Studies on Epidemiology and management of early blight of tomato caused by *Alternaria solani*

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(PLANT PATHOLOGY)

By

RASHMI SINGH

Department of Plant Pathology

Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya,

College of Agriculture, Gwalior (M.P.)

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CERTIFICATE – I

This is to certify that the thesis entitled "Studies on Epidemiology and management of early blight of tomato caused by *Alternaria solani*" submitted in partial fulfillment of the requirements of the degree of **Master of Science in Agriculture** in the **Department of Plant Pathology** of Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior (M.P.) is a record of the bona-fide research work carried out by **Miss. Rashmi Singh**, I.D. No. 18111806 under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee and the Director of Instruction.

No part of the thesis has been submitted for any other degree or diploma or has been published. All the assistance and help received during the course of this investigation have been acknowledged by the scholar.

Place – Gwalior

Date -26.04.2022 Signature

Dr. Rajni Singh Sasode

Chairman of the Advisory Committee

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Chairman	-	(Dr. R. S. Sasode)	.....
Member	-	(Dr. R. K. Pandya)	.....
Member	-	(Dr. P. D. Singh)	.....
Member	-	(Dr. V. B. Singh)	.....

CERTIFICATE – II

Adar:808226996975
I.D. No. 18111806

This is to certify that the thesis entitled "Studies on Epidemiology and management of early blight of tomato caused by *Alternaria solani*" submitted by **Miss. Rashmi Singh**, to the Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior (M.P.) for the degree of **Master of Science in Agriculture** in the **Department of Plant Pathology** has been accepted after evaluation by the external examiner and approved by the Student's Advisory Committee after an oral examination of the same.

Place – Gwalior

Date - Signature

Dr. Rajni Singh Sasode

Chairman of the Advisory Committee

MEMBERS OF THE ADVISORY COMMITTEE

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Member - (Dr. P. D. Singh) .....

Member - (Dr. V. B. Singh) .....

Head of the Department .....

Dean of the College .....

Director of Instruction .....

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Place – Gwalior

Date 26.04.2022 (Miss. Rashmi Singh)

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ABBREVIATIONS

S.N.	Legends	Description
1.	@	At the rate
2.	%	Per cent
3.	0_C	Degree Celsisus
4.	ANOVA	Analysis of variance
5.	C.V.	Coefficient of variation
6.	Cal.	Calculated
7.	CD	Critical difference
8.	Cfu	Colony forming unit
9.	Cm	Centimeter
10.	CRD	Completely Randomized Design
11.	DAT	Day after transplanting
12.	DF	Degree of freedom
13.	EC	Emulsifiable Concentrate
14.	EMS	Error means sum of square
15.	et al.	Allied (and other)
16.	Fig	Figure
17.	G	Gram
18.	i.e.	That is (in reference to)
19.	Kg	Kilogram
20.	mm.	Millimeter
21.	MSS	Mean Sum of Square
22.	No.	Number
23.	PDA	Potato Dextrose Agar
24.	Ppm	Parts Per Million
25.	Q	Quintal
26.	S.N.	Serial number
27.	S.S	Sum of square
28.	S.V.	Source of variance
29.	SE(m)±	Standard error of mean
30.	SL	Suspendable liquid
31.	Spp.& sp.	Species
32.	Tab	Tabulated
33.	t/ha	Tones/hectare
34.	Viz.,	Wide list
35.	WG	Wettable granule
36.	WS	Water Soluble

