STUDIES ON GRAY MOLD OF CASTOR CAUSED BY *Amphobotrys ricini* (N. F. Buchw.) Hennebert

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DEPARTMENT OF PLANT PATHOLOGY

UNIVERSITY OF AGRICULTURAL SCIENCES

BENGALURU- 560 065

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in

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BENGALURU AUGUST, 2018

Affectionately Dedicated to

my beloved Parents,

Teachers, Sisters and

my Guide

DEPARTMENT OF PLANT PATHOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES
BENGALURU- 560 065

CERTIFICATE

This is to certify that the thesis entitled **"STUDIES ON GRAY MOLD OF CASTOR CAUSED BY *Amphobotrys ricini* (N. F. Buchw.) Hennebert"** submitted by **Ms. RAMYA, R. V., ID. No. PALB 6304** in partial fulfillment of the requirements for the award of the degree of **MASTER OF SCIENCE (Agriculture) in PLANT PATHOLOGY** to the University of Agricultural sciences, Bengaluru, is record of *bona fide* research work done by her during the period of her study in this University under my guidance and supervision and that no part of the thesis has been submitted for the award of any degree, diploma, associateship, fellowship or any other similar titles.

AUGUST, 2018
BENGALURU

Dr. K. KARUNA
(Major advisor)

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With regardful memories…

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Bengaluru *RAMYA, R. V.*
August, 2018

STUDIES ON GRAY MOLD OF CASTOR CAUSED BY *Amphobotrys ricini* (N. F. Buchw.) Hennebert

RAMYA, R. V.

ABSTRACT

Castor crop suffers from many diseases of which gray mold is important. Anamorphs of *Botryotinia ricini viz*., *Amphobotrys ricini* and *Botrytis cinerea* are found to incite gray mold. On the basis of morphology of the fungus isolated from the castor capsules collected at Gandhi Krishi Vignan Kendra, Bengaluru which was grown on oat meal enriched medium produced septate and branched mycelium which was thin, hyaline to light brown in colour and produced cylindrical, dichotomously branched conidiophores bearing conidia and this was identified as *Amphobotrys ricini*. Morphological studies revealed that highest radial growth was observed on Malt extract agar medium, but profuse sporulation was in oat meal enriched medium. Maxium dry mycelial weight was observed in Richard's agar. Highest number of sclerotia were produced on Malt extract agar whereas large sized sclerotia were produced on Richard's agar and Malt extract agar. The PCR amplification and sequencing of ITS rDNA region confirmed that *A. ricini* as the cause of gray mold. Among the systemic fungicides Carbendazim, Propiconazole @ 50, 250, 500 ppm showed complete inhibition; contact fungicide Mancozeb @ 1000 ppm; combi fungicide Carbendazim + Mancozeb @ 250, 500, 1000 ppm; nano molecule Silicon dioxide @ 250 ppm were found to be superior in inhibiting the growth. Management of gray mold disease revealed that, least disease index with higher seed yield was recorded in Carbendazim 1.0 g L^{-1} when prophylactic spray was given before the onset of disease and next spray 15 days after first spray.

August, 2018

Dept. of Plant Pathology — **Dr. K. KARUNA**

UAS, G.K.V.K., Bengaluru-65 — (Major advisor)

ಅಂಫೋಬೋಟ್ರಿಸ್ ರಿಸಿನಿ (ಎನ್. ಎಫ್. ಬುಚ್.) ಹೆನ್ನೆಬರ್ಟ್ ಕಾರಣದಿಂದ ಹರಳಿನ ಬೂದು ಬಣ್ಣದ ಬೂಷ್ಟಿನಲ್ಲಿನ ಅಧ್ಯಯನಗಳು

ರಮ್ಯ, ಆರ್. ವಿ.

ಪ್ರಬಂಧದ ಸಾರಾಂಶ

ಹರಳು ಅನೇಕ ರೋಗಗಳಿಂದ ಬಳಲುತ್ತಿದ್ದು, ಅದರಲ್ಲಿ ಬೂದು ಬಣ್ಣದ ಬೂಸ್ಟು ಪ್ರಮುಖವಾಗಿದೆ. ಬೊಟ್ರೊಟೀನಿಯ *ರಿಸಿನಿಯ ಅನಾಮಾರ್ಫ್* ಹಂತಗಳಾದ *ಅಂಫೋಬೋಟ್ರಿಸ್ ರಿಸಿನಿ* ಮತ್ತು ಬೊಟ್ರೈಟಿಸ್ *ರಿಸಿನಿ* ಬೂದು ಬಣ್ಣದ ಬೂಸ್ಟನ್ನು ಉಂಟು ಮಾಡುತ್ತವೆ. ಗಾಂಧಿ ಕೃಷಿ ವಿಜ್ಞಾನ ಕೇಂದ್ರದ ಆವರಣದಲ್ಲಿ ಸಂಗ್ರಹಿಸಿದ ಹರಳಿನ ಬೀಜಕೋಶಗಳಿಂದ ಪ್ರತ್ಯೇಕಿಸಲಾದ ಶಿಲೀಂಧ್ರವನ್ನು ರೂಪವಿಜ್ಞಾನದ ಆಧಾರದ ಮೇಲೆ *ಅಂಫೋಬೋಟ್ರಿಸ್ ರಿಸಿನಿಯೆಂದು ಗುರುತಿಸಲಾಯಿತು.* ಓಟ್ಮೀಲ್ ಸಮೃದ್ಧವಾಗಿರುವ ಮಧ್ಯಮದಲ್ಲಿ ಶಿಲೀಂಧ್ರವು ವಿಭಜಿತ ಮತ್ತು ಕವಚದ ತಂತುಜಾಲವನ್ನು ಉತ್ಪತ್ತಿಮಾಡುತ್ತದೆ. ಈ ತಂತುಜಾಲವು ತೆಳುವಾಗಿದ್ದು, ಇದರ ಬಣ್ಣವು ಪಾರದರ್ಶಕದಿಂದ ತಿಳಿ ಕಂದು ಬಣ್ಣ ಹೊಂದಿರುತ್ತದೆ ಮತ್ತು ಕೊನಿಡಿಯವನ್ನು ಹೊಂದಿರುವ ಕೊನಿಡಿಯೊಫೋರ್ ಎರಡು ಕವಲುಗಳ ರಚನೆಯದ್ದಾಗಿರುತ್ತದೆ, ಈ ರೂಪವಿಜ್ಞಾನದ ಆಧಾರದ ಮೇಲೆ ಶಿಲೀಂಧ್ರವನ್ನು *ಅಂಫೋಬೋಟ್ರಿಸ್ ರಿಸಿನಿ* ಎಂದು ಗುರುತಿಸಲಾಗಿದೆ. ರೂಪವಿಜ್ಞಾನ ಅಧ್ಯಯನದ ಪ್ರಕಾರ ಗರಿಷ್ಠ ತಂತುಜಾಲದ ಬೆಳವಣಿಗೆಯು ಮಾಲ್ಟ್ ಸಾರ ಅಗರ್ ಮಧ್ಯಮದಲ್ಲಿ ಕಂಡುಬಂದಿದೆ, ಆದರೆ ಅತಿ ಹೆಚ್ಚು ಬೀಜಕಗಳ ಉತ್ಪಾದನೆಯು ಓಟ್ಮೀಲ್ ಸಮೃದ್ಧವಾಗಿರುವ ಮಧ್ಯಮದಲ್ಲಿತ್ತು. ರಿಚರ್ಡ್ಸ್ ಬ್ರಾತ್‌ನಲ್ಲಿ ಗರಿಷ್ಠ ಒಣಗಿದ ತಂತುಜಾಲದ ತೂಕವನ್ನು ಗಮನಿಸಲಾಗಿದೆ. ಹೆಚ್ಚಿನ ಸಂಖ್ಯೆಯ ಸ್ಕ್ಲಿರೋಷಿಯವನ್ನು ಮಾಲ್ಟ್ ಸಾರ ಅಗರ್ನಲ್ಲಿ ಉತ್ಪಾದಿಸಿತು, ಆದರೆ ರಿಚರ್ಡ್ಸ್ ಅಗರ್ ಮತ್ತು ಮಾಲ್ಟ್ ಸಾರ ಅಗರ್ನಲ್ಲಿ ದೊಡ್ಡಗಾತ್ರದ ಸ್ಕ್ಲಿರೋಷಿಯಾದ ಉತ್ಪಾದನೆಯಾಗಿದೆ. ಪಿಸಿಆರ್ ವರ್ಧನೆ ಮತ್ತು ಅದರ ಆರ್‌ಡಿಎನ್‌ಎ ಪ್ರದೇಶದ ಕ್ರಮಾಗತಿಯ ಪ್ರಕಾರ *ಅಂಫೋಬೋಟ್ರಿಸ್ ರಿಸಿನಿ* ಬೂದು ಬಣ್ಣದ ಬೂಸ್ಟಿಗೆ ಕಾರಣವೆಂದು ದೃಢಪಡಿಸಿತು. ವ್ಯವಸ್ಥಿತ ಶಿಲೀಂಧ್ರನಾಶಕಗಳಾದ ಕಾರ್ಬೆಂಡಜೀಮ್, ಪ್ರೊಪಿಕೊನಜೋಲ್ ೫೦, ೨೫೦, ೫೦೦ ಪಿಪಿಎಮ್ ಸಾಂದ್ರತೆಯಲ್ಲಿ ಸಂಪೂರ್ಣ ನಿಷೇಧತೆಯನ್ನು ತೋರಿಸಿದೆ; ಸ್ಪರ್ಶ ಶಿಲೀಂಧ್ರನಾಶಕವಾದ ಮ್ಯಾಂಕೊಜೆಬ್ ೧೦೦೦ ಪಿಪಿಎಮ್; ಸಂಯುಕ್ತ ಶಿಲೀಂಧ್ರನಾಶಕಗಳಾದ ಕಾರ್ಬೆಂಡಜೀಮ್ + ಮ್ಯಾಂಕೊಜೆಬ್ ೨೫೦, ೫೦೦, ೧೦೦೦ ಪಿಪಿಎಮ್; ನ್ಯಾನೊ ಅಣುವಾದ ಸಿಲಿಕಾನ್ ಡೈ ಆಕ್ಸೈಡ್ ೨೫೦ ಪಿಪಿಎಮ್ ಶಿಲೀಂಧ್ರದ ಬೆಳವಣಿಗೆಯ ನಿರೋಧಕದಲ್ಲಿ ಉತ್ತಮವಾಗಿದೆ. ಪ್ರತಿ ಲೀಟರ್‌ಗೆ ಒಂದು ಗ್ರಾಂ ಕಾರ್ಬೆಂಡಜೀಮ್‌ನ್ನು ರೋಗನಿರೋಧಕವಾಗಿ ಸಿಂಪಡಿಸಿ ಮತ್ತು ಇದಾದ ೧೫ ದಿನಗಳ ನಂತರ ಎರಡನೇ ಸಿಂಪಡಣೆಯನ್ನು ನೀಡಿದಾಗ ಹೆಚ್ಚಿನ ಬೀಜ ಇಳುವರಿ ಹಾಗೂ ಕನಿಷ್ಠ ಕಾಯಿಲೆ ಸೂಚ್ಯಂಕವನ್ನು ದಾಖಲಿಸಲಾಗಿದೆ.

ಆಗಸ್ಟ್, ೨೦೧೮
ಸಸ್ಯ ರೋಗಶಾಸ್ತ್ರ ವಿಭಾಗ
ಕೃಷಿ ವಿಶ್ವ ವಿದ್ಯಾನಿಲಯ
ಜಿ.ಕೆ.ವಿ.ಕೆ., ಬೆಂಗಳೂರು–೬೫

ಡಾ. ಕೆ. ಕರುಣ
(ಮುಖ್ಯ ಸಲಹೆಗಾರರು)

Studies on gray mold of castor

R. V. RAMYA, PALB 6304 AND K. KARUNA

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Introduction

➢ Castor (*Ricinus communis* L.) is an important non-edible oilseed crop with unique oil features (40-60% of oil) for the chemical industry which belongs to the family Euphorbiaceae.

➢ India ranks first in area and production of castor in the world and its derivatives contributing to 65% share in the world production. The area under castor cultivation in India was 1060.72 ha, with total production of 1751.81 million tonnes and productivity of 1652 kg/ha. (Ministry of Agriculture, Government of India, 2015-16). In Karnataka, it occupies an area of about 0.11lakh ha with a production of 0.08 lakh tonnes and productivity of 685 kg /ha.

➢ Castor oil is characterized by high concentration of triglycerides, mainly ricinolein.

➢ Castor is attacked by many diseases *viz*., wilt, gray mold, root rot, *Cercospora* leaf spot, *Alternaria* blight, powdery mildew and bacterial leaf spot. Among them gray mold caused by *Botryotinia ricini* (Godfrey) Whetzel has been considered as a potentially destructive disease in many parts of the castor growing countries.

➢ Yield losses are extensive due to this disease and are a major threat to commercial cultivation of the crop. Seed yield loss up to 100% was reported from India (Soares, 2012).

Objective

➢ Morphological and molecular characterization of pathogen associated with gray mold of castor.

Material and Methods

➢ The gray mold infected castor capsules collected from GKVK campus and brought to the laboratory for characterization of pathogen associated with gray mold of castor.

Morphological characterization

➢ The collected diseased castor capsules were first microscopically examined to confirm the presence of the fungus causing gray mold. After confirmation on fungal spores, isolation was done by following the standard tissue isolation method. Different media were used for morphological characterization of anamorph of pathogen associated with gray mold of castor (Table 1).

Molecular characterization

➢ The DNA of the anamorph of pathogen causing gray mold was isolated and the Internal Transcribed Spacer (ITS) region of the fungal genome was amplified in a PCR using ITS 1 and ITS 4 primer pair. The amplified region was sequenced and analyzed in a standard Gen Bank BLAST search for fungal species identification.

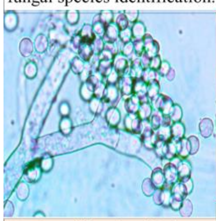

Plate 1: conidia and conidiophores of *Amphobotrys ricini*

Plate 2: Pure culture of *Amphobotrys ricini* on oat meal enriched medium

Results

➢On the basis of morphological and molecular studies, the anamorph of the fungus associated with gray mold of castor was identified as *Amphobotrys ricini.*

➢The radial growth of *A. ricini* was maximum on malt extract agar medium (8.5 cm) and minimum growth was recorded on Richard's agar medium (2 cm). Colony colour varied from white to dirty white, cottony white, brown to light brown in colour.

➢The number of sclerotia produced on various media varied (3-250 per plate) with the type of medium used. Sclerotial production was observed on 10th day after inoculation (DAI)

➢ Based on rDNA sequence comparision using BLAST progarmme , it was confirmed as *A. ricini* as the cause of gray mold of castor.

Plate 3: Typical mold symptoms castor raceme.

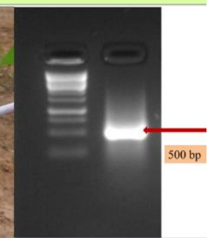

Plate 4: Gel picture of ITS 1 and ITS 4 PCR product

Table 1: Morphological growth characteristics of *A. ricini* on different culture media

Sl. No.	Medium	Radial growth (cm)	Colony colour	Sclerotial number/ plate	Sclerotial initiation (DAI)
1	Potato dextrose agar (PDA)	5.80	Whitish at the centre and brownish at the periphery	55	17
2	Sabouraude agar (SA)	5.70	Whitish at the centre and brownish at the periphery	3	12
3	Castor leaf extract (CL)	7.25	Whitish at the centre and dark brownish at the periphery	75	11
4	Czapek' s Agar (CD)	4.00	Brownish white	-	-
5	V8 Juice agar (V8)	8.20	Cottony white mycelium	-	-
6	Richard's Agar (RA)	2.00	Whitish mycelia growth	37	17
7	Carrot agar (CA)	4.10	Whitish at the centre surrounded by brownish	78	10
8	Malt extract agar (ME)	8.50	Whitish at the inoculation point surrounded by dirty white in colour	250	10
9	Asthana and Hawker's (AH)	4.03	Cottony white mycelium surrounded by light brown	3	14
10	Tochinai' s agar (TA)	7.90	White to dirty white in colour	56	14
11	Oat meal enriched medium (OME)	6.25	Initially white later turn to brown colour	120	12
12	Oat meal agar (OA)	7.00	Initially white later turn to light brown colour	132	10

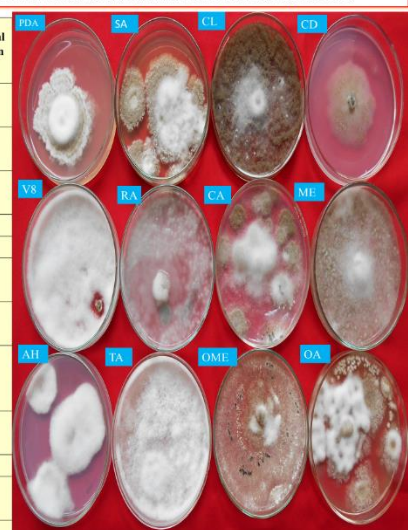

Plate 5: Colony morphology of *A. ricini* on different solid media

Discussion

➢On the basis of morphology, the fungus was identified as *A. ricini.* The fungus on oat meal enriched medium produced septate and branched mycelium which is thin, hyaline to light brown in colour. Conidia were globose, hyaline to pale brown colour which are produced on dichotomously branched conidiophore. The morphological characters obtained are in accordance with the description of Hennebert (1973).

➢Among the different solid media used, the maximum growth of *A. ricini* was recorded on Malt extract agar medium (8.5 cm) and minimum growth was recorded on Richard's agar medium (2 cm).

➢Colony colour differed with different solid media and varied from white to dirty white, cottony white, brown to light brown in colour.

➢The number of sclerotia produced on various media varied (3-250 per plate) with the type of medium used. Sclerotial production observed on 10th day after inoculation (DAI) on Carrot agar, Malt extract agar and Oat meal agar medium and up to 17 DAI on PDA and Richard's agar. Sclerotial formation was not observed on Czapek's agar and V8 juice agar medium.

➢ITS rDNA sequence analysis of pathogen confirmed the anamorph associated with gray mold of castor is *A. ricini.*

Summary

➢ Morphological and molecular studies confirmed that the anamorph of the fungus associated with gray mold of castor as *A. ricini*.

➢ Among solid media tested, highest radial growth of *A. ricini* was noticed on Malt extract agar. The colour of the culture on different media varied from white to dirty white, cottony white, brown to light brown in colour.

➢ Sclerotial number per plate varied from 3-250 based on the type of media used and CA/ MEA supported the maximum production of sclerotia on 10 DAI.

➢ PCR amplification and sequencing of ITS rDNA region of the pathogen confirmed castor proved *A. ricini* as the cause of gray mold of castor in and around Bengaluru.

Advisory committee

Chairman: Dr. K. Karuna

Members: Dr. T. Narendrappa
Dr. M. K. Jayashree
Dr. Veena.S.Anil

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I INTRODUCTION

Castor (*Ricinus communis* L.) a member of Euphorbiaceae family is an important non-edible oilseed crop with immense industrial and medicinal value. It contains 40-60 per cent oil that is rich in triglycerides, mainly ricinolein and plays a vital role in Indian vegetable oil economy. Castor is indigenous to the south-eastern Mediterranean Basin, Eastern Africa and India, but is widespread throughout tropical regions. It is grown in tropical and subtropical climate. World production of castor, on an average, is around 12.5 lakh tonnes (SEA, 2014) and is produced in about more than 35 countries of which India, Brazil, China, Paraguay and Ethiopia are the major producers. India ranks first in area and production of castor in the world and its derivatives contributing to 65% share in world castor production. At present, India is the undisputed world leader in global castor economy in terms of production and export of castor oil and its derivatives. Castor oil is an essential source of raw materials for several industries and is of great economic importance in domestic, medicinal, and veterinary uses. Castor oil and its derivatives are used in the manufacturing of soaps, lubricants, hydraulic and brake fluids, paints, dyes, coatings, inks, cold resistant plastics, waxes and polishes, nylon, pharmaceuticals and perfumes (Mutlu and Meier, 2010). Important products like, octyl alcohols, proteins and active enzymes are also extracted from castor leaves. It plays a vital role in agricultural economy of arid and semi-arid regions of the country. Apart from meeting the internal demands, India exports 4 lakh tonnes of castor oil earning 40 billion rupees in foreign exchange (SEA, 2014). With the introduction and release of high yielding hybrids, castor is now established as a commercial crop.

In India, castor is cultivated in an area of 8.29 lakh ha with 1.42 lakh tonnes of production and the average yield is 1713 kg ha^{-1} (Anon., 2018). The major castor growing states in the country are Karnataka, Andhra Pradesh, Gujarat, Maharashtra, Odisha, Rajasthan and Telangana. In Karnataka it is grown in an area of 0.09 lakh ha with a production of 0.031 lakh tonnes and productivity of 333 kg ha^{-1} (Anon., 2018).

Castor is affected by many biotic stresses. Among them fungal and bacterial diseases are severe at different crop growth stages. About 150 organisms are known to be pathogenic to castor plant. The crop is mainly affected by wilt, gray mold, root rot, seedling blight, *Cercospora* leaf spot, *Alternaria* blight, powdery mildew and bacterial leaf spot. Gray mold caused by the fungus *Amphobotrys ricini* is one of the most destructive disease of castor in India. In the states of Telangana, Andhra Pradesh, Tamil Nadu and Karnataka, the area under castor cultivation is continuously decreasing due to gray mold. In India, gray mold of castor was first reported in Karnataka (Anon., 1921). The anamorphic phase of *Botryotinia ricini* is responsible for disease epiphytotics and heavy yield losses are observed as the inflorescence (raceme) and the capsules are the primary targets of the fungal infection. The disease appeared in epidemic form during the years 1965 and 1987 causing extensive damage to the crop in erstwhile, Andhra Pradesh and Tamil Nadu states. Since then the disease started appearing year after year attaining serious proportions limiting castor production in southern states of India. Several anamorphs of *Botryotinia ricini* are found to incite gray mold in various countries.

Yield losses due to the disease are a major threat to commercial cultivation of the crop. Seed yield loss up to 100% was reported from India (Soares, 2012). A meticulous study of gray mold was conducted in the early 20th century (Godfrey, 1923), but only a few studies on this disease have been conducted recently (Soares, 2012). Consequently, there have been only a few advances in the management of gray mold. Breeding programs have failed to develop resistant genotypes, but genotypes with moderate levels of tolerance have been identified (Anjani, 2012). Inspite of availability of control measures against gray mold in castor, epiphytotic incidences of gray mold are not uncommon.

