STUDIES ON LEAF BLOTCH DISEASE OF TURMERIC (*Curcuma longa* L.)

BY

MUKUL KUMAR

MASTER OF SCIENCE IN AGRICULTURE
(PLANT PATHOLOGY)

DEPARTMENT OF PLANT PATHOLOGY

Dr. RAJENDRA PRASAD CENTRAL AGRICULTURAL UNIVERSITY, PUSA, SAMASTIPUR (BIHAR) – 848125

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A THESIS SUBMITTED TO
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2019

Regd. No. M/PP/209/2017-18

Dedicated

To

My Chacha ji

"Whose faith, Blessings, Sacrifice and Perpetual Affection Always Inspired Me to Attain High Ambition in Life"

Mukul Kumar...

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Date 27 / 06 / **2019**

Certificate

This is to certify that the thesis entitled **"Studies on leaf blotch disease of turmeric (*Curcuma longa* L.)."** submitted in partial fulfilment of the requirements for the award of the degree of **MASTER OF SCIENCE IN AGRICULTURE (PLANT PATHOLOGY)** of the **Faculty of Post-Gradute studies, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur (Bihar)** is a genuine record of ***bonafide*** research work carried out by **MUKUL KUMAR** under my guidance and supervision.

The results of the investigation reported in this thesis have not so far been submitted for any other degree or diploma. The assistance and helps received during the course of this investigation and sources of literature have been duly acknowledged.

27/06/2019

Dr. A. K. Mishra
Chairman
Advisory Committee

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27.06.2019

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Professor and Head
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We, the undersigned, members of the Advisory Committee of **Mr. MUKUL KUMAR, ID. No. 17032604** a candidate for the degree of **MASTER OF SCIENCE IN AGRICULTURE (PLANT PATHOLOGY),** have gone through the manuscript of the thesis and agree that the thesis entitled **"Studies on leaf blotch disease of turmeric (*Curcuma longa* L.)."** may be submitted by him in partial fulfilment of the requirements for the award of the said degree of the Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, (Bihar).

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"Gratitude is the most exquisite form of memory"

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Mukul Kumar
27/06/2019
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LIST OF ABBREVIATION

Symbol		Word Stand for
In vitro	:	In Plates
CD	:	Critical difference
DF	:	Degree of freedom
±	:	Plus or Minus
%	:	Per cent
>	:	Greater than
<	:	Smaller than
µM	:	Micro meter
^{o}C	:	Celcius
BOD	:	Biological Oxygen Demand
Cm	:	Centimetre
cv.	:	Cultivar
d.f.	:	Degree of freedom
DAP	:	Days after planting
DAS	:	Days after spraying
i.e.	:	That is
e.g.	:	For example
et al.,	:	And others
kg	:	Kilogram
g	:	Gram
ha	:	Hectare
h	:	hour
m	:	meter
Max.	:	Maximum
Min.	:	Minimum
ml	:	Millilitre
mm	:	Millimeter
Morn.	:	Morning
NS	:	Non significant
Q	:	Quintal
S	:	Significant
SEm(±)	:	Standard error of mean
Sl. No.	:	Serial Number
Sp.	:	Species (singular)
Spp.	:	Species (plural)
t/ha	:	Tonnes per hectare
Temp.	:	Temperature
Viz.,	:	Namely
R.F.	:	rainfall
R.H.	:	Relative humidity
PDA	:	Potato dextrose agar
@	:	At the rate of
Etc.	:	Etcetra and others
Fig.	:	figure
ICBR	:	Incremental cost benefit ratio
ha	:	Hectare
PDI	:	Per cent disease index

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ABSTRACT

Turmeric (*Curcuma longa* L.) known as golden spice as well as "spice of life" has emerged as low volume high value crop. Among various diseases attacking turmeric, leaf blotch disease caused by *Taphrina maculans* Butler is one of the most serious disease. Turmeric yield losses due to this disease have been recorded upto 37.6-52.9 per cent. Considering the seriousness of this disease, present investigation was carried out on various aspects *viz.*, survey, germplasm screening and evaluation of botanicals, fungicides and resistance inducing chemical against leaf blotch disease of turmeric at T. C. A., Dholi, Muzaffarpur (Dr. Rajendra Prasad central Agricultural University, Pusa, Samastipur, Bihar) during 2018-19. The fungus isolated from the diseased turmeric leaf was confirmed as *Taphrina maculans* by observing and comparing blastospore measurement with previous reports. Survey results indicated that leaf blotch was observed in all the villages of Samastipur and Muzaffarpur district. Muzaffarpur district was found more prone to leaf blotch disease compared to Samastipur district. There was an increase in the disease incidence during September to November. Among 16 turmeric germplasm screened against the leaf blotch disease, three and eight germplasm showed Resistant and Moderately Resistant reaction respectively. Neem (6% aqueous extract) followed by brahmi (6% aqueous extract) were found most effective plant extract under *in-vitro* conditions against *T. maculans*. Under disease management, rhizome treatment alongwith three foliar spray of Tricyclazole 75 WP (0.1%) at fortnightly interval starting from appearance of disease incidence resulted lowest PDI (26.67) and highest rhizome yield (37.85 t ha^{-1}) consequently with 49.99 per cent disease reduction and 55.75 per cent yield increase over control was recorded. Maximum incremental cost benefit ratio (1:16.34) was exhibited by the same treatment.

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CHAPTER - I

INTRODUCTION

1. INTRODUCTION

Turmeric (*Curcuma longa* L.) is known as the "golden spice" as well as the "spice of life". It is also called nature's precious gift and commonly known as 'National Heritage' (Maurya *et al*., 2011). Turmeric has strong associations with the socio-cultural life of the people of the Indian subcontinent. From ancient period turmeric considered as the "Oushadhi" means the healing herb (Jager, 1997). The genus Curcuma was established by Linnaeus (1753). The generic epithet is derived from the Arabic word karkum, meaning yellow, referring to the yellow colour of the rhizome, and Curcuma is the latinized version (Purseglove *et.al*., 1981, Sirirugsa, 1999).

Turmeric (*Curcuma longa* L.) is spice crop native to Asia and India. It is widely cultivated in India, Sri Lanka, Indonesia, China, Peru, Jamaica, and other tropical and subtropical countries. Asian countries contribute about eighty five per cent of total production whereas Europe and North America contributes only upto fifteen per cent of total production (Luthra *et al.*, 2001). India contributes about 78 per cent of the world production and 60 per cent to the total trade (Anon., 2008). According to data base of Horticulture statistic at a glance, 2017, the turmeric production of India was 1052 thousand MT from acreage of 193 thousand ha area. The main turmeric producing states in India are Andhra Pradesh, Tamilnadu, Orissa, West Bengal, Maharashtra, Karnataka, and Kerala. Maximum area under turmeric cultivation is in Andhra Pradesh (71.61 thousand ha), where production is very high *i.e*., 371.64 thousand tones. India, the "Home of spices" has been the largest turmeric producer (80%) and exporter (50%) in the world ranking third (Thiripurasundari and Salvarani, 2014). In Bihar, the turmeric production was 2.60 thousand MT from 2.40 thousand ha area (Horticulture statistic at a glance, 2017).

Turmeric plant is a perennial herb, 60-90 cm tall with a short stem and tufted leaves. The rhizome which are short and thick constitute the turmeric of commercial value. It is used as spice of flavoring agent for coloring, cosmetic purpose besides being extensively used in Indian system of medicine as an ingredient in the preparation of medicinal oils, pastes, ointments, etc.

Turmeric (*Curcuma longa* L.) is a rhizomatous herbaceous perennial monocot plant, belongs to the family-Zingiberaceae, order-Scitaminae having 60-90 cm high, with a short stem and tufted leaf. There are 7 to 12 leaves, the leaf sheaths forms the pseudo stem. The lamina is green above and pale green below and has a length of 30-40 cm and width 8-12 cm. Inflorescence is a central spike of 10-15 cm length. 1-4 flowers are born in axil of the bract opening one at a time. About 30 flowers are produced in a spike. Seeds are produced in capsules and there will be one to numerous sunken capsules in an inflorescence. The rhizome which are short and thick constitute the turmeric of commercial value. Growth and development of turmeric rhizome and leaves are dependent on several factors, such as nutrition, cultivation practices, genotype, and environmental factors. The crop endures an annual average rainfall of 640 to 4200 mm and optimum annual mean temperatures of 18.2 to 27.4°C.

Turmeric (*Curcuma longa* L.) is severely affected by several diseases, mostly fungal, have been recorded on turmeric. Bacterial and viral diseases are of minor importance. The crop is prone to many fungal diseases *viz.*, *Colletotrichum* leaf spot (*Colletotrichum capsici* [(Syd.), Butler and Bisby], leaf blotch (*Taphrina maculans* Butler) and Rhizome rot (*Pythium spp*.) are the most serious diseases resulting in yield losses in different parts of the country (Rathaiah, 1987; Annon, 1996). *Colletotrichum* leaf spot and leaf blotch is the most important among foliar diseases of turmeric which cause severe reduction in yield due to loss of photosynthetic area.

Leaf blotch disease caused by *Taphrina maculans* was reported for the first time reported from Rangapur, East Pakistan (Butler, 1911). Later, it was observed from all turmeric growing regions of the country (Upadhyay & Pavgi 1967). *Taphrina* belongs to the class Ascomycetes. The fungus produces brown leaf spots and the mycelium grows in the subcuticular interspaces of the epidermis (Upadhyay and Pavgi, 1979) and produces cuboid ascogenous cells. The fungus persists during the summer by means of ascogenous cells on leaf debris and as desiccated ascospores or blastospores in the soil and on fallen leaves (Upadhyay and Pavgi, 1967b). Secondary infection was demonstrated under controlled conditions. The ascospores discharged from the successively maturing asci grow into eight-spored microcolonies and infect fresh leaves. Secondary infection causes profuse spotting all over the leaves (Upadhyay and Pavgi, 1966). The disease is characterized by the appearance of several spots on both the surfaces of leaves and being generally more numerous on upper surface (Joshi &

Sharma 1982). The leaf spots first appear as pale yellowish discolouration which become dirty yellow and then deepen to the colour of old gold and sometimes to bay shade (Butler 1911). The individual spots are small, 1-2 mm in diameter and coalesce freely. In severe cases of attack, hundreds of spots appear on both the sides of leaves. The spots are discrete brownish black and mostly confined to lower leaves (Joshi & Sharma, 1982). The early appearance and severity of *T. maculans* depends on the concentration of inoculum in the soil (Upadhyay and Pavgi, 1967c). Primary infection occurs on the lower leaves in October to November at 80 per cent relative humidity and 21 to 23°C. Secondary infection is related to the availability of large inoculum potential periodically produced under cool and humid conditions. The plant debris, rhizomes, etc. of the previously infected crop or soil from infested fields did not serve as a primary source of infection (Ahmad and Kulkarni, 1968b).

The poor productivity of the crop in the state has been attributed to leaf blotch disease caused by *Taphrina maculans* among other factors that hinder its production. Turmeric yield losses due to disease have been recorded up to 37.6-52.9 per cent and becoming so colossal in some areas that turmeric cultivation has become uneconomical, especially where susceptible varieties were grown (Panja *et al.,* 2000; Annon., 2011).

The disease is such that, its rapid spread and spore production are favoured by warm, rainy and/ humid weather. Because of prevalence of such environmental factors almost throughout the year coupled with growing of susceptible commercial variety in Bihar, the disease causes a serious threat to turmeric cultivation.

Though some cultural practices have been advocated to manage the disease, the same has been practically possible by using chemicals. In this context little efforts have been made to screen effective fungicides to manage this disease. Both systemic and non-systemic fungicides have been found effective to the disease by workers in different parts of country and advocated for disease management. Very little information is available on fungicidal spray to control this disease. No effort has been made to manage this disease by using botanicals or by resistance including chemicals.

Keeping in view the above facts and losses caused by this disease, the present investigation has been undertaken with the following objectives.

1. To know the status of leaf blotch disease of turmeric in zone-I of Bihar.
2. To identify the resistance source of turmeric against leaf blotch disease.
3. Evaluation of antifungal efficacy of botanical extract against pathogen (*Taphrina maculans*) *in-vitro*.
4. To find out the efficacy of fungicides and resistance inducing chemical for the management of leaf blotch disease of turmeric.

CHAPTER - II

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Spices constitute an important group of agricultural commodities, which is considered as low volume and high value crops. Spices sector is one of the key areas in which India has an inherent strength to dominate the global markets and plays a significant role in our national economy (Rajesh, 2004). Turmeric (*Curcuma longa* L.) is severely affected by several diseases, mostly fungal, have been recorded on turmeric. Bacterial and viral diseases are of minor importance. The most important of them are rhizome rot, leaf spot and leaf blotch diseases (Rao *et al.*, 1993). Among these, leaf blotch disease caused by *Taphrina maculans* is one of the most serious and destructive disease of turmeric in the field conditions. The poor productivity of the crop in the state has been attributed to leaf blotch disease caused by *Taphrina maculans* among other factors that hinder its production. Turmeric yield losses due to disease have been recorded up to 37.6-52.9 per cent and becoming so colossal in some areas that turmeric cultivation has become uneconomical, especially where susceptible varieties were grown. (Panja *et al.*, 2000, Annon., 2011). The disease is such that, its rapid spread and spore production are favoured by warm, rainy and/ humid weather. Because of prevalence of such environmental factors almost throughout the year coupled with growing of susceptible commercial variety in Bihar, the disease causes a serious threat to turmeric cultivation.

2.1 History and Distribution:

Butler (1911 and 1918) was the first to give the account of leaf spot disease of turmeric (*Curcuma longa* L.) in India and identified its cause to be due to *Taphrina maculans*. Mundkur (1949) also described this disease and its cause on similar lines but slightly elaborated on some aspects not covered by Butler (1918). Besides these two reports, no account on *T. maculans* was forthcoming. In India, the disease has been reported from Madras, Tamil Nadu (Ramakrishnan, 1954), Uttar Pradesh (Pavgi and Upadhyay, 1967), Maharashtra (Patil and Moniz, 1973), West Bengal (Kar and Mahapatra, 1981), Assam (Rathaiah, 1987), Andhra Pradesh (Dakshinamurti *et al.*, 1996) and Bihar (Annon, 1996). Voluminous literature and classical work on *Taphrina*, particularly on *T. deformans* and other species have been published by Mix (1924, 1925, 1929, 1935, 1938, 1954), Ellamartin (1925, 1940), Jaczewski (1927),

Wieben (1927), Eftimiu (1927), Roberts (1946), Klebahn (1924), Goode *et al.* (1954), Yarwood (1940, 1941), Pierce (1900) etc. which deal with several aspects about the fungus including isolation.

Pierce (1900) observed that *T. deformans* can be isolated on extract of malt and beer solutions. Klebahn (1924) showed that culture of *T. tosquinetii* can be obtained on salepager.

Earlier attempts made by Brefeld and Sadebeck, as reported by Mix (1924) revealed that *T. johnsonii* and allied species of Taphrina could grow into conidial stage on nutrient solutions. Mix (1924 and1925) and Martin (1925) did extensive work and obtained pale, pink and yeast like colonies of *T. deformans* on a variety of media like potato sucrose, potato maltose, pea malt and peach leaf agars, stem corn meal, steamed rice, plugs of sweet potato, carrot and beet and on bean pods. They also observed that growth of fungus was restricted on leaf broth corn meal, starch oat and soil dextrose agars and also on beef broth and beef broth gelatin. Both these authors, however, reported that this fungus grew best on cleared potato dextrose agar having pH 4.5. Mix (1924, 1925) and Martin (1925) also reported several methods for getting successful isolations. In addition, Martin (1925) obtained successful isolations from mature asci, ascospores or conidia and also by fastening the small infected portion to the lid of the plate from inside thus allowing ejection of spores on the medium. The optimum temperature for the growth of *T. deformans* as reported by Mix (1924) was around 20 °C, the range being from below 10 °C to 26-30 °C.

Ramakrisnan (1954) observed that the disease become evident when the crop was about four month old *i.e.*, in the months of August- September. However, the infection was favourably influenced by high and continuous humidity of the atmosphere. Joshi and Sharma (1980) observed the disease incidence to be high not only during August and September when there was high and continuous humidity in the atmosphere, but also during October - November in certain areas.

2.2 Status of leaf blotch disease of turmeric in farmers' field:

Koche M. *et al.,* (2009) presented the results of a survey on intensity of foliar diseases of turmeric in 5 districts (Akola, Amravati, Washim, Wardha and Yeotmal) of Vidarbha region of Maharashtra (India) during kharif 2005-06. They also studied on pathogenic behaviour of *C. dematium* in vitro; and field screening of 16 turmeric

cultivars for resistance to leaf spot (caused by *C. dematium*) and leaf blotch (caused by *Taphrina maculans*) diseases in Akola during kharif 2005-06.

Survey conducted during 1994-98 in the district of Muzaffarpur, Vaishali, Siwan, Samastipur and Darbhanga in Bihar. It was observed that leaf blotch was more severe in comparison to leaf spot disease of turmeric (Annon., 1998). A survey conducted in Vaishali district of Bihar during 2009 to 2012 revealed in the range of 12 to 30 and 24 to 54 per cent respectively, (Annon., 2012 & 2013).

Singh *et al.,* (2002) conducted survey and reported that PTS-62 exhibit lowest disease index which was closely followed by ACC-360, JTS-1 and PTS-12. In this study none of the cultivar was found free from infection but PTS-62, ACC-360, JTS-1 were recorded as resistant. High degree of tolerance to leaf blotch were exhibited by Rajendra Sonia and RH-5 and both may be recommended for cultivation in endemic area to leaf blotch in Chhatisgarh. Resistance showing cultivars viz., PTS-62, ACC-360 and JTS-1 may also be recommended for the cultivation and can be used while breeding for higher yielding and resistance line to leaf blotch disease.

Survey conducted in turmeric cultivated area of Samastipur and Muzaffarpur district during 2008-09 and revealed turmeric affected with leaf blotch disease caused by *T. maculans* (Annon., 2009).

A survey conducted in the farmers' field of Vaishali distict of Bihar during 2013-14 revealed the prevalence of leaf blotch and leaf spot disease incidence with disease intensity in the range of 5-20 per cent (mean- 10%) and 10-50 per cent (mean-28.0%) respectively. (Annon., 2014).

