, branching and width of the hyphae were studied with the help of microscope. Colour, shape, size and septation of conidium were recorded under compound microscope after 4-7 days of incubation. The Twenty-Six germplasm of *Aloe vera* were collected and screened. Each germplasm was planted in well prepared field in one line at row-to-row distance 50 cm and plant to plant distance 40 cm. The experimental site falls under sub-tropical climatic of Eastern part of India. The District Faizabad comes under Eastern region of Uttar Pradesh, which is distributed in three seasons viz., rainy, winter and summer. The rainy season occurs from mid June to mid of September. The winter months prevails from November to March with mild to severe cool temperature. The severe cold was recorded in the month of December and occasionally winter rains and frost was also noticed. The summer months occur from April to June. The dry and hot wind waves were also noticed in the months of mid May and June. Experimental layout was prepared as per treatment combinations and replications. Seven plots were laid out for this experiment with a plot size of $3 \times 2.4\text{m}^2$. The main irrigation channel was provided in western of side the experimental field and sub irrigation channels were provided between the replication of the crop. Treatments detail, T₁: Three foliar sprays with Carbendazim 50 WP @ 0.25%, T₂: Three foliar sprays with Mancozeb 75 WP @ 0.25%, T₃: Three foliar sprays with Propiconazole 25 EC @ 0.25%, T₄: Three foliar sprays with Neem leaf extract @ 5% , T₅ : Three foliar sprays with Garlic bulb extract @ 5% , T₆ : Three foliar sprays with Tulsi leaf extract @ 5% , T₇ : Control (Untreated). The leaf and bulb extracts of Neem, Garlic and Ocimum were prepared by cold water extraction method described by Shekhawat and Prasad

(1971). The samples were washed separately in tap water and finally three changes in distilled water. They were crushed in mortar and pestle by adding distilled water @ 2 ml/g fresh weight. The extracts were clarified by passing through two layers of cheese cloth and finally through What Mann No. 1 filter paper. The filtered extracts were quoted in the study as 100 % extract. Five plants from each plot were randomly selected and tagged. Observations were recorded on per cent disease incidence, per cent disease control, leaf yield per hectare. Per cent disease incidence and per cent disease control was calculated as per formula given by (Kushalappa and Ludwig, 1982).

3.RESULTS AND DISCUSSION

Isolation

Isolations were made from infected leaves (Plate-1 & 2). The pathogen was isolated on Modified Potato-Dextrose-Agar medium (Singh and Chaube, 1970) in Petri plate by transferring them after surface sterilization. After the mycelial growth, the fungus was purified through hyphal tip culture method. Subsequently, the culture was maintained on PDA slant for further studies. The pathogen under study was identified as *Fusarium oxysporum* on the basis of its cultural and morphological characters described by Booth (1971).

Purification:

The pure culture of isolated pathogen was done by single spore isolation (Plate-3 & 4). A dilute spore suspension was poured on plain agar petriplate to form a very thin layer on it and spores allowed settling down on the agar surface. Settled spores were separated out from each other, selected under the microscope and enriched with the help of dummy culture in petriplates. They were lifted along with agar blocks and transferred to petriplates containing sterilized 2% PDA. After proper growth of fungus obtained by single spore culture regular sub-culturing was done to check contamination till pure cultures were obtained. These cultures were sub-cultured at monthly intervals and maintained on PDA slant under refrigeration at 6 to 8°C temperature for further studies.

Identification:

White fluffy colony of *Fusarium oxysporum* was seen on PDA and the microscopic studies revealed that the mycelium was septate and hyaline, microconidia were slightly curved and coma shaped and had 0-1 septa while macroconidia were strongly curved or hooked at apex, smooth hyaline and had 1-4 septa. The chlamydospores were oval to globose hyaline and found in solitary or chain form (Plate-5) and leaf spot also caused by *Alternaria alternate* (Plate-6 & 7).

Screening of *Aloe vera* germplasm in natural field condition against leaf rot disease caused by *Fusarium oxysporum*.

Twenty six germplasms of *Aloe vera* were screened for their reaction to *Fusarium oxysporum* by following technique.

The genotypes were grouped in various categories of resistant and susceptible on the basis of percent leaves rotting as described and the results were summarized in Table-1.

Table-1. Reaction of *Aloe* germplasm against leaf rot Fungus (*Fusarium oxysporum*) under field conditions during 2014 and 2015.