Very little information is available regarding its management and seed yield loss up to 100 per cent was reported from this disease. The ambiguity on the anamorph of castor gray mold pathogen still exists as two anamorphs, *viz*., *Amphobotrys ricini*, and *Botrytis cinerea* are found to be associated with this disease (Soares, 2012).

Therefore in view of the importance of the crop and disease management, present investigation on gray mold of castor was conducted with the following objectives.

Objectives:

1. Morphological and molecular characterization of gray mold pathogen of castor.

2. *In vitro* evaluation of fungicides against gray mold pathogen.

3. Management of castor gray mold disease.

II REVIEW OF LITERATURE

Castor (*Ricinus communis* L.) is an important non edible oilseed crop grown in India. This crop suffers from many diseases, among which gray mold disease caused by *Amphobotrys ricini* is the most serious disease causing considerable loss in many castor growing areas.

In this chapter, an attempt has been made to review the work done on various aspects of the pathogen and disease and presented briefly under different headings.

2.1 Distribution and economic importance of gray mold disease

Castor gray mold was first reported in the state of Florida in 1918. Later, the disease was reported from almost all countries where castor has been cultivated (Kolte, 1995), having a worldwide distribution.

Tropova (1928) reported that castor bean inflorescences infection by gray rot pathogen *Botrytis cinerea* in Don region of Russia. Subsequently, occurrence of the disease was reported from Ukraine region of Russia (Bohovic, 1936). In 1932, the disease was reported from Sao Paulo state in Brazil (Goncalves, 1936) and later from Africa, Asia, Europe, America and from almost all countries where castor has been cultivated (Kolte, 1995).

In India, incidence of gray rot of castor was reported for the first time in 1985 and was identified to be caused by *Botrytis ricini*. Later sporadic incidence of disease was observed but its occurrence became a regular feature from 1997-1998 (Anon.,1998).

Godfrey (1919) reported that in susceptible varieties and hybrids of castor the losses were up to 40 per cent in irrigated areas and up to 85% in humid areas due to gray mold incited by *B. ricini*. In Mississippi, the disease affected more than 25% racemes in a commercial field (Stone and Culp, 1959). In India, gray rot incidence occurred at an epidemic level with extensive damage of the crop in Andhra Pradesh (Moses and Reddy, 1989) and Tamil Nadu (Anon., 1995) which led to the decline in castor cultivation (Rao, 1997). The disease is more prevalent in the southern states where the weather conditions are more favourable for disease development and the fungus was responsible for losses up to 100 % of castor yield (Godfrey, 1923). In 1987, an epidemic outbreak of gray mold occurred in southern states of Andhra Pradesh and Tamil Nadu (Dange *et al.,* 2005).

2.2 Pathogen and symptomatology

2.2.1 Pathogen

The causal agent of gray mold of castor was originally reported by Godfrey (1919) as *Sclerotinia ricini* Godfrey, based on the holomorph. Later, Whetzel (1945) transferred the species *S. ricini* to the genus *Botryotinia*, which since then it has been known as *Botryotinia ricini* (Godfrey) Whetzel.

Buchwald (1949) named the anamorphic state of *Botryotinia ricini* as *Botrytis ricini* N.F. Buchw. In 1973, Hennebert erected a new genus, *Amphobotrys,* to accommodate the anamorphic state of *B. ricini*, based mainly on the distinctive pattern of conidiophore ramification, and since then the anamorphic state became known as *Amphobotrys ricini* (N.F. Buchw.) Hennebert (Hennebert, 1973). Even so, several authors used, and still use, the erroneous name "*Botrytis ricini*" attributing its authority to Godfrey (Barreto and Evans, 1998; Batista *et al*., 1998; Lima and Soares, 1990). However, several anamorphs of *Botryotinia ricini* are found to incite gray mold in several countries. Sung-Kee *et al*. (2001) isolated the pathogens associated with gray mold of castor from 25 locations in Korea. Out of the 25 isolates studied, 5 isolates which originated from Wonju were identified as *Botrytis cinerea*, while 20 isolates from Okcheon were identified as *Amphobotrys ricini* based on morphological and cultural characteristics.

2.2.2 Symptomatology

The primary targets of the fungus are the inflorescence and the capsules, in any development stage. (Araujo *et al*., 2007; Dange *et al*., 2005; Goncalves, 1936 and Lima *et al*., 2001). Drumond and Coelho (1981) and Batista *et al*. (1996) reported that the male flowers are the first to be infected, but Soares (2012) reported that it is not always the case because any part of the inflorescence can be infected. However, the female flowers being the preferential target.

The initial symptoms on the inflorescence are visible as bluish spots on both female and male (before anthesis) flowers, and also on developing fruits. On fruits, the symptoms can be observed as circular or elliptic, sunken, dark coloured spots that can result in rupture of the capsule (Araujo *et al*., 2007).

The symptoms of gray mold of castor was first described by Godfrey (1923). Initial symptoms are seen as small bluish spots on panicles, leaves and stems from which yellowish drops of liquid exude. The inflorescence or 'spike' is covered with a moldy growth which is gray at first and then olive gray. As the affected inflorescence becomes older, the mass become darker in colour and more compact. The racemes are covered by tan to gray coloured fungal growth. As the infection spreads upwards, the entire racemes including flowers, capsules and seeds become infected resulting in total destruction of the affected parts.

Under favourable weather conditions, profuse sporulation is observed when the infection starts on immature capsules, they become rotten, if the infection starts later, with fully developed capsules, the seeds usually became hollow, with coat discoloration and weight loss (Dange *et al*., 2005).

On the leaves, the disease is usually traceable to small dots where the inoculum is deposited from infected inflorescence. From this central point the fungus spreads, producing a light brown dead spot on the leaf accompanied by gray mold. The lesions are circular to sub-circular and vary in size from 5 to 9 mm in diameter affecting large portions of the leaf. As the lesions coalesce, they increase in area causing extensive necrosis of leaves. Infection also occurs apically or marginally and near or around the base of the leaf where dew or rain usually accumulates. The lesions are pale brown and under humid conditions become covered with a delicate web of mycelium. At times, large blighted areas on the leaves show zonations which mark the periodic advance of the disease (Godfrey, 1923; Orellana, 1959; Cook, 1981 and Moses and Reddy, 1989).

Infection on the stalks and branches do not readily occur, the tissue being more mature and consequently more resistant (Godfrey, 1923). However, Moses and Reddy (1989) reported that the disease appeared on tender shoots and peduncles as water soaked lesions which turned dull white and the destruction of tissues in the affected portions resulted in breaking of stems and spikes.

Sung-Kee *et al*. (2001) reported gray mold of castor caused by *Botrytis cinerea* and *Amphobotrys ricini* in Korea. Gray mold symptoms on leaves of castor observed in Wonju area appeared as oval to irregular grayish spots. Lesions enlarged and became grayish brown blight with abundant conidial mass in humid conditions. However, gray mold of castor observed in Okcheon slightly differed from that found in Wonju in terms of spotted lesions on the leaves. The symptoms were circular to oval spots on the leaves at the early stage of infection. As symptoms progressed, lesions enlarged and became pale brown blight with dark blue to purple borders at the margins of the leaves. Conidial mass developed on the lesions at late stage of the disease, which was very similar to that observed in Wonju. The pathogen associated with gray mold in Wonju and Okcheon in Korea were identified as *Botrytis cinerea* and *Amphobotrys ricini*, respectively based on morphological and cultural characters.

Differential susceptibility of castor cultivars to *B. ricini* was studied by Thomas and Orellana (1963). They found that the structure of the flower raceme of castor bean is important for its susceptibility to *B. ricini*. Cultivars with short internodes, compact inflorescences were more severely attacked than loose inflorescenses, and staminate flowers were more susceptible. The compact inflorescences and short internodes favoured surface water retention and hence improved the likelihood of infection.

2.3.1 Morphological characterization of gray mold pathogen of castor

Botryotinia ricini belongs to the phylum Ascomycota (Family: Sclerotiniaceae, Order: Helotiales) and is characterized by its dark, plane-convex, elongated sclerotia, which give rise to cinnamon brown to chestnut brown, long stipitate apothecia, with cylindrical to cylindro-clavate asci containing eight ascopores, ellipsoidal, often sub-fusoid, one-celled, bi-guttulate and hyaline, paraphyses hyaline, filiform, septate

(Godfrey, 1919). Its anamorphic phase is characterized by cylindrical, straight, dichotomously branched, pale brown conidiophores with conidiogeneous cell not inflated, thin-walled, conidia globose, maturing synchronically, on short denticles, smooth, one-celled, sub-hyaline to pale brown (Godfrey, 1919; Hennebert, 1973 and Lima *et al*., 2008).

Species of *Botrytis* to date have been delimited primarily on the basis of morphological and cultural characteristics coupled with host specificity (Hennebert, 1973). Characters such as sclerotial size, form and size of conidium are useful in delimiting some species, though many species are morphologically similar with growth conditions significantly influencing variation.

Goto *et al*. (1980) classified the grapevine isolates of *Botrytis cinerea* into five types (SI-SV) based on the presence and mode of sclerotia formation on PDA. Among them 60% belonged to SIII and formed large sclerotia. Classified as types W or L (weakly or strongly pathogenic, respectively), 77% of isolates were strongly pathogenic (L). A fairly good correlation was observed between the type of sclerotium, colony and pathogenicity. Most SI and SII isolates formed colonies of type CI or CII and were of pathogenicity type weakly (W), most SIII isolates formed CIII or CIV colonies and were of strong pathogenic type.

Cook (1981) reported that the gray mold incited by *Botryotinia ricini* (Godfrey) Whetzel produced dichotomously branched conidiophores bearing globose shaped, hyaline microconidia (2-3.5 m μm). It produced black, irregular sclerotia that germinate by production of apothecia 5-30 mm in height and 1-7 mm in diameter with curved margins, which produces cylindrical asci (6-10 × 50-100 μm). The ascospores are ellipsoidal, hyaline measuring 4-5 × 9-12 μm.

Bryk (1985) studied the variability of monoconidial culture of *Botrytis cinerea* on morphological characters and mycelial radial growth and they observed that better mycelial growth was observed on potato dextrose agar (PDA) than on Czapek's medium.

But more sclerotia production was observed on Czapek's medium, however smaller in size than on PDA medium.

Moses and Reddy (1989) observed sclerotia formation of *Botryotinia* on axis and peduncles of old inflorescences of castor. Sclerotia were found to be black, rough, elongated, irregular, 1-25 mm long (usually 3 to 9 mm), sub-erumpent to superficial on inflorescence stalks.

Sung-Kee *et al*. (2001) described the growth characters of *Botrytis cinerea* and *Amphobotrys ricini* based on morphological and cultural characteristics. *B. cinerea* on potato dextrose agar (PDA) produced pale gray colonies with abundant aerial mycelium with black, irregular and large sclerotia. Conidiophores were erect and usually over 1.0 mm high. Stipes were slender, cylindrical, pale brown below, paler near the apics, 12.0-23.0 μm wide, some with swollen basal cell, and branches alternating at about three-fourths of the height from the basal portion and branched again two to three times. Conidiogenous cells were inflated at apices producing conidia on sterigma. Conidia were globose to subglobose or ellipsoidal, unicellular, pale brown, smooth, and measured 7.5-13.3 x 7.0-10.5 μm.

Amphobotrys ricini on PDA produced effuse, pure white, cottony mycelium, and produced small, black and irregular sclerotia. Conidiophores scattering over the infected plant parts were single, erect, and usually 1.2-1.3 mm high. Stipes were long, slender, cylindrical, pale brown, and 10.0-12.5 μm wide, with unswollen basal cells, branches bifurcating at about two-thirds height from the basal portion and branched dichotomously again two to three times. Each apical cell of the branches and branchlets was delimited by a septum. Conidiogenous cells were ampulliform, somewhat inflated at the apices, 6.3-8.8 μm wide, and produced conidia on cylindrical sterigma measuring 2.0-2.5 μm in length. Conidia were globose, unicellular, pale brown, smooth, and measured 5.0-10.8 μm (av. 8.1 μm) in diameter. Microconidia produced in PDA culture were globose, hyaline, and measured 2.0-2.7 μm in diameter.

Mirzaei *et al*. (2007) characterized 355 isolates of *Botrytis* from various gray mold infected plants in Isreal and identified the species based on morphological characters such as conidiophore length, conidial and sclerotial dimensions. Conidiophore length was 662-2999 μm, conidial dimension fell in the range of 6-13 × 4-8 μm and sclerotial dimension was 1- 11 × 1-8 mm.

Tanovic *et al*. (2009) studied the morphological growth characters of *Botrytis cinerea* isolates on PDA and Malt agar (MA) medium. They reported that faster growth and more sclerotia were recorded on MA medium, but the number of isolates that produce sclerotia on PDA medium was higher than on MA medium, more sporulation was also observed on MA medium.

Hosen *et al*. (2010) studied the effect of different culture media on colony growth, sporulation and sclerotia formation of *Botrytis cinerea*. They reported that Chickpea dextrose agar medium supported the highest mycelial radial growth of 79.17 mm. The quickest sclerotia initiation was recorded on Chickpea destrose agar (in 5 days) and lentil dextrose agar (LDA) culture media, while the highest number of spores (2.5×10^4 m L^{-1}) were recorded on LDA medium.

Raluca and Emilia (2010) studied the morphology of *Botrytis cinerea* on PDA medium. The conidia were ovate, ellipsoidal or sometimes globose to subglobose. They were smooth, often with a slightly protuberant hilum and usually unicellular but occasionally septate conidia were observed. The sclerotia were black, shiny black or white at first, becoming black with the age. They were variable in shape and size. Sclerotia were formed in rings, surface of the medium, edges of the plates and irregularly distributed. Number of sclerotia per plate fell in the range of 36- 190.

Vasilica *et al*. (2012) studied the morphological characteristics of *Botrytis cinerea* isolates on three different media like Potato Dextrose agar (PDA), Czapek's agar and Malt agar (MA) medium. The majority of the colonies grown on the PDA and MA media reached maximum development on sixth day, on Czapek's medium the fungus had a slower development. The highest sporulation was observed on Czapek's medium. The colony colour varied on different media with different growth pattern. The colonies were fluffy, radial or in concentric rings, warty with different colors ranging from white, dirty white, grayish white or hyaline and later becoming light gray, dark gray. Conidia were ellipsoidal or sometimes globose, smooth, often with a slightly protuberant hilum and unicellular. Sclerotia formed on 12th day after inoculation and formed in concentric rings, along the edges of the Petri dish or scattered irregularly.

Ahmed *et al*. (2014) reported that the highest mycelial growth of *Botrytis cinerea* was recorded on malt extract agar medium with an average colony diameter of 81.26 mm followed by chickpea dextrose agar medium 81.11 mm.

Tanovic *et al*. (2014) based on colony morphology of *Botrytis cinerea* on PDA medium, the isolates were sorted into eight groups, described by Martinez *et al*. (2003). Among the isolates those forming large, irregularly placed sclerotia were prevalent (81.5 %), while the mycelial type isolates with mycelial masses were the least frequent (0.7 %).

Zada *et al*. (2016) reported that maximum radial growth of *Botrytis cinerea* was recorded on Czepk’s and Malt extract medium (85 mm) and minimum growth was observed on PDA (74.1 mm). The colony color was varied from whitish to black grayish with fluffy texture. Fungus produces gray mycelium with branched conidiophores having rounded apical cells bearing cluster of colorless or gray one celled, ovoid conidia. The conidiophores and cluster of conidia resembles a grape-like cluster.

2.3.2 Molecular characterization of gray mold pathogen of castor

The phylogenetic approach to systematics has been boosted by the advances in DNA sequencing, but for *Botrytis* this approach is in its infancy. The ITS rDNA region has been widely used to discriminate the fungi at species-level, but the variation in the ITS region within *Botrytis* is low. An example for the application of modern methods to species recognition in *Botrytis* has been provided for the neck-rotting species of onion.

Thompson and Latorre (1999) characterized the 29 isolates of *Botrytis cinerea* from table grapes in Chile using RAPD-PCR. The DNA was amplified using the primers

OPA4 and OPA11, these primers distinguished isolates of *B. cinerea* from other epiphytic fungi found on table grapes, including *Alternaria alternata*, *Aspergillus niger*, *Cladosporium herbarum*, *Epiccocum nigrum*, *Rhizopus stolonifer*, *Penicillium* sp.

Universal-primed polymerase chain reaction (UP-PCR) fingerprinting, coupled with restriction analysis of ITS rDNA regions, allowed five groups to be distinguished: *B. cinerea*, *B. squamosa*, *B. byssoidea*, and two groups in *B. aclada* (AI and AII) (Nielsen *et al*., 2001).

Munoz *et al*. (2002) evaluated the genetic diversity of *B. cinerea* in Chile, isolates were collected from grapes, tomato, kiwifruit and blueberry and they were analysed using molecular markers. RAPD analysis revealed a high degree of genetic diversity, dendrograms, analysis of molecular variance revealed the existence of genetic differentiation between isolates from the different host plants. PCR-RFLP markers also showed that isolates sampled from grapes and tomato were genetically differentiated.

Staats *et al*. (2005) conducted molecular phylogenetic analyses of 22 *Botrytis* species and one hybrid using ITS 1 and ITS 4 primers. Based on the morphological characters together with molecular data nearly all obtained sequences corresponded with *Botrytis cinerea*.

Rigotti *et al*. (2006) designed primers, BC108 and BC563, for the detection of *Botrytis cinerea* strains which used to amplify a DNA fragment of 0.48 kb for the main group of 26 *B. cinerea* strains. Other closely related *Botrytis* species such as *B. allii* and *B. fabae* were not amplified with these primers, confirming their specificity for *B. cinerea.*

Khazaeli *et al*. (2010) characterized 30 isolates of *Botrytis cinerea* collected from rose greenhouses using specific primer pairs C729. Based on the morphological identification, *i.e*., conidiophore and conidial length, all the isolates were found to be *Botrytis cinerea*. Using specific primer pairs (5’-AGCTCGAGAGAGATCTCTGA-3’; 5’- CTGCAATGTTCTGCGTGGAA-3’), a DNA fragment of 700 bp was amplified for most of the isolates, thus confirming the presence of *Botrytis cinerea* on rose samples.

Kwon *et al*. (2011) reported gray mold of blueberry caused by *Botrytis* sp. for the first time in Korea. A detailed description of the fungus is given based on morphological characters and amplification of rDNA using ITS primers 1 (5'-TCCGTAGGTGAAC CTGCGG-3') and ITS 4 (5'-TCCTCCGCTTATTGATATGC-3'). The fungus was identified as *Botrytis cinerea* based on morphological characteristics and molecular data.

Sarita Kumari *et al.* (2014) collected 79 isolates of *Botrytis cinerea* from different host plants and different locations of India and Nepal. All the isolates were identified as *B. cinerea* based on morphological features and were confirmed using *B. cinerea* specific primers. Differentiation among the isolates was assessed using morphological, genetic and biochemical approaches. To analyze morphological variability, differences in conidial size, presence or absence of sclerotia and their arrangement were observed. However, isolates could not be grouped on the basis of a single approach which provides evidence of their wide diversity and high evolution potential.

Saito *et al*. (2014) identified 526 isolates of *Botrytis* sp. based on PCR-RFLP of a fragment of the *Bc-hch* gene. Four isolates studied showed distinctive restriction banding pattern associated with *B. pseudocinerea.* The identity of these four isolates was further confirmed by sequencing portions of four genes, internal transcribed spacer region, glyceraldehyde-3-phosphate dehydrogenase (G3PDH), heat-shock protein 60 (HSP60), and DNA-dependent RNA polymerase subunit II (RPBII). BLAST analysis showed that sequences of all four genes of the four isolates were 99.8 to 100% similar to those of *B. pseudocinerea*.

Zhang *et al*. (2018) identified 135 isolates of *B. cinerea* using PCR-RFLP of a fragment of the Bc-*hch* gene. All the isolates were heterothallic, belonged to two genetic clusters. Seventy one isolates belonged to the MAT1-1 type and 64 were characterized to MAT1-2 type.

2.4 *In vitro* evaluation of fungicides against gray mold pathogen

Dispite of many ill effects of chemical control, it is still a first line of control to tackle several destructive plant disease. The infectious fungal pathogens which are seed, soil, air borne in nature can be effectively controlled by using fungicides as seed, soil and foliar applications.

2.4.1 *In vitro* evaluation of fungicides against gray mold pathogen

Iqbal *et al*. (1992) tested fourteen fungicides (Antracol, Bayton, Benlate, Calixin-M, Cobox, Daconil, Dithane M-45, Liromenzeb, Polyram Combi, Ridomil, Tecto-60, Topsin-M, Tri-Miltox Forte and Vitavax) against *Botrytis cinerea* the causal fungus of lentil blight by poisoned food technique. Banlate, Calixin-M and Tecto-6- were the most effective in suppressing the growth of the fungus**.**

Singh *et al*. (2008) evaluated the efficacy of eight fungicides (systemic and non systemic) *viz*., Carbendazim, Benomyl, Mancozeb, Chlorothalonil, Iprodione, Zineb, Vinclozolin and Quintal against *Botrytis gladiolorum* under *in vitro* condition using detached leaf technique. All the test fungicides, except Carbendazim and Benomyl showed good efficacy with lower per cent disease index.

Hosen *et al*. (2010) tested the efficacy of seven different fungicides *in vitro* against *Botrytis cinerea* of chickpea. Among the fungicides tested Carbendazim, Thiophanate methyl and Iprodione were the most effective to inhibit the mycelial radial growth of *B. cinerea* at 500 mg L^{-1} concentration.

Koycu *et al*. (2012) tested the sensitivity of *Botrytis cinerea* isolates to Cyprodinil + Fludioxonil, Fenhexamid, Procymidone, Pyrimethanil and Tebuconazole under laboratory conditions. Results showed that the isolates were more sensitive to Cyprodinil + Fludioxonil than to Fenhexamid, Imazalil, Procymidone, Pyrimethanil and Tebuconazole.

In vitro efficacy of nine different fungicides systemic and non systemic *viz*., Amistar, Score, Bavistin, Contaf, Hilnate (50, 100, 150 and 200 ppm), Avtar, Cabrio top, Quintal and Antracol (150, 500,750 and 1000 ppm) were evaluated against *Botrytis* *cinerea*. Complete mycelial inhibition was observed in Quintal, Contaf @ 50, 100, 150 and 200 ppm followed by Avtar (91.16 %) and Score (63.99 %) respectively (Kaur, 2015).