Chapter - I

INTRODUCTION

Chapter – I

INTRODUCTION

Tomato [*Lycopersicon esculentum* Mill (2n=24)] is considered as “Poor man’s orange” in India. It is the most important and useful member of the family Solanaceae and is grown in tropics as well as subtropics during *Rabi* and *Kharif* season. Tomato is an annual vegetable crop grown over the world is considered as protective food as because of its nutritional value and its wide production (Somappa*et al*., 2013). India ranks second in area and production. In Madhya Pradesh area and production of tomato is 65.72 ha and 1937.37 tons respectively with productivity 29.5 tons/ha (Anonymous, 2014). There are several diseases known in tomato which caused by fungi, bacteria, viruses and nematodes. Early blight of tomato among all fungal diseases is one of the most important disease (Munde *et al.* 2013). It is very destructive in temperate humid climates. Although the disease is called early blight, but can occur on the plant at all stage of development. Early blight can cause decrease in fruit quantity and quality (Kumar and Srivastava, 2013). Epidemics can also occur in semiarid climates where frequent and prolonged nightly dews occur (Roten and Reichert 1964). Epidemic of early blight having coefficient of disease index (CODEX) 71.66% was noticed to cause a remarkable loss up to 78.5% in the yield of tomato (Datar and Mayee, 1981). It is most prevalent and destructive throughout the tomato growing areas causing loss of millions of Dollar annually worldwide including India (Datar and Mayee, 1982). China, India, U.S.A., Italy, Turkey, Mexico, Russian Federation are Japan are major tomato growing countries. China precedes India, United State of America, Turkey, Egypt and Iran in production and productivity. M.P., A.P. Karnataka, Gujrat, Odisha, C.G., W.B., Bihar, Maharstra, T.N., U.P. Haryana and Telangana are major tomato producing states of India. These thirteen states account for about 90% of total production of the country. As per report received from different states total area during 2019-20 sown is 7.87 lakh hectares in the country as against 7.58 lakh hectares of last year while all India expected production in 2019-20 is estimated to be 186.08 lakh tonnes (Anonymous, 2020).

The fruit is a excellent source of nutrients which are vital for human health (Wilcox *et. al.,* 2003). Tomato in large portion is used to prepare soup, juice, ketchup, puree, paste and powder. The pigments lycopene and carotenes are responsible for red and yellow color respectively in tomato fruits. It is stated to be useful against mouth cancer and blood purifier. Dried tomato juice keeps vitamin C. It is one of the best vegetables which keep our stomach and intestinein good order. The caratenoid present in tomato is known to prevent cardiovascular as well as certain types of cancers (Perkins-Veazie*et al.,* 2006).

Tomato crop affected by several biotic stresses and it is estimated that it has increased 30% production cost (Lopes and Santos, 1994). Major economically important fungal diseases of tomato India are early blight (*A. solani*), late blight (*P. infestans*), Sclerotinia rot (*S. sclerotiorum*), Fusarium wilt (*F. oxysporum*), Bacterial leaf spot (*X. campestris*), bacterial wilt (*R. solanacearum*), Tomato yellow leaf curl (*Tomato leaf curl virus*) and Root-knot (*Meloidogyne* sp.)

Alternaria blight is most destructive disease and most serious in warm humid regions. Its cause nearly 78% loss in quality and quantity fruit yield (Sherf and MacNab,1986) and in semi-arid areas where frequent and prolonged night dew (Rotem and Reichert, 1964).

In the present scenario use of chemical based fungicides is the major concern for the environmentalist. So there is need to find our novel approaches which are eco-friendly as well as economically sound for farmers.

In search of effective management of the epidemiological evidence and study of related factors is one of the main reasons to focus on this aspect. The epidemics of Alternaria leaf blight are strongly favored by the environmental condition and causes very much economic losses. Therefore the epidemiological study was taken. The epidemiological studies on Alternaria leaf blight of tomato will give an idea for relationship between weather variables for occurrence of Alternaria leaf blight disease, survival and dissemination of the pathogen in nature. In presently studies will help for

devising forecasting methods and to develop an appropriate model for conformation of the disease. Therefore, keeping this background in view the present studies are as follows:

1. Isolation, purification, identification and pathogenicity of pathogen.
2. Role of weather parameters on development of early blight.
3. *In-vitro* evaluation of botanicals against *Alternaria solani*.
4. *In-vitro* evaluation natural farm product against *Alternaria solani*.
5. *In-vitro* evaluation of fungicides against *Alternaria solani.*
6. *In-vivo* evaluation of botanicals, natural farm products and fungicides against early blight of tomato.

Chapter - II

REVIEW OF LITERATURE

Chapter- II

REVIEW OF LITERATURE

Early blight of tomato caused by *A. solani* is a three-phase disease, which produce leaf spots, stem canker and fruit rot, but the foliar phase is the most common and destructive part of the disease responsible for significant economic losses and it occurs in mild to severe form in different parts of India (Hassanein *et. al*., 2008). It occurs to some extent every year wherever tomatoes are grown. In spite of its name, the disease may occur at any time during the growing season (Vloutoglou and Kalogerakis, 2000). Large numbers of reports are available in literature on different aspects of this disease. However, reviews relevant to the aspects investigated gated have been included here.

2.1. Symptomatology

Datar and Mayee (1981) showed that *A. solani* could attack fruits in the green and ripe stages at the stem end growth cause cracks and other wounds.