A survey conducted in the farmers' field of Darbhanga distict of Bihar during 2015-16 revealed the prevalence of leaf blotch disease incidence ranged from 5 to 55% with mean disease incidence of 26 % and leaf spot disease incidence with disease intensity in the range of 0 to 15 % with mean disease incidence of 26 per cent (Annon., 2016).

A survey conducted at Pundibari centre during (2008-09) in two blocks of Coochbehar (Coochbehar I and II) and some places of Dinhata of Coochbehar district to identify the disease occurring in the area and to assess the severity of different disease on turmeric. The report revealed that leaf blotch disease severity was highest

in Coochbehar I block (average 34.47%) followed by Coochbehar II block (average 31.08%) and Dinhata (average 29.11%). (Annon., 2009).

A field survey on incidence of major disease of turmeric was conducted in turmeric growing areas of Coimbatore district during the year 2009-10 and it was observed that prevelance of maximum leaf spot noticed at Thondamathur and Sennanur (42.0 PDI) and the minimum intensity of the disease was noticed at Narasipuram (26.0 PDI). In the case of leaf blotch, the maximum disease intensity of 20 PDI was noticed at Mathampatty and the minimum intensity of 10 PDI was at Boluvampatty. (Annon., 2010) In Erode district, survey was conducted in 15 places during 2009-10 and it was observed that minimum leaf spot disease was noticed at Lakampatty (10.0 PDI) and the maximum at Kadathur (32.0 PDI). Leaf blotch disease incidence was noticed minimum at Kadathur (8.0 PDI) and maximum (12 PDI) at Kavundapadi (12.0 PDI). (Annon., 2010). In Tirupur district, survey was conducted at ten different places for disease occurance and it was observed that prevelance of minimum leaf spot disease was at Unity Nagar (8 PDI) and the maximum was at Kunnathur (28 PDI), leaf blotch was minimum disease intensity of 10 pdi was noticed at Unity Nagar and maximum was noticed at Thekkalur (20 PDI). In Salem district sixteen places were surveyed. The minimum leaf spot disease was noticed at Kothampadi (12 PDI) and the maximum of 32 PDI was noticed at Karipatty (Aathur). The leaf Blotch disease intensity ranged from 8-12 PDI. In Namakkal district, the minimum disease intensity of leaf spot (12 PDI) was noticed at Pallpalayam amd maximum was noticed at Attayampalayam (20 PDI). The leaf blotch disease intensity ranged from 8-12 PDI. In Karur district five places were surveyed. The minimum leaf spot disease intensity was noticed at Vengamedu (12 PDI) and the maximum was noticed at Velayuthampalayam (32 PDI). The leaf blotch disease intensity ranged from 10-14 PDI.

Highest incidence of leaf spot was observed in Coimbatore district (42 PDI) while the lowest incidence was noticed in Erode district (10 PDI). A similar trend of the highest leaf blotch disease incidence (20 PDI) was observed in Coimbatore district while the least incidence was noticed in Salem, Namakkal, Erode district (8 PDI).

A field survey on turmeric disease was conducted in different turmeric growing districts of Tamil Nadu viz., Coimbatore, Erode, Salem, Namakkal and

Tiruppur district during 2010-11. In Coimbatore district, totally eleven places were surveyed with different cropping pattern. In the case of leaf spot, the maximum intensity was noticed at Mettupalyam (54.20 PDI) and the minimum intensity was recorded at Thennamanallur (28.20 PDI). For the leaf blotch, the maximum intensity of 28.20 PDI was noticed at Narasipuram and minimum at Sundarapuri (12.03 PDI). In Erode district, tptally seventeen places were surveyed with different cropping pattern. Among the places surveyed, the maximum leaf spot intensity of 56.60 PDI was recorded at Palaiyur and minimum at Gana pathypalayam (14.0 PDI). In Salem district totally 5 places were surveyed. The maximum leaf spot intensity of 48.32 PDI was recorded at Manakadu and the minimum intensity was recorded at Vazhapadi (32.0 PDI). The leaf blotch intensity was maximum at Yethapur (26.67 PDI) and minimum at Selliyampalayam (20.21 PDI). In Namakkal district, the maximum leaf spot intensity was observed at Rasipuram (42.30 PDI) and minimum at Nasianur (28.32 PDI). The leaf blotch intensity was minimum at Nasianur (18.67 PDI) and maximum at Rasipuram (24.67 PDI). In Tiruppur district, maximum leaf spot intensity of 44.00 PDI was observed at Thekkalur and Minimum at Perumanallur (28.20 PDI). In the case of leaf blotch, the Maximum intensity of 63.13 PDI was observed at Kunnathur and minimum at Thekkalur (24.60 PDI) (Annon; 2011).

A survey was conducted in two blocks of Coochbehar (Coochbehar I and II) and some places of Dinhata of Coochbehar district to identify the disease occurring in the area and to asses the severity of different disease of turmeric in these area. Twelve well distributed location within those places were selected for the survey. In each location the survey was done in at least two different places. Three major disease of turmeric were found to be prevalent in this area, Namely, Leaf blotch (*T. maculans*), *Colletotrichum* leaf spot (*Colletotrichum* spp.) and *Helminthosporium* lea spot (*Helminthosporim sp*.). Most of the area is coverd with local varieties which are highly susceptible to leaf Blotch disease and some of the area is highly susceptible to leaf spot disease too. In survey it was found that leaf blotch disease severity was highest in Coochbehar II block (average 36.08%) followed by Coochbehar I block (average 35.13%) and Dinhata (32.11%) (Annon; 2011).

2.3 Resistance source of turmeric against leaf blotch disease:

Earlier evidences to control *Colletotrichum* leaf spot disease of turmeric mainly indicate the cultural measure. Disease could be checked by burning all diseased plant, rotation of crop, showing of healthy seed rhizome, good drainage, early sowing, clean cultivation and use of nitrogenous fertilizer (Mc Rae, 1908). Use of healthy seed for sowing was most important measure for getting a healthy crop (Park, 1941 and Bertus, 1942).

Nybe and Nair, (1979) reported that ginger cultivar Taffingiva was highly tolerant to the disease followed by Maran, Bajpai, and Nadia under Kerala conditions. Cultivars Maran and Karakkal were found to be comparatively resistant whereas, cultivars Wynad-Mananthody, Wynad-Kunnamangalam, Arippa, Narasapattom, Thingpuri, Burdwan, Vengara, Tura, Wynad-Local, Jugijan Emad-Chernad and Taiwan were highly susceptible under Kerala conditions (Premanathan *et al*, 1982).

Maurya (1990) selected RH10 from *Curcuma longa* germplasm collected in Balmiki Nagar, West Champaran and conducted an experimental trial with 10 lines and the control variety Dholi Local, and found that RH10 is resistant to leaf blotch but susceptible to leaf spot.

Panja *et al.,* (2000) conducted an experimental trail to assess the yield losses caused by leaf blotch disease (*Taphrina maculans*) in West Bengal, India during 1996-98. They selectd turmeric genotypes like RH-5, Rajendra Sonia, PCT-13, Nagaland Local, Tall Clone Assam, PCT-14, Sonajuli Local, Sugandham and Meghalaya Local. Percent disease index (PDI), dry biomass yield and fresh rhizome yield of nine turmeric genotypes differed significantly under both control and treated conditions. Moderately susceptible and highly susceptible genotypes against the disease exhibited PDI values of 55.3-66.2 and 84.1-88.8 under the control, and 8.7-11.8 and 37.6-52.9 under treated conditions, respectively.

Panja (2000) conducted a trial to assess the yield losses caused by leaf blotch disease (*Taphrina maculans*) in west Bengal, India during 1996-98. Mainplot treatments consisted of fungicides sprayed and unsprayed (control); and subplot treatments were turmeric genotypes RH-5, Rajendra Sonia, PCT-13, Nagaland Local, Tall clone Assam, PCT-14, Soajuli Local, Sugandham and Meghalya Local. Per cent disease index (PDI), dry biomass yield and fresh rhizome yield of nine turmeric

genotypes differed significantly under both control and treated conditions. Moderately susceptible and highly susceptible genotypes against the disease exhibited PDI values of 55.3-66.2 and 84.1-88.8 under the control, and 8.7-11.8 and 37.6-52.9 under treated conditions, respectively. Per cent loss in dry biomass yield (19.7-32.5), and fresh rhizome yield (11.6-32.0) of genotypes could not be related to PDI increament. However, the rate of loss (amount of loss per unit PDI increament) in dry biomass yield (0.37-0.72) remained parallel to the rate of loss of fresh rhizome yield (0.36-0.79) for all genotypes except RH-5. Highly susceptible genotypes exhibited not only as a loss of dry biomass (21.6-32.5%) and fresh rhizome yield (0.64-0.79%) than other genotype.

With respect to rhizome yield, it is apparent that fungicides like Blitox-50, Bavistin, indofil M-45, Captaf and Topsin-M were statistically *at par* and superior to Ziram, Difolatan and Kawach. The present finding are in consonance with the findings of Rao (1980) and Srivastav *et al.*, (1989).

Treatment with Metalaxyl (500ppm) resulted in the lowest PDI (31.50) and the highest fresh rhizome yield (27.67 t/ha). PDI and yield values for turmeric treated with Thiophanate-methyl were found at par with Metalaxyl (35.90 and 26.67 t/ha respectively). The cost of fungicidal treatment was found to be lowest with the Thiophanate- Methyl spray (Singh *et al.*, 2000).

Panja, and Majumdar (2001) evaluated fifteen turmeric (*C. longa*) cultivars during 1996-99 to identify the best-suited high yielding, leaf blotch disease (*T. maculans*)-resistant cultivars for the tarai region of West Bengal, India. They found that Nagaland Local, Tall Clone Assam, PCT-14, Sonajuli Local, Sugandham and Meghalaya Local were highly susceptible, RH-5, Rajendra Sonia and PCT-13 were moderately susceptible; and PTS-62, ACC-360, ACC-361, Roma, BSR-1 and Kasturi were highly resistant to leaf blotch disease.

Singh (2002) they evaluated the ten turmeric cultivars, namely Rajendra Sonia, RHS-5, PTS-43, PTS-12, PTS-62, ACC-360, ACC-361, JTS-1, JTS-2 and RTS-1 for their resistance to the leaf blotch disease caused by *Taphrina* sp. during kharif 1997 and 1998 in Chhattisgarh, Madhya Pradesh, India and found that none of the cultivars was free from infection, but PTS-62, ACC-360 and JTS-1 were considered as resistant, while Rajendra Sonia and RH-5 were highly susceptible.

Kumari *et al.,* (2002) planted turmeric rhizomes of different sizes, i.e. full mother (80-100 g), half-mother (50-80 g), small full mother (50-60 g), fingers (two, 10-30 g), finger (one, 10 g) and finger (half, 5-10 g), in May 1999 in Bihar to know, the effect on disese incidence. They observed that planting of full mother rhizome resulted in minimum leaf blotch (caused by *Taphrina maculans*) disease severity (3.2) and maximum rhizome yield (220 q/ha), which was closely followed by half mother rhizome. In the case of small full mother rhizome treatment, the disease severity was higher and the yield was lesser than in full mother rhizome and half mother rhizome treatments.

Prasadji *et al.,* (2004) found propiconazole (0.1%, Bitertenol (0.1%) and Chlorothalonil (0.2%) to be at par and significantly Superior to other fungicides treatments in reducing disease severity. The highest yield was obtained in Bitetenol (0.1%), and Chlorothalonil (0.2 %) treated plots in both the years. These earlier attempts on management of leaf blotch disease in turmeric were limited to use of protectant fungicides like Zineb, Copper oxychloride, Captan, Cumin (Nirwan *et al.,* 1974) and Dithane Z-78, Dithane M-45, Bavistin (Srivastav and Gupta, 1977).

Spraying the crop with Blitox-50 recorded least intensity of disease which was statistically at par with Bavistin, Topsin-M and Indofil M-45. Other fungicides like Captaf, Ziram, Difolatan and Kawach indicated the similar pattern of disease intensity and proved significantly superiority to control (Kumar *et al.,* (2005)

In a disease management study carried out by Devi (2008) Thiophanate methyl 0.1% (PDI 33.9) and Carbendazim 0.1% (PDI_33.7) were found to be the most effective fungicides in reducing the disease incidence followed by Mancozeb 0.3% (PDI 36.6). However, Carbendazim 0.1% ad Propiconazole 0.1% showed highest fresh rhizome yield (15.8 kg/ha and 15 kg/ha) followed by Thiophanate methyl @ 0.1% and Hexaconazole @ 0.1% (13.5 kg/ ha)

Yamgar *et al.,* (2006) screened the segregating population in Digraj, Maharashtra, for yield potential and curcumin content, and resistance to diseases and pests and found that the green and cured yields of DTS-222 were lower than those of Salem (by 3.6 and 3.7%, respectively), but higher than those of Rajapuri (by 31.4 and 31.3%). DTS-222 had a lower cured yield than Salem (by 5.4%) but had a higher cured yield than Rajapuri (by 12.0%). The curcumin contents of DTS-222 (5.19%)

were higher than those of Salem (4.14%) and Rajapuri (3.65%). DTS-222 was less susceptible to rhizome fly and leaf-eating larvae, rhizome rot, leaf spot and leaf blotch, and superior in quality parameters, such as core colour of the finger rhizome (deep yellow orange), curing recovery (22.0%), and average length (8.7 cm) and weight (35.13 g) of finger rhizomes. In terms of consumer preference, DTS-222 was more or less equal to Salem.

An experiment was conducted by Singh (2007) during *kharif* 2004-05 and 2005-06 at Raigarh, Madhya Pradesh, India to screen turmeric entries/cultivars against different disease under natural conditions. The entries were neither found highly resistant nor highly susceptible against *Taphrina* leaf spot and *Colletotrichum* leaf spot. Roma, Rashmi Sugana, Suroma, Sudarshana, TCP-56, Pratibha and TCP-11 were found moderately resistant to Taphrina leaf spot and average disease intensity from 12.7 to 16.0. ACC-573 was resistant to Taphrina leaf spot and Moderately resistant to *Colletotrichum* leaf spot. Local control IT-1 was found resistant to Taphrina leaf spot but it was moderately resistant to *Colletotrichum* leaf spot. Roma, Rashmi, Sugana, Suroma, Sudarshana, Tcp-56, Pratibha and TCP-11 were found resistant to both diseases.

Mina koche *et al.,* (2009b) found three varieties viz., Sudarshan, Alleppy and Salem showing moderately resistant reaction. The varieties like Rajapuri, Krishna, Brahmni, Suroma, Roma and Suguna were Suguna were susceptible to the leaf spot caused by Colletotrichum dematium. In case of leaf blotch caused by Taphrina maculans ten varieties viz., Allepy, Suguna, Sudarshana, Roma, Suroma, Bramhani, Rajpuri, Nandini, Clt-320 and Tekurpetha have showed moderately resistant action.

In a screening experiment was conducted by Khalko and Chowdhary (2011) and found that turmeric cultivars viz., PTS-12, PTS-63 and ACC-360 resistant to leaf blotch and leaf spot diseases.

Khalko (2011) evaluated five ginger cultivars viz., SG-536, V1S1-8, ACC-64, V3S1-8, Garubathan and nine turmeric cultivars viz., RH-5, Rajendra Sonia, PTS-12, PTS-43, PTS-62, ACC-360, ACC-361, JTS-1 and JTS-2 for their resistance to major fungal diseases and yield performance under West Bengal condition during 2001-02. Results observed that none of the cultivars of ginger and turmeric tested was free from infection though the degree of infection varied. Among five cultivars of ginger, V1S1-

8 and V3S1-8 showed lowest incidence of leaf spot and rhizome rot, respectively, while highest yield was obtained from Garubathan. Out of nine cultivars of turmeric, PTS-12, PTS-63 and ACC-360 were found to be resistant to leaf blotch and leaf spot diseases. However, RH-5 and Rajendra Sonia though susceptible to the leaf blotch and leaf spot appreciably recorded higher yield than other cultivars.

Singh (2011a) conducted field experiment in Chhattisgarh, India, in 2006-07 and 2007-08, for the screening of available entries against the leaf blotch diseases (caused by *Taphrina maculans*) of turmeric. Results showed that the disease severity was maximum in the first 5 lower leaves and minimum in upper/newer leaves. Maximum yield and disease resistance were recorded in the TCP-11 cultivar, followed by TCP-82, TCP-56 and TCP-2.

2.4 Efficacy of antifungal plant extracts for the management of leaf blotch disease of turmeric:

Mina *et al.,* (2009a) tested three botanicals viz., *Azadirachta indica* seed extract (5%), *Ageal marmalos* leaf extract (5%) and Eucalyptus leaf extract (5%) against foliar diseases of turmeric. *Azadirchta indica* seed extract was effective among botanicals to check the mycelial growth of *C. dematium* (74.69%) as compared to control. Amongst botanicals *Azadirchta indica* seed extract (41.58% and 32.08%) and Eucalyptus leaf extract (49.03% and 32.08%) were found effective to check these two leaf spot diseases of Turmeric.

Okigbo *et al.,* (2009) evaluated the extract of different plants against some pathogenic fungi and found that extracts of *Allium sativum* and *Ocimum gratissimum* were inhibitory to mycelia growth of all tested fungi including *B. theobromae* and *M. phaseolina.*

Kayalvizhi *et al.,* (2016) Methanol extract of ocimum sanctum demonstrated good antimicrobial activity and inhibitory effect on key virulence factors of *streptococcus mutans*.

Gwa and Nwankiti (2017) tested the botanicals to find out the efficacy of some plant extracts (*Piper nigrum, Zingiber officinale, Azadirachta indica, Carica papya and Nicotiana tabacum*) at 5 % concentration under *in-vitro* condition on inhibition of *Colletotrichum sp*. mycelia at Advanced Plant Pathology Laboratory, Federal University of Agriculture, Makurdi, Nigeria. The results obtained showed

that all the plant extracts at all concentrations significantly ($p<0.05$) inhibited the mycelia growth of *Colletotrichum sp*.