Rautela and Singh (2017) evaluated the fungicides against mycelial growth of *B*otrytis *cinerea* @ three different concentration of 25, 50, and 100 μgML^{-1}. Among the fungicides tested Azoxystrobin + Tebuconazole and Azoxystrobin + Chlorothalonil were more effective showing 100 per cent inhibition of mycelial growth of *B. cinerea*, which were on par with Captan + Hexaconazole (96.67 %). Whereas Chlorothalonil was found to be least effective showing 45.19 per cent inhibition at 25 $\mu gM\ L^{-1}$.

2.4.2 *In vitro* evaluation of nano particles against gray mold pathogen

Maekawa *et al*. (2003) reported that hyphal growth of rice blast fungus was very slow on agar plates containing liquid silicon.

Kim *et al*. (2009) investigated the antifungal activity of silver nano particles against *Raffaelea* sp. It was observed that growth of *Raffaelea* was significantly inhibited in the presence of silver nano particles in a dose-dependent manner.

Fayadh and Aledani (2011) reported that silicon elements were completely inhibited the growth of the pathogenic fungus *Rhizoctonia solani* at 30, 200 and 500 ppm concentration.

He *et al*. (2011) reported that zinc oxide nano particle at concentrations greater than 3 mmol L^{-1} significantly inhibit the growth of *Botrytis cinerea.*

Kaur *et al*. (2012) reported fungicidal properties of silver nano particles used as an agent for antifungal treatment of various seed borne plant pathogens *viz*., *Rhizoctonia solani*, *Aspergillus flavus* and *Alternaria alternata* and observed the antifungal activity of the silver nanoparticles (100 $\mu g\ ml^{-1}$) by inhibition of mycelial growth of the fungi.

Elamawi *et al*. (2013) studied the effect of silver nanoparticles (20-30 nm) against rice leaf blast caused by *Magnaporthe grisea*. Under laboratory conditions, the application of four concentrations *viz*., 25, 50, 100 and 200 ppm of silver nano particles showed significant inhibition of both hyphal growth and number of colonies formed in a dose-dependent manner.

Khan *et al*. (2013) reported silicon in the form of sodium silicate was found to be effective in reducing mycelial growth of *Macrophomina phaseolina* and also observed gradual reduction of mycelial growth with increase in concentration of silicon in the agar medium.

Saharan *et al*. (2013) reported that Cu-chitosan nanoparticles were most effective at 0.1% concentration and showed inhibition of *Alternaria alternata* (89.5 %), *Macrophomina phaseolina* (63.0 %) and *Rhizoctonia solani* (60.1 %) under *in vitro* condition. At the same concentration, Cu-chitosan nano particles also showed maximum of 87.4 per cent inhibition rate of spore germination of *A. alternata*.

Shantamma (2016) reported colloidal silver suspension at the concentration of 500 ppm inhibited the growth of *Phytophthora infestans* by 86.13 per cent and at 25 ppm concentration inhibited the growth of *Xanthomonas axonopodis* pv. *punicae* by 100 per cent.

Zalewska *et al*. (2016) studied the effect of nano particles on growth of *Septoria carvi* colonies on caraway *in vitro* using fertilizers containing nano particles of copper, silver and chitosan and they reported that Viflo Cal S at the concentration of 1.00 gcm^{-3} totally inhibited the mycelial growth of *S. carvi*.

Nejad *et al*. (2017) tested the *in vitro* efficacy of silver nano particles against *Rhizoctonia solani* which causes sheath blight in rice. They observed that at 50 ppm concentration of silver nano particles highest inhibition level against sclerotia formation (92 %) and mycelia growth (85 %) was recorded.

2.5 Management of castor gray mold disease

Rathi (1994) evaluated the six fungicides against *Botrytis* gray mold of chickpea. The results revealed that Bavistin and Ronilan gave the highest protection to crop with least disease severity and other fungicides *viz*., Dithane M-45, Dithane Z-78, Captan and Fytolan reduced the disease severity significantly over control.

Elad *et al*. (1995) reported that fungicides *viz*., Dicarboximide (Iprodione or Procymidone) were applied alone decreased the gray mold disease by 40-80 per cent and also the disease was effectively suppressed by the application of Dicarboximide with Thiram, Dichlofluanid or Tebuconazole. Similar results of disease suppression was also achieved by mixtures of Tebuconazole + Dichlofluanid and Carbendazim + Diethofencarb.

Mlikota *et al*. (1996) conducted an experiment to study the effectiveness of fungicides in controlling gray mold on grapes. All the fungicides which were tested *i.e*., Iprodione, Vinclozoline, Procymidone, Dichlofluane and Folpet gave a significant reduction in disease.

Pande *et al*. (1998) evaluated the ten fungicides Carbendazim, Balyeton, Belltone, Captaf, Dithane M-45, Kavach, Baycor, Celest, Chlorothalonil and Cyperconazole for control of foliar infection of *Botrytis* gray mold and results revealed that all the fungicides which were tested were very effective in controlling foliar infection.

Chaurasia and Joshi (2001) reported that three sprays of Bavistin @ 0.2 per cent is effective in reducing the gray mold of chickpea.

Rosslenbroich and Stuebler (2000) carried out biochemical studies on newly introduced Botryticides *viz*., the Anilinopyrimidines Pyrimethanil, Cyprodinil and Mepanipyrim, Phenylpyrrol Fludioxonil and the Hydroxyanilide Fenhexamid, which indicated that Fenhexamid is an effective tool for management of *Botrytis* which is having anti-resistance management strategies.

Singh *et al*. (2008) tested the efficacy of fungicides *viz*., Mancozeb, Chlorothalonil, Iprodione @ 0.2 per cent, Benomyl and Bavistin @ 0.1 per cent under field condition against *Botrytis gladiolorum* of gladiolus. Out of five fungicides tested, three fungicides namely Mancozeb, Chlorothalonil, Iprodione provided very good control of the disease with reduced foliar infection and enhanced cormel yield significantly over the control.

Jung *et al*. (2010) studied the effect of nano silver liquid against white rot of green onion caused by *Sclerotium cepivorum* and observed reduced disease severity and increased biomass of plants under greenhouse condition.

Lamsal *et al*. (2011) reported that successful control of cucumber and pumpkin powdery mildew using silver nano particles at 100 ppm concentration observed the highest inhibition rate for both before and after the outbreak of disease on cucumbers and pumpkins under field condition.

Pandey (2011) carried out an experiment for the management of *Botrytis* blight in gladiolus through chemical means. They tested both systemic and non systemic fungicides. The results were showed that the fungicides Stroby and Flint were found to be the most effective against *Botrytis* blight under field conditions and also the fungicides Kavach and Dithane-M-45 also showed very promising efficacy.

Koycu *et al*. (2012) tested the sensitivity of *Botrytis cinerea* isolates to Cyprodinil + Fludioxonil, Fenhexamid, Procymidone, Pyrimethanil and Tebuconazole under *in vivo* condition. Results of efficacy tests showed that Cyprodinil + Fludioxonil and Tebuconazole were the most effective fungicides (100 %) on isolates both resistant and sensitive to these fungicides. No lesion on berries was observed on the fungicide applied and inoculated fruits.

Rashid *et al*. (2014) evaluated six fungicides against *Botrytis* gray mold of chickpea under field condition. Among the fungicides Companion was most effective, resulting in the lowest disease severity (3.33 score on 1–9 scale) and the highest increase (38 %) of grain yield.

Bhat and Rajasri (2015) tested the efficacy of different fungicides against gray rot incidence, among the fungicides tested Propiconazole @ 1 ml L^{-1} was found to be highly effective in controlling the disease with least disease incidence (9.6 %) and higher yield of 2473.6 kg ha^{-1} followed by Carbendazim as compared to control.

Shahiduzzaman (2015) carried out an experiment to evaluate the most effective fungicides in controlling *Botrytis* gray mold of chickpea during 2011-12 and 2012-13. Among the five different fungicides used Fenamidone + Mancozeb (Secure 600WG) sprayed at the rate of 1 g L^{-1} with seven days interval recorded lowest *Botrytis* gray mold score of 3.80 and 4.00 in 1-9 scale, with highest yield of 1547 and 1443 kg ha^{-1}, respectively during the two consecutive years over untreated control plot with *Botrytis* gray mold score of 6.26 and 6.33 and produced the lowest yield of 988 and 853 kg ha^{-1} during the two consecutive years.

III MATERIAL AND METHODS

The present investigation was undertaken during 2017-18 at Zonal Agricultural Research Station, University of Agricultural Sciences, GKVK, Bengaluru. The experimental site is located in the eastern tract (Zone 5) of Karnataka at 12^0 58' N latitude and 77^0 35' E longitude and at an altitude of 930m above mean sea level.

3.1 General procedure

3.1.1 Glassware and cleaning

For all the laboratory experimental studies Borosil glassware's were used. The glass wares were kept in the cleaning solution containing 60g of potassium dichromate ($K_2Cr_2O_7$) and 60 ml of concentrated sulphuric acid (H_2S0_4) in one litre of water for 24 hours and then washed with detergent followed by cleaning in running tap water and rinsing in distilled water.

3.1.2 Sterilization

All the glassware's as well as both solid and liquid media were sterilized in an autoclave at 1.1 $kgcm^{-2}$ pressure at 121.6 ^{0}C for 15 minutes. Inoculation needles, forceps, cork borers and blades were sterilized by passing through the flame after dipping in alcohol.

3.1.3 Equipment and apparatus used

Incubators were used for incubating cultures at different temperatures. The cultures were stored in a refrigerator. Haemocytometer was used for recording spore count and adjusting the spore concentration. Samples were weighed using a single pan electronic balance. The other equipment and apparatus used in the present study for various experiments included inoculation needles, microscope *etc*.

3.1.4 Media preparation

Oat meal enriched medium was used for the isolation of the pathogen, maintenance of culture and also in most of the experimental studies. The composition of oat meal enriched medium was as follows

Oat meal	:	72.00 g
Castor pericarp extract	:	20.00 %
Agar agar	:	20.00 g
L-asparagine	:	1.00 g
Gallic acid	:	1.00 g
Yeast extract	:	1.50 g
Distilled water (Volume to make up)	:	1000.00ml

The medium was prepared by boiling 200 g of fresh castor capsules in 500 ml of water in a metallic container for about 25 minutes and filtered through a muslin cloth into an another container. In other container with 500 ml water, 72 g of oat meal powder, 20 g of agar, 1.5 g of yeast extract, 1 g of L-aspargine and1g of gallic acid were added and melted. The final volume of the medium was made up to 1000 ml by adding required water and the pH of the medium was adjusted to 6.0 before autoclaving by using 1N HCl or 1N NaOH as the case may be. Known quantity of such medium was dispensed into a number of conical flasks and plugged with non-absorbent cotton and finally wrapped with brown paper. The flask containing dispensed medium was autoclaved at 15 *psi* (121.6 ^{0}C) for 15 minutes.

3.2.1 Isolation of pathogen

The gray mold infected capsules were collected from GKVK campus and brought to the laboratory for isolation of the pathogen causing gray mold. Gray mold infected capsules were microscopically examined for confirmation of the fungus. Sections of the diseased capsules were made with the help of a sharp blade on a clean glass slide having a drop of lactophenol. The specimen was then covered with a cover slip and observed under microscope.

After confirmation of the fungus as *Amphobotrys*, infected capsules exhibiting typical gray mold symptoms were selected and pathogen was isolated by following standard tissue isolation method.

The infected capsules were cut into small pieces and were surface sterilized by immersing in 0.1 per cent mercuric chloride for 30 seconds and placed on to the Petri plates containing oat meal enriched medium after thorough washings in sterile distilled water. The plates were incubated at 23±1^{0}C temperature for 3 to 4 days. The fungal growth emerging from diseased capsule pieces were observed. A loop full of fungal culture developed on oat meal enriched medium in Petri plates was taken on a glass slide and observed under microscope for presence of conidia.

Single spore isolation technique was followed for the purification of pathogen. The spore suspension of the fungal isolate was prepared in sterile distilled water and one ml of the suspension from fungal isolate spread gently on 15 ml of molten two per cent water agar. Petri plates were observed for the presence of conidia under compound microscope.

The spore along with water agar was picked and transferred on oat meal enriched medium plates and slants. Petri plates were incubated at room temperature (23±1 ^{0}C) and observed for fungal growth and the pure culture so obtained was preserved on oat meal enriched medium slants in the refrigerator for further use.

3.2.2 Proving the pathogenicity of the isolated fungus

Pathogenicity test was carried out to establish the isolated fungus capability of producing typical symptoms of gray mold under artificial inoculation condition on castor and also to re-isolate the pathogen to confirm Koch's postulates of pathogenicity. The detached spikes of the castor raceme (DCS 9 or Jyothi) was placed in a closed polythene humid chamber and these detached spikes were kept in conical flask containing 2 per cent sucrose solution. The detached spikes were thoroughly sprayed with sterile distilled water using hand atomizer to ensure free water droplets on the surface of the capsules before inoculation.

Inoculum of the pathogen was prepared by using six day old culture of *A. ricini* grown on oat meal enriched medium. Conidia of the pathogen were harvested by flooding sporulating cultures with sterile distilled water and gently scraping the surface with a sterile needle. The resultant suspension was filtered through a muslin cloth and the conidial concentration was adjusted to 10^6 conidia ml^{-1} using a haemocytometer. Conidial suspension (10^6 conidia ml^{-1}) was spray inoculated on detached racemes of castor by using an atomizer sprayer.

The inoculated racemes were placed in a growth chamber at 25±1 ^{0}C temperature and above 90 per cent humidity and observation was made at regular intervals for the symptom development.

The symptoms appeared after 5-6 days of inoculation. The organism was re-isolated from these infected detached spikes showing gray mold symptoms and the culture obtained was compared with the original culture for confirmation.

3.2.3 Maintenance of pure culture

The pure culture was sub-cultured on oat meal enriched medium slants and allowed to grow for one week at 23±1 ^{0}C. Such slants were stored in a refrigerator at 4 ^{0}C and periodically sub-cultured once in a month to keep the pathogen in rejuvenated and viable condition.

3.3 Morphological and molecular characterization of gray mold pathogen of castor

3.3.1 Morphological characterization of gray mold pathogen of castor

3.3.1.1 Effect of different media on growth of gray mold pathogen of castor

Different media were used for morphological characterization of the pathogen. Fifteen ml of medium was poured in each Petri plate and allowed to solidify. Such plates were inoculated with 5 mm discs of the pathogen cut from periphery of the culture and incubated at room temperature (23±1°C). Each treatment was replicated thrice. Colony diameter was recorded by averaging the linear growth of colony in two directions for each plate. Colony colour, sclerotia number per plate, size of sclerotia and formation pattern of sclerotia were also noted. The data on radial growth was analyzed statistically. The fungus was identified based on morphological characteristics, *viz*., colour, growth pattern of the pathogen (Soares, 2012 and Sung-Kee *et al*., 2001). Comparision of morphological characters of anamorphs of *Botryotinia ricini* are represented in the Table 1 (Sung-Kee *et al*., 2001).

Table 1: Comparision of morphological characters of anamorphs of *Botryotinia ricini*

Sl. No.	Characters	*Botrytis cinerea*	*Amphobotrys ricini*
1	Colony colour and mycelium	Pale gray with abundant aerial mycelium	Effuse, pure white and cottony mycelium
2	Size of sclerotia	Large	Small
3	Branching of conidiophore	Dichotomously branched and branches alternating at about three-fourths of the height from the basal portion and branched again two to three times	Conidiophore branches bifurcating at about two-third height from the basal portion, and branched dichotomously again two to three times.
4	Size of the conidia	7.5-13.3 x 7.0-10.5 µm.	5.0-10.8 µm

Composition and preparation of the media were obtained from Ainsworth and Bisby's 'Dictionary of the fungi' by Hawksworth *et al*., (1983). The composition of the media are given below.

1. Oat meal enriched medium composition was mentioned in heading 3.1.4.

2. Potato Dextrose Agar (PDA)

Peeled and sliced potatoes	:	200.00 g
Dextrose ($C_6H_{12}O_6$)	:	20.00 g
Agar agar	:	20.00 g
Distilled water (Volume to make up)	:	1000.00 ml

Two hundred grams of peeled and cleaned potatoes were cut into small pieces. Later these pieces were boiled in distilled water and then extract was collected by filtering through muslin cloth. Dextrose 20 g and agar 20 g each were dissolved in potato extract and the final volume was made up to 1000 ml with distilled water. Known quantity of such medium was dispensed into a number of conical flasks and plugged with non-absorbent cotton and finally wrapped with brown paper. The flask containing dispensed medium was sterilized at 1.1 kg/cm^2 pressure for 15 minutes.

3. Castor leaf extract medium

Castor leaf bits	:	200.00 g
Dextrose ($C_6H_{12}O_6$)	:	20.00 g
Agar agar	:	20.00 g
Distilled water (Volume to make up)	:	1000.00 ml

The medium was prepared by boiling 200 g of fresh castor leaf bits in 500 ml of water in a metallic container for about 25 minutes. In another container 20 g of agar was melted in 500 ml of water. Both of these were filtered through a muslin cloth into another container and 20 g of dextrose was added to the filtrate. The final volume of the medium was made up to 1000 ml by adding distilled water.

4. V8 juice agar medium

V8 juice extract	:	8.30 g
L-aspargine	:	10.00 g
Calcium carbonate	:	2.00 g
Glucose	:	2.00 g
Yeast extract	:	2.00 g
Agar agar	:	20.00 g
Distilled water (Volume to make up)	:	1000.00 ml

44.3 g of V8 juice agar powder was suspended in 1000 ml distilled water and sterilized at 1.1 kg/cm^2 pressure for 15 min.

5. Oat meal agar

Oat meal	:	72.00 g
Agar agar	:	20.00 g
Distilled water (Volume to make up)	:	1000.00 ml

72 g of Oat meal powder was boiled in 400 ml of distilled water for 20 min and the extract was filtered through a muslin cloth. Agar agar was melted separately in 400 ml of distilled water. Both the solutions were mixed thoroughly. The volume was made up to 1000 ml with distilled water and sterilized at 1.1 kg/cm^2 pressure for 15 min.

6. Czapek's Agar

Sucrose ($C_{12}H_{22}O_{11}$)	:	30.00 g
Sodium nitrate ($NaNO_3$)	:	2.00 g
Potassium dihydrogen phosphate (KH_2PO_4)	:	1.00 g
Magnesium sulphate ($MgSO_4$. $7H_2O$)	:	0.50 g
Potassium chloride (KCl)	:	0.50 g
Ferrous sulphate ($FeSO_4$. $7H_2O$)	:	0.01 g
Agar agar	:	15.00 g
Distilled water (Volume to make up)	:	1000.00 ml
Final pH (at 25°C)	:	7.3±0.2

Agar agar was melted in 500 ml of distilled water. All the other ingredients were mixed in 400 ml of distilled water. The two solution were mixed thoroughly. The volume was made upto 1000 ml by adding distilled water and sterilized at 1.1 kg/cm^2 pressure for 15 min.

7. Richard's Agar

Potassium nitrate (KNO_3)	:	10.00 g
Potassium dihydrogen phosphate (KH_2PO_4)	:	5.00 g
Magnesium sulphate ($MgSO_4$. $7H_2O$)	:	2.50g
Potassium chloride (KCl)	:	0.02 g

Sucrose	:	50.0 g
Agar agar	:	15.0g
Distilled water (Volume to make up)	:	10000.0 ml

All the above ingredients except Potassium dihydrogen phosphate and agar-agar dissolved in 450 ml of distilled water. Agar-agar was melted separately in 500 ml of distilled water and was mixed with the above solution. The volume was made upto 950 ml. Potassium dihydrogen phosphate was dissolved in 50 ml of distilled water. The two solutions were sterilized at 1.1 kg/cm^2 pressure for 15 min and subsequently mixed together.

8. Carrot agar

Fresh carrots	:	200.00 g
Agar agar	:	20.00 g
Dextrose	:	20.00 g
Distilled water (Volume to make up)	:	1000.00 ml

First, Carrot were peeled off and cut into small pieces. Then they were boiled and extract was filtered through the muslin cloth. The dextrose was dissolved in the solution.

9. Malt extract agar

Malt extract	:	30.00 g
Mycological peptone	:	5.00 g
Agar	:	15.00 g
Distilled water (Volume to make up)	:	1000.00 ml
Final pH (25^0 C)	:	5.4 ±0.2

Malt extract was dissolved in 400 ml of distilled water. Agar agar was melted separately in 400 ml of distilled water. Both the solutions were mixed thoroughly volume was made up to 1000 ml with distilled water and 1.1 kg/cm^2 pressure for 15 min.

10. Sabouraud's agar

Dextrose ($C_6H_{12}O_6$)	:	40.00 g
Peptone	:	10.00 g
Agar	:	20.00 g
Distilled water (Volume to make up)	:	1000. 00 ml
Final pH	:	5.6

Agar agar was melted in 400 ml of distilled water. All other **i**ngredients were dissolved in 400 ml of distilled water. The two solutions were mixed thoroughly and the volume was made up to 1000 ml by adding distilled water. This was sterilized at 1.1 kg/cm^2 pressure for 15 min.

11. Tochinai's agar

Potassium dihydrogen phosphate (KH_2PO_4)	:	0.50 g
Potassium nitrate (KNO_3)	:	2.00 g
Magnesium sulphate ($MgSO_4 . 7H_2O$)	:	1.00 g
Ferric chloride ($FeCl_3 . 6H_2O$)	:	Trace
Sucrose ($C_{12}H_{22}O_{11}$)	:	30.00 g
Agar-agar	:	15.00 g
Distilled water (Volume to makeup)	:	1000.00 ml

Agar agar was melted in 400 ml distilled water. The other ingredients were dissolved in 400 ml of distilled water. The two solutions were mixed thoroughly and the volume was made up to 1000 ml by adding distilled water and sterilized at 1.1 kg/cm^2 pressure for 15 min.

12. Asthana and Hawker's medium

Glucose ($C_6H_{12}O_6$)	:	5.00 g
Potassium nitrate (KNO_3)	:	3.50 g
Potassium dihydrogen phosphate (KH_2PO_4)	:	1.70 g
Magnesium sulphate ($MgSO_4 . 7H_2O$)	:	0.70 g

Agar-agar	:	15.00 g
Distilled water (Volume to make to makeup)	:	1000.00 ml

Agar agar was melted in 400 ml of distilled water. The other ingredients were dissolved in 400 ml of distilled water. The two solutions were mixed thoroughly and the volume was made up to 1000 ml by adding distilled water. Sterilized at 1.1 kg/cm^2 pressure for 15 min.