Locke (1949) observed that early blight on tomato was characterized by the appearance of brown to dark leathery necrotic spots first on leaflets producing target board effect.

Mayee and Datar (1986) reported that early blight disease of potato was characterized by the appearance of brown to dark brown colour necrotic spots. Appearance of concentric rings inside the spots produced target board effect.

Mathur and Shekhawat (1986) reported that the early blight disease in tomato appears on leaves, stems, petiole, twig and fruits under favourable conditions resulting in defoliation, drying off of twigs and premature fruit drop and thus causing loss from 50 to 86 per cent in fruit yield.

Ramakrishnan *et al*. (1971) observed cankerous spots on tomato stems. They were especially injurious when they occurred at the juncture of the stem and side branches. Collar rot, another symptom on tomato occurred as stem lesions on seedlings at soil line extending above and below that point to form cankers which resulted in girdling of the plants (Basu, 1971 and Mc Carter *et al*., 1976).

Singh (1987) reported that the spots were oval to angular in shape measuring up to 0.3-0.4 cm in diameter and usually with a chlorotic zone around the spot.

Walker (1952) reprted that the spots were oval or angular in shape upto0.3or 0.4 cm diameter and there was usually a narrow chlortic zone around the spot which later faded in to the normal green colour. Older leaves of tomato were affected first as a role and the diseases progressed upwords. Finally the leaves dried up and dropped down.

2.2. Pathogen:

Aruna *et. al.,* (2006) observed the genus *Alternaria* was first recognised by Nees in 1817. In 1836, Berkeley identified the causal fungus on plants belonging to family *Brassicaceae* as *Macrosporium brassicae* Berk, which was later renamed as *Alternaria brassicae* (Berk.) Sacc. Thereafter, Elliot studied the taxonomy of Alternaria in detail. Wiltshire pioneered the basic studies of this group of *Hyphomycetes*. His descriptive literature was fundamental to the prevailing concepts of *Alternaria, Macrosporium* and *Stemphylium*. Later, Neergaard made an extensive study on the taxonomy, parasitism and economic significance of this genus. The morphological variations of *Alternaria* species were described by Joly and later he divided these in three sections and proposed a simple key for identification and determination of the most common species

Bose and Som, (1986) found that early blight of tomato caused by A.soloni was first recoreded in 1882 in New Jersey, USA the mycelium consisted of septet, branched, light brown hyphae, which turned darker with age. The condiphores were short, 50 to 90 um and dark colored. Condia were 120-296 x 12-20um in size, beaked, muriform dark colored and borne singly. However in culture they formed short chains.

Early blight of tomato caused by *Alternaria solani* was first recorded in 1882 in New Jersey, USA (Bose and Som, 1986). The genus *Alternaria* belongs to the sub-division Fungi Imperfecti (Deuteromycotina), class Hyphomycetes, order

Hyphales, family Dematiaceae (Agrios, 2005). Species of the genus are cosmopolitan, surviving both as saprophytes as well as weak parasites. The genus is characterized by the formation of polymorphous conidia either singly or in short or longer chains and provided with cross, longitudinal as well as oblique septa and having longer or short beaks. The spores of these polyphagus fungi occur commonly in the atmosphere and also in soil. An Ascomycete fungus, *Pleospora solani*, has been claimed by Esquivel (1984) as telomorphs (sexual stage) of *A. solani* and placed in the class *Loculoascomycetes* of sub-division *Ascomycotina*, in which sleeper- shaped, muriform ascospores are produced in bitunicate asci.

According to morphological characters and physiologic analysis, *A. solani* belongs to large, long beaked (Simmons, 2000) and characterized by separate conidia borne singly on simple conidiophores (Neergaard, 1945). The conidia of *A. solani* are muriform and beaked (Neergaard 1945; Ellis and Gibson, 1975). Like other members of the genus *Alternaria*, *A. solani* has transverse and longitudinal septate conidia, multinucleate cells, and dark- coloured (melanized) cells (Rotem, 1994) and mycelium consisted of septate, branched, light brown hyphae, which turned darker with age. The conidiophores were short, 50 to 90 µm and dark coloured. Conidia were 120- 296 x 12-20 µm in size, beaked, muriform dark coloured and borne singly. However in culture they formed short chains. According to Singh (1987) the conidia contained 5-10 transverse septa and 1-5 longitudinal septa.