S. No.	Grade	Name of germplasm	Number of germplasms	Reaction
1	0	Nil	0	Immune

2	1	IC-111280, IC-112531, IC-112513	3	Highly Resistant
3	2	IC-111279, IC-112518, IC-285626, IC-283655	4	Resistant
4	3	IC-310618, IC-471886, IC-310904, IC-112569, IC-112519, IC-112532, IC-310611, IC-310617, IC-310596	9	Moderately resistant
5	4	IC-283610, IC-283945, IC-112512, IC-112527, IC-283943, IC-310517	6	Susceptible
6	5	IC-310609, IC-112521, IC-285629, IC-471882	4	Highly Susceptible

It is clear from results presented in Table-1 (Plate-8 & 9) that during 2014 and 2015, out of 26 germplasm of *Aloe vera* screened, none of germplasm were found immune, three germplasm viz., IC-111280, IC-112531 and IC-112513 were found highly resistant, four germplasm viz., IC-111279, IC-112518, IC-285626 and IC-283655 were showed resistant, nine germplasm viz., IC-310618, IC-471886, IC-310904, IC-112569, IC-112519, IC-112532, IC-310611, IC-310617 and IC-310596 were showed moderately resistant, six germplasm viz., IC-283610, IC-283945, IC-112512, IC-112527, IC-283943 and IC-310517 were showed moderately susceptible and four germplasm viz., IC-310609, IC-112521, IC-285629 and IC-471882 were showed highly susceptible against *Aloe vera* leaf rot disease. So, growing resistant germplasm is one of the cheapest and most economic method than other preventing measures.

Management of leaf rot disease through fungicides and botanicals.

Disease incidence

All the treatments delayed the appearance of disease symptoms with respect to per cent disease incidence. Results presented in Table- 2, indicated that all treatments were found significantly superior over control during 2014 and 2015 respectively. However, minimum disease incidence were recorded in treatment T₃= Propiconazole 25 EC @ 0.25% PDI (28.24, 26.61 and pooled 27.42) in years 2014 and 2015, followed by T₂= Mancozeb 75 WP @ 0.25% PDI (33.06, 29.26 and pooled 31.16) and T₁= Carbendazim 50 WP@ 0.25% (36.47, 36.39 and pooled 36.43). While among the botanicals T₄= Neem leaf extract @ 5% (36.19, 34.51 and pooled 35.35), followed by T₅= Garlic bulb extract @ 5% PDI (39.38, 38.66 and pooled 39.02) and T₆= Tulsi leaf extract @ 5% (40.91, 39.34 and pooled 40.12). All the treatments were found significantly superior per cent disease intensity during both the years.

Table 2. Effect of fungicides and botanicals on per cent disease incidence of *Aloe vera* leaf rot

S.No.	Treatments	Per cent Disease Incidence		
		2014	2015	Pooled
1	T ₁ = Carbendazim 50 WP @ 0.25%	36.47 (37.15)	36.39 (37.10)	36.43 (37.13)
2	T ₂ = Mancozeb 75 WP @ 0.25%	33.06 (35.08)	29.26 (32.75)	31.16 (33.92)
3	T ₃ = Propiconazole 25 EC @ 0.25%	28.24 (32.10)	26.61 (31.05)	27.42 (31.57)
4	T ₄ = Neem leaf extract @ 5%	36.19	34.51	35.35

		(36.98)	(35.96)	(36.47)
5	T ₅ = Garlic bulb extract @ 5%	39.38 (38.86)	38.66 (38.44)	39.02 (38.65)
6	T ₆ = Tulsi leaf extract @ 5%	40.91 (39.76)	39.34 (38.81)	40.12 (39.29)
7	Control (Untreated)	82.64 (65.38)	78.71 (62.53)	80.67 (63.95)
	SEm±	0.67	1.01	0.60
	CD at 5%	2.06	3.11	2.10
	CV	2.85	4.43	3.70

Per cent disease control

Data elaborated in Table-3 revealed that in case of chemical control the maximum per cent disease control were recorded in treatment T₃ = Propiconazole 25 EC @ 0.25% PDC (65.20, 65.71 and 65.46) followed by T₂ = Mancozeb 75 WP@ 0.25% PDC (61.05, 62.28 and pooled 61.67), and T₁ = Carbendazim 50 WP@ 0.25% PDC (59.36, 60.28 and pooled 59.82) during 2014 and 2015. Among the botanicals maximum per cent disease control were recorded T₄ = Neem leaf extract @ 5% PDC (58.26, 59.16 and pooled 58.71) followed by T₅ = Garlic bulb extract @ 5% PDC 51.81, 51.79 and pooled 51.80) and T₆ = Tulsi leaf extract @ 5% PDC 44.99, 45.84 and pooled 45.41) during both the years 2014 and 2015, respectively.

Table No.3. Effect of fungicides and botanicals on Per cent disease Control of *Aloe vera* leaf rot.