3.3.2 Molecular characterization of gray mold pathogen of castor

3.3.2.1 DNA extraction of *Amphobotrys ricini* by CTAB method

The mycelium collected from the liquid cultures in potato dextrose broth after 7-8 days of incubation was filtered through Whatman No.40 filter paper. The mycelia were then dried completely by pressing in between folds of pre-autoclaved filter papers. The DNA extraction method was standardized and certain steps were optimized to produce good concentration of DNA using plant DNA isolation kit (CTAB method).

- Mycelium (0.5 g) was taken and ground in a Pestle and Mortar with nine ml of CTAB extraction buffer, mixed gently by inversion. For 60-90 minutes the tubes were incubated, with occasional inversion at 65 ^{0}C. The samples were allowed to cool by keeping the tubes in a trough of water at room temperature.
- Five ml of chloroform:isoamyl alcohol (24:1) was added, the tubes were gently to mix the content for five min.
- The samples were subjected to spinning in a centrifuge for 15 min at 6500 rpm at room temperature.
- The aqueous layer was transferred to a fresh tube and 25 µlRNaseA (20 mg ml^{-1}) was added.
- The samples were mixed gently by inversion and incubated for 30 min at room temperature.
- Six ml isopropanol was added to each tube and mixed gently by inversion until a white fluffy DNA precipitate appeared.

- The contents were centrifuged at 6500 rpm for 15 min to pellet the DNA. After 2-3 min, eight ml of cold wash buffer was added and incubated for 20 min at room temperature.
- The tubes were centrifuged to pellet the DNA at 6500 rpm for 15 min.
- The supernatant was discarded and eight ml of cold 70 per cent ethanol was added to the tube containing the DNA pellet.
- One ml of elution buffer was added and mixed gently to dissolve the pellet and kept at 4 ^{0}C overnight.
- The DNA solution appeared to be turbid after standing overnight at 4 ^{0}C and the samples were heated to 65 ^{0}C for centrifugation at 6500 rpm for 15 min and the clear supernatant containing DNA was transferred to a fresh 1.5 ml tube discarding the pellet.

3.3.2.2 Qualitative and quantitative analysis of DNA

The quality and quantity of DNA was analyzed by running 2 µl of sample mixed with 2 µl of 10x loading dye in 1.5 per cent agarose gel. The DNA from the sample prepared produced clear sharp bands in 1.5 per cent agarose gel indicating good quality of the DNA. The DNA was quantified by comparing with the 1 kb size marker (Genei Pvt. Ltd. Bengaluru). The gel was observed under UV light and documented using BIO RAD gel documentation unit.

3.3.2.3 PCR amplification of ITS region

The ribosomal DNA (rDNA) unit contains genic and non-genic or spacer region. Each repeat unit consists of a copy of 18S, 5.8S and 28S like rDNA and its like Internal Transcribed Spacers (ITS) and Inter-Genic Spacers (IGS). The rDNA have been employed to analyze evolutionary events because it is highly conserved, whereas ITS rDNA is more variable. Hence, it has been used for investigating the species level relationships.

The primers for amplification were custom synthesized at Bengaluru Genie Pvt. Ltd. and supplied as lyophilized products of desalted oligos. Primer sequences used were as follows

Organism	Sequence	
Fungal ITS	ITS-1-F	5´-TCCGTAGGTGAACCTGCGG -3´
	ITS-4-R	5´-TCCTCCGCTTATTGATATGC -3´

3.3.2.4 PCR reaction mixture

Reaction mixture	**Quantity**
Template DNA(40 ng)	2 µl
Primers-F and R	4 µl
dNTP´s (2 mM)	2 µl
Taq buffer A (10X)	2 µl
Taq DNA polymerase (3U/ µl)	0.3 µl
$Mgcl_2$	1 µl
Sterile Water	13.7 µl
Total	**25 µl**

3.3.2.5 PCR condition for ITS region amplification

Step	**Temp (^{0}C)**	**Duration**
Initial denaturation	95	4 min
Denaturation	95	30 sec
Annealing	58	30 sec
Extension	72	1 min
Final extension	72	7 min
Hold	4	20 min
No. of cycles Denaturation Annealing Extension	35	

3.3.2.6 Separation of amplified products on agarose gel

Agarose gel electrophoresis was performed to resolve the amplified product using 1.5 per cent agarose in IX TAE (Tris Acetate EDTA) buffer, 0.5 ug ml^{-1} of Ethidium bromide and loading buffer (0.25 % Bromophenol Blue in 40 % sucrose). Four µl of the loading dye was added to 20 µl of PCR product and loaded to the agarose gel. Electrophoresis was carried at 65 V for 1.5 h. The gel was observed under UV light and documented using gel documentation unit.

3.3.2.7 Sequencing of ITS region and in silico analysis

The ITS region was sequenced to confirm organism. The PCR product was sequenced using forward and reverse primers at Sakhala enterprise Bengaluru. Homology search done using BLAST algorithm available at the VA3T.ncbi.nlm.nih.gov. Phylogenetic tree was constructed using MEGA7 software.

3.4 *In vitro* evaluation of fungicides and nano particles against gray mold pathogen

The efficacy of eleven fungicides and five nano particles listed in Table 2 were evaluated against *Amphobotrys ricini* at different concentrations on oat meal medium using poisoned food technique (Vincent, 1947). Sterilized oat meal agar was prepared and autoclaved. The medium was cooled to 50 ^{0}C. Molecules were dissolved in sterilized water to make the stock solution. Appropriate quantity of stock solution was added to oat meal agar, to get the desired concentration of the molecule, the flasks were agitated gently to aid in uniform dispersion of the fungicidal solution into the medium. About 15 to 20 ml of poisoned oat meal agar was poured into 90 mm Petri plates and allowed to solidify. Three replications were maintained for each treatment. One 5 mm disc of the actively growing test fungal culture of *Amphobotrys ricini* were obtained with sterilized cork borer and transferred to the centre of the poisoned medium in each of the Petri plates. Control was maintained with the pathogen under similar conditions on oat meal agar medium without poisoning. Inoculated plates were incubated at 24±1^{0}C. The diameter of fungal colony was measured in each of the treatment when the fungal colony growth in control plate was full. The colony diameter inhibited in fungicide treated plates as compared

Table 2: The details of chemicals evaluated under *in vitro* and in field conditions

No.	Common Name	Trade Name	Chemical Name
Systemic fungicides			
1.	Carbendazim	Bavistin 50 WP	Methyl-2-benzimidazole carbamate ($C_9H_9N_3O_2$)
2.	Azoxystrobin	Amistar 23 % EC	Methyl(2E)-2-(2-{[6-(2-cyanophenoxy)pyrimidin-4-yl]oxy}phenyl)-3-methoxyacrylate
3.	Trifloxystrobin	Compass50%WDG	Methyl2-methoxyimino-2-[2-[[1-[3-(trifluoromethyl)phenyl]ethylideneamino] oxymethyl] phenyl] acetate ($C_{20}H_{19}F_3N_2O_4$)
4.	Propiconazole	Tilt 25 % EC	1-2-(2-chloro-4-(4-dichlorophenoxy))-4-propyl-1,3-dioxolan-2yl methyl)- 1H-1, 2, 4-triazole
5.	Tebuconazole	Folicure 250 EC	(RS)-1-(4-Chlorophenyl)-4,4-dimethyl-3-(1H, 1,2,4-triazol-l-ymethyl)pentan-3-ol
Contact fungicides			
1.	Captan	Captaf 50 % WP	(3a*R*,7a*S*)-2-[(Trichloromethyl)sulfanyl]-3a,4,7,7a-tetrahydro-1*H*-isoindole-1,3(2*H*)-dione ($C_9H_8Cl_3NO_2S$)
2.	Thiram	Thiram 75% DS	Dimethylcarbamothioylsulfanyl N, N-dimethylcarbamodithioate (C6H12N2S4)
3.	Mancozeb	Dithane M- 45 75 %WP	Manganese ethylenebis (dithiocarbamate) (polymeric)
Combi products			
1.	Carbendazim 12 % + Mancozeb 63 % WP	Saaf	Methyl 2 benzimidazole carbomae 1,2 + Manganese ethylene bis dithiocarbonate plus zinc 63 $C_8H_{12}MnN_4S_8Zn$
2.	Tebuconazole 50 % WG + Trifloxystrobin 25 % WG	Nativo	4-chlorphenyl-4, 4-dimethyl-3-[1,2,4]triazol-l-ylmethyl-pentan-3-ol+(E,E)-methoxyimino-{2-{1-{3-trifluromethyl-phenyl-ethyllideneaminooxymethyl]-phenyl}acetic acid methyl ester
3.	Hexaconazole 4 % + Zineb 68 % WP	Avtar	(Zinc ethylene Bis Dithiocarbamate + Hexaconazole) $C_4H_6N_2S_4Zn$+$C_14H_{17}C_{l2}N_3O$
Nano particles		Silicon dioxide, Titanium dioxide, Zinc oxide, Boron nitride, Pure silver nano particles	

to control was taken as a measure of fungitoxicity. Per cent inhibition over control was calculated by following the equation (Vincent, 1947).

$$\mathrm{I} = \frac{\mathrm{C} - \mathrm{T}}{\mathrm{C}} \times 100$$

Where,

I = Per cent inhibition of mycelial growth

C = Radial growth of pathogen in control (mm)

T = Radial growth of pathogen in treatment (mm)

3.5 Management of castor gray mold disease

Field experiment was conducted at Zonal Agricultural Research Station, GKVK, Bengaluru during *Kharif* 2017-18 for the management of gray mold of castor.

Plot size	:	3.0 m x 3.0 m
Variety	:	DCS-9 (Jyothi)
Space	:	(90 x 45) cm
Date of sowing	:	14- 08-2017
Design	:	RCBD
Treatment	:	8
Replication	:	3

Standard agronomic practices were followed to grow the crop. Fungicides tested include three systemic (Carbezdazim, Azoxystrobin, Tebuconozole), two combi products (Tebuconazole + Trifloxystrobin and Carbendazim + Mancozeb) and two nano particles (Silicon dioxide and Pure silver) with one untreated control. First spray was given before the onset of disease *i.e*., 80 days after sowing (DAS) and second spray was given 15 days after first spray (95 DAS).

Treatment details

T_1: Carbendazim @ 1.0 gL^{-1}

T_2: Tebuconazole + Trifloxystrobin (Nativo) @ 1.0 %

T_3: Azoxystrobin @ 0.5 %

T_4: Silicon dioxide @ 200 ppm

T_5: Tebuconazole @ 2mlL^{-1}

T_6: Carbendazim + Mancozeb (Saaf) @ 2.0 gL^{-1}

T_7: Pure silver @ 200 ppm

T_8: Control (Untreated)

Disease incidence in all the spike orders was recorded on 110 days after sowing using 0-9 scale.

Table 3: Key for assessment of disease severity

Disease grade	**Intensity of infection (%)**
0	No infection
1	1 to 10 % raceme area infected
3	11 to 20 % raceme area infected
5	21 to 30 % raceme area infected
7	31 to 50 % raceme area infected
9	>51 % raceme area infected

The per cent disease index (PDI) was calculated by using McKinney (1923) infection index. The data on castor gray mold incidence and yield was recorded in net plot and converted to $kgha^{-1}$. The data were analyzed statistically.

$$\text{PDI} = \frac{\text{Sum of individual ratings}}{\text{Total number of spikes observed} \times \text{Maximum disease grade}} \times 100$$

3.6 Statistical analysis

The field experimental data was analysed statistically by Fischer´s method of analysis of variance by Panse and Sukhatme (1967). The level of significance used in F test was P= 0.05. Critical difference was worked out wherever F- test was significant.

IV RESULTS AND DISCUSSION

Castor is an important non edible oil seed crop which contains 40-60 per cent of oil having unique features, rich in triglycerides, mainly ricinolein. India ranks first in area and production of castor in the world. Diseases are the major biotic stresses for the successful production of castor. Among these gray mold caused by *Amphobotrys ricini*, a necrotrophic pathogen has been considered as a most destructive pathogen which has been reported to cause potential seed yield loss up to 100 per cent. Experiments were conducted on various aspects of gray mold of castor with reference to its morphological and molecular characterization, *in vitro* and field evaluation of chemicals at GKVK, Bengaluru, during 2017-18. The results obtained are presented and discussed below.

4.1 Symptomatology

The symptoms first appeared as bluish spots on flowers, developing fruits, panicle, leaves and stem from which yellowish drops of liquid exude. On fruits the symptoms appeared as circular or elliptic, sunken, dark coloured spots which result in rupture of the capsule. The inflorescence or 'spike' is covered with a moldy growth (Plate 1). These descriptions are similar to the symptoms described by Araujo *et al*. (2007) and Godfrey (1923).

4.2 Isolation and identification of the pathogen

4.2.1 Isolation of *Amphobotrys ricini* from capsules of castor

Isolations made from naturally infected capsules of castor showing typical symptoms of gray mold revealed the presence of *Amphobotrys ricini*. Colonies of the fungus on oat meal enriched medium were irregular, initially light brown in colour which later turned dark gray. The pure culture of the fungus was obtained and purified by single spore isolation method on oat meal enriched medium (Plate 2a). The purified colonies were stored in oat meal enriched medium agar slants (Sung-Kee *et al*., 2001).

4.2.2 Morphological identification of gray mold pathogen of castor

The fungus on oat meal enriched medium produced septate and branched mycelium, dichotomously branched conidiophores bearing globose, hyaline to light brown coloured conidia (Plate 2b). The morphological characters obtained are in accordance with the descriptions of Hennebert (1973).

4.3 Pathogenicity test

The pathogenicity test was conducted in a closed polythene humid chamber as described in material and methods. The detached spikes of castor variety, DCS 9, were kept in conical flasks containing 2 per cent sucrose solution and spray inoculated with spore suspension (10^6 conidia ml^{-1}). Initial symptoms were observed on 3^{rd} day of inoculation. The first symptoms of the disease were visible as bluish spots on the capsules. These spots enlarged covering the entire castor raceme with grayish moldy growth within six days after inoculation (Plate 3). Godfrey (1923) observed small bluish spots on panicles, leaves and stems of castor infected with castor gray mold. The growth of gray to olive gray mycelium on infected racemes was described as a spider's web by him. The pathogen was re-isolated from the infected capsules on oat meal enriched medium and the morphological characters were compared with the original culture which was similar in all aspects.

4.4 Morphological and molecular characterization of gray mold pathogen of castor

4.4.1 Morphological characterization of gray mold pathogen of castor

The fungus on oat meal enriched medium produced septate and branched mycelium which was thin, hyaline to light brown in colour. Conidiophores were produced individually or in clusters, cylindrical, hyaline to pale brown, branched dichotomously, which developed from conidiogenous cells. Conidia were globose, unicellular, smooth, hyaline to pale brown in colour.

On the basis of morphology, the fungus was identified as *Amphobotrys ricini*. The morphological characters obtained are in accordance with the descriptions of Hennebert (1973). Similar observations were made by Sung-Kee *et al*. (2001) on potato dextrose

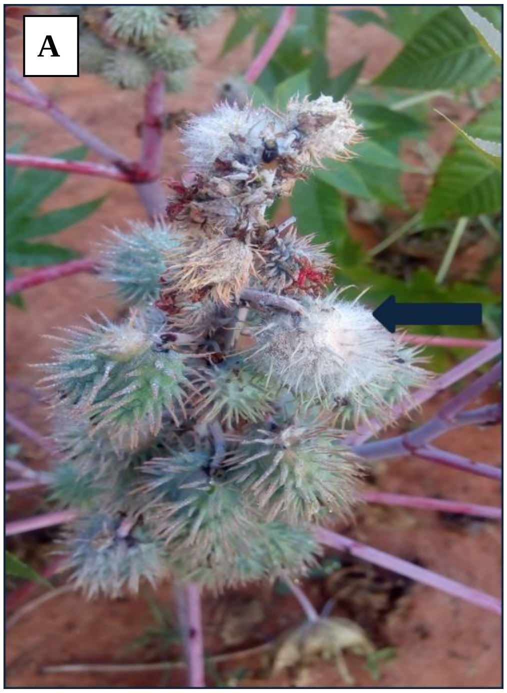

Plate 1a. Whitish mycelial growth on capsules

Plate 1b. Initiation of sclerotia on pericarp of dried capsules

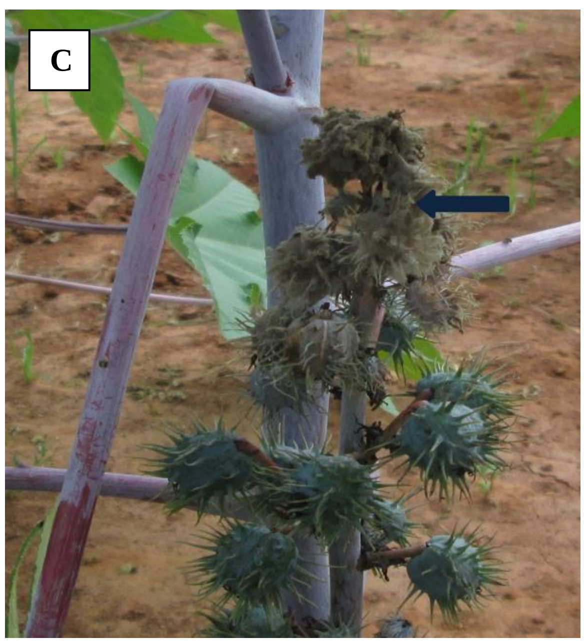

Plate 1c. Gray mold growth on capsules

Plate 1. Symptoms of gray mold on castor

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Plate 2a. Pure culture of *Amphobotrys ricini* on oat meal enriched medium

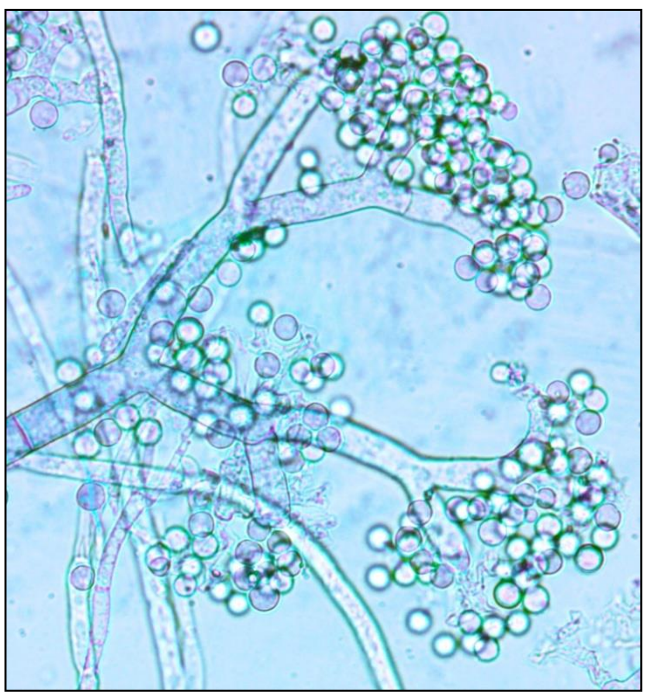
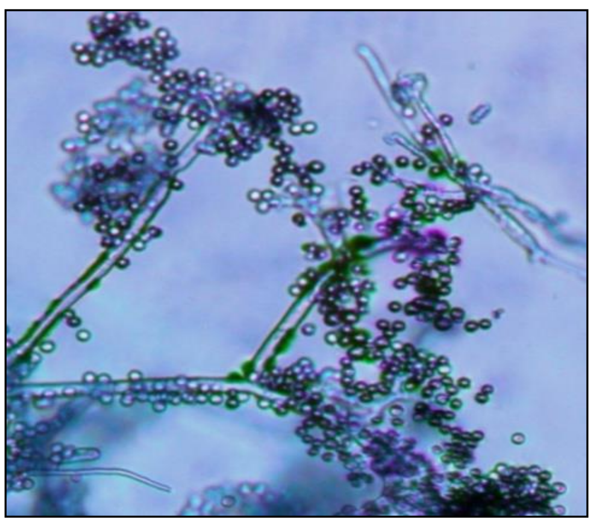


Plate 2b. Photomicrograph showing conidia and conidiophores of *Amphobotrys ricini* (40x) bearing clumps of conidia

Healthy raceme	Inoculated with conidia of *A. ricini*



Re-isolated conidia with dichotomously branched conidiophore (10x)

Plate 3. Pathogenicity of *Amphobotrys ricini* on detached castor spike on DCS 9

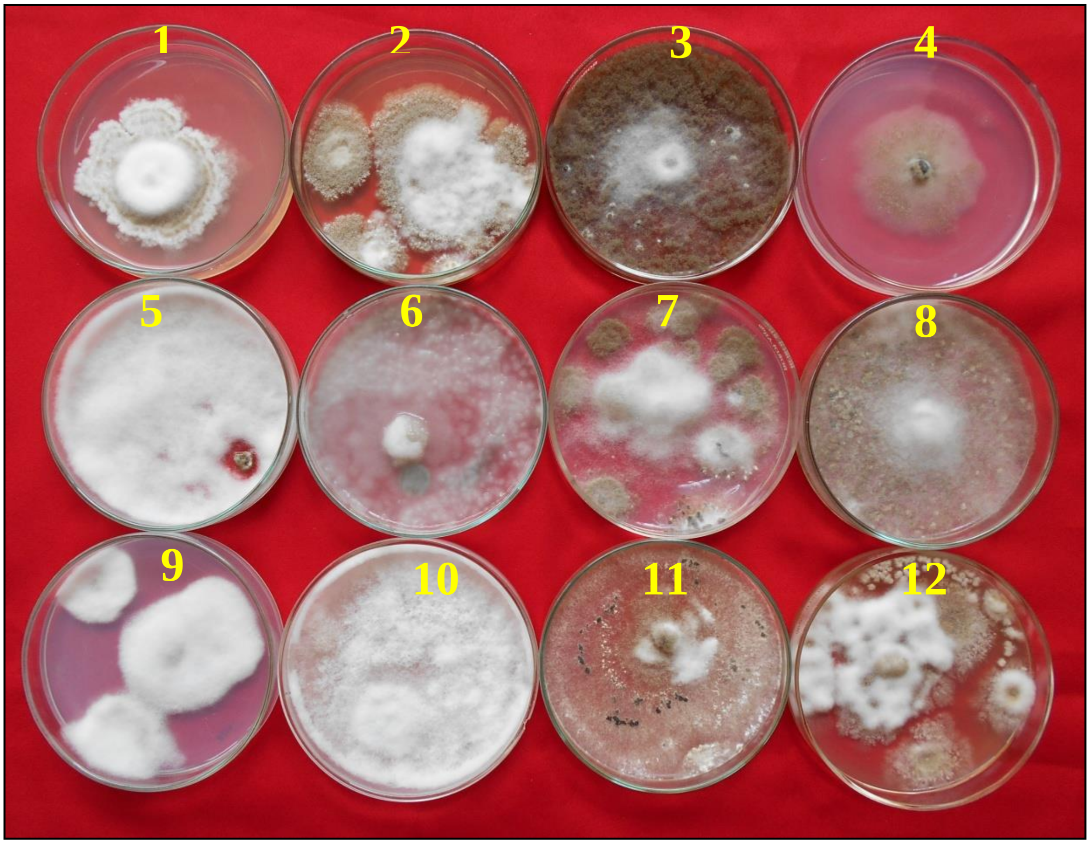


Plate 4. Colony morphology of *Amphobotrys ricini* on different solid media

Treatment	Medium	Treatment	Medium
1	Potato dextrose agar	7	Carrot agar
2	Sabouraude agar	8	Malt extract agar
3	Castor leaf extract	9	Asthana and Hawker’s
4	Czapek’s agar	10	Tochinai’ s agar
5	V8 Juice agar	11	Oat meal enriched medium
6	Richard’s agar	12	Oat meal agar

agar. The conidiophores were single, erect, pale brown colour and branched dichotomously. The conidia were globose, unicellular, pale brown, smooth. These descriptions are in accordance with the present findings.