Dhal *et al*. (1997) observed the association of *A. alternata* with blossom end rot of tomatoes for the first time in Orissa, India.

Shahi and Shyam (1993) isolated *A. solani* and *A. alternata* f. sp. *lycopersici* from tomato plants in Himachal Pradesh, India. In laboratory test, A. *alternata* f. sp *lycopersici* produced symptoms on leaflets, stems and branches following inoculation, while *A. solani* produced only on leaflets.

2.3. Host Range

Akhtar *et al.,* (2004) observed *Alternaria alternate* causing leaf blight disease in tomato field at the Nuclear Institute for Agriculture and Biology, Faisalbad,

Pakistan.

Bhatt *et al.* (2000) reported that *Alternaria alternate* showing the leaf blight symptoms on tomato, capscicum and spinach from Kumaon hills of Uttar Pradesh.

Dragomir (1995) reported that the tomato plants are attacked by *Alternaria porri f. sp. solani* in the vidra area, Romania.

Joshi (1981) reported the incidence of early blight in Lab-lab bean caused by *Alternaria solani*.

Subramanian (1954) and Dutta (1979) reported the occurrence of fruit rot of chillies caused by *Alternaria solani.*

Walker (1952) in his book Plant Pathology mentioned that besides potato and tomato, *Solanum aviculare*, *S.carolinensis, S.nigrum*, *Lycopersicon pimpinellifolium* are known to be host of *Alternaria solani*.

2.4. Pathogenicity

Andrus *et al.* (1945) confirmed the pathogencity on tomato by using myelia fragments of *A. solani* as inoculum.

Barksdale (1968) and Dhiman *et al.* (1980) used suspension containing 20000 spores/ml distilled water for proving pathogenicity of early blight of tomato caused by *A. solani.* Further, they atomized the culture suspension on three leaf stage seedling at the rate of 30 ml per seedling for successful inoculation.

Brock (1950) Henning and Alexander (1959) used the suspension of mycelia fragments of *A. solani* to inoculate the leaves of field or green house grown plant.

Locke (1949) used blended mycelial fragments of *A.soloni* for puncture inoculation.

Manjula *et al.* (2004) observed that the scanning electron microscopy of wild beet and cotton leaves infected by an aggressive isolate of Alternaria alternate revelved that condiophores of the pathogen emerged only from necrotic area of leaf tissues. Sporulation occurred on leaves only during periods of high relative humidity

(60% at 22-25^{o}C), mycelium penetrated into internal tissue of the leaf or emerged through the stomata.

Tippeswamy *et al.* (2010) confirmed the pathogenicity of early blight of tomato by spraying 104 condial suspension of *A. solani* to one month seedling (30 days), before flowering (60days) and after flowering (90 days).

2.5. To study the epidemiological aspects of the disease development.

Aruna *et. al.* (2006) reported that the *A. solani* produced maximum growth at 25 to 30 °C temperature followed by 25°C, 20°C, 35°C, 15°C, 40°C, 10°C.

Champawat and Sharma (2009) studied the influence of environmental factors such as temperature, relative humidity and rainfall on the development of Alternaria blight of tomato from Rajasthan. The multiple regression analysis revealed that weather parameters contribute 77 per cent towards disease incidence. Maximum and minimum temperature has positive while maximum and minimum RH have negative significant correlations with appearance of Alternaria blight of tomato. The linear regression coefficients for temperature were positively significant while the both RH was negatively significant.

Chaerani *et al*. (2007) reported that early blight prefers a cool, wet environment and spreads rapidly when conditions alternate between wet and dry.

Murumkar *et al*. (2008) studied the weather in relation to development of Alternaria leaf spot of safflower in Solapur for four years (2004-05, 2005-06, 2006-07 and 2007-08). Rain fall, minimum temperature and relative humidity (minimum and maximum) had a positive correlation with the disease development and rains coupled with high humidity above 80 per cent and temperature in the range of 21 to 33^{0} C favoured the primary infection of *Alternaria carthami*.

Sidlauskiene *et al.* (2003) determined the effect of environmental conditions on the development of *Alternaria* sp. and observed favorable temperatures, 20-30^{0} C for *A. alternata*, 24-30^{0} C for *A. brassicae* and *A. solani*, 24-26^{0} C for *A. brassicicola* and 20-22^{0} C for *A. tenuissima* and *A. cucumerina*.