Sharma *et al.,* (2017) *Ocimum sanctum* leaves was evaluated in the form of crude (10%), powdered (10%), boiled (10%) and ethanol (1%) extracts against ten fungal pathogens viz., *Rhizoctonia solani, R. bataticola, Phoma sorghina, Colletotrichum gloeosporioides, Fusarium oxysporium* f.sp. *pallidoroseum, F. oxysporium* f.sp. *ciceri, Sclerotium rolfsii, Sclerotinia sclerotiorum, Alternaria solani* and *A. alternate.* The growth of the species of *Phoma sorghina, Colletotrichum gloeosporioides, Fusarium oxysporum f.sp. ciceri, and Fusarium oxysporium f.sp. pallidoroseum* was more effectively inhibited under its crude form than the other four forms, while the powdered form of *Ocimum sanctum* leaf extract was found more suitable for the control of *Sclerotinia sclerotiorum.*

Pawan *et al.,* (2018) found that Garlic+Neem+Datura and only garlic extract induced 100% growth reduction at 10% and 20% concentrations for both the pathogens followed by Neem oil up to 69% for *Fusarium oxysporum.*

2.5 Efficacy of fungicides and resistance inducing chemical for the management of leaf blotch disease of turmeric:

Singh *et al.,* (2000a) sprayed different fungicide with different concentration like 0.3% Blitox-50 (copper oxychloride), 500 ppm Ridomil (metalaxyl), 0.1% thiophanate-methyl, 0.1% carbendazim, 0.3% mancozeb or 0.3% Antracol (propineb) followed by 2 more sprays at monthly intervals on a local variety of turmeric showing the first symptoms of *Taphrina maculans* leaf blotch, during the kharif seasons of 1997-98. They observed that all fungicides reduces the disease severity compared with the control but treatment with Ridomil resulted in the lowest percent PDI (31.50%) and the highest fresh rhizome yield (27.67 t/ha). PDI and yield values for turmeric treated with thiophanate-methyl were on a par with Ridomil (35.90% and 26.67 t/ha, respectively.

Panja *et al.,* (2001) conducted an experiment during 1998/99-1999/2000 in west bengal, to determine a suitable, cheap and effective control measure for leaf blotch disease of turmeric The treatments were 0.15% captan, 0.15% ziram, 1% copper sulfate, 0.15% dodine, 0.1% edifenphos, 0.1% carbendazim, and 75 ppm griseofulvin. They reported that none of the fungicides except copper sulfate was

effective against leaf blotch disease with respect to reduction of percent disease index and increment of aerial dry biomass and fresh rhizome yield per plot. The highest net return and benefit cost ratio were obtained with copper sulfate treatment.

Singh *et al.,* (2003) conducted an experiment in Himachal Pradesh, during 1995-96 and 1998-99 to investigate the effects of eight fungicides on leaf blotch (*Taphrina maculans*) infecting a local clone of turmeric. The fungicides used were dithane M-45 (mancozeb) at 0.25%, Score (difenoconazole) at 0.10%, Biltox-50 (copper oxychloride 50 WP), Antracol (propineb) at 0.25%, SAN 619 (cyproconazole) at 0.10, Baycor (bitertanol) at 0.10%, Contaf (hexoconazole) at 0.10% and Tilt (propiconazole) at 0.10%. They applied the fungicides between the end of August and the first week of September, when the symptoms were evident. The most effective fungicides, Score, Tilt and Dithane M-45 were applied at 1, 2 and 3 sprays at 20-day intervals starting from the first appearance of symptoms. They found that all fungicides significantly reduces the disease severity. The lowest disease severity (24.4%) and the highest yield (208.1 q/ha) were obtained in the 3-spray treatment of Score. Score and Tilt had disease reduction of 67.6 and 55.2%, respectively. Tilt produced a yield of 182.5 q/ha. A single spray of Score was considered economical according to cost benefit ratio analysis.

Mowlick *et al.,* (2007) conducted an experiment in the hilly region of Khagrachari, Bangladesh, to control the leaf blotch disease (*Taphrina maculans*) of turmeric with the fungicide bavistin (carbendazim) during 2005-06. 1, 2, 3, 4, 5 and 6 sprays of Bavistin (0.15%) were given. The five sprays of bavistin were found to be the most effective and economic treatment for controlling the disease. The highest yield (20.37 t/ha) and minimum percentage disease index (5.73) were recorded from this treatment.

Singh (2009) conducted field experiments in Himachal Pradesh, during 2004-05 and 2005-06 to select the effective fungicides for controlling *Taphrina maculans* infecting turmeric. The fungicides comprised Indofil M-45 (mancozeb + thiophanate-methyl), Tilt (propiconazole), Stuff (mancozeb + carbendazim), Folicur (tebuconazole), Bavistin (carbendazim), Kavach (chlorothalonil), Melody Duo (iprovalicarb + propineb) and Contaf (hexaconazole). Tilt gave the lowest mean disease severity and the highest mean crop yield.

Mina *et al.*, (2009a) studied effect of various diseases management tools as a part of suitable option for management of foliar diseases of Turmeric. Five fungicides viz., Propiconazole (0.1%), mancozeb (0.25%), carbendazim (0.1%), hexaconazole (0.1%), penconazole (0.1%) tested against foliar diseases of turmeric. Amongst fungicides propiconazole (91.98%), hexaconazole (87.55%), penconazole (85.62%) and carbendazim (84.02%) were effective to inhibit the growth of *Colletotrichum dematium* (91.98-84.02%). Significant inhibition was recorded in propiconazole (91.98%) as against control (80.33 mm). Propiconazole was effective against *C. dematium* (11.68%) and *Taphrina maculans* (12.34%) with added benefit of rhizome yield. This fungicide was found best against foliar diseases under field conditions.

Singh (2011b) conducted field studies in Chhattisgarh, in 2006-07 and 2007-08, for the assessment of fungicides against the leaf blotch of turmeric (*Taphrina maculans*). The fungicides, mancozeb (0.25%), carbendazim (0.1%), SAFF a mixture of 63% mancozeb + 12% carbendazim (0.25%), copper oxychloride (0.25%), hexaconazole (0.1%), propiconazole (0.1%) and tricyclazole (0.1%) were tested. Results showed that the best fungicide treatment was recorded in SAFF, followed by carbendazim.

CHAPTER - III

MATERIALS AND METHODS

3. MATERIALS AND METHODS

Leaf blotch disease caused by *Taphrina maculans* is the most important among foliar diseases of turmeric which cause severe reduction in yield due to loss of photosynthetic area.The poor productivity of the crop in the state has been attributed toleaf blotch disease caused by *Taphrina maculans* among other factors that hinder its production. Turmeric yield losses due to disease have been recorded up to 37.6-52.9 per centand becoming so colossal in some areas that turmeric cultivation has become uneconomical, especially where susceptible varieties were grown. Because of prevalence of favorable environmental factors almost throughout the year coupled with growing of susceptible commercial variety in Bihar, the disease causes a serious threat to turmeric cultivation. The present investigation on leaf blotch of turmeric (*Taphrina maculans*) has been carried out on survey of disease incidence in the field, symptomatology, pathogenicity, germplasm screening and its management through the use of chemicals and resistance inducing chemical. The material used and the methods followed during entire research programme have been described below.

3.1 Survey of leaf blotch in turmeric growing areas of north Bihar:

An extensive survey was conducted for recording the occurrence of leaf blotch disease starting after first appearance of symptoms till harvest. Survey was conducted two times during the crop period *i.e.*, first at the time of disease initiation (in September) and second during the month of November when the disease attains its maximum intensity. A number of diseased leaf sample of turmeric showing characteristic symptoms of leaf blotch were collected from the turmeric fields of two districts i.e., Samastipur and Muzaffarpur of north Bihar during *Kharif*, 2018.

Survey was conducted at farmers' field in five locations in each five villages of each Muzaffarpur and Samastipur district of Bihar during 2018 for assessing the occurrence of disease table.

3.2 Materials:

The material used were ingredient of media, fungicides, turmeric rhizomes, diseased turmeric leaf/rhizome samples, polythene bags, sulphuric acid, $K_2Cr_2O_7$,

antibiotic, non-absorbent cotton, $HgCl_2$, alcohol etc., glass wares like Petri dishes, beakers, funnels, pipettes, Erlenmayer flasks, culture tubes, measuring cylinder and different equipments *viz*., Hot air oven, LPG gas burner, autoclave, B.O.D. incubator, laminar air flow, instruments like cork borer, inoculation needle, scalpel, forceps, spirit lamp, Bunsen burner, enamel tray etc.

3.3 Cleaning and sterilization:

3.3.1 Cleaning of glasswares:

Borosil/Corning made glasswares were used during the present investigation. Different glasswares, *viz*., Petri-dishes, flasks, culture tubes, pipettes, slides and funnels, etc. required for various experiments were cleaned with freshly prepared cleaning solution containing mixture of 80 g potassium dichromate ($K_2Cr_2O_7$), 400 ml concentrated sulphuric acid (H_2SO_4) and 300 ml water. Concentrated H_2SO_4 was poured gently with inner wall of the beaker containing 300 ml water and 80 g potassium dichromate and final volume was made 3.5 litres by adding water and mixing thoroughly. All the glasswares were dipped overnight in the cleaning solution and then rinsed with several changes of water and air dried.

3.3.2 Sterilization of glasswares:

The air dried glasswares were sterilized in hot air oven at 170°C for 90 minutes.

3.3.3 Sterilization of inoculating needles, forceps, cork-borers and working table:

Clean inoculating needle, forceps and cork-borer were sterilized by dipping the loop of needle in methylated spirit and heating over the flame until red hot. The process was repeated 2-3 times. The working table of laminar flow was disinfected by sweeping with cotton soaked in absolute alcohol and exposing it to UV light for 30 minutes.

3.3.4 Sterilization of culture media:

All the solid media were sterilized in an autoclave at a pressure of 15 lbs psi for 15 minutes. Liquid media were sterilized at 10 lbs psi for 10 minutes and process was repeated after 24 hours.

3.4 Pathological investigation:

3.4.1 Collection of samples:

The infected leaves of turmeric plants showing the typical leaf blotch symptoms in turmeric variety were collected from farm of Tirhut College of Agriculture, Dholi. Infected leaves were brought into the laboratory, first observed under microscope and then placed in blotting papers under pressure with herbarium press and preserved for further inspection. The symptoms and signs both were critically observed from the naturally infected fields and recorded.

3.4.2 Preparation of culture media:

For isolation of the fungus *Taphrina maculans* B., the following culture media was used.

(A) Potato dextrose agar (PDA) media amended with yeast extract

Constituents		Quantity
Peeled potato	:	250 g
Dextrose	:	20 g
Agar-agar	:	20 g
Yeast extract	:	20 g
Distilled water to make final volume	:	1000 ml

3.4.2.1 Method of preparation:

For preparation of PDA, 250 g peeled potatoes were cut into slices and boiled in 500 ml of distilled water in a beaker of one litre for 15 minutes. The extract was strained through two folds of muslin cloth and 20 g dextrose was added in it. The 18g agar-agar melted separately in 500 ml of distilled water in another beaker separately, was mixed in potato dextrose solution and final volume was made up to one litre by adding distilled water. Two per cent yeast extract was amended and then medium was poured in 1000 ml conical flask. Desired pH (4.6) of the culture media was adjusted by adding a few drops of N/10 HCl or N/10 NaOH. PDA was poured in flasks plugged with non-absorbent cotton plugs and finally covered with butter paper and tied with thread. The flasks containing medium were sterilized in an autoclave. To avoid bacterial contamination streptomycin sulphate @1 g/l to the sterilized medium was added just prior to pour the medium into petri dishes.

3.4.3.1 Preparation of slants:

For preparation of slants, 2 to 3 ml of medium was poured in each culture tube, plugged with non-absorbent cotton plugs and sterilized in an autoclave at 15 lbs psi for 15 minutes. Later on tubes were kept in slanting position on wooden support and allowed to solidify. These slants were stored in refrigerator for further use.

3.4.3.1 Sterilization of media:

All the media were sterilized in an autoclave at 15 lbs psi for 15 minutes and process was repeated after 24 hours.

3.4.4 Isolation and purification of pathogen:

Freshly infected leaves of turmeric showing typical symptoms were used to isolate the pathogen from the infected area.Leaf samples of turmeric showing characteristic symptoms of leaf blotch were washed thoroughly in tap water for five minutes to remove dust and dirt. Small pieces of infected tissues of 1-2 mm dimension from advancing margin of the spot, adjacent to healthy portion were cut with blade. These bits were surface sterilized by dipping in 0.1 per cent mercuric chloride solution for 30 seconds followed by washing in three changes of sterilized distilled water. The bits were then transferred aseptically on PDA slants with the help of inoculating needle and incubated at 20 ± 2°C. After seven days of incubation, the fungus was transferred to sterilize petri-plates containing PDA medium and incubated in the same manner. After ten days of incubation, a bit of hyphal growth from growing tips was transferred aseptically to fresh PDA slants. Pure culture was made following single spore isolation method.

3.4.5 Identification of the pathogen:

The pathogen isolated on the culture media was identified with the help of description made by Pagvi and Upadhyay (1964) and Kulkarni and Ahmed (1968). The pathogen *Taphrina maculans* produced colonies that were easily mistaken for yeast or bacterial colonies. As in all other species studied so far, *T. maculans* produced only conidia in cultures without any mycelial growth and represented the asexual stage.

On sub-culturing the growth was restricted to the line of inoculation but soon very small pinkish colonies appeared on the part of the medium that was not touched

by the inoculating needle. These colonies remained isolated for a long time. This peculiarity was observed while sub-culturing, the fungus produced bud conidia or blastospores which might have been sometime forcefully thrown out of the colony developing into individual colonies.

3.4.6 Maintenance and preservation of culture:

Pure culture was maintained on PDA slants by sub-culturing it at 30 days interval. For preservation of cultures the plugged end of the culture tubes were dipped in melted wax and stored at 7.5 ± 1°C in a refrigerator.

3.4.7 *In vitro* evaluation for the efficacy of plant products against leaf botch of turmeric caused by *Taphrina maculans*:

Five plants extracts namely, Tulsi, Brahmi, Neem, Pipli and Surpgandha were evaluated against *T. maculans in vitro* by following the poison food technique. List of plant extracts used in the present study with their common name, scientific name, plant part used and concentrations used are given in Table.

Table 1: Common name, scientific name, plant part used and concentrations used of the plant extracts

Common name	Scientific name	Plant part used	Concentration used
Tulsi	*Ocimum sanctum*	Leaf	6%, 8%, 10%
Brahmi	*Bacopa monieri*	Leaf	6%, 8%, 10%
Neem	*Azadirachta indica*	Leaf	6%, 8%, 10%
Pipli	*Piper longum*	Leaf	6%, 8%, 10%
Surpgandha	*Rauwolfia serpentina*	Leaf	6%, 8%, 10%

Cold water extract of the leaves of Tulsi, Brahmi, Neem, Pipli and Sarpgandha were evaluated against *T. maculans in vitro* to evaluate their inhibitory effect on the growth of the fungus. For preparation of cold water extracts, fresh leaves were washed with tap water followed by distilled water. It was then processed with distilled water in 1:1 ratio, *i.e.* 100 gram leaf tissue in 100 ml distilled water. The plant parts were crushed in mortar and pestle and strained through double layer muslin cloth. This formed the standard extract solution (100%).

The plant extracts were incorporated into PDA medium at three different concentrations, i.e. 6, 8and 10 per cent. For obtaining 6, 8 and 10 per cent concentrations of plant extracts in the medium 6, 8 and 10 ml of plant extracts, respectively, were added in PDA to make volume 100 ml. Streptomycin @ 30 ppm and penicillium @ 125 ppm were also added to the medium before pouring in the Petri-plates to prevent bacterial contamination. PDA not amended with extract served as check. The amended PDA @ 20 ml/plate was poured into 90 mm sterilized Petri-plates, aseptically. Three plates were poured for each treatment. All the Petri-plates were inoculated with 5 mm mycelia disc of ten days old culture of *Taphrina maculans* and incubated at 20 ± 2°C for ten days and observations were recorded on radial growth. The data were converted in per cent inhibition of growth over check by using the formula:

$$\text{Per cent growth inhibition } I = \left(\frac{C - T}{C} \right) \times 100$$

Where,

I = Per cent growth inhibition

C = Colony diameter (mm) in check plate

T = Colony diameter (mm) in the treated plate

3.5 Field experiments:

Field experiments were conducted to identify the resistance source of turmeric against leaf blotch disease and to find out the efficacy of fungicides and resistance inducing chemical for the management of leaf blotch disease of turmeric. The details of the field trials conducted are as given in the following text.

3.5.1 Location:

A Field experiment was conducted during *Kharif* 2018-19 at the Experimental Farm of Tirhut College of Agriculture, Dholi (Muzaffarpur), Bihar. The Experimental farm is situated at an altitude of 52.0 meter above mean sea level, at a latitude of 25.98^0 N and longitude of 85.67^0 E. The climate is sub-humid type and monsoon receiving an average annual rainfall of 1234.7 mm mainly during the months June to

October. The experimental plot had a uniform topography. Soil is sandy loam well drained and with medium fertility

3.5.2 Rhizome material:

Rhizome material of different turmeric cultivars were available under AICRP on spices Department of Horticulture Tirhut College of Agriculture, Dholi, Muzaffarpur, used in all the field experiments. Healthy rhizomes with prominent buds were selected for experimental purpose.

3.5.3 Irrigation:

A total of 2 irrigations were given as and when required by flooding method at different interval.

3.5.4 Cultural operations:

A post-emergence weedicide, Butachlore were applied at 4.0 l/ha by mixing in sand along with the first irrigation after planting of rhizomes. Additionally two hand weedings at 40 and 80 days after planting were done to check the weeds.

3.5.5 Screening of turmeric germplasm against leaf blotch disease of turmeric:

A field experiment was conducted to screen various germplasm against leaf blotch of turmeric. Sixteen number of germplasm *viz*., RH-3, RH-6, RH-14, RH-80, RH-81, RH-414, RH-418, RH-421, RH-426, RH-429, RH-430, RH-434, RH-436, RH-439. RH-441 and RH-2/80 along with a susceptible check variety Morangia were screened for their reaction against leaf blotch disease under artificial inoculated condition. Each test germplasm under the investigation was grown in plot size of 3m x 1m with a spacing of 30 x 20 cm. After planting of every two test germplasm plot, Morangia (susceptible check) was planted in same plot size and spacing as that of test germplasm to exert maximum possible inoculum pressure to the test germplasm. Observation on disease severity was taken on the basis of per cent leaf area infected at 180-190 days after planting (DAP). Fifteen plants leaving the plants in the borders from each plot were considered for data recording.