4.4.2 Effect of different soild media on the growth of *Amphobotrys ricini*

The variation in cultural characters of *Amphobotrys ricini* was studied on twelve different growth media as described in material and methods. The radial growth of *A. ricini* on different media ranged from 20.00 to 85.00 mm. The radial growth of *A. ricini* was maximum on Malt extract agar medium with mean colony diameter of 85.00 mm. This was followed by V8 juice agar medium (82.66 mm), Tochinai's agar (79.00 mm), Castor leaf extract (72.50 mm). The growth of *A. ricini* was minimum in Richard's agar medium (20.00 mm) (Table 4, Plate 4 & Fig. 1).

Though the radial growth of the pathogen was highest on Malt extract agar, profuse sporulation was observed in Oat meal enriched medium, more than 100 conidia per microscopic field was observed, followed by PDA, Sabouraud's agar, Malt extract agar and oat meal agar (80-100 conidia per microscopic field). Sporulation was minimum in Carrot agar (60 conidia per microscopic field) and no sporulation was observed in V8 juice agar, Richard's agar, Asthana and Hawkers agar and Tochinai's agar (Table 4 & Plate 4).

The perusal of the data presented in Table 5 and Fig. 2 shows that the dry mycelial weight of the pathogen varied from 84.24 mg to 604.13 mg on different liquid media. Maximum dry mycelial weight of 604.13 mg was recorded in Richard's broth followed by Tochinai's broth (456.20 mg), Oat meal enriched broth (325.57 mg). Dry mycelial weight of the pathogen was minimum in Asthana and Hawkers broth (84.24 mg).

The variation in the colony characters of *A. ricini* on different media are presented in Table 6 & Plate 4. Colony colour on different media varied from white to dirty white, cottony white, brown to light brown in colour. Colony colour on PDA, Sabouraud's agar, Castor leaf extract and carrot agar were whitish at the centre and brownish at the periphery. Brownish white mycelium was observed on Czapek's agar, cottony white mycelium was observed in V8 juice agar and it is surrounded by light brown colour mycelium in Asthana and Hawkers agar medium. On Richard's agar medium the fungus produced whitish mycelial growth, on Tochinai's agar medium colony appeared as white to dirty white in colour. Colony colour was whitish at the inoculation point surrounded by dirty white in colour on Malt extract agar medium. On Oat meal enriched medium and Oat meal agar medium colony was initially white later turned to brown colour.

On different media tested the sclerotia number per plate, size, formation pattern and also the sclerotia initiation varied. The number of sclerotia produced on various media varied from 3-250 per plate. Highest number of sclerotia was observed on Malt extract agar (250 per plate) followed by Oat meal agar (132 per plate), Oat meal enriched medium (120 per plate). Least sclerotial production was noticed in Sabouraud's agar (3 per plate).

Small (< 2.00 mm), medium (2.00-5.00mm) and large (> 5.00 mm) sized sclerotia were observed on different media. On Carrot agar, Asthana and Hawkers agar and Oat meal agar medium the fungus produced small sclerotia. Medium sized sclerotia was observed on PDA, Sabouraud's agar, castor leaf extract, Tochinai's agar and Oat meal enriched medium. Large sized sclerotia were produced on Richard's agar and Malt extract agar medium.

Production of sclerotia was observed on 10th day after inoculation on Carrot agar, Malt extract agar and Oat meal agar medium, whereas sclerotia production initiated after 17th day after inoculation on PDA and Richard's agar medium. Sclerotia formation was not observed on Czapek's agar and V8 juice agar medium.

The pattern of sclerotia formation also varied with type of medium used. On PDA sclerotia were formed in concentric rings, whereas, in Richard's agar, Carrot agar, Asthana and Hawker's agar, Tochinai's agar and Oat meal enriched medium the sclerotia formed irregularly. Sclerotia formed irregularly and scattered throughout the plate on Sabouraud's agar, Castor leaf extract and Malt extract agar medium. On Oat meal agar

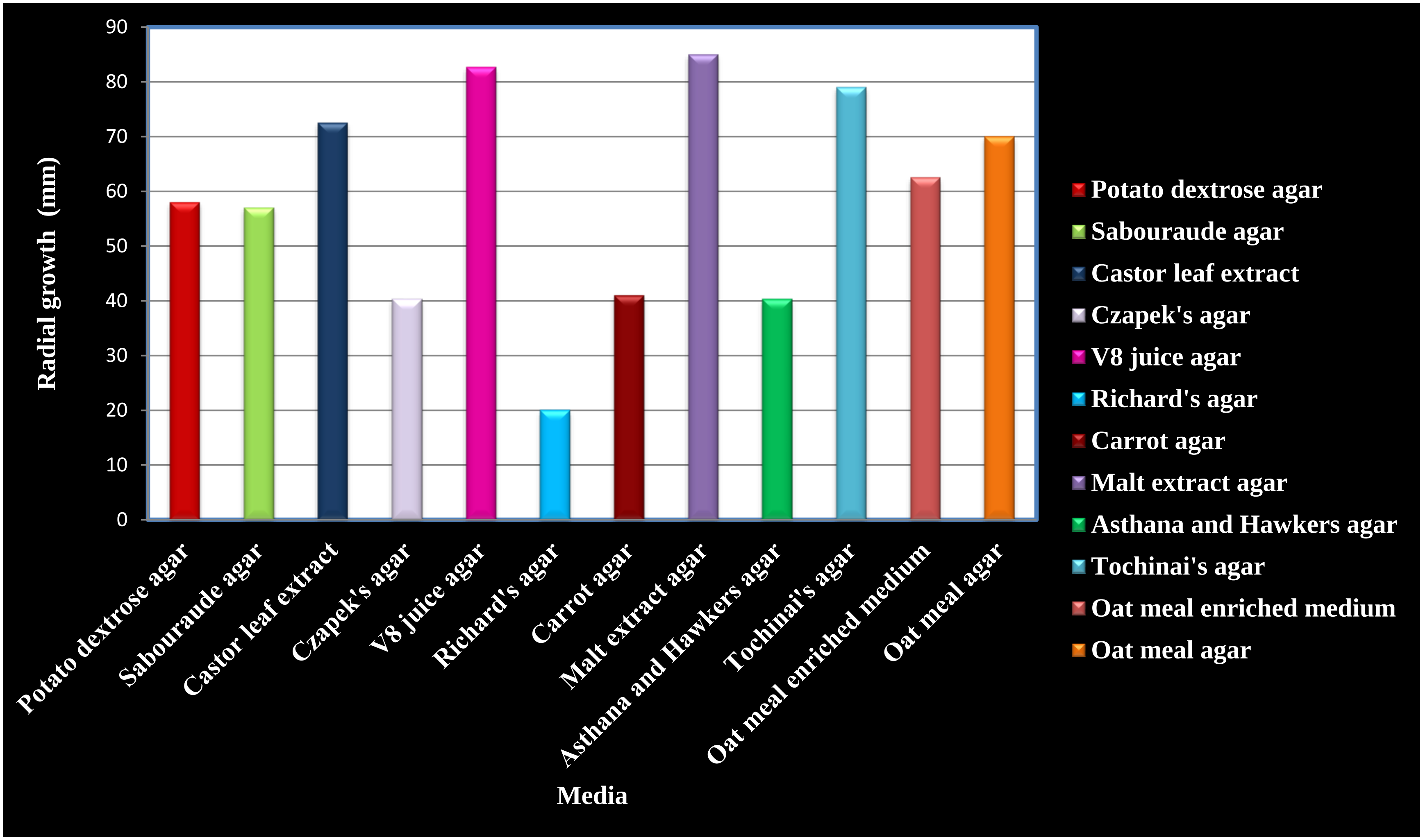


Fig. 1. Effect of different media on growth of *Amphobotrys ricini*

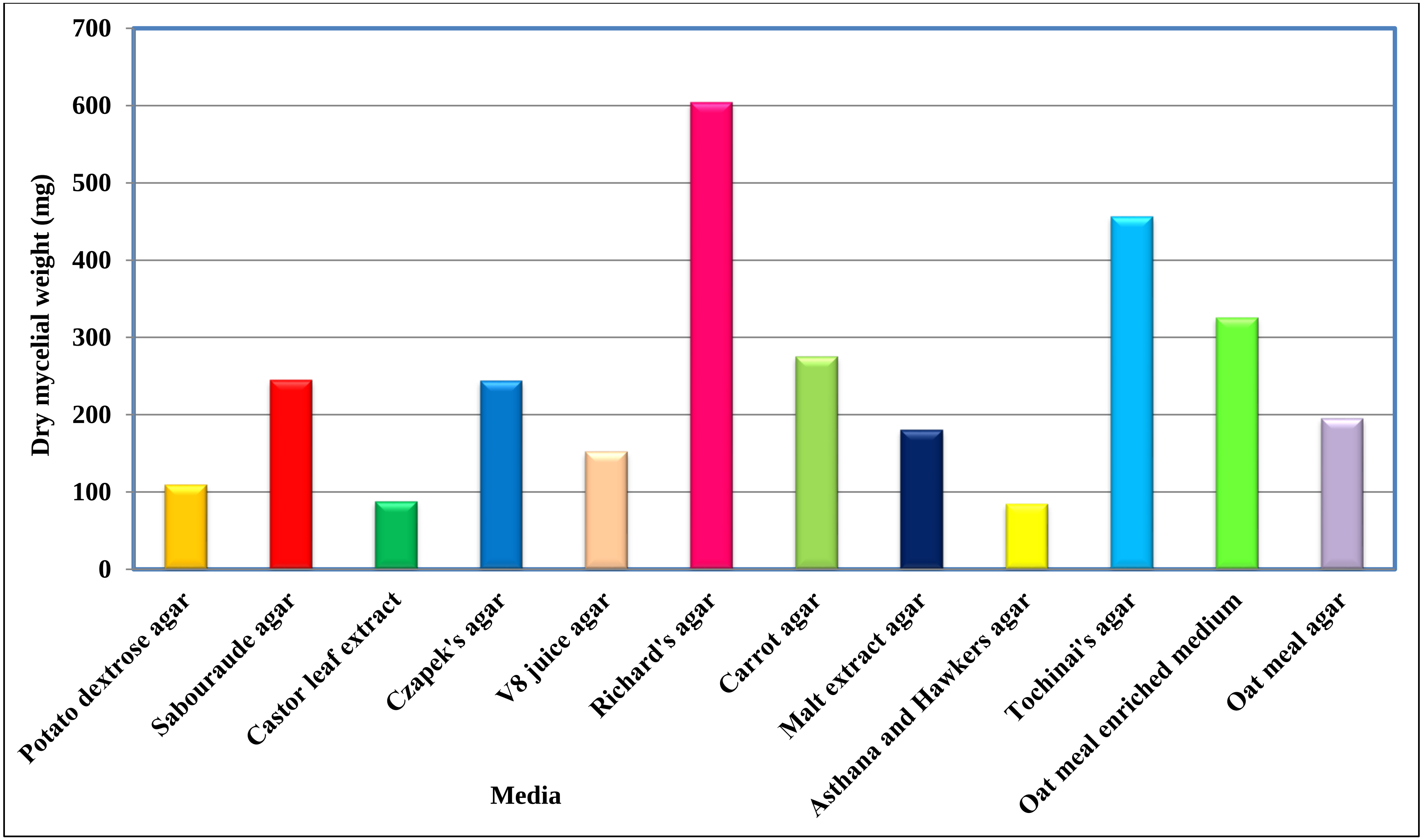


Fig. 2. Effect of different media on dry mycelial weight of *Amphobotrys ricini*

medium sclerotia formed in concentric rings and later scattered throughout the plate (Plate 4).

Similar type of results were obtained by Sung-Kee *et al*. (2001) who reported that *A. ricini* on PDA produced pure white cottony mycelium. Similarly, Mirzaei *et al.* (2007) characterized 355 isolates of *Botrytis cinerea* and identified the species based on morphological characters such as conidial and sclerotial dimensions, conidiophore length. Different kinds of growth pattern were observed on PDA at 20 ^{0}C under light. Colonies were white, dirty white, grayish white, or hyaline at first but soon becoming light gray, dark gray to dark brown in colour.

The morphological characters obtained are in agreement with the findings of Raluca and Emilia (2010) who reported that *Botrytis cinerea* on PDA produced globose conidia. Scleroria were black and were formed in rings, on suface of the medium, edges of the plate and irregularly distributed. Number of sclerotia per plate varied from 36-190. Similarly, Tanovic *et al*. (2009) reported that the growth of *B*otrytis *cinerea* was maximum and more sclerotia were produced on Malt agar (MA) medium. Morphological types of colonies on PDA and MA media were similar to those observed by Martinez *et al*. (2003).

Zada *et al*. (2016) reported that colony colour of *Botrytis cinerea* on PDA, Czepk’s and Malt extract medium varied from whitish, slightly white, grayish white and grayish black.

Hennebert (1973) reported that *Botrytis* taxonomy has traditionally been based on morphological and cultural characteristics coupled with host specificity. However, cultural conditions could considerably modify taxonomic characters such as dimension and shape of conidia (Jarvis, 1977). Despite these, morphological characters, in recent year’s molecular markers have been used to identify *Botrytis* species (Mirzaei *et al*., 2007).

Table 4: Effect of different solid media on growth and sporulation of *Amphobotrys ricini*

Mediu Medium	Radial colony growth (mm)*	Sporulation
Potato dextrose agar	58.33	+++
Sabouraud’s agar	57.00	+++
Castor leaf extract	72.50	++
Czapek's agar	40.33	++
V8 juice agar	82.66	-
Richard's agar	20.00	-
Carrot agar	41.00	+
Malt extract agar	85.00	+++
Asthana and Hawkers agar	40.30	-
Tochinai's agar	79.00	-
Oat meal enriched medium	62.50	++++
Oat meal agar	70.00	+++
S.Em ±	**0.67**	
C.D. (P=0.01)	**2.67**	

*Mean of three replications

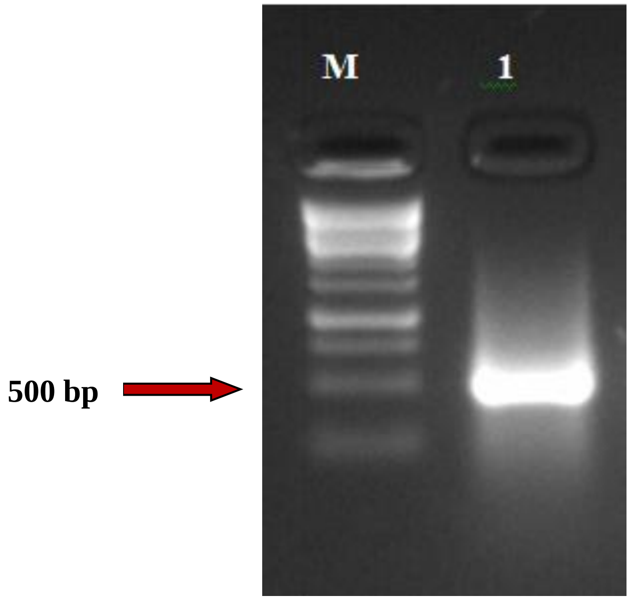
++++ : > 100 conidia per microscopic field

+++ : 80-100 per microscopic field

++ : 60-80 conidia per microscopic field

+ : < 60 conidia per microscopic field

- : No sporulation



Ladder: M. 1kb; 1. *Amphobotrys ricini*

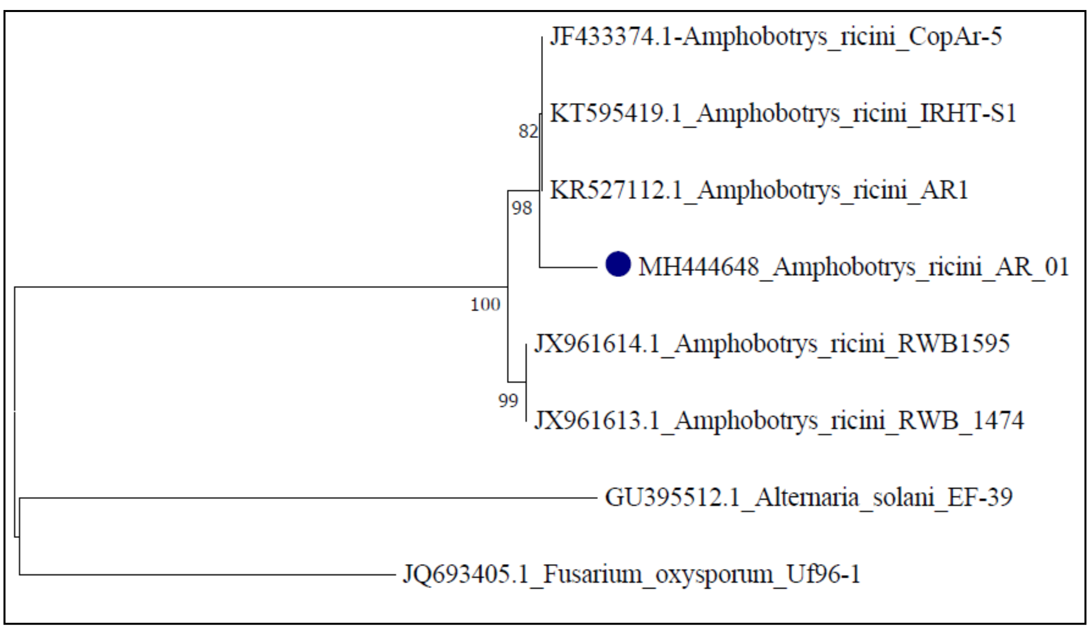


Plate 5: ITS rDNA region amplification and Phylogenetic tree analysis

Table 5: Effect of different liquid media on dry mycelial weight of *Amphobotrys ricini*

Medium	**Dry mycelial weight (mg)***
Potato dextrose broth	109.00
Sabouraud’s broth	244.63
Castor leaf extract broth	87.50
Czapek's broth	243.86
V8 juice broth	151.96
Richard's broth	604.13
Carrot broth	275.20
Malt extract broth	180.23
Asthana and Hawkers broth	84.24
Tochinai's broth	456.20
Oat meal enriched broth	325.57
Oat meal broth	195.04
S.Em ±	**0.59**
C.D. (P=0.01)	**2.35**

*Mean of three replications

Table 6: **Colony morphology of *Amphobotrys ricini* on different media**

Medium	Colony characters	Sclerotia characters		
	Colony colour	Number /plate	Size	Formation pattern
Potato dextrose agar	Whitish at the centre and brownish at the periphery	55	Medium	Formed in concentric circular ring (17 DAI)
Sabouraud’s agar	Whitish at the centre and brownish at the periphery	3	Medium	Formed irregularly, scattered throughout the plate (12 DAI)
Castor leaf extract	Whitish at the centre and dark brownish at the periphery	75	Medium	Formed irregularly, scattered throughout the plate (11 DAI)
Czapek’ s Agar	Brownish white	-	-	-
V8 Juice agar	Cottony white mycelium	-	-	-
Richard's Agar	Whitish mycelial growth	37	Large	Formed irregularly (17 DAI)
Carrot agar	Whitish at the centre surrounded by brownish	78	Small	Formed irregularly (10 DAI)
Malt extract agar	Whitish at the inoculation point surrounded by dirty white in colour	250	Large	Formed irregularly, scattered throughout the plate (10 DAI)
Asthana and Hawker’s agar	Cottony white mycelium surrounded by light brown	3	Small	Formed irregularly (14 DAI)
Tochinai’ s agar	White to dirty white in colour	56	Medium	Formed irregularly (14 DAI)
Oat meal enriched medium	Initially white later turn to brown colour	120	Medium	Formed irregularly (12 DAI)
Oat meal agar	Initially white later turn to light brown colour	132	Small	Formed in concentric rings and later scattered throughout the plate (10 DAI)

DAI: Days after inoculation; Small= < 2.00 mm; Medium= 2.00-5.00 mm; Large= > 5.00 mm

4.4.3 Molecular characterization

The DNA of the anamorph of pathogen causing gray mold was isolated and the Internal Transcribed Spacer (ITS) region of the fungal genome was amplified in a PCR using ITS1 and ITS 4 primer pairs. The amplified region was typically 500 bp in length with a concentration of around 70 $ng\mu g^{-1}$. The amplified product was checked by electrophoresis in 1.5 per cent agarose gel (Plate 5). The amplified region was sequenced and the sequence of amplified region was

5^1CCCCAAGCCTAAACTCCCCACCCTTTTTTACCTGTTGCTTTGGCAGATGCAA
GTCTGCCAGGCTTAAACTCTGTGTTTTGTTGTCTGAGTGTGGCTTTTGGAATGT
TAAAACTTTCAACAATGGATCTCTTGGCTCTGGCATCGATGAAGAACGCAGC
GAAAAGCGATATGTAATGTGAATTGCAGAATTTAGTGAATCATCGAATCTTT
GAACGCACATTGCGCCCTGTAGCATTCTACAGGGCATGCCTGTTCGAGTGTCA
TTTCACCCTCAAGCATAGCTTGGTCTTGAATCTAGTCGTACGACTGATTTTAA
AAATAGTACTGGCTTCGTAGGTGCTAAACGTAATAACTTTCGTTCCAGCGCCT
CATCTTAGCTGCGAAATGGACTTTTTGGAGGTGAACTCAAAGCAGGGACGGA
AACCC 3^1.