Under free moisture or near-saturated humidity at a wide range of

temperatures (8°–32°C), conidia germinate to produce one or more germ tubes. These subsequently penetrate the host epidermal cells directly by means of appressoria or they enter through stomata or wounds by hyphal growth (Sherf and MacNab, 1986; Perez and Martinez, 1999; Agrios, 2005).

2.6. In-vitro evaluations of fungicide and plant extracts against *Alternaria solani.*

Aruna *et al.* (2010) reported that propiconazole at 1000 ppm was most effective with 53.51 per cent inhibition of *Alternaria* sp. in tomato.

Aruna *et al.* (2006) observed that among the systemic fungicides evaluated against *A. solani* causing early blight of tomato, propiconazole (84.57%) gave maximum inhibition of the mycelial growth of pathogen. He also reported that among ten plant extracts *Clerodendron inerme* Gaertn leaf extract (57.22%) was found effective inhibiting mycelia growth followed by *Eucalyptus globes* Labill (53.69%), *Eupatorium oduratum* L. (42.98%). Least inhibition was observed in *Glyricidia maculata* L. (28.00%).

Ahmed and Grainage (1982) reported that plant products to be gaining importance in crop protection in view of their selective properties (as insecticides, fungicides and anti-viral), low cost and safely to ecosystem.

Babu *et al.* (2001) found mancozeb (0.2%) followed by captafol (0.2%) were effective against *A. solani* causing tomato leaf blight disease.

Choulwar and Datar (1994) studied the tolerance of *A. solani* (Early blight of tomato) to fungicides like Mancozeb, Captofol, Thiophenate methyl and Carbendazim. These were tested at 1000, 1500, 2000, 2500 ppm *in-vitro*. The results indicated that *A. solani* could tolerate 2500 ppm of all the fungicides tested.

Dushyant *et al.* (2014) evaluated the efficacy of different fungicides against *A. solani* at three different concentrations *viz*., 100, 200 and 300 ppm under laboratory condition. Minimum mycelial growth of *A. solani* was observed in Carbendazim + Mancozeb (8 mm) followed by Mancozeb (10.33 mm) and Iprodione + Carbendazim (12.67 mm) at 300 ppm. Carbendazim + Mancozeb were found most effective among all tested fungicides.

Dubey *et al.* (2000) tested nine fungicides against *Alternaria alternata* causing Alternaria blight of broad bean and reported that blitox-50 inhibited maximum mycelial growth followed by kavach and bavistin.

Kumar and Singh (2017) tested plants extracts and fungicides against *A. solani in vitro*. They found *A. sativum* @ 5% was most effective which exhibited maximum inhibition in mycelium growth (45.15%) followed by *Crotalaria juncea* @ 5% (44.40%) and among fungicides, most effective fungicides was found Hexaconazole 5% EC which exhibited 100.00 percent inhibition in mycelium growth at 100 ppm followed by Thiafluzamide 24% SC at 500ppm.

Koley *et al.* (2015) reported growth inhibitory activity of botanicals against *A. solani* causing early leaf blight of tomato. Aqueous leaf extract of *D. stramonium* was the best followed by *A. indica* oil and *L. camara* leaf extract showing fungus growth inhibition of 57.03%, 51.35% and 48.02%, respectively. The efficiency of the botanicals was significantly at 15% concentration for all the botanicals as compare to lower concentration 5% and 10%.

Maya and Thippanna (2015) tested aqueous extracts of botanicals against *A. solani* using poison food technique. Maximum mycelial inhibition of 80.70% was recorded in Rhizome extracts of *Zingiber officinalea* followed by *Azadirachta indica* (73.64%), *Psidum guajava* (71.75%) and *Pongamia pinnata* (68.92 and least mycelial inhibition (13.74%) was recorded in *Cassia tora*.

Maya and Thippanna (2013) evaluated ten plants extracts were evaluated against *A. solani* by poison food technique. The results revealed that leaf and seed extracts of *Azadirachta indica* recorded maximum mycelial inhibition with 78.83% followed by *Lantana camara* with 59.9% and *Eucalyptus globules* with 59.7%.

Mesta *et al.* (2009) conducted study on evaluation of new molecules of fungicide against causal agent of alternaria blight of tomato. A combi product of iprodione + carbendazim was found most effective which inhibited 58.39 per cent spore germination and 65.18 percent mycelial growth. Systemic fungicides, hexaconazole (63.12 % and 72.87 % respectively) and propiconazole (61.80 and 76.53 %) were most effective.