S.No.	Treatments	Per cent Disease Control		
		2014	2015	Pooled
1	T ₁ = Carbendazim 50 WP @ 0.25%	59.36 (50.40)	60.28 (50.93)	59.82 (50.67)
2	T ₂ = Mancozeb 75 WP @ 0.25%	61.05 (51.39)	62.28 (52.11)	61.67 (51.75)
3	T ₃ = Propiconazole 25 EC @ 0.25%	65.20 (53.88)	65.71 (54.20)	65.46 (54.04)
4	T ₄ = Neem leaf extract @ 5%	58.26 (49.76)	59.16 (50.30)	58.71 (50.03)
5	T ₅ = Garlic bulb extract @ 5%	51.81 (46.04)	51.79 (46.03)	51.80 (46.03)
6	T ₆ = Tulsi leaf extract @ 5%	44.99 (42.12)	45.84 (42.61)	45.41 (42.37)
7	Control (Untreated)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	SEm±	0.79	1.12	0.68
	CD at 5%	2.43	3.45	2.37
	CV	3.26	4.58	3.98

Aloe vera is the oldest medicinal plant grown worldwide. The plant was believed to be originated in African continent specifically in Egypt (Daodu, 2000). *Aloe vera* (*Aloe barbadensis*) is a perennial, drought resisting succulent plant belongs to the family Liliaceae. *Aloe vera* is cultivated in fairly large area in many parts of India viz., Uttar Pradesh, Chhattisgarh, Maharashtra, Madhya Pradesh and Gujarat, Tamil Nadu and Andhra Pradesh. The objectives of the present investigation were to bring together a comprehensive update of the research on the leaf rot disease of *Aloe vera* and the management strategies in Indian context.

Therefore, the present study was undertaken on isolation, purification, identification and maintenance of culture, screening of cultivars/germplasm, epidemiological studies and management of disease through fungicides and botanicals were studied at the Department of Plant Pathology, Narendra Deva University of Agriculture and Technology, Kumarganj, Faizabad. An attempt has been made to discuss the findings of present study with the help of reports available in the literature.

Isolation, Purification and Identification

The pathogen was isolated from infected plant leaf on modified Potato Dextrose Agar medium in Petri plate by transferring them after surface sterilization, after mycelial growth, the fungus was purified through hyphal tip culture method. The culture was maintained on PDA slant. The pathogen under study was identified as *Fusarium oxysporum* on the basis of cultural and morphological characters of the *Fusarium oxysporum*. White fluffy colony of *Fusarium oxysporum* was seen on PDA and the microscopic studies revealed that the mycelium was septate and hyaline. Microconidia were slightly curved and coma shaped and has 0-1 septa. Macroconidia were strongly curved or hooked at apex, smooth hyaline and have 1-4 septa. The chlamydospores were oval to globose hyaline and found in solitary or chain form. These cultural and morphological characters of *Fusarium oxysporum* were also similar as described by Booth (1971), Singh (2006), Ji *et al.* (2007), Chavan and Korekar (2011) and Hirooka *et al.* (2007).

4. SUMMERY & CONCLUSIONS

The present investigation entitled “A Case Study on the Isolation, Purification, Identification & maintenance of the Pathogen Causing Leaf Rot in *Aloe vera* (*Aloe barbadensis* Miller)” was carried out in the Department of Plant Pathology, Horticulture farm, Narendra Deva University of Agriculture and Technology, Narendra Nagar, Kumarganj, Faizabad (U.P.). Some diseases have been reported on *Aloe barbadensis* that can lead to loss in quality of leaves, mucilage, gel content and yield, if not managed properly in time. *Aloe vera* leaf rot is an important disease caused by *Fusarium oxysporum* which causes quantitative as well as qualitative losses. Keeping in view, the importance of *Aloe vera* and its medicinal value and visualizing the seriousness of the disease, the present studies were carried out to know as first is the Isolation, Purification, Identification second is Screening of germplasms, third is Epidemiological studies and fourth is management of disease through fungicides and botanicals. The salient findings of the present investigation are as follows:

The pathogen was isolated on Modified Potato-Dextrose-Agar medium in Petri plate by transferring them after surface sterilization after the mycelial growth, the fungus was purified through hyphal tip culture method. Subsequently, the culture was maintained on PDA slant for

further studies. The pathogen under study was identified as *Fusarium oxysporum* on the basis of its cultural and morphological characters.

Settled spores were separated out from each other, selected under the microscope and enriched with the help of dummy culture in petri-plates. They were lifted along with agar blocks and transferred to petriplates containing sterilized 2% PDA. After proper growth of fungus obtained by single spore culture regular sub-culturing was done to check contamination till pure cultures were obtained. These cultures were sub-cultured at monthly intervals and maintained on PDA slant under refrigeration at 6 to 8 °C temperature for further studies.

Chemical control the maximum per cent disease control were recorded in treatment T₃= Propiconazole 25 EC @ 0.25% PDC (65.20, 65.71 and pooled 65.46) followed by T₂= Mancozeb 75 WP@ 0.25% PDC (61.05, 62.28 and pooled 61.67), and T₁= Carbendazim 50 WP@ 0.25% PDC (59.36, 60.28 and pooled 59.82) during 2014 and 2015. Among the botanicals maximum per cent disease control were recorded T₄= Neem leaf extract @ 5% PDC (58.26, 59.16 and pooled 58.71) followed by T₅= Garlic bulb extract @ 5% PDC 51.81, 51.79 and pooled 51.80) and T₆= Tulsi leaf extract @ 5% PDC 44.99, 45.84 and pooled 45.41) during both the years 2014 and 2015, respectively.

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