The amplified region was compared using bioinformatics tools like NCBI (National Centre for Bioinformatics) BLAST programme. BLAST search has shown 98% similarity of the query sequence with *Amphobotrys ricini* (Table 7). The results of this study confirmed that *A. ricini* as the cause of gray mold of castor. ITS rDNA sequence of *Amphobotrys ricini* was deposited in NCBI and GenBank accession number MH444648 was obtained.

The ITS rDNA region has been extensively used for the species-level discrimination of fungal species. Universal-primed polymerase chain reaction (UP-PCR) fingerprinting, coupled with the restriction of ITS rDNA regions helps in the identification of five groups of *Botrytis i.e*., *B. cinerea*, *B. squamosa, B. byssoidea* and two groups of *B. aclada* (AI and AII) (Nielsen *et al*., 2001).

Table 7: Sequence comparision of *Amphobotrys ricini* strain AR 01 with that available in NCBI GenBank

Sl. No.	Sequence isolate	GenBank accession number	GenBank reference accession number	NCBI BLAST Hit results	Maximum Identity (%)
1	AR 01	MH444648	JF433374.1	*Amphobotrys ricini* strain CopAr-5 internal transcribed spacer 1	98 %

Inter and intra-specific variation within the ITS regions have been reported in several fungi and are mostly due to deletion or insertion events in the ITS 1 and ITS 4 regions (Carbone and Kohn, 1993; Chen *et al*., 1992).

Kwon *et al*. (2011) reported the gray mold of blueberry first time in Korea which is caused by *Botrytis* sp**.** A detailed description of the fungus is given based on morphological characteristics and amplification of rDNA internal transcribed spacer sequence using ITS 1 and ITS 4. The fungus was identified as *Botrytis cinerea* based on mycological characteristics and molecular data.

First report of a gray mold on *Lilium cernuum* Komar, caused by *Botrytis cinerea* in China was reported by Chen *et al*., 2017. Based on the morphological and molecular analyses using ITS primers 1 and 4, they confirmed that *Botrytis cinerea* as the causal agent of gray mold on *Lilium cernuum.*

4.5 *In vitro* evaluation of fungicides on growth of *Amphobotrys ricini*

Availability of different mode of action of fungicides necessitates their evaluation under *in vitro* to provide useful and preliminary information regarding their efficacy against pathogens within a short period of time and therefore, serves as a guide for field testing. *In vitro* evaluation of different systemic fungicides, contact fungicides, combi products and nano particles against *A. ricini* was done by poisoned food technique and the results revealed the following.

The efficacy of five systemic fungicides at three different concentrations of 50, 250 and 500 ppm were tested against *A. ricini*. The results presented in the Table 8, Plate 6 & Fig. 3 revealed that all the systemic fungicides used in the experiment inhibited the growth of *A. ricini*. The growth of *A. ricini* varied significantly in different fungicides tested. Of the five systemic fungicides tested complete inhibition (100%) of growth of the pathogen over control was observed in two fungicides Carbendazim and Propiconazole at 50, 250 and 500 ppm concentrations. In Tebuconazole complete inhibition was observed at 250 and 500 ppm concentration,whereas it was 97.22 % at 50 ppm concentration. Minimum inhibition was observed in Trifloxystrobin (20.84 %) and the least inhibition was observed in Trifloxystrobin at 50 ppm (6.10 %).

Three contact fungicides and three combi products were evaluated at concentrations of 250, 500 and 1000 ppm. Among the contact fungicides Mancozeb (59.27 %) recorded the maximum inhibition of mycelial growth followed by Thiram (52.52 %). Among the three concentration tested 1000 ppm was found to be highly effective with mean per cent inhibition of 86.18. Interaction between fungicides and concentration was significant *i.e.*, complete inhibition of mycelia growth was observed at 1000 ppm of Mancozeb. The minimum mycelial inhibition was observed in Captan at 250 ppm (5.13 %) (Table 9, Plate 7 & Fig. 4).

Among the combi products tested *viz*., Carbendazim + Mancozeb, Tebuconazole + Trifloxystrobin and Hexaconazole + Zineb. 100 % inhibition of growth was recorded in Carbendazim + Mancozeb followed by Tebuconazole + Trifloxystrobin (98.64 %). Out of three concentrations tested 1000 ppm was highly effective with mean per cent inhibition of 86.18. All the combi products tested gave the superior results in inhibition of growth of *A. ricini* (Table 9, Plate 7 & Fig. 4).

Five nano particles *viz*., Silicon dioxide, Titanium dioxide, Zinc oxide, Boron nitride and Pure silver were evaluated for their efficacy against *A. ricini* under *in vitro* condition. Among these better mycelial inhibition of pathogen was observed in Silicon dioxide (36.05 %) followed by Pure silver (28.86 %), Zinc oxide (20.81 %). Maximum inhibition was recorded in Silicon dioxide (58.26 %) at 250 ppm concentration followed by pure silver (51.19 %) at same concentration. Pure silver and Boron nitride at 50 ppm concentration there was no inhibition of pathogen. Among the concentrations tested 250 ppm was found to be effective with mean per cent inhibition of 35.51. Per cent inhibition of pathogen increased with the increase in concentration of the molecules (Table 10, Plate 8 & Fig. 5).

Rautela and Singh (2017) conducted *in vitro* evaluation of fungicides against gray mold of tomato and results revealed that Azoxystrobin + Tebuconazole and Azoxystrobin + Chlorothalonil were more effective showing 100 per cent inhibition of mycelial growth of *Botrytis cinerea*.

Iqbal *et al*. (1992) reported that Banlate, Calixin-M and Tecto-6 were the most effective in suppressing the growth of the *Botrytis cinerea.*

He *et al*. (2011) reported that zinc oxide nano particle at concentrations greater than 3 mmolL^{-1} significantly inhibit the growth of *Botrytis cinerea.* Similarly, Derbalah *et al.* (2012) reported that silver nano particles showed a high efficacy against *B .cinerea.*

The antifungal activity of nano particles against *Botrytis cinerea* is that nanoparticles may adhere to the cell surface, thereby altering membrane properties. In support of this mechanism, silver nano particles have been reported to degrade lipopolysaccharide molecules, accumulate inside the membrane by forming “pits” and cause large increases in membrane permeability (Sondi and Salopek-Sondi 2004). Other proposed mechanisms include the penetration of silver nano particles into the microbial cell, resulting in DNA damage, and the release of antimicrobial silver ions due to the dissolution of silver nano particles (Morones *et al*., 2005).

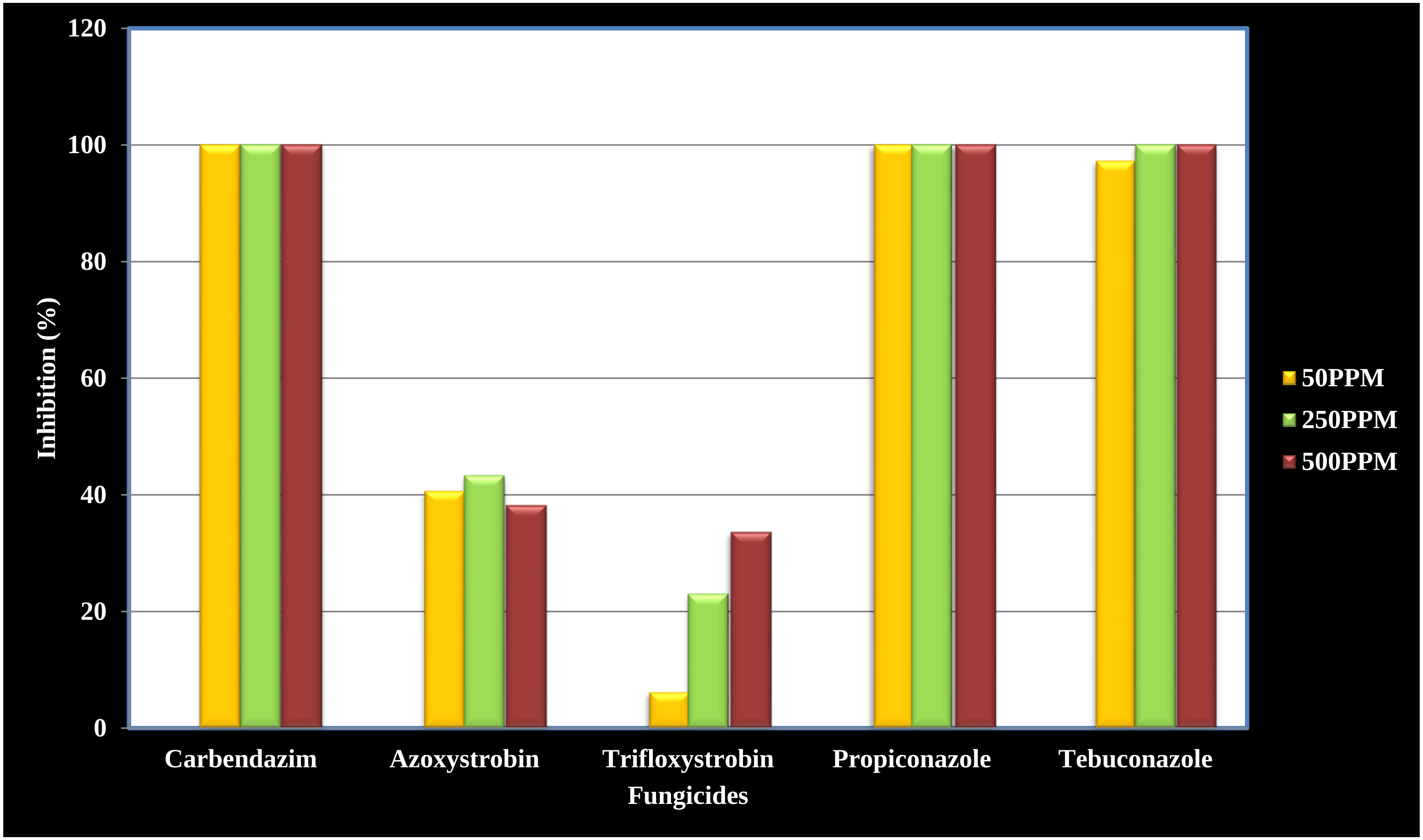
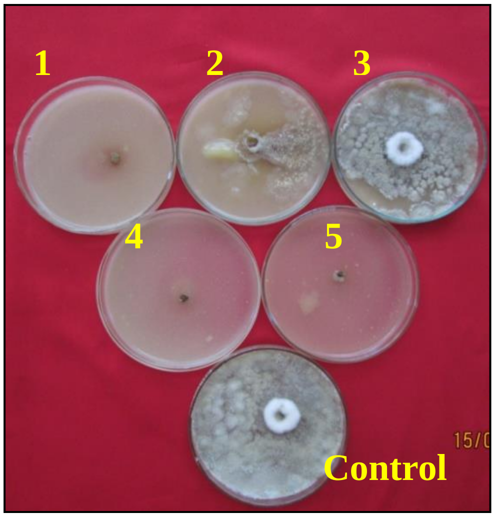
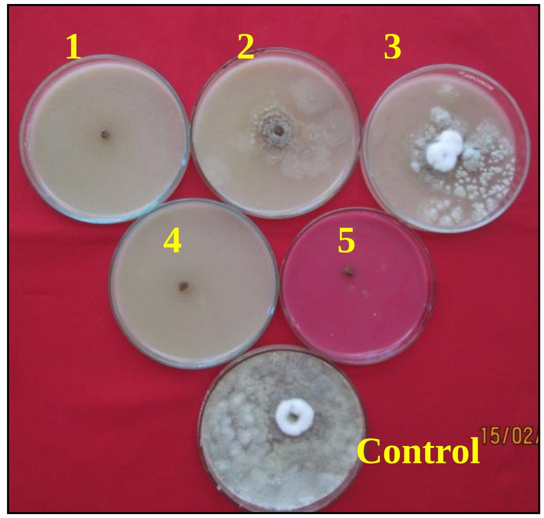


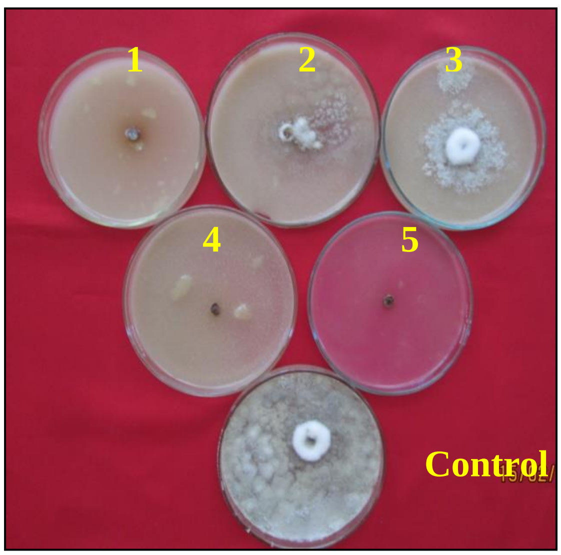
Fig. 3. Effect of systemic fungicides on growth of *Amphobotrys ricini*



50 ppm



250 ppm



500 ppm

Treatments

1. Carbendazim
2. Azoxystrobin
3. Trifloxystrobin
4. Propiconazole
5. Tebucanozole

Plate 6: *In vitro* evaluation of systemic fungicides on radial growth of *Amphobotrys ricini*

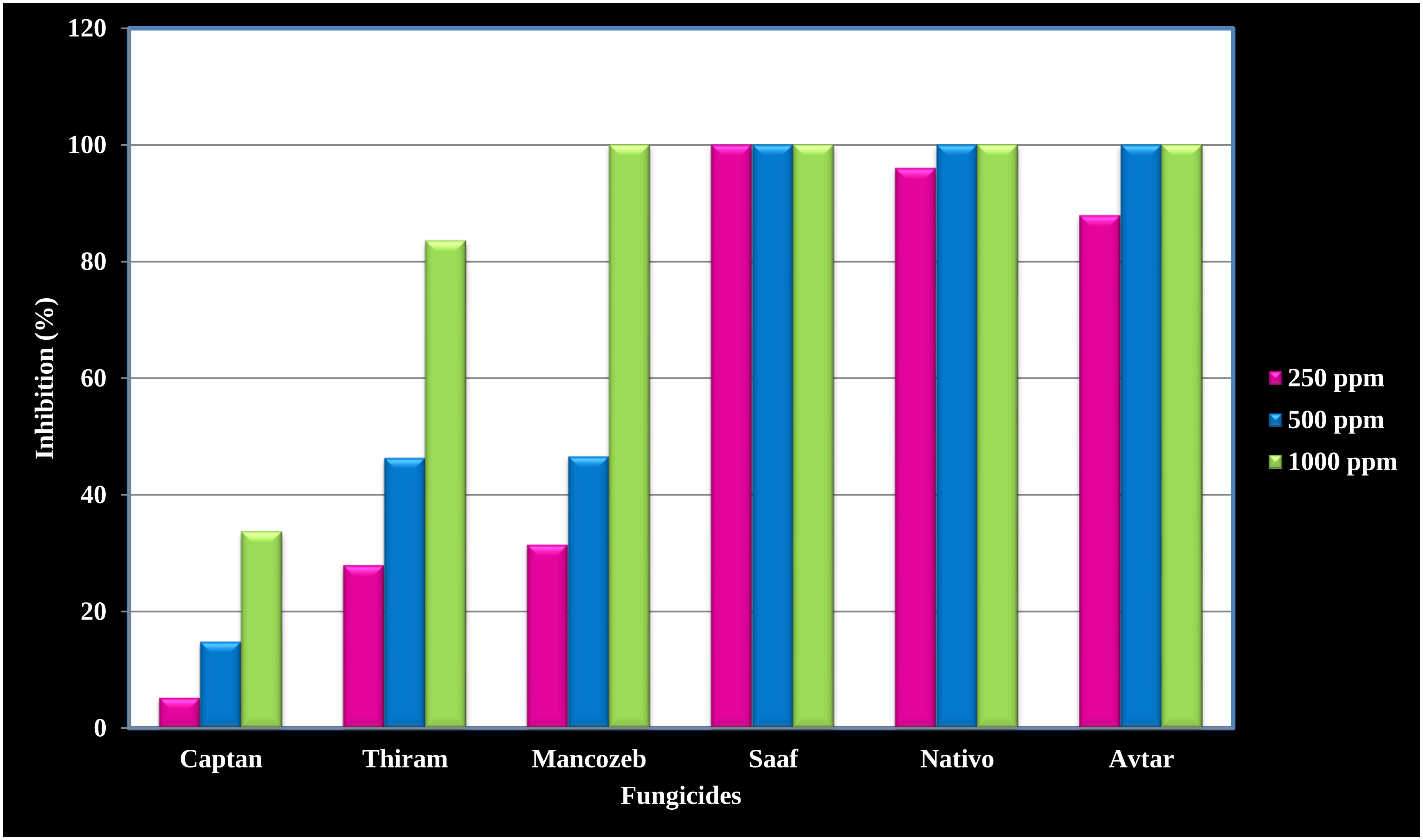
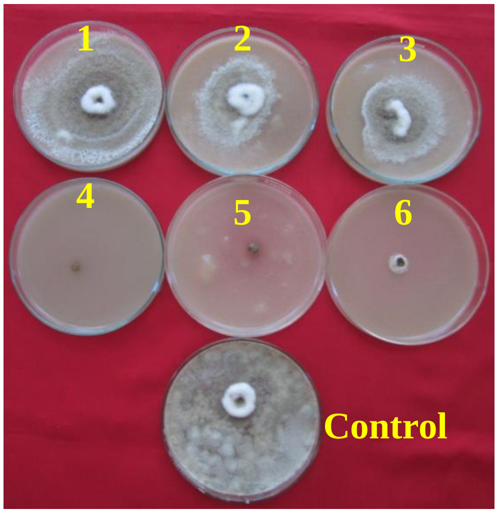
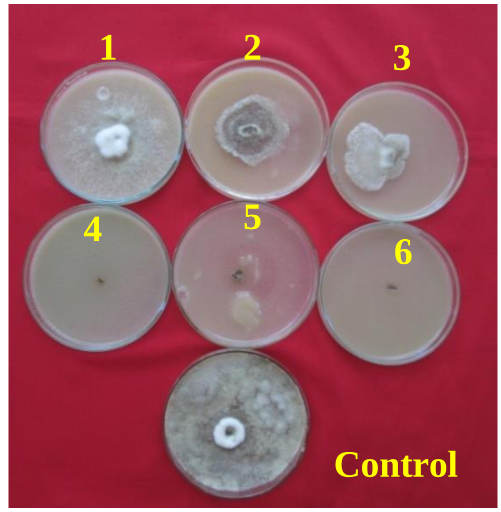


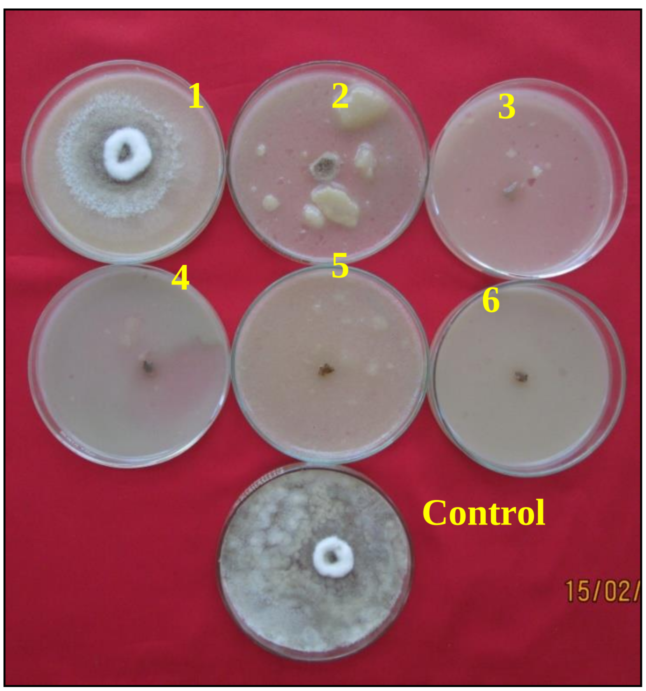
Fig. 4. Effect of contact and combi fungicides on growth of *Amphobotrys ricini*



250 ppm



500 ppm



1000 ppm

Treatments

Contact fungicides

1. Captan
2. Thiram
3. Mancozeb

Combi products

4. Carbendazim + Mancozeb
5. Tebuconazole + Trifloxystrobin
6. Hexaconazole + Zineb

Plate 7: *In vitro* evaluation of contact and combi fungicides on radial growth of *Amphobotrys ricini*