Nashwa and Abo-Elyousr (2012) evaluated antimicrobial activity of six plant

extracts *viz*. *Ocimum basilicum* (Sweat Basil), *Azadirachta indica* (Neem), *Eucalyptus chamadulonsis* (Eucalyptus), *Datura stramonium* (Jimsonweed), *Nerium oleander* (Oleander) and *Allium sativum* (Garlic) for controlling *Alternaria solani in vitro* and *in vivo*. In *in vitro* condition the leaf extracts of *D. stramonium, A. indica,* and *A. sativum* at 5% concentration caused 44.4, 43.3 and 42.2% reduction of mycelial growth of *A. solani*. Under greenhouse experiments the highest reduction of disease severity and increased the fruit yield was achieved by the extracts of *A. sativum* at 5% concentration 46.1 and 76.2%, respectively as compared to the control.

Nashwa (2011) reported that in *in vitro* study of leaf extracts of *Datura stramonium, Azadirachta indica,* and *Allium sativum* at 5% concentration caused the highest reduction of mycelial growth of *A. solani* (44.4, 43.3 and 42.2%, respectively). Whereas, in greenhouse experiments the highest reduction of disease severity was achieved by the extracts of *Allium sativum* (5%) and *Datura stramonium* (1% and 5%). The greatest reduction of disease severity was achieved by *Allium sativum* at 5% (45.2%) and the smallest reduction was obtained when tomato plants were treated with *Ocimum basilicum* at 1% and 5% (46.1 and 45.2%, respectively).

Patel and Choudhary (2010) evaluated the efficacy of different systemic and contact fungicides against early blight of tomato. Among systemic fungicides, Difeconazole inhibited maximum growth of *A. solani* and in contact fungicides; mancozeb gave highest per cent inhibition.

Prasad and Naik (2003) evaluated fungicides and botanicals against *A. solani* causing early blight tomato *under in vitro* conditions Among the fungicides, iprodione (0.2%) and mancozeb (0.2%) and among botanicals garlic bulb and Prosopis leaf extract were found most effective.

Rani *et al.* (2017) evaluated fungicides and plant extracts against *A. solani*. Among plant extracts, maximum mycelial growth inhibition was exhibited by *D. stramonium* (20%) followed by *L. camara* (20%) and *A. indica* (20%).

Raza *et al.* (2016) evaluated five plant extracts were used *viz*; *A. indica* (Neem), *A. sativum* (Garlic), *P. hysterophorus* (Chatak Chandni), *D. stramonium*

(Datura) *and Eucalyptus camaldulensis* (Safeda) against this disease. Mycelial growth and inhibition percentage of *A. solani* were recorded after 3, 5 and 7 days post application. All the tested plant extracts significantly inhibited the mycelial growth of the pathogen when compared with control. However, among all five tested plant extracts *A. indica* (69.65%) was significantly superior over other treatments followed by *A. sativum* (66.15%), *P. hysterophorus* (59.94%) and *D. stramonium* (49.46%). Least inhibition was observed in *E. camaldulensis* (49.31%).

Rahman *et al.* (2015) evaluated some botanical against *A. porri*. *Adhatoda vasica* extract @ 5% showed the maximum (91.11%) inhibition of mycelial growth of *A. porri* followed by *Azadirachta indica* (60 %) and *Ocimum sanctum* (55.33%).

Sadana and Didwania (2015) studied some plant extracts against *A. solani* under *in vitro* conditions. Fresh aqueous extract of *Eucalyptus obliqua* @ 15% was most effective which exhibited 88 percent inhibition of mycelial growth of *A. solani* strain A1 followed by *Datura stamonium* and *Azadirachta indica.*

Sahu *et al.* (2014) evaluated antifungal activities of 9 plant extracts against *A. solani* causing early blight of tomato. All tested plant extracts produced some antifungal activities, whereas *Azadirachta indica* (neem), *Datura stramonium* (datura) and *Withania somnifera* (ashwagandha) showed significant antifungal activities. The leaf extract of *W. somnifera* was most effective in inhibiting the mycelial growth of *A. solani* (62.56%) followed by *D. stramonium* (34.65%) and *A. indica* (25.27%).

Sharma *et al*. (2007) reported that the leaf extract of neem show maximum inhibition of the radial growth of *A. solani* (43.3% and 26.7% respectively at 0.1% and 0.01%).