Finally, the turmeric germplasm under test were categorizal into different group of resistance or susceptibility by following 0-4 disease scoring scale suggested by Annon, 2004 as described below:

Disease score	% leaf area infected	Disease Reaction
0	0-1	HR
1	1.1-10	R
2	10.1-20	MR
3	20.1-50	S
4	>50	HS

3.5.6 Field Efficacy of fungicides and resistance inducing chemical for the management of leaf blotch disease of turmeric:

Quality and quantity of turmeric is adversely affected by leaf blotch disease caused by *T. maculans* results in considerable losses of market value of the produce. Therefore, to minimise the economic loss of turmeric it is required to find out the most suitable chemicals for the management of the disease.

The experiment was laid out during *kharif*, 2018 in randomized block design with ten treatments and three replication. The susceptible cultivar Morangia was grown in plot size of 3 x 1 m with a row to row spacing of 30 cm and plant to plant spacing of 20 cm under each treatment.

To find out the most suitable chemicals two fungicides *i.e.*, zineb (0.25%) and tricyclazole (0.1%) and a resistance inducing chemical, salicylic acid (400 µM) were tested under field condition. The details of treatments are as follows:

T_1 = Rhizome treatment and one foliar spray with zineb 75WP @ 0.25%

T_2 = Rhizome treatment and two foliar spray with zineb 75WP @ 0.25%

T_3 = Rhizome treatment and three foliar spray with zineb 75WP @0.25%

T_4 = Rhizome treatment and one foliar spray with tricyclazole 75WP @ 0.1%

T_5 = Rhizome treatment and two foliar spray with tricyclazole 75WP @ 0.1%

T_6 = Rhizome treatment and three foliar spray with tricyclazole 75WP @ 0.1%

T_7 = Rhizome treatment and one foliar spray with salicylic acid @ 400µM.

T_8 = Rhizome treatment and two foliar spray with salicylic acid @ 400µM.

T_9 = Rhizome treatment and three foliar spray with salicylic acid @ 400µM.

T_{10} = Control

One to three sprays of the fungicides and resistance inducing chemical were done, based on treatment specification first foliar spray was done after 7 days of appearance of the disease incidence and subsequently at fortnightly interval.

The efficacy of these fungicides and resistance inducing chemical were compared with the control plot, which was sprayed with water only.

For recording the observation, fifteen plants from each of the plot were selected. Observation pertaining to disease intensity was recorded under each treatment, after fifteen days of last (IIIrd) foliar spray. Based on per cent leaf area infected, PDI was calculated as follows:

$$\textbf{PDI} = \frac{\text{sum of all numerical rating}}{\text{Total number of plants graded x Maximum grade}} X100$$

Finally, the yield / plot was recorded at the time of harvest of turmeric rhizome and subsequently it was converted in tone / hectare. The incremental cost benefit ratio (ICBR) was also calculated as below:

$$\textbf{ICBR} = \frac{\text{Income from the yield over control per hectare}}{\text{Expenditure incurred for spraying per hectare}}$$
$$(\text{cost of fungicide/ chemicals} + \text{cost of labour charge})$$

3.6 Statistical analysis:

The statistical analysis was done with the aid of off-campus OP-STAT analyses package and the method as suggested by Gomez and Gomez (1984). Critical difference (CD) was calculated at 5 and 1 per cent level of significance for comparison of treatment

3.7 Collaboration with other department:

The study was carried out with collaboration with Department of Horticulture, Entomology and Agricultural Statistics, Tirhut college of Agriculture, Dholi, Muzaffarpur, Bihar.

CHAPTER - IV

RESULTS AND DISCUSSION

4. EXPERIMENTAL FINDINGS

4.1 The Disease

4.1.1 Collection of disease specimen

The infected leaves of turmeric plants showing the prominent leaf blotch symptoms in turmeric variety, Morangia were collected from research plot situated besides Agriculutural meteorology field, Tirhut College of Agriculture, Dholi, Muzaffarpur campus of Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur Bihar and brought to the laboratory. Presence of naked asci of *T. maculans* was observed when examined under microscope. Fresh diseased sample was brought every time as and when required.

4.1.2 Symptomatology

The disease was observed in all the turmeric cultivar that was used in the studied. Symptoms of the disease were first observed on the lower most leaves of the plants during the last week of August, 2018 and subsequently the disease symptom spread to upper leaves also. *Taphrina maculans* is an fungal species producing spots on leaves of turmeric (*Curcuma longa*) wherever it is cultivated. *T. maculans* was established by Butler (1911) as a parasite of *Curcuma longa* L. (turmeric leaves) in India.

The pathogen produces yellowish to orange coloured minute spots often in large number on both the surfaces of leaves, generally more numerous on the upper surface and hampers the normal process of assimilation resulting in smaller rhizomes fetching low price and causing loss in yield. Initially symptom appeared as minute dot sized (1 to 2 mm diameter) pale yellow specks on the portion of leaf lamina neared to petiole region. In later stage of disease development, the colour of these pale yellow specks turned to dirty yellow and deepen to colour of old gold, finally to dark orange brown colour surrounded by the dried region. The specks or spots coalesced freely in advance stage as they increases in size, giving the leaf blotched appearance (Plate –I & Plate-II). The development of the leaf blotches is a continuous and progressive process, can be arbitrarily divided into three stages; (i) initial stages of development prominently marked out by yellowing and discolouration of leaves; (ii) middle stages

A. Leaf blotch symptom on leaf and Petiole

Progressive stage of Development of leaf blotch on turmeric leaves

Plate I- Typical symptom of leaf blotch caused by *Taphrina maculans* Butler.

Plate II: Turmeric field infected with leaf blotch caused by *T. maculans*

of development where the spots start to become dull yellow in colour and (iii) final stages of development when the spots turn light brown to chocolaty brown which is accompanied by necrosis and drying of the infected regions. Blotch appears as irregular discoloration due to pustules produced by the pathogen following infection. The infection progresses from the bottom leaves to the top leaves in a successive fashion. Late in the season a month before harvest infection was found to extend in the petiolar canal also (Plate-I). The appearance of symptoms, progression and symptomatology matched with the description provided by earlier researchers (Butler, 1918; Mundkar, 1949; Kulkarni and Ahmed, 1968).

The nomenclature pertaining to the investigated disease is not uniform as in some earlier study it referred as a "leaf spot" (Butler, 1911; McRae, 1917; Upadhyay and Pagvi, 1966 and Ahmed and Kulkarni, 1968a), while in the recent studies reports it referred as "leaf blotch" (Nambir, 1979; Rao and Rao, 1987; Rao, 1995, Prasadji, 2001; Singh *et al.,* 2003 and Singh, 2011). Hence, there is need to fix the nomenclature of the disease and also make a distinction from the other foliar disease of turmeric like, "leaf spot" caused by *Colletotrichum capsici* (Sydow) (Butler and Bisby). Since the observations recorded on the symptoms in this study as well as the literature of earlier workers satisfy the definition of blotch *i.e.*, irregular discolouration due to pustules produced by the pathogen following an infection, it is more appropriate to call the infection as a leaf blotch caused by *T. maculans* on the turmeric leaves.

4.2 The Pathogen

4.2.1 Isolation and identification of the Pathogen

The pathogen was isolated from infected leaves inoculated on PDA media amended with 2 per cent yeast. The isolated culture was brought in pure culture by employing the technique described in previous chapter "materials and methods. Morphology of the pathogen was ascertained on the basis of observation made on cultural characteristics of blastospore White and pink colonies of the fungus were obtained. Potato dextrose agar (pH 4.5) was found to be the suitable growth medium to the pathogen than nutrient agar (NA) and turmeric leaf decoction agar.

The fungus on the modified culture media *i.e*., PDA supplemented with 2 per cent yeast extract was identified based on the morphological descriptions made by earlier researchers. The fungus in culture was yeast like. No filamentous growth of the fungus was observed in the culture. The individual cell *i.e*., the shape of blastospore of the culture was found ellipsoidal to ovoid. The length and breadth of blastospore from culture were measured by using a microscope with stage and occular micrometers. Similar observations were also made on conidia obtained from naturally blotch infected leaves. The average length of blastospore obtained from culture media were ranged from 5.89 µm to 6.38 µm and average breadth ranged from 2.87 µm to 3.24 µm. The average length of the blastospore obtained from naturally blotch infected leaves was ranged from 4.26 µm to 5.35 µm and average breadth was ranged from 2.10 µm to 2.53 µm.

4.2.2 Pathogenicity test:

For pathogenicity test, fresh leaves of tutmeric cv. Morangia and blastospore suspension (7×10^4 blastospore/ml) obtained from the pure culture of *T. maculans* was taken. Inoculation was done by sprinkling the spore suspension on the lower leaves of four months old turmeric plants with the help of atomizer. Small pin head size spots appeared on the inoculated leaves after 3-4 days. Mostly the spot were found on the upper surface with very few on the under surface of the leaves. After 10- 15 days of inoculation the spot varied in size and coalesced to form big patches.

4.3 Survey

4.3.1 Survey of leaf blotch disease in turmeric growing area of North Bihar (Zone-I)

To find out the status of leaf blotch disease of turmeric caused by *Taphrina maculans* in North Bihar (Zone-I), two prime turmeric growing districts *viz*., Samastipur and Muzaffarpur were considered. Disease severity (%) were recorded during survey done twice in crop season *i.e*., first during initiation of disease in the first week of September and secondly during second fortnight of November when disease severity attains its maximum dimensions (plate III).

Plate III: Survey of leaf blotch disease of turmeric in farmers field

The results of survey carried out in five different locations or farmers' field in each five villages under Samastipur and Muzaffsrpur district during September presented in Table 2. No disease incidence was recorded in some location of four village *viz*., Somnaha, Tehra, Hashanpur and Wajidpur under Samastipur district. Minimum range of disease severity (0.00-10.00 %) and maximum range of disease severity (5.00-25.00 %) with mean disease severity of 7.50 and 12.50 per cent was recorded in village Tehra and Warisnagar respectively. Similarly, in muzaffarpur district also it was observed that some farmers' field was free from the disease incidence *viz*., Rajkhand, Gopalpur, khotahi and Bakhari but per cent Disease severity was more in Muzaffarpur District than Samastipur. Range of minimum disease severity (0.00-15.00 %) and maximum disease severity (5.00- 20.00%) was observed during the first week of November. Mean disease severity ranged from 0.00- 25.00 and 0.00- 20.00 per cent with disease severity mean of 9.00 and 9.50 per cent across twenty five location of five villages considered in Samastipur and Muzaffarpur district respectively under Study.

The results of survey carried out in five different locations or farmers' field in each five villages under Samastipur and Muzaffsrpur district during November is presented in Table 3. During the survey in month of November minimum disease severity range from (15.00-55.00 %), and maximum diease severity ranges from (40.00-65.00%) with mean disease severity of 35.00 recorded in Somnaha village and 52.50 was recorded in Wajidpur village of Samastipur District. Similarly in Muzaffarpur District, minimum disease severity ranged from 25.00 to 60.00 per cent and maximum disease severity ranged from 30.00-65.00 per cent in Rajkhand village with minimum mean disease severity of 42.50 per cent in Khotahi and Bakhari and maximum 47.50 per cent in Rajkhand.

4.4 Screening of turmeric germplasm

4.4.1 Reaction of various turmeric germplasm to leaf blotch disease.

As many as sixteen turmeric genotype (germplasm) alongwith a susceptible check (Morangia) grown under open field conditions were evaluated for their relative susceptibility to leaf blotch disease caused by *Taphrina maculans* in *kharif*, 2018. A general view of experimental plot under germplasm screening against leaf blotch

Table 2: Status of leaf blotch disease of turmeric (*Taphrina maculans* L.) in zone-I of Bihar during September, 2018

District :- Samastipur					District :- Muzaffarpur				
Village	**Variety/ cultivar**	**Disease Severity (%)**	**Disease Severity Range (%)**	**Disease Severity Mean (%)**	**Village**	**Variety/ cultivar**	**Disease Severity (%)**	**Disease Severity Range (%)**	**Disease Severity Mean (%)**
Somnaha	Hirotia	0.00	0.00-15.00	7.50	Rajkhand	Hirotia	15.00	0.00-15.00	7.50
	Deshla	5.00				Deshla	5.00		
	Deshla	10.00				Deshla	10.00		
	Unkown	10.00				R. Sonia	0.00		
	Deshla	15.00				Deshla	10.00		
Tehra	Deshla	10.00	5.00-10.00	7.50	Gopalpur	Deshla	20.00	0.00-20.00	10.00
	Deshla	5.00				Deshla	5.00		
	Deshla	0.00				Morangia	10.00		
	Unkown	5.00				Hirotia	0.00		
	Deshla	10.00				Deshla	5.00		
Hashanpur	Hirotia	5.00	5.00-15.00	10.00	Khotahi	R. Sonali	0.00	0.00-15.00	7.50
	Deshla	0.00				unknown	10.00		
	Deshla	5.00				Deshla	10.00		
	Unkown	5.00				Hirotia	5.00		
	Hirotia	5.00				Hirotia	15.00		
Wajidpur	Deshla	5.00	0.00-15.00	7.50	Bakhari	Unkown	20.00	0.00-20.00	10.00
	R. sonali	0.00				R. sonali	0.00		
	Morangia	10.00				Deshla	15.00		
	Hirotia	15.00				Unkown	10.00		
	R. Sonia	10.00				Deshla	25.00		
Warisnagar	Deshla	20.00	5.00-25.00	12.50	Narauli	Hirotia	5.00	5.00-20.00	12.50
	R. sonali	10.00				R. Sonia	5.00		
	Morangia	15.00				Deshla	20.00		
	Hirotia	15.00				Unkown	15.00		
	R. Sonia	5.00				Hirotia	10.00		
Mean			0.00-25.00	9.00				0.00-20.00	9.50

Table 3: Status of leaf blotch disease of turmeric (*Taphrina maculans* L.) in zone-I of Bihar during November, 2018

District :- Samastipur					District :- Muzaffarpur				
Village	**Variety/ cultivar**	**Disease Severity (%)**	**Disease Severity Range (%)**	**Disease Severity Mean (%)**	**Village**	**Variety/ cultivar**	**Disease Severity (%)**	**Disease Severity Range (%)**	**Disease Severity Mean (%)**
Somnaha	Hirotia	35.00	15.00-55.00	35.00	Rajkhand	Hirotia	40.00	30.00-65.00	47.50
	Deshla	15.00				Deshla	35.00		
	Deshla	45.00				Deshla	55.00		
	Unkown	55.00				R. Sonia	30.00		
	Deshla	50.00				Deshla	60.00		
Tehra	Deshla	65.00	25.00-65.00	45.00	Gopalpur	Deshla	50.00	25.00-65.00	45.00
	Deshla	25.00				Deshla	30.00		
	Deshla	45.00				Morangia	65.00		
	Unkown	65.00				Hirotia	45.00		
	Deshla	30.00				Deshla	25.00		
Hashanpur	Hirotia	15.00	15.00-60.00	37.5	Khotahi	R. Sonali	20.00	20.00-65.00	42.50
	Deshla	20.00				unknown	45.00		
	Deshla	60.00				Deshla	65.00		
	Unkown	45.00				Hirotia	35.00		
	Hirotia	55.00				Hirotia	20.00		
Wajidpur	Deshla	55.00	40.00-65.00	52.50	Bakhari	Unkown	60.00	25.00-60.00	42.50
	R. sonali	40.00				R. sonali	35.00		
	Morangia	65.00				Deshla	25.00		
	Hirotia	50.00				Unkown	60.00		
	R. Sonia	50.00				Deshla	55.00		
Warisnagar	Deshla	60.00	30.00-60.00	45.00	Narauli	Hirotia	40.00	25.00-65.00	45.00
	R. sonali	35.00				R. Sonia	25.00		
	Morangia	55.00				Deshla	50.00		
	Hirotia	40.00				Unkown	65.00		
	R. Sonia	30.00				Hirotia	60.00		
Mean			15.00-65.00	43.00				20.00-65.00	44.50

disease of turmeric is presented in plate IV. The genotypic variation was assessed on the basis of per cent disease severity and rhizome yield (t/ha) and finally these genotype were categorized into different group of resistant or susceptible turmeric germplasm. In terms of disease severity, the turmeric genotypes under test varied significantly in their reaction to leaf blotch disease during crop season Table 4 & Fig.1

Per cent disease severity varied from 2.50 to 57.50 per cent. Among the different turmeric germplasm tested, RH-434 recorded lowest disease severity (2.50%) which was statistically *at par* to RH-81, RH- 14, RH-6 registering disease severity of 5.00, 7.50, 10.00 per cent and RH-430, RH- 436 experiencing disease severity of 12.50. 57.50 per cent against recorded in susceptible check, Morangia. Among remaining genotypes only eight genotypes *viz*., RH-80, RH-426, RH-2/80, RH-421, RH-439 and RH-418 recorded disease severity ranging from 17.50 to 27.50 per cent and they were found statistically *at par* to each other. Remaining germplasm under study recorded disease severity more than 32.50 per cent but they were found significantly superior over susceptible check Morangia, having disease severity of 57.50 per cent

These germplasm were also assessed on the basis of their rhizome yield obtained under unprotected condition. The relevant data presented in table 4 revealed all the germplasm to give statistically higher yield against susceptible check variety, Morangia. Rhizome yield varied significantly from 22.80 to 47.22 t/ha with minimum and maximum being recorded with RH-418 and RH- 434 respectively. Among the germplasm under test RH-434 gave highest rhizome yield (47.22 t/ha) which was found statistically superior over all the germplasm except RH-80 giving yield of (43.97 t/ha).