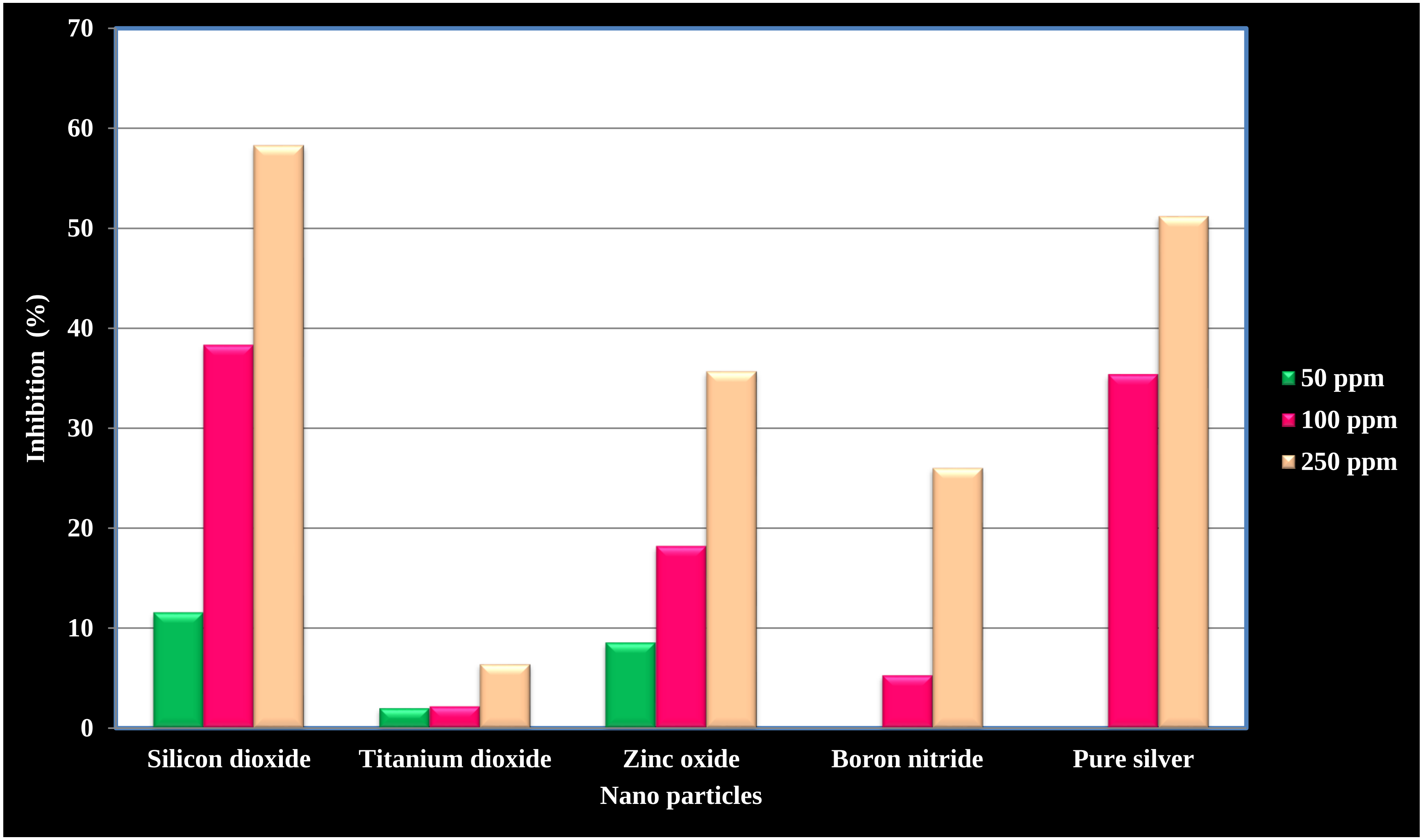
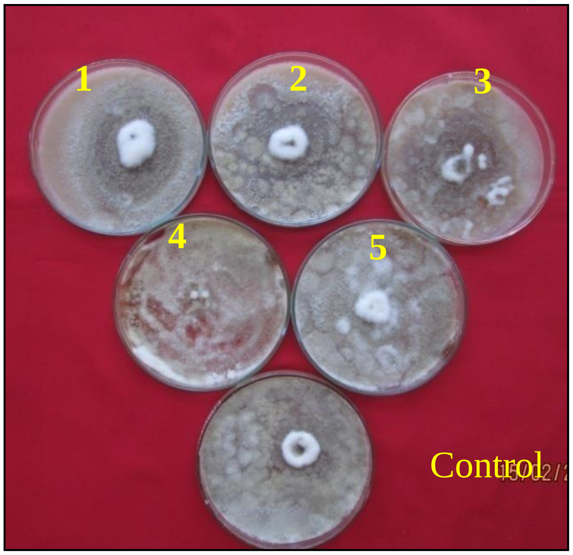
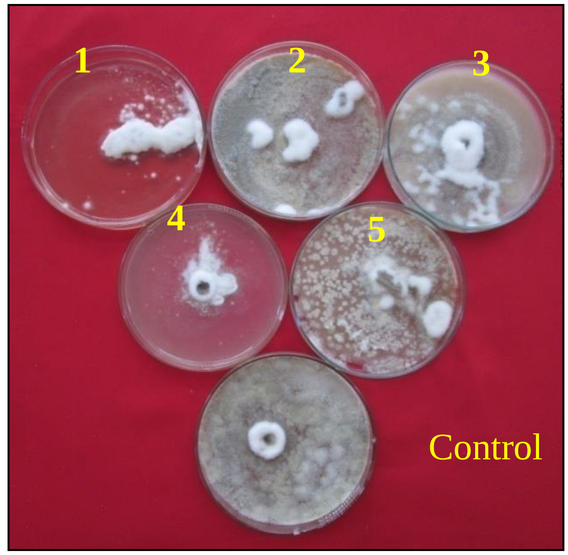


Fig. 5. Effect of nano particles on growth of *Amphobotrys ricini*



50 ppm



100 ppm

250 ppm

Treatments

1. Silicon dioxide
2. Titanium dioxide
3. Zinc oxide
4. Boron nitride
5. Pure silver

Plate 8: *In vitro* evaluation of nano particles on radial growth of *Amphobotrys ricini*

Table 8: Effect of systemic fungicides on growth of *Amphobotrys ricini*

Treatments	**Per cent inhibition of colony growth**			**Mean**
	50 ppm	**250 ppm**	**500 ppm**	
Carbendazim	100.00 (90.00)*	100. 00 (90.00)	100.00 (90.00)	**100.00 (90.00)**
Azoxystrobin	40.55 (39.56)	43.22 (41.11)	38.11 (38.13)	**40.62 (39.60)**
Trifloxystrobin	6.10 (14.31)	22.90 (28.6)	33.52 (35.39)	**20.84 (27.17)**
Propiconazole	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	**100.00 (90.00)**
Tebuconazole	97.22 (80.41)	100 (90.00)	100.00 (90.00)	**99.07 (84.48)**
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	**0.00 (0.00)**
Mean	**68.77 (56.03)**	**73.22 (58.84)**	**74.32 (59.56)**	**72.11 (58.13)**
	Fungicides (F)	**Concentration (C)**	**F×C**	
S.Em ±	0.59	0.53	1.02	
C.D. (P=0.01)	2.26	1.75	3.92	

*Figures in the parenthesis are arc sine transformed values.

Table 9: Effect of contact and combi fungicides on growth of *Amphobotrys ricini*

Treatments	Per cent inhibition of colony growth			Mean
	250 ppm	500 ppm	1000 ppm	
Captan	5.13 (13.11)*	14.73 (22.58)	33.60 (35.43)	**17.82 (24.98)**
Thiram	27.83 (31.85)	46.22 (42.84)	83.52 (66.06)	**52.52 (46.45)**
Mancozeb	31.33 (34.04)	46.50 (43.00)	100.00 (90.00)	**59.27 (50.35)**
Carbendazim + Mancozeb (Saaf)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	**100.00 (90.00)**
Tebuconazole + Trifloxystrobin (Nativo)	95.94 (78.38)	100.00 (90.00)	100.00 (90.00)	**98.64 (83.33)**
Hexaconazole + Zineb (Avtar)	87.86 (69.61)	100.00 (90.00)	100.00 (90.00)	**95.95 (78.40)**
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	**0.00 (0.00)**
Mean	**58.01 (49.62)**	**67.90 (55.50)**	**86.18 (68.19)**	**70.70 (57.24)**
	Fungicides (F)	**Concentration (C)**	**F×C**	
S.Em ±	**0.14**	**0.11**	**0.25**	
C.D. (P=0.01)	**0.55**	**0.39**	**0.96**	

*Figures in the parenthesis are arc sine transformed values.

Table 10: Effect of nano particles on growth of *Amphobotrys ricini*

Treatments	Per cent inhibition of colony growth			Mean
	50 ppm	100 ppm	250 ppm	
Silicon dioxide	11.58 (19.90)*	38.31 (38.25)	58.26 (49.76)	**36.05 (36.91)**
Titanium dioxide	1.97 (8.08)	2.16 (8.46)	6.39 (14.65)	**3.50 (10.8)**
Zinc oxide	8.56 (17.02)	18.19 (25.25)	35.7 (36.7)	**20.81 (27.15)**
Boron nitride	0.00 (0.00)	5.26 (13.27)	26.03 (30.68)	**10.43 (18.85)**
Pure silver	0.00 (0.00)	35.38 (36.51)	51.19 (45.69)	**28.86 (32.50)**
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	**0.00 (0.00)**
Mean	**4.42 (9.00)**	**19.86 (24.34)**	**35.51 (35.49)**	**19.93 (26.52)**
	Nano particles (N)	**Concentration (C)**	**N×C**	
S.Em ±	**0.13**	**0.11**	**0.24**	
C.D. (P=0.01)	**0.53**	**0.41**	**0.92**	

* Figures in the parenthesis are arc sine transformed values.

4.6 Management of gray mold

Field experiment was conducted to evaluate the relative efficacy of chemicals against gray mold of castor during 2017-18 at Zonal Agricultural Research Station (ZARS), Gandhi Krishi Vignana Kendra, Bengaluru (Plate 9).

The effect of different treatments on gray mold incidence was recorded using 0-9 scale and the results are presented in Table 11 and Fig. 6 & 7. Results revealed that all the treatments reduced the disease significantly compared to the unsprayed control.

Per cent disease index on different treatments varied from 12.03- 85.65 %. Least disease index of 12.03 % was recorded in T1 (Carbendazim, 1 g L^{-1}) followed by T6 (Carbendazim + Mancozeb, 2 g L^{-1}) 16.16 %, T2 (Tebuconazole + Trifloxystrobin 1%) 20.38 %, T5 (Tebuconazole, 2 ml L^{-1}) 22.25 %, T3 (Azoxystrobin, 0.5 %) 28.13%. Per cent disease index in control plants (T8) was 85.65.

Yield differed significantly among the treatments sprayed with different molecules. Maximum seed yield of 2410.22 kg ha^{-1} was recorded in treatment Carbendazim followed Carbendazim + Mancozeb (2372.00 kg ha^{-1}), Tebuconazole + Trifloxystrobin (2108.00 kg ha^{-1}). While unsprayed control treatment recorded lowest seed yield of 1101.10 kg ha^{-1}.

In a similar study Bhat and Rajasri (2015) reported that propiconazole @ 1 ml L^{-1} resulted least disease incidence (9.6 %) and higher yield (2473.60 kg ha^{-1}) which was followed by Carbendazim 1 g L^{-1} with yield of 2327.00 kg ha^{-1}. The results obtained are in agreement with the Rathi (1994) who reported that Bavistin gave the highest protection to the crop with least disease severity of *Botrytis* blight on chickpea.

Similarly, Bezerra (2007) reported that Carbendazim and Azoxystrobin are effective against the gray mold pathogen. But, Chagas (2009) reported that Azoxystrobin to be ineffective against *B. ricini*, while Carbendazim and other fungicides *viz*., Tebuconazole, Iprodione and Procymidone are highly effective.

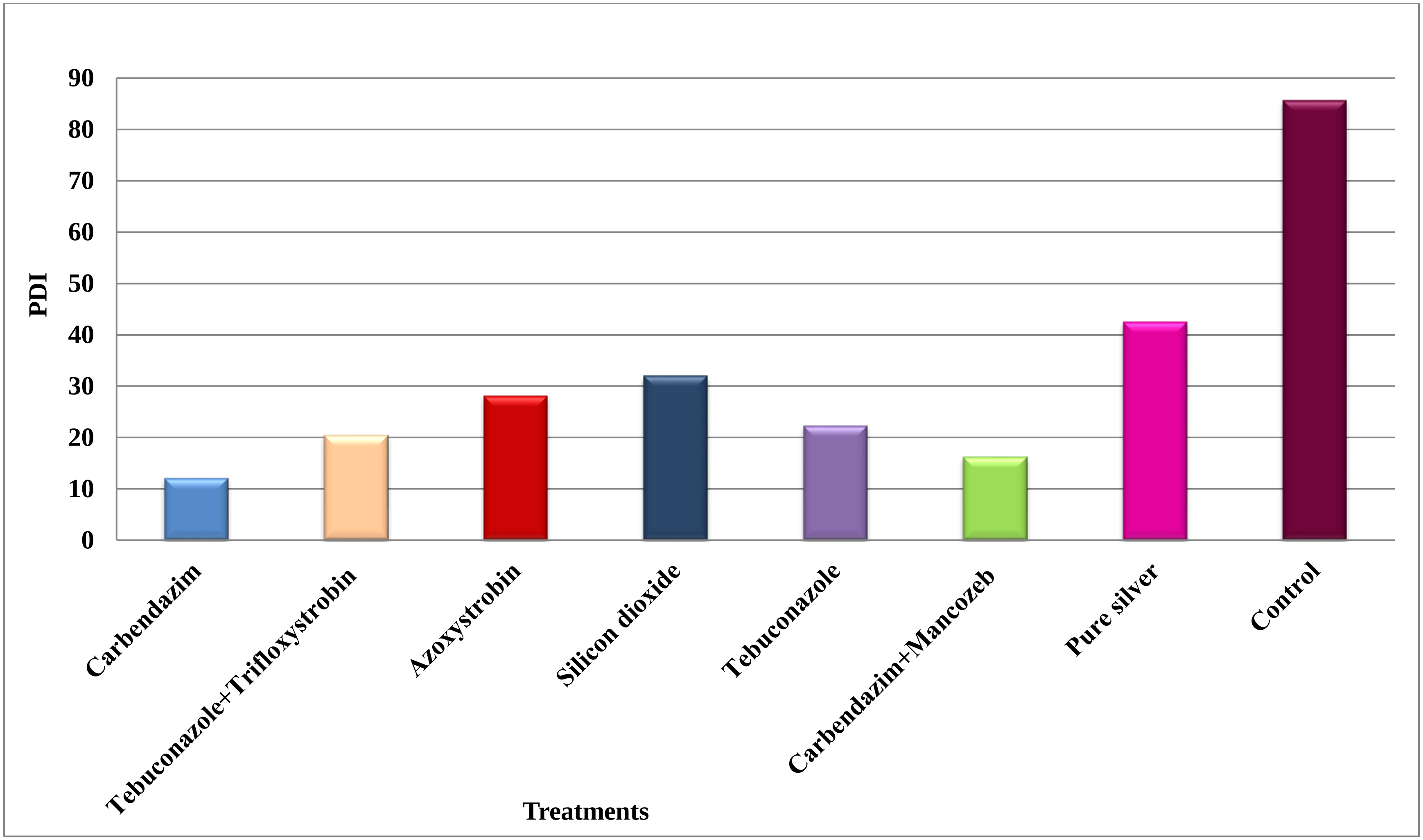


Fig. 6. Effect of different chemicals on per cent disease index of gray mold of castor

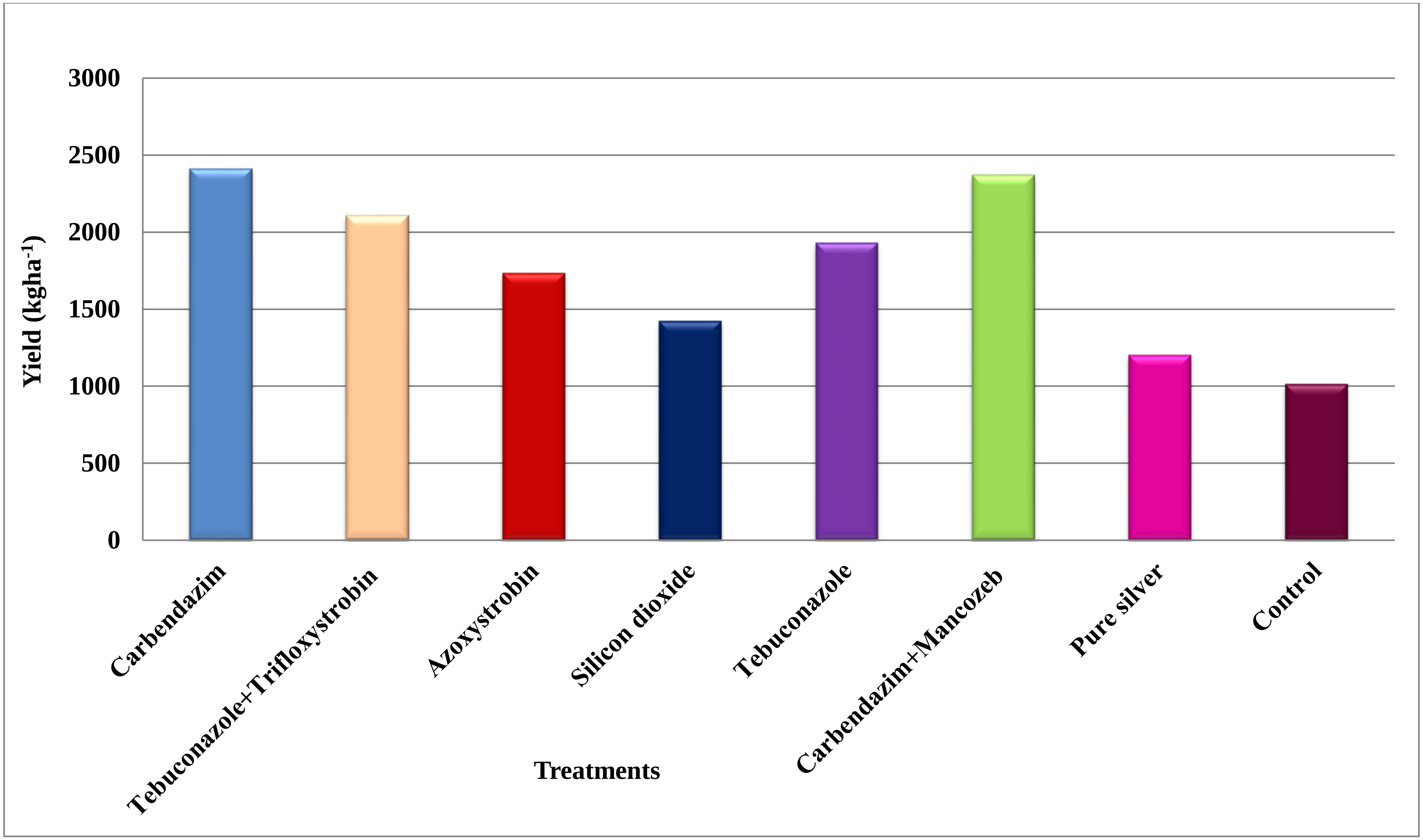


Fig. 7. Effect of different chemicals on the yield of castor

Plate 9: Field view of gray mold management trial

Nejad *et al*. (2017) in the glass house studies observed that silicon nano particle at 50 ppm concentration effectively suppressed the lesion development on rice leaves.

Zalewska *et al*. (2016) reported that under field condition, the formulations containing nano particles *viz*., Copper, silver and Chitosan *i.e*., Viflo Cal S, significantly limited the severity of septoriosis and powdery mildew in comparison to the severity of these diseases in the control plants, which contributed to an increase in the size and quality of the crop of caraway schizocarps.

Benzimidazole fungicides such as Benomyl and Thiophanate methyl allowed a higher level of *Botrytis* control than the protectants. According to Maiti *et al*. (1988), application of Carbendazim (0.05%) or Thiophanate-methyl (0.05%) twice at 15 days interval after emergence of racemes gave good control of castor gray mold. Similar results were obtained by Siva Kumar *et al*. (2011) who reported Carbendazim as the most effective treatment with low incidence of *Botrytis* gray mold on detached castor racemes under green house conditions. Dange *et al*. (2005) recommended two prophylactic sprays with Carbendazim (0.05%), the first at 50 per cent of flowering, and the second, when the first disease symptoms appear, reduce castor gray mold effectively.

Table 11*: Field evaluation of chemicals against gray mold of castor*

Treatments		Conc.	PDI	Yield ($kgha^{-1}$) #
T_1	Carbendazim	1.0 g L^{-1}	12.03# (20.30)*	2410.22
T_2	Tebuconazole + Trifloxystrobin (Nativo)	1.0 %	20.38 (26.85)	2108.00
T_3	Azoxystrobin	0.5 %	28.13 (32.04)	1733.33
T_4	Silicon dioxide	200 ppm	32.10 (34.52)	1421.33
T_5	Tebuconazole	2ml L^{-1}	22.25 (28.15)	1928.14
T_6	Carbendazim + Mancozeb (Saaf)	2.0 g L^{-1}	16.16 (23.71)	2372.00
T_7	He Pure silver	200 ppm	42.48 (40.68)	1200.14
T_8	Control (Untreated)	-	85.65 (67.75)	1011.10
S.Em ±			**0.12**	**3.52**
C.D. (P=0.05)			**0.36**	**10.68**

Mean of three replications

* The values in the parenthesis are arc sine transformed

V SUMMARY

Amphobotrys ricini, a well known necrotrophic pathogen and considered as a most destructive and known to cause potential seed yield loss up to 100 per cent. Present investigations was made to characterize *A. ricini*, *in vitro* and field evaluation of chemicals at ZARS, UAS, GKVK, Bengaluru, during 2017-18 and the results obtained thereon are summarized in this chapter.

The pathogen causing gray mold on castor initially produces bluish spots on flowers, developing fruits, panicle and leaves. On fruits circular, sunken, dark colored spots are produced which results in rupture of the capsules and the inflorescence or 'spike' is covered with moldy growth.

The pathogen causing gray mold was isolated from infected castor racemes showing typical gray mold symptoms on oat meal enriched medium through standard tissue isolation technique and pure culture was obtained through single spore isolation method.

The colonies of *A. ricini* on oat meal enriched medium are light gray in colour. The fungus produces septate and branched mycelium which is thin, hyaline to light brown in colour produces dichotomously branched conidiophores bearing conidia which are globose, smooth, hyaline to pale brown colour.

Highest radial growth was recorded on Malt extract agar medium but profuse sporulation was observed on Oat meal enriched medium and maximum dry mycelial weight was recorded on Richard's agar medium. The colony colour on different media varied from white to dirty white, cottony white, brown to light brown in colour. Maximum sclerotia production was observed on Malt extract agar medium (250 per plate). Other characters such as sclerotia size, pattern of sclerotia formation and sclerotia initiation differed with the media composition. Large sized sclerotia were produced on Richard's agar and Malt extract agar medium. Sclerotia initiation was observed on 10^{th} day after inoculation.

The PCR amplification and analyses of sequences using ITS rDNA sequence, comparision using BLAST progarmme, it was confirmed as *A. ricini* as the cause of gray mold of castor.

In vitro studies revealed that complete inhibition of *A. ricini* was observed in two systemic fungicides Carbendazim and Propiconazole at 50, 250 and 500 ppm concentrations, followed by Tebuconazole (99.07 %). Likewise among the contact fungicides maximum inhibition was observed in Mancozeb (59.27 %). Among the combi products complete inhibition was observed in Carbendazim + Mancozeb. Silicon dioxide nano particle showed better inhibition of pathogen (36.05 %).