Singh *et al.* (1990) reported that Ajoene a compound derived from garlic inhibited spore germination of some fungi including *Alternaria solani*, *Alternaria tenuissima*, *Alternaria triticina, Colletotrichum, Curvularia* and *Fusarium* sp. which cause serious diseases in many important crop plants in India.

Srivastava and Lal (1997) studied fungicidal properties of aqueous leaf extract of *Calotropis procera, Azadirachta indica, Lantana camara* and *Ocimum basilicum* against *Alternaria alternata* under *in vitro* conditions. Leaf extract of

Ocimum basilicum inhibited conidial germination of *Alternaria alternata*.

Verma and Verma, (2010) reported that application of fungicides is the most effective method of Alternaria blight control and found that Tetra methyl thiram disulphide (TMTD), Dithane M-45,Bavistin, Dithane Z-78, Difoltan, Blitox, Captafol and Bordeaux mixture fungicides effectively manage the disease

2.7. Field evaluations of selected fungicide and botanicals for the management of Alternaria blight disease

One of the most effective methods for disease control is the use of fungicides and botaicals. Many workers had done lot of works on management of early blight of tomato through chemicals and botanicals.

Abhinandan *et al*. (2004) evaluated the efficacy of fungicides *viz*., mancozeb @ 0.25%, kavach @ 0.25%, iprodione @ 0.20%, copper oxychloride @ 0.25%, dodine @ 0.3%, propineb @ 0.15%, propiconazole @ 0.05% and penconazole @ 0.05% in controlling the Alternaria leaf blight disease in tomato. Among these mancozeb followed by kavach were found effective in controlling the disease compared to the control.

Babu *et al*. (2000) reported the efficacy of plant extracts, oils and neem products (Neem leaf, neem seed kernel and neem cake) against early blight of tomato in the field trails. Among the plant extracts, *Acacia concinna* pod extract resulted in the lowest percent disease index (23.1%) followed by neem oil (30.9%).

Bharadwaja *et al*. (1995) The fungicide spray schedules and staking taken of by increased the marketable tomato fruit yield by 65.4 and 25.4 per cent when compared to unsprayed control and no staking, respectively.

Brammatta (1993) reported that application of chlorothalonil (0.2%) decreased early blight severity. However, application did not affect the yield of any cultivar, nor were fungicide cultivar interactions detected. Cultivar differed in the extent of defoliation resulting from early blight. Area under disease progress curve in the cultivar celebrity was not affected by fungicide application. Generally, weight, number of fruit harvested and the mean fruit weight raised significantly among

cultivars.

Bhardwaja (1991) reported that sequential application of captofol, mancozeb and copper oxychloride (all at 0.25%) at 40, 55, 70 days after transplanting, increased yield by 50.5% by reducing the incidence of *A. solani.*

Bashi (1979) noticed that application of daconil @ 3 kg/ha twice a week reduced incidence of *A. solani* on potato.

Choulwar and Datar (1992) evaluated nine fungicides i.e. copper oxychloride, zineb, ziram, mancozeb, carbendazim, dithionon, thiophenate methyl, iprodione and captafol against early blight of tomato (*Alternaria solani*) on cultivar pusa ruby. They found mancozeb was the most effective in reducing disease intensity and increasing the yield followed by captofol and zineb.

Choulwar and Datar (1988) reported six early sprays of mancozeb at 0.2% followed by six late and five early sprays gave minimum per cent disease intensity both at pre and postharvest stages with increased yield. They concluded that early sprays were most effective than equal number of late sprays.

Chourasiya *et al.* (2013) studied the effect of certain botanicals against early blight of tomato caused by *A. solani* (Ellis and Martin) under field condition by spraying three times at an interval of 15 days starting from the initiation of the disease. Lowest per cent disease incidence was observed in neem leaf extract (30.66 PDI) followed by garlic bulb extract (32.44 PDI) and Eucalyptus leaf extract (34.07 PDI) were also effective in reducing disease incidence and increasing fruit yield by 170.60, 154.40 and 164.40 q/ha, respectively. The highest cost benefit ratio was obtained with neem leaf extract (1:2.88) fallowed by garlic bulb extract and eucalyptus leaf extract which were promising in obtaining higher returns up to 1:2.79 and 1:2.61, respectively.

Dushyant *et al.* (2014) were tested different fungicides against early blight of tomato. All the fungicides were found to be significantly superior over control in management of the disease. Minimum disease intensity (8.27%) was observed with carbendazim + Mancozeb @ 0.2% conc. followed by with Mancozeb @ 0.2%

(11.47%), Iprodione + Carbendazim @ 0.2% (15.2%).