Table 4. Reaction of various germplasm on leaf blotch disease of turmeric caused by *Taphrina maculans*

Sl. No.	Germplasm	Disease Severity (%)	Rhizome yield ($Kg/3m^2$)	Rhizome yield (t/ha)
1	RH-3	37.50 (37.73)	11.70	39.04
2	RH-6	10.00 (17.84)	11.90	39.68
3	RH-14	7.50 (15.67)	10.23	34.13
4	RH-80	17.50 (24.66)	13.19	43.97
5	RH-81	5.00 (9.21)	11.9	39.81
6	RH-414	40.00 (39.18)	11.92	39.75
7	RH-418	27.50 (31.59)	6.84	22.8
8	RH-421	20.00 (26.83)	10.99	36.66
9	RH-426	17.50 (24.66)	11.66	38.88
10	RH-429	37.50 (37.73)	10.68	35.62
11	RH-430	12.50 (20.60)	12.00	40.00
12	RH-434	2.50 (6.45)	14.16	47.22
13	RH-436	12.50 (19.73)	12.33	41.11
14	RH-439	20.00 (26.38)	11.16	37.20
15	RH-441	32.5 (34.72)	10.33	34.44
16	RH-2/80	17.5 (24.66)	12.49	41.66
17	Morangia (Susceptible check)	57.5 (49.29)	8.16	27.20
	S. Em(±)	4.66	1.75	1.24
	CD(p=0.05)	10.64	2.48	3.82
	CV(%)	18.91	5.41	10.11

Note: Figure in parenthesis indicates the angular transformed value of corresponding data.

4.4.2 Categorization of turmeric genotype in different disease reaction group of leaf blotch disease of turmeric.

On the basis of per cent disease severity, these turmeric genotypes were categorized in different group of resistance or susceptiblity based on disease severity scale (0-4) as suggested by Annon., 2004. The overall picture on categorization of turmeric germplasm so obtained has been summarized in the table 5. Out of sixteen turmeric germplasm, none of the genotype showed highly resistant (HR) reaction

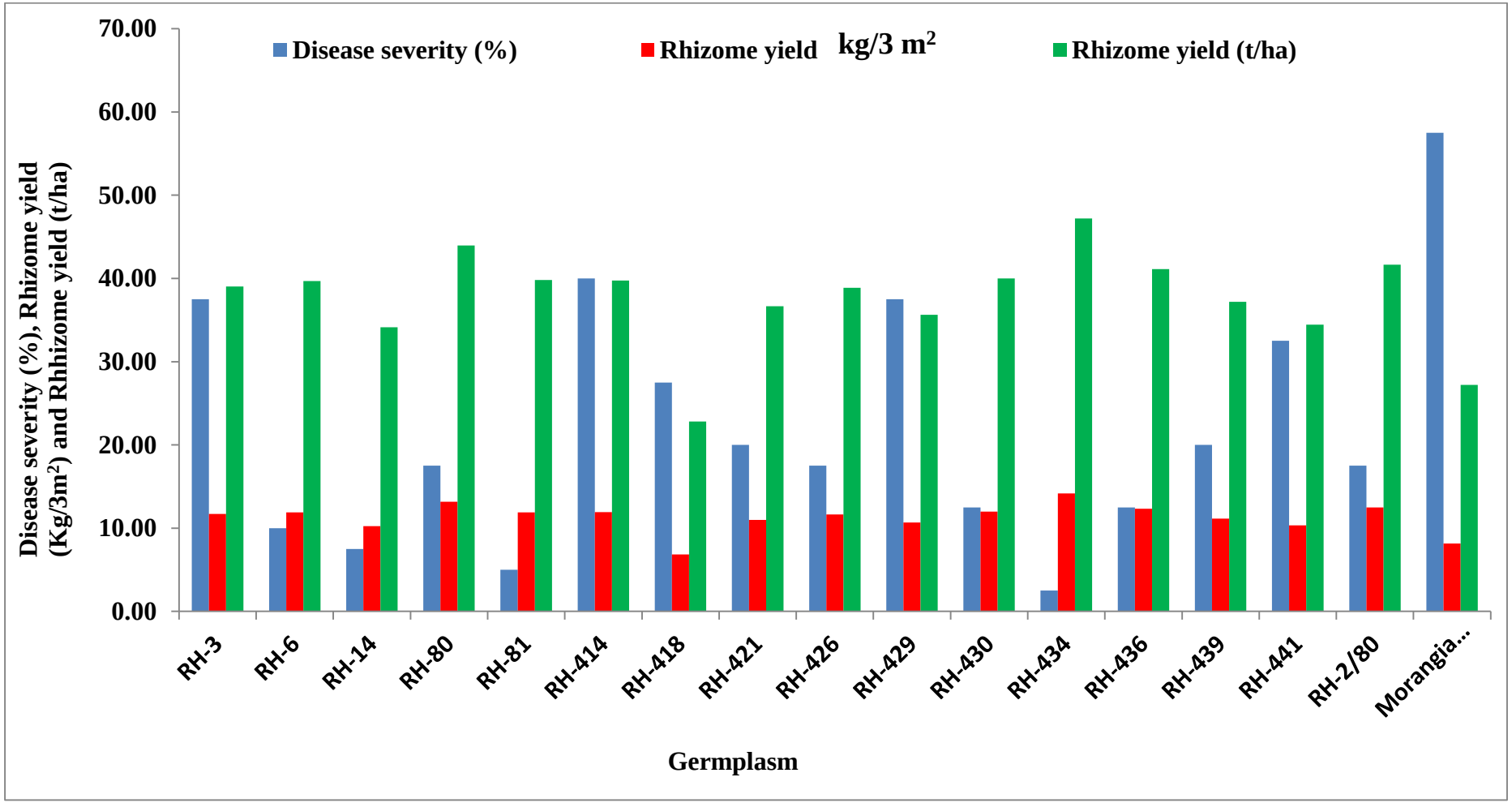


Figure 1: Reaction of various germplasm on leaf blotch disease of turmeric caused by *Taphrina maculans*

Plate IV: General view of field varietal screening experiment at T. C.A. Dholi, Muzaffarur, Bihar

while, three germplasm viz., RH-14, RH-81 & RH-434 and eight germplasm *viz.*, RH-6, RH-80, RH-421, RH-426, RH-430, RH-436, RH-439, RH-2/80, showed resistant and moderately resistant (MR) disease reaction against leaf blotch disease of turmeric. Among rest five germplasm showed susceptible (S) reaction to the disease while Morangia used as susceptible check showed Highly Susceptible (HS) reaction.

Table 5: Categorization of turmeric germplasm in different disease reaction group.

Disease Reaction	Disease Score	Disease Severity (%)	Germplasm
HR	0	0-1	-
R	1	1.1-10	RH-14, RH-81, RH-434,
MR	2	10.1-20	RH-6, RH-430, RH-2/80, RH-421, RH-426, RH-436, RH-80 and RH-439
S	3	20.1-50	RH-3, RH-414, RH-418, RH-429 and RH-441
HS	4	>50	Morangia (susceptible check)

HR= Highly Resistant; R= Resistant; MR= Moderately Resistant; S= Susceptible HS= Highly Susceptible

4.5 Evaluation of efficacy botanical extract against *Taphrina maculans*

Five Botanical extract were tested under laboratory condition for their efficacy against *Taphrina. Maculans* causing leaf blotch disease of turmeric.

Aqueous leaf extract (1:1 V/V) of tulsi, brahmi, neem pipli and sarpgandha of strength 6, 8, and 10 per cent were used to see the antagonistic effect of these botanicals on pathogen, *T. maculans.* The data on radial growth of pathogen culture (*T. maculans*) with respect to different botanical aqueous extract (6%) at time intervals of 72 hrs. starting from 72 to 360 hrs. is presented in table 6. The data indicated that all the botanicals significantly inhibited the fungus growth compared to control in all observations made at different time intervals. Neem leaf extract exhibited minimum radial growth of pathogen in all the days of observation thus giving maximum inhibitory effect on pathogen. Effect of neem leaf extract was found statistically *at par* with tulsi at 72 hrs., brahmi at 216 and 218 hrs. and finally with pipli at 360 hrs. of observation. The next best botanical in terms of conferring inhibitory effect were found sarpgandha, pipli, tulsi and brahmi at 72, 144, 236, 288

and 360 hrs of observation respectively. Based on the last (final) observation, the descending order of fungitoxicity to *T. maculans*. The descending order of fungitoxicity to *T. maculans* by plant extract was as follows:

Neem leaf extract > Pipli leaf extract > Brahmi leaf extract > Tulsi leaf extract > Surpgandha leaf extract.

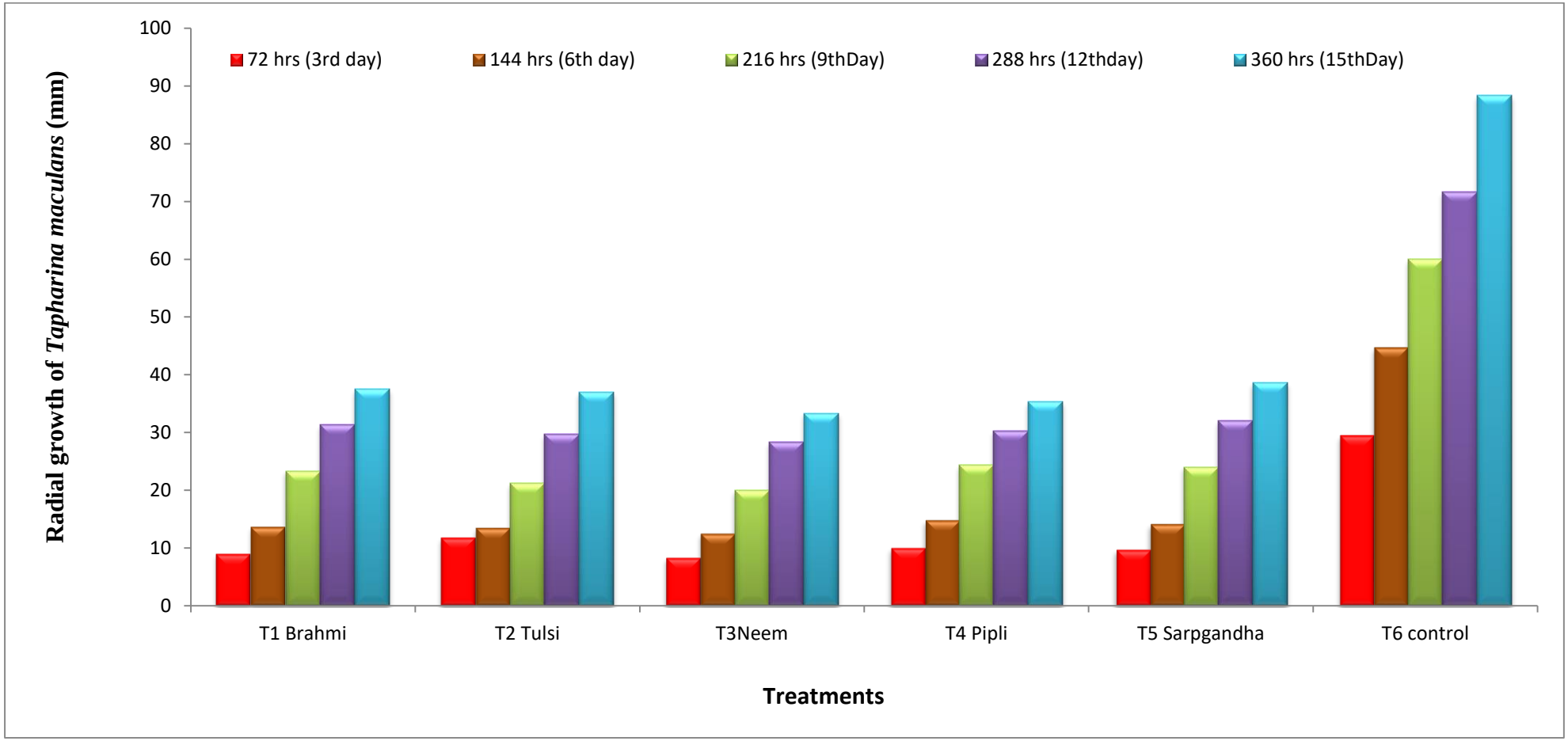
Aqueous leaf extract of all the botanicals at 8 and 10 per cent strength showed complete suppression of radial growth and pathogen during the period of observation *i.e.*, upto 360 hrs. of inoculation.

Table 6. Effect of Neem, Tulsi, Brahmi, Pipli and Sarpgandha extract (6%) on radial growth (mm) of *Taphrina maculans* at different time intervals.

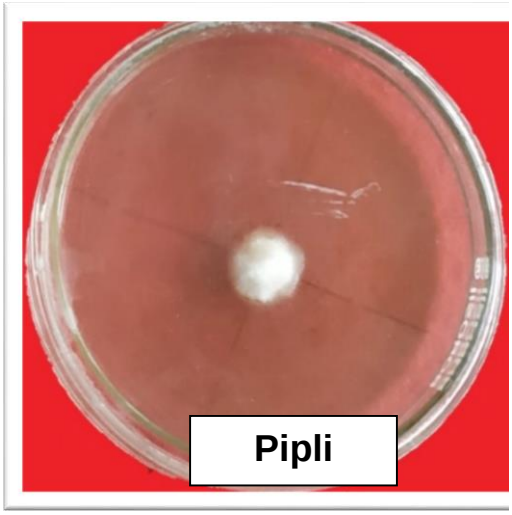
Treatments	**72 hrs (3rd day)**	**144 hrs (6th day)**	**216 hrs (9th Day)**	**288 hrs (12th day)**	**360 hrs (15th Day)**
T_1 Tulsi	9.00	13.67	23.33	31.33	37.50
T_2 Brahmi	11.80	13.50	21.17	29.67	37.00
T_3 Neem	8.30	12.50	20.00	28.33	33.33
T_4 Pipli	10.0	14.83	24.33	30.33	35.33
T_5 Sarpgandha	9.70	14.17	24.00	32.00	38.67
T_6 control	29.40	44.67	60.00	71.67	88.33
SEm (±)	0.33	0.26	0.46	0.92	0.93
CD (*p*=0.01)	1.05	0.82	1.43	2.87	2.90
CV (%)	4.46	2.42	2.76	4.24	3.53

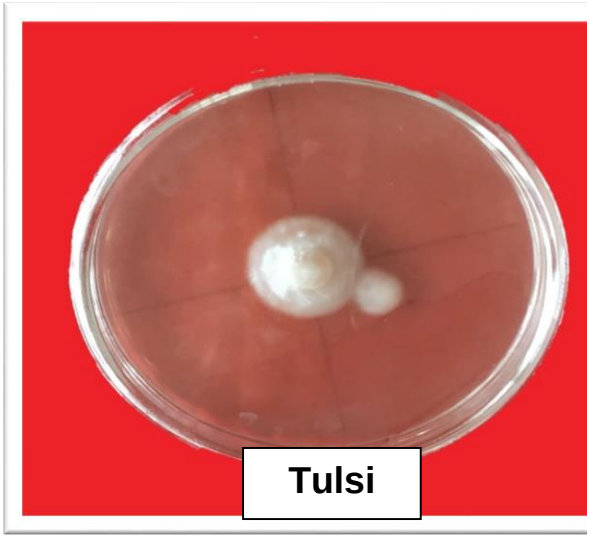
*Data is average of 3 replications.

Table 7 depicts the inhibition of mycelial growth (%) of pathogen by botanicals at different time intervals (Plate V and VI).The data revealed that aqueous extract of tulsi followed by brahmi gave maximum inhibition of mycelial growth (%) across all the days of observation registering 59.28 and 55.08 per cent mycelial growth inhibition on final day of observation (15 day after inoculation).



. Figure 2: Effect of Neem, Tulsi, Brahmi, Pipli and Sarpgandha extract (6%) on radial growth (mm) of *Taphrina maculans* at different time intervals





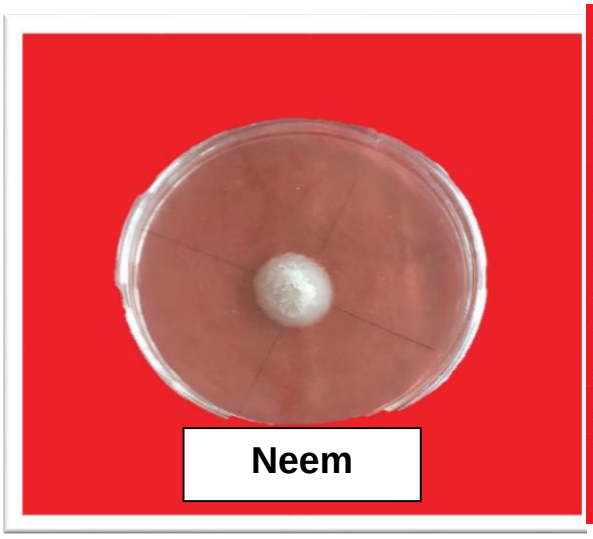


Plate V: Effect of botanical on radial growth of *T. maculans* at 72 hr interval

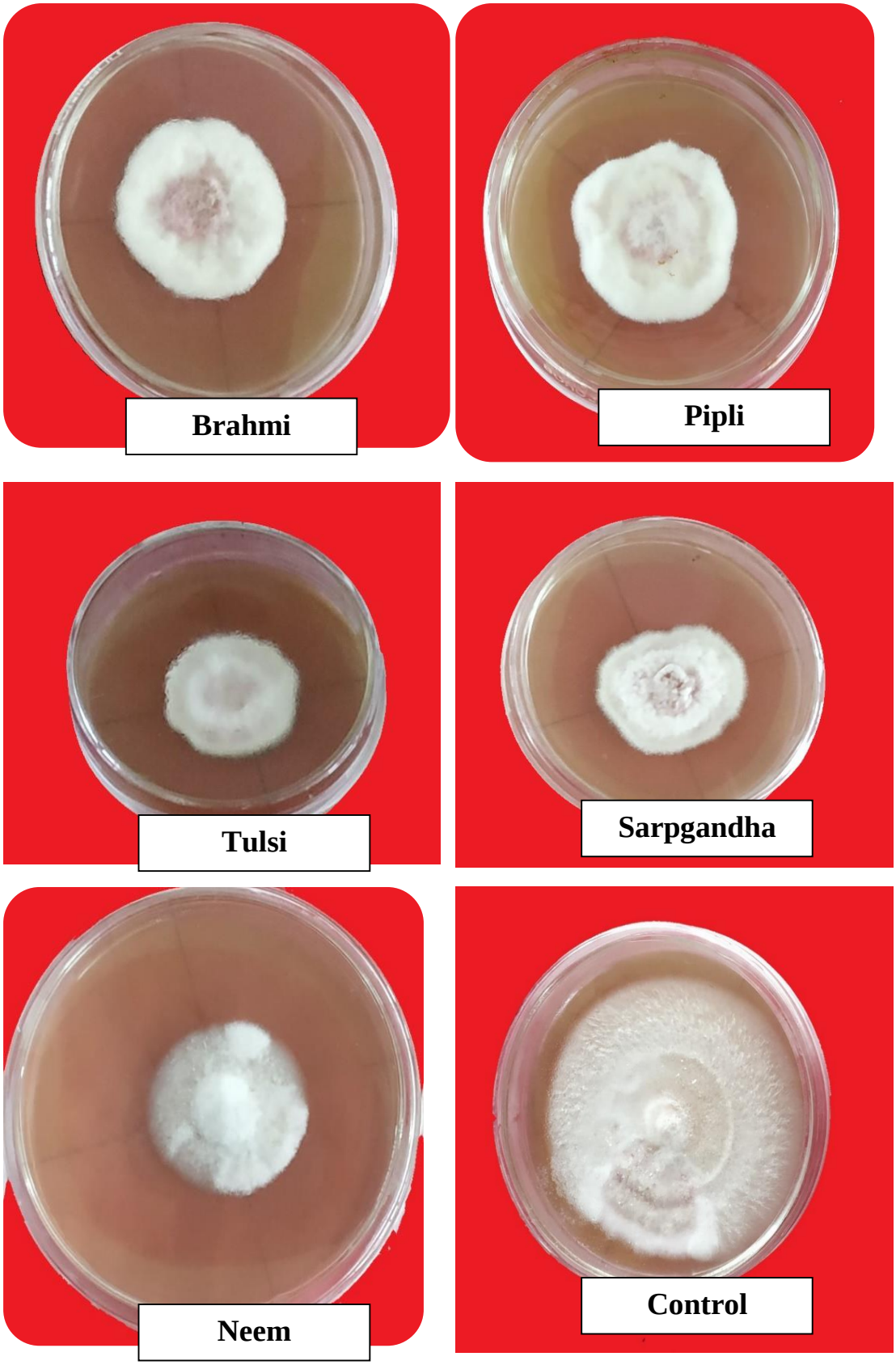


Plate VI: Effect of botanical on radial growth of *T. maculans* at 360 hr interval

Table 7. Effect of Neem, Tulsi, Brahmi, Pipli and Sarpgandha extract (6%) on inhibition of mycelial growth (%) of *Taphrina maculans* at different time intervals.