Carbendazin was highly effective in controlling the gray mold in the field with least percent disease index of 12.03 and higher yield of 2410.22 kg ha^{-1}.

VI REFERENCES

AHMED, A. U., ZAMAN, S., MAZID, M. A., RAHMAN, M. M., SARKAR, M. M. R., ARBIA, L., UD-DEEN, M. M. AND KABIR, G., 2014, Studies of *Botrytis cinerea* causing *Botrytis* gray mold disease in Chickpea (*Cicer arietinum* L.). *J. Bio-Sci*., **22**: 69-76.

ANJANI, K., 2012, Castor genetic resources: A primary gene pool for exploitation. *Industrial Crops and Products*, **35**: 1-14.

ANONYMOUS, 1921, Inflorescence and stem rot. Bangalore, Karnataka. 2nd meeting of Mycological workers in India.

ANONYMOUS, 1995, Annual Progress Report of Castor, *All India Coordinated Research Projects on Oil seeds*. Directorate of Oilseeds Research, Rajendranagar, Hyderabad.

ANONYMOUS, 1998, Annual Progress Report of Castor, *All India Coordinated Research Projects on Oil seeds.* Directorate of Oilseeds Research, Rajendranagar, Hyderabad.

ANONYMOUS, 2018, Director's report. ICAR-IIOR., Rajendranagar, 36 pp.

ARAUJO, E. A., SUASSUNA, D. N. AND COUTINHO, M. W., 2007, *Doenças e seu Manejo. In: O Agronegocio da Mamona no Brasil*, D.M.P. Azevedo and N.E. de M. Beltrao, (Eds.), Embrapa Informacao Tecnologica, Brazil , 283-303.

BARRETO, R.W. AND EVANS, H. C., 1998, Fungal pathogens of *Euphorbia heterophylla* and *E. hirta* in Brazil and their potential as weed biocontrol agents. *Mycopathologia*, **141**: 21- 36 pp.

BATISTA, F. A. S., LIMA, E. F., MOREIRA, J. A. N., AZEVEDO, D. M. P., PIRES, V. A., VIEIRA, R. M. AND SANTOS, J. W., 1998, Avaliacao da resistência de genótipos de mamoneira, *Ricinus communis* L., ao mofo cinzento causado por *Botrytis ricini* Godfrey. Embrapa Algodao. *Comunicado Tecnico* 73, Campina Grande, Brazil.

BATISTA, S. A. F., LIMA, F. E., SOARES, J. J. AND AZEVEDO, P. M. D., 1996, Doenças e pragas damamoneira (*Ricinus communis* L.) e seu controle. *Embrapa Algodao, Circular Tecnica* 21, Campina Grande, Brazil, 213-218.

BEZERRA, S. C., 2007, Estrutura genética e sensibilidade a fungicidas de *Amphobotrys ricini* agente causal do mofo cinzento da mamoneira. *M.Sc. Dissertation. Universidade Federal do Rio Grande do Norte,* Natal, Brazil.

BHAT, B. N AND RAJASRI, M., 2015, Management of *Botrytis* gray mold of castor with chemicals and biocontrol agents. *Int. J.* Agril. *Sci. Res.*, **10**(2): 129-131.

BOHOVIC, V. I., 1936, Diseases of the newly introduced oliferous crops in the Ukraine. *Pl. Prot.,* pp: 1114-1235.

BRYK, H., 1985, The variability of morphological characters and mycelia growth rate of monoconidial culture of *Botrytis cinerea* Pers. *Acta Agrobotanica*., **38**(2): 137-145.

BUCHWALD, N. F., 1949, Studies in the Scletoriniaceae: Taxonomy of the Sclerotiniaceae. *Kongelige Veterinær- og Landbohojskole, Aarsskrift*. **32**:1-116.

CARBONE, I. AND KOHN, L. M., 1993, Ribosomal DNA sequence divergence within internal transcribed spacer 1 of *Sclerotiniaceae. Mycologia*, **85**: 415-427.

CHAGAS, H. A., 2009, Control de mofo-cinzento (*Amphobotrys ricini)* da mamoneria (*Ricinus communis* L.) pormetodosquimico, biologic e com oleosessenciais. *MSc Dissertation, (Agronomy), Universidade Estadual paulista "Julionde Mesquitafilho"*, Botucatu, Brazil.

CHAURASIA, C. P. P. AND JOSHI, S., 2001, Management of *Botrytis* gray mold (*Botrytis cinerea* Pers. Ex. Fr.) of chickpea at Tarahara, *Nepal Agric*. *Res*. *J*., **5**: 37-41.

CHEN, L. J., YIN, Y. Y., SUN, S. K. AND SUN, J., 2017, first report of a gray mold of *Lilium cernuum* Komar, leaves caused by *Botrytis cinerea* in Liaoning province of China. *J*. *Pl*. *Pathol*., **99**(1): 287-304.

CHEN, W., HOY, J. W. AND SCHNEIDER, R. W., 1992, Species–specific polymorphisms in transcribed ribosomal DNA of five *Pythium* species. *Exp*. *Mycol*., **16**: 22-34.

COOK, A. A., 1981, Diseases of tropical and subtropical field, fiber and oil plants. Mac Millan Publishing Company Incorporation, New York, pp: 134.

DANGE, S. R. S., DESAI, G. A. AND PATEL, I. S., 2005, Diseases of castor. In:Saharan, G. S., Mehta, N and Sangwan (Eds.), Diseases of Oilseeds crops. New Delhi, pp: 211-234.

DERBALAH, A. S., ELKOT, G. A. E. AND HAMZA, A. M., 2012, Laboratory evaluation of botanical extracts, microbial culture filtrates and silver nano particles against *Botrytis cinerea*. *Ann*. *Microbiol*., **62**: 1331-1337.

DRUMOND, A. O. AND COELHO, J. S 1981, Doencas da mamoneira. *Informe Agropecuario*, **7**(82): 38-42.

ELAD, Y., GULLINO, M. L., SHTIENBERG, D. AND ALO, C., 1995, Managing *Botrytis cinerea* on tomatoes in greenhouses in the Mediterranean. *Crop Prot*., **14**(2): 105-109.

ELAMAWI, RABAB, A. M. AND EL-SHAFEY, S. A. R., 2013, Inhibition effects of silver nano particles against rice blast disease caused by *Magnaporthe grisea*. *Egypt*. *J*. *Agric*. *Res*., **91**(4): 1271.

FAYADH, A. M. AND ALEDANI, A. M., 2011, Effect of some microelements and biological control agents in control of tomato seedling damping-off caused by *Rhizoctonia solani* Kuhn. *Basra. J. Agric. Sci*., **24**(1): 53-69.

GODFREY, G. H., 1919, *Sclerotinea ricini* parasitic on castor bean (*Ricinus communis* L.*). Phytopathol*., **9**: 565-567.

GODFREY, G. H., 1923, Gray mold of castor bean. *J. Agri. Res*., **23**(9): 679 -715.

GONCALVES, R. D., 1936, Mofo cinzento da mamoneira. *O Biologico*, **2**(7): 232-235.

GOTO, S., TERABAYASHI, T. AND YOKOTSUKA, I., 1980, Identification, cultural properties and pathogenicity of gray mold of grape *Botrytis cinerea*. *J. Agric. Chem*. Japan, **54**(2): 117-121.

HAWKSWORTH, D. L., SUTTAN, B. C. AND AINSWORTH, G. C., 1983, Ainsworth and Bisby's Dictionary of fungi. VII ed. *Commonwealth mycological Institute*. Kew, Survey, England, pp.445.

HE, L., LIU, Y., MUSTAPHA, A. AND LIN, M., 2011, Antifungal activity of zinc oxide nano particles against *Botrytis cinerea* and *Penicillium expansum*. *Microbial Research*, **166**: 207-215.

HENNEBERT, G. L., 1973, *Botrytis* and *Botrytis*-like genera. *Persoonia*, **7**(2): 183-204.

HOSEN, M. I. AHMED, A. U. AND ISLAM, M. R., 2010, Physiological variability and *in vitro* antifungal activity against *Botrytis cinerea* causing *Botrytis* gray mold of chickpea (*Cicer arietinum* L.). *Spanish J. Agril. Res*., **8**(3): 750-756.

IQBAL, S. M., HUSSAIN, S. AND MALIK, B. A., 1992, *In vitro* evaluation of fungicides against *Botrytis cinerea* of Lentil [*Lens culinaris*]. *AGRIS.*, **32**: 283-302.

JARVIS, W. R., 1977, *Botryotinia and Botrytis* species: Taxonomy, physiology and pathogenicity, A guide to the Literature. Monograph No. 15, Canada Department of Agriculture, Ottawa, Canada.

JUNG, J. H., KIM, S. W., MIN, J. S., KIM, Y. J., LAMSAL, K., KIM, K. S. AND LE, Y. S., 2010, The effect of nano-silver liquid against the white rot of the green onion caused by *Sclerotium cepivorum*. *Mycobiol*., **38**(1): 39-45.

KAUR, G., 2015, Variability and management studies of *Botrytis cinerea* causing gray mold of gladiolus. *Ph.D.* (*Pl. Pathol.*) *Thesis*, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, 111 pp.

KAUR, P., THAKUR, R. AND CHOUDHARY, A., 2012, An *in vitro* study of the antifungal activity of silver/chitosan nano formulations against important seed borne pathogens. *Inter. J. Sci. Tech. Res.*, **l**: 83-86.

KHAN, M. O, AZIZUDDIN AND SHAHZAD, S., 2013, Use of silicon in growth of *Macrophomina phaseolina*. *Pakistan J. Bot.*, **45**(4): 1469-1472.

KHAZAELI, P., ZAMANIZADEH, H., MORID AND BAYAT, H., 2010, Morphological and molecular identification of *Botrytis cinerea,* causal agent of gray mold in rose greenhouses in central regions of Iran. *Int. J. Agril. Sci. Res*., **1**(1): 19-24.

KIM, WOO, S., KIM, S. K., LAMSAL, K., KIM, J. Y., KIM, B. S., JUNG, M., SIM, S. J., KIM, H. S., CHANG, S. J., KIM, J. K. AND YOUN SU LEE, Y. S., 2009, An *In vitro* study of the antifungal effect of silver nano particles on Oak wilt Pathogen *Raffaelea* sp. *J. Microbiol. Biotechnol*., **19**(8): 760–764.

KOLTE, S. J., 1995, Castor: Diseases and Crop Improvement. Shipra Publications, Delhi, pp 119.

KOYCU, N. D., OZER, N. AND DELEN, N., 2012, Sensitivity of *Botrytis cinerea* isolates against some fungicides used in vineyard. *African J. Biotechnol.*, **11**(8): 1892-1899.

KWON, J. H., CHEON, M. G., CHOI, O. AND KIM, J., 2011, First report of *Botrytis cinerea* as a postharvest pathogen of Bluebertry in Korea. *The Korean Soc***.** *Mycol.*, **39**(1): 52-53.

LAMSAL, K., KIM, S. W., JUNG, J. H., KIM, Y. S., KIM. K. S. AND LEE, Y. S., 2011, Inhibition effects of silver nano particles against powdery mildews on cucumber and pumpkin. *Mycobiol.*, **39**(1): 26-32.

LIMA, B. V., SOARES, D. J., PERREIRA, O. L. AND BARRETO, R. W., 2008, Natural infection of *Acalypha* hispida and *Jatropra podagrica* inflorescences by *Amphobotrys ricini* in Brazil. *Australasian Pl. Dis. Notes*, **3**: 5-7.

LIMA, E. F. AND SOARES, J. J., 1990, Resistência de cultivares de mamoneira ao mofo cinzento, causado por *Botrytis ricini*. Fitopatologia Brasileira, **15**(1): 96-98 pp.

LIMA, E. F., ARAUJO, A. E. AND BATISTA, F. A. S., 2001, Doencas e seu Controle. In: O Agronegocio da Mamona no Brasil, D. M. P. Azevedo and E. F. Lima (Eds.), *Embrapa Informacao Tecnologica*, Brazil, 192-212.

MAEKAWA, K., WATANABE, K., KANTO, T., AINO, M. AND SAIGUSA, M., 2003, Effect of soluble silicic acid on suppression of rice leaf blast. *Japanese J. Soil Sci. plant Nutri.*, **74**: 293-299.

MAITI, S., HEGDE, M. R. AND CHATTOPADHYAY, S. B., 1988, *Hand book of Annual Oilseed Crops*. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, pp: 325.

MARTINEZ, F., BLANCARD, D., LECOMTE, P., LEVIS, C., DUBOS, B. AND FERMAUD, M., 2003, Phenotypic differences between *vacuma* and *transposa* subpopulations of *Botrytis cinerea*. *European J. Pl. Pathol.*, **109**: 479-488.

MCKINNEY, H. H., 1923, A new system of grading of plant diseases. *J. Agric. Res.*, **26**: 185-218.

MIRZAEI, S., GOLTAPEH, E. M. AND SHAMS-BAKHSH, M., 2007, Taxonomical studies on the genus *Botrytis* in Iran. *J. Agri. Technol.*, **3**(1): 65-76.

MLIKOTA, F., MALES, P. AND CVJETKOVIC., 1996, Effectiveness of five fungicides on grapevine grey mold and their effects on must fermentation. *J*ournal *of Wine Research*, **7**(2): 103-110.

MORONES, J. R., ELECHIGUERRA, J. L., CAMACHO, A., HOLT, K., KOURI, J. B., RAMIREZ, J. T. AND YACAMAN, J., 2005, The bactericidal effect of silver nano particles. *Nanotechnol.*, **16**: 2346–2353.

MOSES, G. J. AND REDDY, R. R., 1989, Gray rot of castor in Andhra Pradesh. *J. Res.*, Andhra Pradesh Agricultural University, **17**: 74-75.

MUNOZ, G., HINRICHSEN, P., BRYGOO, Y. AND GIRAUD, T., 2002, Genetic characterisation of *Botrytis cinerea* populations in Chile. *Mycol. Res.*, **106**(5): 594-601.

MUTLU, H. AND MEIER., 2010, Castor oil as a renewable resource for the chemical industry. *European Journal of Lipid Science and Technology*, **112**(1): 10–30.

NEJAD, M. S., BONJAR, G. H. S., KHATAMI, M., AMINI, A. AND AGHIGHI, S., 2017, *In vitro* and *in vivo* antifungal properties of silver nano particles against *Rhizoctonia solani*, a common agent of rice sheath blight disease. *IET Nanobiotechnol.*, **11**(3): 236-240.

NIELSEN, K., JUSTESEN, A. F., JENSEN, D. F. AND YOHALEM, D. S. 2001, Universally primed polymerase chain reaction alleles and internal transcribed spacer restriction fragment length polymorphisms distinguish two subgroups in *Botrytis aclada* distinct from *B. byssoidea*. *Phytopathol.*, **91**: 527-533.

ORELLANA, R. G., 1959, *Bortytis* leaf blight of *Ricinus communis* L. *Pl. Disease Report*, **43**: 363-364.

PANDE, S., BAKR, M. A. AND JOHANSEN, C., 1998, Recent advances in research and management of *Botrytis* gray mold of chickpea. *International Crops Research Institute for the Semi-Arid Tropics*, Patancheru**,** Andhra Pradesh, 12 pp.

PANDEY, S. P., 2011, Management of *Botrytis* blight (*Botrytis gladiolorum* Timm.) in gladiolus through cultural and chemical means. *M.Sc. (Agri.) Thesis*, Punjab Agric. Univ., Ludhiana, 26 pp.

PANSE, V. G. AND SUKHATME, P. V., 1967, Statistical methods for agricultural workers. ICAR, *Agri*. *Res*., New Delhi.

RALUCA, M. AND EMILIA, P. C., 2010, Phenotypic differences between isolates of *Botrytis cinerea* Pers. from different host plants. *Bulletin UASVM Agriculture*, **67**(1): 212-214.

RAO, H. C., 1997, Castor area declines in South Coastal Andra Pradesh. *DOR Newsltr*., **3**: 3.

RASHID, M. H., HOSSAIN, M. A., KASHEM, M. A., SHIV KUMAR, RAFII, M. Y. AND LATIF, M. A., 2014, Efficacy of combined formulations of fungicides with different modes of action in controlling *Botrytis* gray mold disease in Chickpea. *The Scientific World Journal*, 231-235.

RATHI, Y. P. S., 1994, *Botrytis* gray mold of chickpea: survival and management. *Final Techinical Report*, 36 pp.

RAUTELA, P. AND SINGH, R. P., 2017, Evaluation of fungicides against Gray mould (*Botrytis cinerea* Pers. Fr) and Leaf mould [*Fulvia fulva* (Cooke) Cif. (*Cladosporium fulvum* Cooke)] of tomato. *Int. J. Chemical Studies*, **5**(5): 400-404.

RIGOTTI, S., VIRET, O. AND GINDRO, K., 2006, Two new primers highly specific for the detection of *Botrytis cinerea* Pers. *Fr*. *Phytopathologia Mediterranea*, **45**(3): 253-260.

ROSSLENBROICH, H. J. AND STUEBLER, D., 2000, *Botrytis cinerea* - History of chemical control and novel fungicides for its management. *Crop prot*., **19**: 557-561.

SAHARAN, V., MEHROTRA, A., KHATIK, R., RAWAL, P., SHARMA, S. S. AND PAL, A., 2013, Synthesis of chitosan based nano particles and their *in vitro* evaluation against phytopathogenic fungi. *Int*. *J*. *Bio*. *Macromol*., **62**: 677-683.

SAITO, S., MICHAILIDES, T., J. AND XIAO, C. L., 2014, First Report of *Botrytis pseudocinerea* causing gray mold on blueberry in North America. *Pl*. *Dis*., **98**(12): 1743.

SARITA KUMARI, TAYAL, P., SHARMA, E. AND KAPOOR, R., 2014, Analyses of genetic and pathogenic variability among *Botrytis cinerea* isolates. *Microbio*. *Res*., **169**: 862–872.

SEA, 2014, Castor crop survey 2013-14. The Solvent Extractors Association of India, Mumbai, pp: 4.

SHAHIDUZZAMAN, M., 2015, Efficacy of fungicides in controlling *Botrytis* gray mold of chickpea (*Cicer arietinum* L.). *Bangladesh J. Agril. Res*., **40**(3): 391-398.

SHANTAMMA., 2016, Efficacy of colloidal silver particles against selected plant pathogens. *Ph.D. (Agri.) Thesis*, Univ. Agric. Sci., Bengaluru, 87 pp.

SINGH, P. G., KUMAR, R. AND SIDHU, G. S., 2008, Efficacy of fungicides against *Botrytis gladiolorum* of gladiolus. *Pl. Dis. Res*., **23**(2): 19-23.

SIVA KUMAR, V., MEENA KUMARI, K. V. S., PRASAD, R. D., NARAYANA REDDY, P. AND ANKAIAH, R., 2011, Efficacy of fungicides and bioagents against *Botrytis* grey mold on detached castor racemes. *Indian J. Pl. Prot.*, **39** (4): 321-322.

SOARES, D. J., 2012, Gray mold of castor: A review. In: Cumagan, C. J. R. (ed.) *Pl. Pathol.*, pp: 219-240.

SONDI, I. AND SALOPEK-SONDI, B., 2004, Silver nano particles as antimicrobial agent: a case study on *E. coli* as a model for gram-negative bacteria. *J. Coll. Inter. Sci.*, **275**:177–182.

STAATS, M., BAARLEN, P. V. AND KAN, J. A. L. V., 2005, Molecular phylogeny of the plant pathogenic genus *Botrytis* and the evolution of host specificity. *Mol. Biol. Evol*, **22**(2): 333-346.

STONE, W. J. AND CULP, T. W., 1959. Effects of diseases on castor beans in Mississippi. *Plant Disease Reporter*, **43**(7): 827-829.

SUNG-KEE, H., WAN-GYU, K., WEON-DAE, C. AND HONG-GI, K., 2001, Occurrence of gray mold in castor bean caused by *Botrytis cinerea* and *Amphobotrys ricini* in Korea. *Pl. Pathol. J.*, **17**(6): 357-360.

TANOVIC, B., DELIBASIC, G., MILIVOJEVIC, J. AND NIKOLIC, M., 2009, Characterization of *Botrytis cinerea* isolates from small fruits and grapevine in Serbia. *Arch. Biol. Sci.*, Belgrade, **61**(3): 419-429.

TANOVIC, B., HRUSTIC, J., MIHAJLOVIC, M., GRAHOVAC, M. AND DELIBASIC, G., 2014, *Botrytis cinerea* in raspberry in Serbia I: Morphological and molecular characterization. *Pestic. Phytomed.*, Belgrade, **29**(4): 237-247.

THOMAS, C. A. AND ORELLANA, R. G., 1963, Nature of predisposition of castor beans to *Botrytis.* II. Raceme compactness, internode length, position of staminate flowers and bloom in relation to capsule susceptibility. *Phytopathol.*, **53**: 249-251.

THOMPSON, J. R. AND LATORRE, B. A., 1999, Characterization of *Botrytis cinerea* from table grapes in Chile using RAPD-PCR. *Pl. Dis*., **83**:1090-1094.

TROPOVA, A. T., 1928, Fungal diseases of newly introduced crops and endeavors to find means for their control. Proceedings of Pan-Soviet Congress of Botanists in Leningard, pp 188.

VASILICA, R., ALEXANDRA, L. AND EMILIA, C., 2012, *In vitro* studies regarding the morphology of *Botrytis cinerea* Pers. isolated from geranium plants. *Pro Environment*, **5**: 60- 66.

VINCENT, J. M., 1947, Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*, **159**: 239-241.

WHETZEL, H. H., 1945, A synopsis of the genera and species of the Sclerotiniaceae, family of stromatic inoperculate discomycetes. *Mycologia*, **37**: 648-714.

ZADA, N., IRSHAD, G., NAZ, F., GULZAR, A., SHAHID, M., ASLAM, M. F. AND GHUFFAR, S., 2016, Morphological and cultural characterization of *Botrytis cinerea* causing gray mold disease of lentil crop from Pakistan. *Pak. J. Phytopathol*., **28**(2): 249-254.

ZALEWSKA, E. D., STEFANIAK, Z. M. AND KROL, E. D., 2016, Antifungal activity of nano particles against chosen fungal pathogens of caraway. *Acta Sci. Pol. Hortorum Cultus,* **15**(6): 121-137.

ZHANG, Y., LI, X., SHEN, F., XU, H., LI, Y. AND LIU, D., 2018, Characterization of *Botrytis cinerea* isolates from grape vineyards in China. *Pl. dis*., **102**(1): 40-48.