Treatments	Per cent inhibition of mycelial growth				
	72 hrs 3rd day	144 hrs 6th day	216 hrs 9th day	288 hrs 12th day	360 hrs 15th day
T1	71.68	72.01	66.65	60.40	59.28
T2	59.77	69.77	64.73	57.58	55.08
T3	69.40	69.40	61.10	56.37	54.54
T4	66.01	66.79	59.43	53.42	51.43
T5	67.11	68.28	59.99	55.36	54.24
T6	0.00	0.00	0.00	0.00	0.00

4.6 Management of leaf blotch disease of turmeric through fungicides, resistance inducing chemicals used as rhizome treatment and foliar spray

4.6.1 Relative efficacy of fungicides and resistance inducing chemicals against disease incidence of leaf blotch on turmeric caused by *Taphrina maculans*.

A general field view of experimental plot under management aspect of leaf blotch disease of turmeric is presented in Plate VII

To find out the suitable fungicide or resistance inducing chemicals to control leaf blotch disease two fungicide *viz*., zineb (0.25%), tricyclazole (0.1%) and one resistance inducing chemical *viz*., salicylic acid (400µM) used as foliar spray. Fungicides as well as resistance inducing chemical were used for rhizome treatment before planting as rhizome tretment and also for three levels of foliar spray *i.e.*, one, two and three times at fortnightly interval starting from first appearance of disease incidence (85 DAP). All the treatments were found to influence the reduction in disease intensity and yield increase of turmeric over control. The mean disease intensity (%) varied significantly from 26.67 to 53.33 per cent (Table 8).

Minimum PDI (26.67%) followed by 30.00 per cent with consequently maximum reduction in disease intensity (49.99%) and 43.74 per cent was recorded with treatment of rhizome treatment and three foliar spray of tricyclazole 75 WP

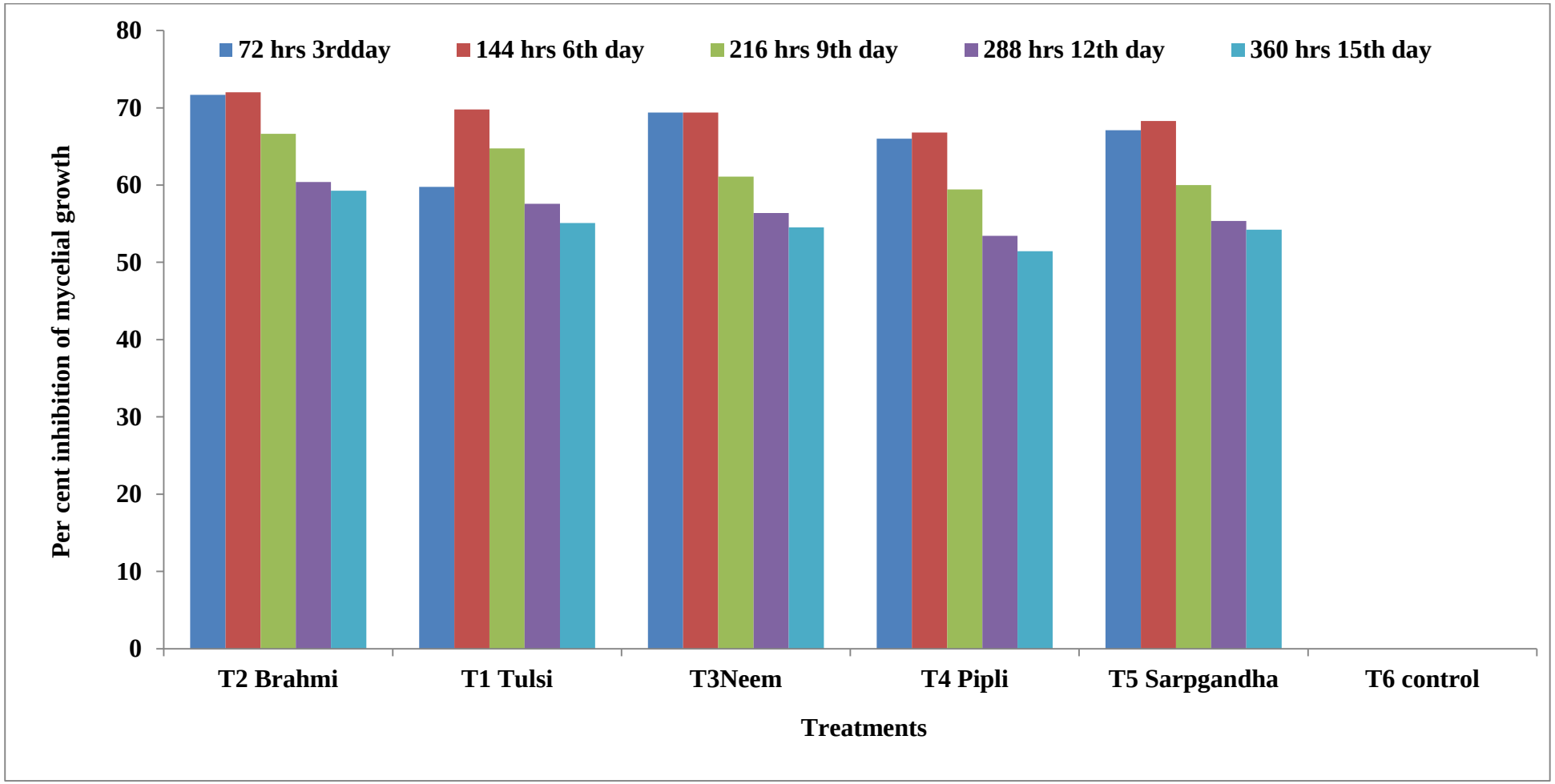


Figure 3: Effect of Neem, Tulsi, Brahmi, Pipli and Sarpgandha extract (6%) on inhibition of mycelial growth (%) of *Taphrina maculans* at different time intervals

@0.1 per cent and rhizome treatment alongwith two times foliar spray with tricyclazole 75 WP @ 0.1 per cent over control respectively.

But both of these treatment along with treatment of rhizome treatment and three times foliar spray with zineb 75 WP @ 0.25 per cent giving PDI (31.67 per cent) and disease reduction over control of 40.61 per cent were found statistically *at par* in their effect. Statistically significant with respect to best treatment next best treatment in terms of recording PDI (33.33%) and consequently 37.50 per cent disease reduction over control was recorded in treatment of rhizome treatment with same fungicide (tricyclazole 75 WP @ 0.1%) with lowest number of foliar spray *i.e.*, once after initiation of disease incidence. The treatment was also found statistically *at par* in its effect on reducing disease incidence to all the treatment except rhizome treatment as well as one foliar spray with zineb 75 WP @ 0.25 per cent and treatment of rhizome treatment and one as well as two time foliar spray of salicylic acid @ 400 µM over control. The efficacy of different fungicides and resistance inducing chemical was also accessed on the basis of rhizome yield of turmeric (var. Morangia). All the treatments were found to confer statistically significant effect on rhizome yield against control except the treatment of rhizome with one foliar spray of zineb 75 WP @ 0.25 per cent and rhizome treatment with one or two foliar spray of salicylic acid @ 400 µM. the range of rhizome yield varied from 25.20 to 37.85 t/ha as influenced by different treatment effect. Highest rhizome yield (37.85 t/ ha) with maximum increase in rhizome yield over control (55.75%) followed by rhizome yield (36.85 t/ ha) with yield increase over control (40.80%) were registered in treatment *viz.*, rhizome treatment with three foliar spray of tricyclazole 75 WP @ 0.1 per cent and rhizome treatment with thrice foliar spray of zineb 75 WP @ 0.25 per cent in place of of earlier treatment of tricyclazole 75 WP respectively. Both of these treatment and treatment comprising rhizome treatment along with two foliar spray with tricyclazole 75 WP @ 0.1 per cent was found statistically at par to each other in their effect. The next best statistically significant treatment in its effect on recording yield to the tone of 31.33 t/ ha with consequent 36.49 per cent increase in yield over control was recorded in rhizome treatment alongwith twice foliar spray of zineb 75 WP @ 0.2 per cent. This treatment was found statistically at par in its effect to one foliar spray with zineb 75 WP @ 0.25 per cent and one or two foliar spray with tricyclazole 75 WP @

Plate VII General view of the experimental plot under management of leaf blotch disease of Turmeric

Table 8: Relative efficacy of fungicides and resistance inducing chemicals on disease incidence of leaf blotch of turmeric and rhizome yield

Treatments	PDI (%)	Disease reduction over control (%)	Rhizome yield (kg/3m^2)	Rhizome yield (t/ha)	Rhizome yield increase over control (%)
T_1 Rhizome treatment and one foliar spray with Zineb 75WP @ 0.25%	46.67(42.10)^{cde}	12.48	8.52	28.70^{bcd}	22.41
T_2 Rhizome treatment and two foliar spray with Zineb 75WP @ 0.25%	41.67(40.15)^{bc}	21.86	9.50	31.33^{bc}	36.49
T_3 Rhizome treatment and three foliar spray with Zineb 75WP @ 0.25%	31.67(34.134)^{a}	40.61	9.80	36.85^{a}	40.80
T_4 Rhizome treatment and one foliar spray with Tricyclazole 75WP @ 0.1%	33.33(35.15)^{ab}	37.50	9.39	28.35^{bc}	34.91
T_5 Rhizome treatment and two foliar spray with Tricyclazole 75WP @ 0.1%	30.00(33.14)^{a}	43.74	10.25	33.25^{ab}	47.27
T_6 Rhizome treatment and three foliar spray with Tricyclazole 75WP @ 0.1%	26.67(30.93)^{a}	49.99	10.64	37.85^{a}	55.75
T_7 Rhizome treatment and one foliar spray with Salicylic acid @ 400µM.	50.00(44.98)^{e}	6.24	7.76	25.87^{d}	11.49
T_8 Rhizome treatment and two foliar spray with Salicylic acid @ 400µM.	48.33(44.01)^{d}	9.37	8.15	27.17^{cd}	17.09
T_9 Rhizome treatment and three foliar spray with Salicylic acid @ 400µM.	40.00(39.13)^{bc}	24.99	9.06	26.43^{c}	30.17
T_{10} Control	53.33(46.93)e	-	6.96	25.20^{d}	-
C.D	8.19			5.33	
SEm(±)	2.73			1.780	
C.V.	12.13			10.271	

Note: the values followed by different alphabelts differ significantly.

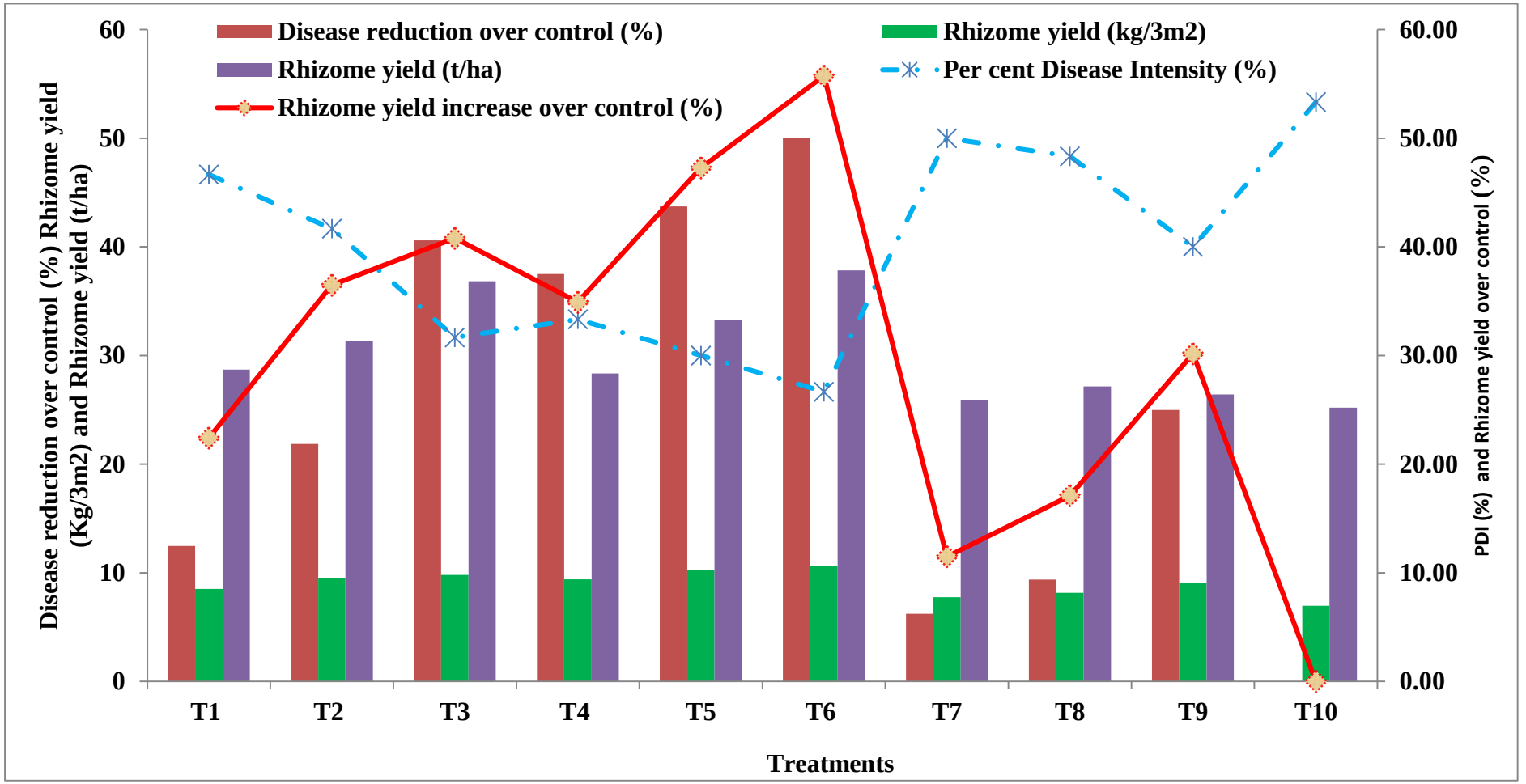


Figure 4: Relative efficacy of fungicides against disease incidence of leaf blotch on turmeric and rhizome yield

0.1 per cent in addition to rhizome treatment with some fungicide used for foliar spray.

4.6.2 1Economics of fungicides and resistance inducing chemicals for the management of leaf blotch disease of turmeric

Field efficacy of various fungicides and resistance inducing chemical under test was finally accessed and compared on the basis of benefits realized in monitary term and the relevant data pertaining to their economic parameters are presented in table 9. The gross highest income obtained under different treatment was highest (Rs 1,26,500.00 ha^{-1}) followed by Rs. 1,16,500 ha^{-1} while the lowest gross income (Rs. 6700.00 ha^{-1}) was registered in treatment of thrice foliar spray of tricyclazole 75 WP @ 0.1 per cent, zineb 75 WP @ 0.25 per cent and one foliar spray with salicylic acid alongwith rhizome treatment with respective chemicals in each of treatment respectively

Remaining treatments occupied intermediate position with wide differences in respect of gross income ranging from Rs. 12,300.00 ha^{-1} to 80500 ha^{-1}. The net profit derived out of different treatments got affected since the cost involved in these treatments ranges from a minimum of Rs. 1972 ha^{-1} to 8460.00 ha^{-1}. Consequently, there was considerable differences in ICBR among the various treatments (Fig.). The highest ICBR (1: 16.34) was recorded in rhizome treatment and three foliar spray of Tricyclazole @ 0.1 per cent followed by rhizome treatment and two spray of tricyclazole @ 0.1 per cent (1: 15.60). Among remaining treatments, highest ICBR OF 1: 13.77 and lowest (1:2.07) was found in treatment of rhizome and thrice foliar spray with zineb 75 WP @ 0.25 per cent and treatment of rhizome and thrice foliar spray with salicylic acid @ 400µM respectively

Table 9: Economics of fungicides and resistance inducing chemicals for the management of leaf blotch disease of turmeric

Treatments	Additional yield over control (t/ha)	Price of additional yield (Rs/ha)	Cost of treatments (Rs/ha)	Net profit/loss over control (Rs/ha)	ICBR
T_1= Rhizome treatment and one foliar spray with Zineb 75WP @ 0.25%	3.50	35000	2820	32180	1:12.41
T_2= Rhizome treatment and two foliar spray with Zineb 75WP @ 0.25%	6.13	61300	5640	55660	1:10.80
T_3= Rhizome treatment and three foliar spray with Zineb 75WP @ 0.25%	11.65	116500	8460	108040	1:13.77
T_4= Rhizome treatment and one foliar spray with Tricyclazole 75WP @ 0.1%	3.15	31500	2580	28920	1:12.21
T_5= Rhizome treatment and two foliar spray with Tricyclazole 75WP @ 0.1%	8.05	80500	5160	75340	1:15.60
T_6=Rhizome treatment and three foliar spray with Tricyclazole 75WP @ 0.1%	12.65	126500	7740	118760	1:16.34
T_7= Rhizome treatment and one foliar spray with Salicylic acid @ 400µM.	0.67	6700	1972	4728	1:3.39
T_8=Rhizome treatment and two foliar spray with Salicylic acid @ 400µM.	1.97	19700	3945	15755	1:4.99
T_9=Rhizome treatment and three foliar spray with Salicylic acid @ 400µM.	1.23	12300	5918	6382	1:2.07
T_{10}= Control					

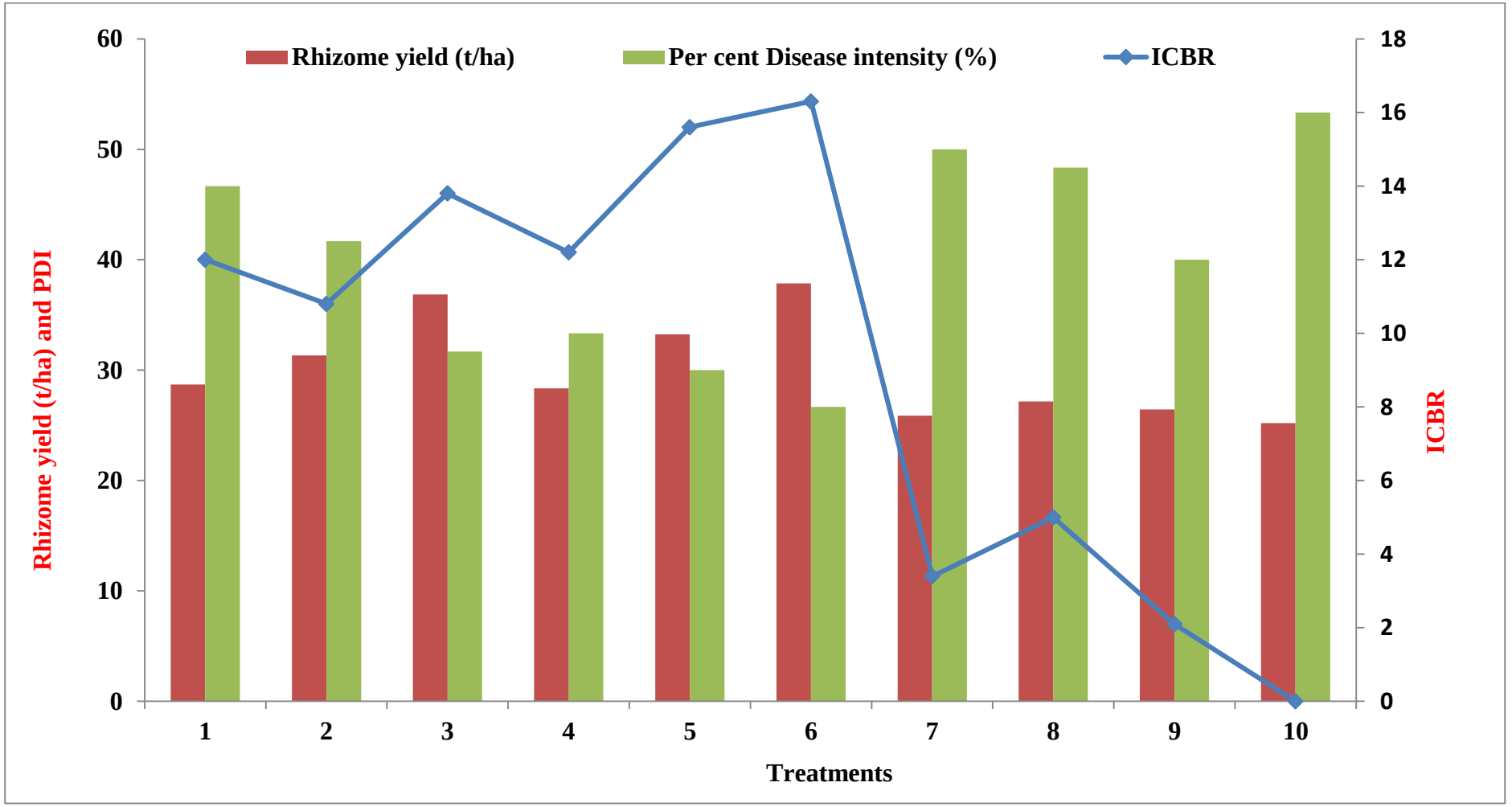


Figure 5: Effect of different treatments on the incidence of Leaf Blotch disease (yield and PDI) of turmeric (cv. Morangia) with ICBR

CHAPTER - V

DISCUSSION

5. DISCUSSION

The fungus on the modified culture media *i.e.,* PDA amended with 2 per cent yeast extract was identified based on the morphological descriptions made by earlier researchers. The fungus in culture was yeast like. No filamentous growth of the fungus was observed in the culture. The individual cell, that is, the shape of blastospore of the culture were ellipsoidal to ovoid. The length and breadth of blastospore from culture were measured by using a microscope with stage and occular micrometers. Similar observations were also made on conidia obtained from naturally blotch infected leaves. The average length of blastospore obtained from culture media were ranged from 5.89 µm to 6.38 µm and average breadth ranged from 2.87 µm to 3.24 µm. The dimensions of blastospore from pure culture of *T. maculans* as reported by Kulkarni and Ahmed (1968) were 6 µm to 7 µm (length) and 2.5 µm to 4 µm (breadth). The measurement made in this study fall within this range.

The average length of the blastospore obtained from naturally blotch infected leaves was ranged from 4.20 µm to 5.3 µm and average breadth was ranged from 2.1 µm to 2.5 µm.

The dimensions of the blastospore reported by Butler (1918) and Kulkarni and Ahmed (1968) were, 4 µm to 6.5 µm (length) and 2 µm to 2.8 µm (breadth). The range of measurements of blastospore observed in present study fall within the above mentioned range.

The pink and yeast like colony characteristics of *T. maculans* observed in this study also matched with descriptions of Pagvi and Upadhyay (1964) and Ahmed and Kulkarni (1968a). The naked asci with intercellular mycelium and yeast like growth of *T. maculans* in culture further confirmed the dimorphic nature of the fungus (Mix, 1924; Romano, 1966).

Thus the identity of the pathogen as *Taphrina maculans* Butler was confirmed. This result is in confirmation with the finding of Prasadji *et al.* (2004). The fungus was isolated from the diseased turmeric Plants tissues. Pathogenicity of this pure culture of *T. maculans* on the plants of susceptible turmeric cultivar morangia was tested and proved in same field and established the etiology of the pathogen.

The pathogenic ability of such pure cultures of *T. maculans* on turmeric plants were also established by Pagvi and Upadhyay (1964) and Ahmed and Kulkarni (1968a).

Agriculture is an important source of income for indian people. Farmers can grow variety of crops but the crop disease hamper the growth of crops. One of the major factor responsible for the crop destruction is plant disease. In the present study, the disease appears on leaves which can reduce both the quality and quantity of crops and their further growth. Survey on plant disease gives an idea or information of plant disease appearing or prevailing in a particular area. Survey also awares regarding adoption of suitable disease management practices keeping in view the devastating consequences of plant diseases. Survey on appearance and severity of leaf blotch disease of turmeric caused by *Taphrina maculans* was taken during Kharif, 2018 in two main turmeric growing districts of Bihar *viz*., Samastipur and Muzaffarpur of Bihar. Survey of farmers' field growing turmeric was done twice *i.e*., first during initiation of disease (September) and second during November, when the disease attains almost its maximum severity. Considering the overall mean of five villages disease severity observed was more in Samstipur (0.00-25.00 %) than Muzaffarpur (0.00-20.00 %) but mean disease severity was slight more (9.50 %) in Muzaffarpur compared to Samastipur (9.00 %) during the initial disease appearance stage *i.e*., in September.

Survey conducted during when the disease attains the status of maximum severity, it was seen that disease severity ranged almost same dimension *i.e*., 15.00-65.00 and 20.00-65.00 per cent in Samastipur and Muzaffarpur respectively but the mean disease severity was observed slightly more (44.50 %) in Muzaffarpur compared to Samastipur (43.00%). In general, the range and mean disease severity was seen close in both the district during survey. Mean disease severity was found below 45.00 per cent in both the district. Disease severity data indicates the leaf blotch disease as a major disease of turmeric in the studied area. Although the disease severity was observed below 50 per cent in surveyed area, above which the diseased crop is declared as most susceptible germplasm/ variety/ cultivar, The mean disease severity recorded near 45.00 per cent which indicates leaf blotch disease as a major disease of turmeric in the studied area.

Farmers' growing improved high yielding varieties released from this University (RPCAU, Pusa) were found mostly free from disease or with low disease severity during September 2018 whereas, local cultivar (Deshla, Hirotia), susceptible variety, Morangia or Unknown cultivar were found to have more disease severity. Survey of such disease were also carried out earlier in Samastipur, Muzaffarpur and Vaishali district of Bihar during 2008-09, 2011-12 and 2016-17 wherein leaf blotch disease of turmeric was observed in the range of 0.00-56.11 per cent (Annon., 2009a; 2012; 2013a; 2014; 2016a and 2017a).

In the present study undertaken, an attempt was made to identify resistance source in turmeric germplasm by screening them against leaf biotch disease of turmeric caused by *T. maculans* under artificial condition. During Kharif 2018, the germplasm screening indicated that the maximum disease severity was recorded in Morangia (57.50 %) used as susceptible check, and minimum disease severity of 2.50 per cent was recorded in only one germplasm *viz*., RH-434, next minimum disease severity of 5.00 per cent was recorded in germplasm RH-81.Considering the yield parameter, germplasm RH-434 giving the lowest disease severity was able to yield maximum (47.22 t/ha). This is because of universal fact of indirectly proportional relationship between disease and yield.The above principle can't be ruled out always as evident from findings of present study. Here germplasm RH-81 having the second lowest numerical value of disease severity didn't resulted in second highest rhizome yield. Likewise, germplasm RH-80 having second highest rhizome yield didn't exhibited second lowest disease severity. This suggest that between disease severity and rhizome yield always indirect proportion relationship doesn't exist always. Moreover, it also implies that yield parameter is not always influenced by disease. There are some other factor responsible for influenceing the yield besides disease being one of the major player influencing yield.

Out of seventeen germplasm including one susceptible check (Morangia), none of the germplasm could be categorized in the category of highly resistant (HR), while three and eight germplasm were grouped in the category of resistant (R) and moderately resistant (MR) respectively. Based on their relation to leaf blotch disease, disease pressure was sufficient to cause disease as evidence by Morangia (susceptible check) to categorise it under group of most susceptible (MS) disease relation. The study indicates that under natural selection, no germplasm can completely escape or

counter the virulence of pathogen rather they response to varied degree of susceptibility or resistance.

The finding of present study can be substantiated by the results of germplasm screened earlier at same location *i.e*., Dholi. Germplasm, RH-14, RH-81, and RH-421 were found R and moderately resistant MR respectively. In the present study they were also found to fall in either category of Rt or MR during study period of 2011-12 to 2018-19 consistently. Germplasm, RH-430 and RH-6 showing R in the present study were found to exhibited either R or MR in studies done during 2011-12 to 2018-19 except 2012-13 and 2013-14 in case of RH-430 and except during 2013-14 and 2015-16 in RH-6. Among the rest germplasm categorized in other group of resistance in the present study , germplasm RM-80 was found to fall in either of resistance group time to time during the study period of 2014-15, 2016-17 to 2017-19, likewise, germplasm RH-434, RH-436, RH-439 and RH-2/80 were found to fall either under R or MR category during the study period of 2015-16 to 2018-19 at Dholi (Bihar) either Germplasm , RH-439 was observed to show either R to MR disease reaction against leaf blotch disease of turmeric during 2016-17 to 2018-19. (Anon.,2012; 2013a; 2014; 2015; 2016a;, 2017a; 2018a; 2019). Disease reaction showed by these germplasm in the category of either of resistant group strengthen the credibility of germplasm screened under either of resistant group in the present study.

Though the results of the present investigation are from a single site, it may be hoped that information presented here will be of interest to turmeric breeders and that in the future good use might be made of these important genetic sources. The interaction between the inherent virulence of the pathogen and the weather factors is avery complex phenomenon, which was dealt with monistic approach in the present investigation. Nevertheless, it is an important topic, which warrants future investigation particularly in the case of those germplasm which shows signs of different degree of resistance in the initial screening against the disease.

Searches are and to screen the available turmeric germplasm resistant or moderately resistant against leaf blotch disease in turmeric growing area of country. The resistant source of germplasm of any crop is invaluable for the plant pathologist as well as plant breeders.

A greater attention has been paid from All India co-ordinated Research Project on Spices, Calicut, Kerala to evaluate turmeric germplasm against this disease in different co-ordinating centres across the country including Dholi. At our centre (Dholi, Bihar), pooled data of three years of field trial carried from 2015-16 to 2017-18 indicates germplasm *viz*., RH-7, CL-54, TCP-14, TCP-129, NDH-10, and NDH-128 to be R. (Annon; 2016a; 2017a and 2018a). The same set of germplasm screened in different geographical location of country showed germplasm CL-54, RH-7 and RH-129 to be R at Solan (Himachal Pradesh) and Coimbatore (Tamil Nadu) while TCP-14 & TCP-129 and CL-54 & RH-7 showed disease reaction as MR at Pundabri (West Bengal) and HS at Kamarpally (Andhra Pradesh) respectively. At Dholi, germplasm RH- 406, RH-410, CL-32, CL-52, TCP-161, NDH-40, Rajendra Sonia and Rajendra Sonali were found as MR against leaf blotch Disease.

The disease reaction of above germplasm at different locations of country were found as R or S. Under R category, only one germplasm *viz*., CL-52 could make its existence at Pundibari and Solan (Himachal Pradesh) while, the germplasm *viz*., CL-32, CL-52, RH-406, RH-410, TCP-161 and NDH-40 were found in the Category of S at Kammarpally, Pundibari and Kumarganj (Annon., 2016; 2017 and 2018)

The germplasm *viz*., TCP-129, TCP-14 screened as R at Dholi was shown in the category of MR at Pundibari. Likewise, germplasm CL-32, CL-52, RH-406, RH-410, TCP-161 and NDH-40 showing MR reaction at Dholi (Bihar) has shifted their resistance towards susceptibility at Kumarganj, Pundibar and Kammarpally. This might be primarily due to change in environmental condition in terms of unfavourable to host and congenial for the pathogen to become more aggressive. Moreover, the scale used for categorizing the germplasm in different groups of resistance or susceptibility has the range of 1-10.0 per cent; 10.1-20.0 and 20.1-50.0 per cent for designating a particular genotype/ germplasm under R, MR and S category respectively. A particular germplasm categorized by earlier one worker under R might have the mean disease severity of 9.0 or near 9.0 per cent and 19.0 or near 19.0 per cent in case of germplasm categorized as MR. While making visual observation by another worker, the mean disease severity may come 11 or 21 per cent of same germplasm screened by earlier worker. So, subsequently these germplasm categorized by earlier worker as R (disease severity= 9.0 %) and MR (disease severity= 19.0%) may be categorized as MR (disease severity= 11.0%) and S (disease severity= 21.0%)

respectively by merely enhance of 2.0 per cent disease severity while visual observation made by another worker.

Besides screening of turmeric germplasm under AICRP network system, some other workers from different parts of country also made efforts to screen turmeric germplasm against foliar disease of turmeric. In this context, Maurya (1990) screened germplasm RH-10 (Rajendra Sonia) as R to leaf blotch at Dholi location of Bihar, while Singh (2002) at Chattisgarh and Khalko (2011) at west Bengal evaluated Rajendra Sonia as susceptible. In a study conducted during 1996-99 in trai region of west Bengal, india, turmeric germplasm *viz*., RH-5, Rajendra Sonia and PCT-13 were found moderately susceptible (MS) and PTS-62, ACC-360, ACC-361, Roma, BSR-1 and Kasturi were ranked as HR to leaf blotch disease. Maximum yield and disease resistance were recorded in germplasm, TCP-11 followed by TCP-82, TCP-56 and TCP-2 at Chhattisgarh, India during a study conducted from 2006-07 to 2007-08. Khalko (2011) also suggested turmeric cultivars, PTS-12, PTS-63 and ACC-340 to be R against leaf blotch disease.

Results of present study are in the close agreement with the reports made earlier at different places like Pottangi (Odisha) and Coimbatore (Tamil Nadu), Kumarganj (Uttar Pradesh), Guntur (Andhra Pradesh), Pundibari (West Bengal), Solan (Himachal Pradesh).

In the present investigation, the aqueous extract of tulsi, brahmi, neem, pipli and sarpgandha were evaluated at 6, 8 and 10% concentration against *Taphrina maculans* by poison food technique. Initially the minimum (8.30mm) growth of the test- fungus was found in case of neem (6% extract) after 72 hours (3rd day) which further developed slowly and reached to 33.33 mm after 360 hours (15th day) while, in case of Tulsi (6% extract) radial growth observed after 72 are 9.00 mm and reached to 37.50 mm after 360 hours followed by sarpgandha 6% extract in which 9.70 mm radial growth observed which were reached to 38.67mm after 15th day. Present investigation clearly indicate that the extract of Neem, Tulsi and sarpgandha used at 6%, were promising in controlling *T. maculans* at 6 % concentration. However, at 8% and 10% all the five Botanicals show cent per cent inhibition. Singh et al., (2009) revealed that Maximum inhibition of fungal growth was recorded with extracts (25%) of *Allium sativum* (82.01%), followed by *Azadirachta indica* (79.90%), *Curcuma*

longa (79.88%) and *Zingiber afficinales* (79.82%) in ethanol solvent. The least inhibition was recorded at 10% concentration of *A. cepa* (36.87%), followed by *Ocimum sanctum* (37.00%) in distilled water.

Indiscriminate use of chemical has been a major concern in the present day's concern. People are much more concerned than even before for health hazards, environmental hazards, water and air pollution etc. Judicious application of chemicals therefore has been advocated in such circumstances to minimize the yield loss of crop. Present study was an attempt to minimize the use of chemicals upto the possible extent. As leaf blotch disease of turmeric cause by *Taphrina maculans* is an air borne disease very littile option are left to manage the disease caused by means other than chemicals. Though various cultural practices have been advocated (Rathore and Pal, 2010), their practical utilty is limited as these are sometimes difficult to carry out and are labour intensive that is why, such type of cultural practices., burning of crop and crop debris, crop rotation, sowing of healthy rhizome, resistant variety, proper drainage, timely (early) sowing, clean cultivation, optimum use of nitrogenous fertilizers are rarely seen in farmers' field. In the present study, two fungicides *viz.,* Zineb, Tricyclazole and one resistant inducing chemical *viz.,* Salicylic acid were taken in consideration based on their merit on disease control(Annon., 2009; Annon., 2014; Annon., 2015; Mandal *et al.,* 2009; Hadi and Balali, 2010). The effect of these chemicals in relation to management of leaf blotch disease of turmeric, rhizome yield of turmeric and most importantly ICBR were determined by rhizome treatment and foliar spray with these chemicals at different levels of sprays *i.e*., once, twice or thrice at fortnightly intervals starting from first initiation of disease symptom.

In the present study, three spray of Tricyclazole @0.1% proved to be the best fungicide followed by three spray of Zineb @0.25% in terms of recording lowest disease severity of 26.67, 30.00 and 31.67 per cent and reducing disease severity to the extent of 49.00, 43.74 and 40.61 per cent respectively. Though some efforts has been made to screen the fungicide in Indian context but information on these aspects are very limited in other turmeric growing countries. However, the credibility of best fungicide *i.e*., Tricyclazole as observed in the present study can also be substantiated by the findings of work performed at different coordinating centres under All India Coordinated Research Project on Spices in the country. At Dholi (Bihar) during 2010-11 and 2014-15 foliar spray of Tricyclazole @ 0.1 per cent twice at 45 and 90 days

after sowing (DAS) was able to reduce the disease and thus disease severity of 7.44 to 26.09 per cent with 49.68 per cent reduction in disease severity was recorded (Annon., 2010; 2011 and 2015). Similar observation were also made from study carried out at different geographical location of the country *viz.*, during 2008-09. Study carried out at Coimbatore (Tamil Nadu), Jagital (Andhra Pradesh) and Kumarganj (Uttar Pradesh) revealed role of twice foliar spray of Tricyclazole at 45 and 90 days after sowing (DAS) in reducing the leaf blotch severity 28.55 to 46.37 per cent (Annon., 2008-09). Similarly, disease severity of 27.12 to 31.10 per cent with reduction in PDI 13.74 to 36.27 per cent was observed in studies carried over at Coimbatore in (2008-09); Raigarh in 2010-11 and Kumarganj 2012-13. (Annon., 2009; 2011 and 2013).

Efficacy of some other systemic, non-systemic fungicides and botanicals were authenticated at different turmeric growing areas of the Countary. Foliar application of carbendazim +mancozeb (0.1%) at 45 and 60 days after sowing (DAS) resulted in disease severity of 13.12 to 27.74 per cent in field study conducted at Pundibari (West Bengal) and Pottangi (Odisha) during 2008-09; Chintapalle (Andhra Pradesh), Raigarh (Chhattisgarh) and Kamarpally (Andhra Pradesh) during 2010-11 and at Raigarh during 2015-16.

The finding of our study can also be supported by the outcome of similar trials taken up in prime turmeric growing areas of country *viz.,* Chintapalle (Andhra Pradesh) during 2008-09 and Coimbatore (Tamil Nadu) during 2010-11 and 2017-18 where in foliar spray done with propiconazole belonging to the same group of fungicide (Triazoles) as that of the best fungicide (Tricyclazole) screened in our present study. (Annon., 2009a; 2011a and 2018). In an another study carried out at Kumarganj (Uttar Pradesh) during 2009-10 also indicated a fungicide (Hexaconazole) belonging to Triazoles group of fungicide to minimise the leaf blotch disease upto 52.90 per cent. At Kumarganj (Uttar Pradesh) initiative taken during 2015-16 and 2017-18 access the efficacy of some botanicals *viz.*, Argimone and Jatropa oil (1%), it was seen that foliar spray of Argimone (1%) could able to reduce the leaf blotch disease as evidenced by recording PDI in the range of 25.07 to 27.30 per cent. (Annon., 2015-16 and 2017-18).

Few other systemic fungicides belonging to Triazole group also showed its effectiveness in controlling the leaf blotch disease incidence as evidenced by the work of Singh (2009), Mona *et. al*., (2009) and Singh *et. al.*,(2013) A combination of two group of fungicide (Dithiocarbamate +Phenylamide and systemic benzoic) marketed in the chemical name of Ridomil M-Z was considered by Singh *et.al*., (2000) in his field study showed it to be promising in controlling leaf blotch disease by registering PDI of 31.50 per cent. Panja *et.al*., (2001) conducted an experiment during 1998-99 to 1999-2000 in West Bengal on management of leaf blotch disease of turmeric and copper sulphate (1%) was found most effective in reduction of per cent disease index among the fungicides used in the study. Findings of different workers as discussed above are in close agreement with the finding of present study.

Effect of different treatment on yield parameter in the present study indicates that with respect to first three top rankers of treatment with respect to yield parameter are the same as recorded in case of reducing disease severity *i.e*., rhizome treatment alongwith three, two and three foliar spray of Tricyclazole @ 0.1 and Zineb @ 0.25 per cent respectively. It also infers that these treatments were able to protect or increase the yield over control proportionally by reducing the disease severity. It also refers that reduction in disease severity is one of the major factor directly influencing the yield parameter in most of the cases. In support of above statement Tricyclazole @ 0.1 per cent was found to increase the rhizome yield by registering the range of yield from 10.55 to 38.50 t/ha in studies carried out at different location of country *i.e*., Coimbatore, Jagital, Kumarganj during 2009; Dholi; Coimbatore during 2010; Raigarh during 2011 and Kumarganj during 2013 (Annon., 2009; 2010; 2011; and 2013). Some other chemical not considered under present study *viz*., carbendazim + Mancozeb (0.1%) was found to record more yield over control *i.e*., 14.08 to 20.68 t/ ha, highest being recorded in study at Pottangi during 2008-09 and lowest at Raigarh in 2016 (Annon., 2009 and 2016). Foliar spray of Propiconazole (0.1%) was found to increase the yield recorded (38.50 t/ha) in our study (Annon., 2010-11). At Kumargunj during 2015-16 and 2017-18, rhizome yield upto 18.0 t/ha was recorded. by spraying Jatropha oil @ one per cent (Annon., 2015-16 and 2017-18). But the yield recorded in above case is almost half than the yield recorded in our study.

Keeping pace with our study, some other fungicide *viz*., Ridomil, Difenconazole, Carbendazim were found to record higher yield upto the tune of 20.37

t ha^{-1} in studies carried out by Mowlick *et. al.,* (2007) at Bangladesh, Singh (2009) and More *et. al.,* (2009) in India.

Based on effect of a treatment on disease reduction and yield enhancement, credibility of a particular treatment can be judged for academic purpose. But feasibility of a treatment for adoption at farmers level can be adjudged by considering the economics or income obtained from additional yield obtained over central in terms of incremental cost benefit ratio (ICBR) resulting due to effect of a particular treatment. In the present study, lowest disease severity, highest yield and consequently maximum ICBR of 1:16.34 was recorded in treatment comprising of rhizome treatment along with thrice foliar spray with Tricyclazole 75 wp @ 0.1 per cent at fortnightly interval. Although, the second highest additional yield of 11.65 t ha^{-1} was recorded in treatment (T_3), rhizome treatment and three foliar spray with Zineb 75wp @ 0.25 per cent at fortnightly interval but the second highest ICBR of 1:15.60 was recorded with the treatment (T_5) *i.e.*, rhizome treatment + twice foliar spray of Tricyclazole @ 0.1% registering third highest additional yield (8.05 t ha^{-1}) over concentration. The fact behind it is quite clear that due to one more no. of foliar spray involving labour cost, more quantity of fungicide needed owing to its higher dose (0.25%) and moreover more cost of fungicide per unit resulted higher cost of treatment *i.e.*, Rs. 8460.00 ha^{-1} compared to T_5 (Rs. 5160.00 ha^{-1}). Finding pertaining to ICBR in the present study is in close agreement with the findings of earlier similar study carried at Dholi (Bihar) during 2014-15 where ICBR of 1:23.04 higher tan present study was registered by foliar spray of Tricyclazole (0.1%) at 45 & 90 DAS. However, comparatively lower ICBR of 1:1.17 than present study was recorded at Dholi itself by two foliar spray of Tricyclazole (0.1%), it might be due to comparatively less income received from the additional yield owing to less sale price of produc during particular period or time.

On contrary, to present study a higher ICBR of 1:22.22 was realized at Dholi during 2014-15 in by hizome treatment along with three foliar spray of Zineb wherein in present study ICBR of 1:13.77 was recorded in the treatment with some fungicide with some no. of foliar spray. This might be due to primarily hike in labour wages with pace of time besides the fact of increase in fungicide cost and probably the prevailing less sale price of produce at that time. In consonance to the outcome of present study, more return and benefit cost ratio was realized by some others workers

by fungicidal treatment (Panja *et. al.*, 2001; Singh *et. al.*, 2003 & Singh, 2009). Comparatively a lower ICBR in the range of 1:2.0 to 1: 4.60 was calculated in work of management of leaf blotch disease of turmeric carried out at different locations of the country *viz*., at Coimbatore during 2008-09, 2009-10, 2010-11 at Dholi and Pottangi during 2008-09. (Annon., 2009; 2010 & 2011).

CHAPTER - V

SUMMARY AND CONCLUSION

6. SUMMARY AND CONCLUSION

Turmeric leaf blotch caused by *Taphrina maculans* Butler, was found severe in Samastipur and Muzaffarpur district of North Bihar (Zone-I). Thus it is proved as the major hindrance for the successful economic production of turmeric. Considering the seriousness of the disease, present investigation was carried out on various aspects *viz.*, survey, germplasm screening and evaluation of botanicals and fungicides against leaf blotch disease of turmeric under in-vitro and in-vivo condition in experimental lab and farm of Tirhut college of Agriculture, Dholi, Muzaffarpur (Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar) during the year 2018-19 to generate scientific information on this important pathological problem and to develop suitable management strategies to prevent the crop losses due to turmeric leaf blotch.. Well levelled plot with satisfactory drainage system was selected for the experiment. Turmeric (C*urcuma longa* L.) known as haldi and Indian saffron, is one of the most important and ancient spices of India.

To ascertain the pathogenic behaviour of *Taphrina maculans* butler. Koch's postulate were proved by blastospore suspension method of inoculation. The incubation period varied between 7-9 days. The symptoms appeared on younger leaves on both the surfaces of leaves as small oily looking translucent spot on the lower leaves when the plants are in 3-4 leaf stage in large numbers, generally more numerous on the upper surface. Soon they become dirty yellow and deepen to the colour of old gold and sometimes to bay shade. On maturity spot turn brownish and ultimately dark brown, surrounded by dried regions, the whole leaf then dries off giving burning type appearance. The adjacent individual leaf spots of 1-2 mm in diameter coalesces forming reddish brown blotches leading to varaying degree of leaf blight (Butler, 1911). The disease hastens defoliation of plants. (Disease Management of Spice Crop)

The development of the leaf blotches is a continuous progressive process but can be arbitrarily divided into 3 stages; (i) initial stages of development is externally marked out by yellowing and discolouration of leaves; (ii) middle stages of development are dull yellow in colour and (iii) final stages of development are light

brown to chocolate brown colour and accompanied by necrosis and drying of the Infected regions.

In the earlier report fungus is supposed to be an obligate parasite. Isolation of the pathogen was difficult as it requires rigid culture and environmental conditions. Pavgi and Upadhyay (1964), by applying special technique, successfully cultured the pathogen in artificial medium. Mature lesion from the field were soaked in water for 30 minutes, frozen for 48 hr and incubated at room temperature for 24 hr. small leaf pieces were then suspended from inside the petri dish lid so that the forcibly liberated ascospore germinate on the nutrient agar medium and the developing colonies later transferred to potato-dextrose agar. It was isolated at a temperature of 20˚C on PDA with pH adjusted to 4.5, which helped to prevent bacterial contaminations.

It was reported from earlier study that the hyphae of the parasite are intercellular, embedded in the cuticle and cell walls of the epidermis and adjacent cells of the leaves. In a young spot, the first formed hyphae are found embedded in in the cuticle, running mainly in in the furrows, between the adjacent epidermal cells. Development of asci takes place on both the surfaces of leaves when the spots are fully developed. The central portion of the spots is occupied by a well developed hyphal mass in the cuticle and vertical walls of the epidermis. The outer cells of these hyphal layers grow out in cylindrical or club shaped, thin walled projection which rupture the cuticle and develop into asci. All the outer cells become asci. These ascogenous cells do not mature simultaneously; they mature in groups. (Disease of Betelvine and Spices) Biphasics diurnal development of asci has been studied by Upadhyay and Pavgi (1979). Below each ascus there is at least one basal cell, there being sometimes 2 or 3 in row. The asci are sac- like or clavate in appearance, round or flattened above, narrowed below, measuring 20-30 µm in diameter. Normally, eight ascospore are hyaline, unicellular, ovoid and 4-6 µm x 2-2.5 µm in diameter (Butler, 911).

The detailed morphological study was carried out to confirm the organism as *Taphrina maculans*, the causal agent of leaf spot disease of turmeric. The identity of fungus isolated from the turmeric leaf blotch infected tissues was confirmed by comparing blastospore measurements with previous reports and by proving its pathogenicity. The average length of blastospore obtained from culture media were

ranged from 5.89 µm to 6.38 µm and average breadth ranged from 2.87 µm to 3.24 µm. The average length of the blastospore obtained from naturally blotch infected leaves was ranged from 4.26 µm to 5.35 µm and average breadth was ranged from 2.10 µm to 2.53 µm.

Survey results indicated that leaf blotch was observed in all the villages of Samastipur and Muzaffarpur district. All the five popular varieties grown by farmer's *viz.*, Hirotia, Deshla, Rajendra Sonia, Rajendra sonali and Morangia were found susceptible in surveyed area. In all the five location surveyed, 0.00 -15.00 per cent disease severity was observed during the month of September and 20.00 - 65.00 per cent disease severity was recorded in the month of November. Muzaffarpur district was found more prone to leaf blotch compared to Samastipur district. There was an increase in the disease incidence during September to November. The pernnation of this fungus is mainly by means of ascogenous cell on leaf debris and dessicated ascospore and blastospores in the soil and among fallen leaves (Upadhyay and Pavgi, 1967b). as per the report of Ahmed and Kulkarni (1968b), plant debris, rhizomes, etc., of the previously infected crop or soil from turmeric fields do not serve as a primary source of infection. The early appearance of the disease and its severity depend on the concentration of inoculum in the soil and is further enhanced by warm and humid weather (Upadhyay and Pavgi, 1967b). primary infection occurs on the lower leaves in October- November and at 80% relative humidity and 21-33^{o}C. the ascospore discharged from the successively maturing asci grow into eight spored microcolonies and infect fresh leaves without dormancy.

Amongst the 16 genotypes (germplasm) of turmeric screened against the leaf blotch disease, none of the germplasm showed highly resistant (HR) reaction. Three germplasm *viz.*, RH-14, RH-81 and RH-434 showed resistance reaction against the disease. Germplasm RH-6, RH-80, RH-421, RH-426, RH-430, RH-436, RH-439 and RH-2/80 showed moderately resistant reaction. RH-3, RH-414, RH-418, RH-429 and RH-441 showed susceptible reaction while, Morangia used as susceptible check showed highly susceptible reaction against the leaf blotch at Tirhut college of Agriculture, Dholi, Muzaffarpur Bihar during the study year *i.e.*, 2018. Though RH-414 found susceptible but it was found to produce more yield than a resistant genotype RH-14.

Neem was found most effective at 6% concentration among the plant product under *in-vitro* conditions against *T. maculans* followed by Brahmi.

At 8 % and 10 % concentration each botanical extract exhibit 100 % mycelial growth inhibition.

Rhizome treatment and three foliar spray of Tricyclazole @ 0.1 % found best among all the fungicides treatment taken in study and results in lowest PDI *i.e.*, 26.67 % which results in maximum disease reduction over control (49.99 %) followed by rhizome treatment and two foliar spray of Tricyclazole which exhibit (30.00% PDI) and it also rank second in disease reduction over control (43.74%).

Rhizome treatment and three foliar spray of Tricyclazole gave highest amount of rhizome yield (37.85 t ha^{-1}) which results into maximum rhizome yield increase over control (50.19 %) but rhizome treatment and three foliar spray of Zineb @ 0.25% rank second in terms of rhizome yield (t ha^{-1}). This means that yield trait is not directly governed by single factor, it may be associated with other factor also.

Maximum incremental cost benefit ratio (1:16.34) exhibited by rhizome treatment and three foliar spray of Tricyclazole @ 0.1 % followed by two foliar spray of Tricyclazole @ 0.1% which was (1: 15.60) and rhizome treatment and three foliar spray of Zineb @ 0.25% (1: 13.77) which was statistically at par with each other.

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