Datar and Mayee, (1986) and Sinha and Prasad, (1991) reported that Dithane M-45 proved best for controlling early blight of tomato and potato.

Kumar *et al*. (2007) reported that hexaconazole @ 0.05% and azoxytrobin @ 0.2% was very effective in managing early blight of tomato.

Kapsa and Osowski (2003) conducted a study during 1997-2001 to evaluated the efficacy of fungicides *i.e.* mancozeb and chlorothalonil) and mixtures (Zoxamide + Mancozeb) against early blight on potato incited by *Alternaria* sp. Spraying with fungicides control the disease and increased tuber yield ranging from 21.9 to 60.9% for bonin and from 13.0 to 101.9% for stare olesno surveys. The mixture of zoxamide with mancozeb showed the greatest efficacy.

Lodha and Prasad (1975) found that dithane Z-78 very effective against *A. solani* in tomato crop under *in-vivo* condtions.

Mora (1978) obtained best results in controlling early blight of tomato caused by *A. solani* with the application of daconil and difolaton at intervals of 7, 10 and 13 days from the beginning of flowering stage.

Maheswari *et al.* (1991) conducted field trials to test six fungi toxicants to controlling early blight of tomato caused by A. solani. They observed copper oxy chloride (64.7%) was most effective followed by mancozeb (61.7%).

Mathur and Shekhawat (1986) reported that copper oxychloride and mancozeb were most effective against early blight of tomato caused by *A. solani*.

Maeso (1986) evaluated efficacy of fungicides *i,e*, captafol (Difolaton). Chlorothalonil (Daconil) and Fentin acetate (Breston) during 1981-82 for the control of early blight of tomato for two years and recorded good control of the disease by sprays of Chlorothalonil (Daconil), Fentin acetate + maneb (Brome) copper oxychloride + maneb + zineb, copper salt + mancozeb (Trimiltox Forte) and mancozeb alone (Dithane M-45) were the most effective in 1982-83. Different ways of spray of chlorothalonil (Bravo), mancozeb (Dithane M-45) and their mixtures were

tested in 1983-84 and 1984-85. Preventive spray schedules gave the best results for all the compounds tested, while chlorothalonil was also effective after symptom establishment.

Neto and Oliveira (1980) tested seven fungicides against *A. solani.* They found that maneb and propineb were most toxic to pathogen.

Patel and Choudhary (2010) reported the efficacy of foliar spray of contact fungicide mancozeb 75WP (0.2%) against *A. Solani* gave maximum fruit yield (245.30q/ha). among systemic fungicides Difeconazole (0.1%) was effective in Controlling the disease.

Prasad and Naik (2003) evaluated the efficacy of fungicides and botanicals in controlling the early blight disease of tomato caused by *A. solani*. Among the tested fungicides mancozeb treatment gave the highest cost-benefit ratio of 1:11.4 in addition to reducing the disease incidence and among the tested botanicals garlic and neem was also found most effective.

Sallam and Kamal (2012) reported the efficacy of botanical extracts of *Ocimum basilicum* @ 5%, *Azadirachta indica* @ 5%, *Eucalyptus chamadulonsis* @ 5 %, *Datura stramonium* @ 5%, *Nerium oleander* @ 5% and *Allium sativum* @ 5% against early blight disease of Tomato. They recorded all botanicals reducing the disease severity (25.1%-45.2%) and increased the yield (33.33-76.2%) of tomato as compared to control.

Sali *et al*. (2010) reported that two sprays of mancozeb @ 0.3% or propiconazole @ 0.05% at 15 days interval were found effective for reducing disease Intensity of early blight of tomato from 54.32 per cent (control) to 35.27 and 35.32 percent respectively.

Sobolewski and Robak (2004) tested the efficacy of fungicides i.e. Unikat 75 WG (zoxamide (mancozeb), ethaboksam (ethaboxam). MC 72.5 WP (mancozeb) cymoxanil and Acrobat MZ 69 WP, dimethomorphyl (mancozeb) to control late blight (*Phytophthora infestans*) early blight (*A. solani*) in tomato. The organic products grevit 200 SL and crop set, applied alternatively at 7-day intervals with

Acrobat MZ 69 WP gave satisfactory control of the complex fungal and bacterial diseases.

Sattar and Kaseem (1991) studied the effectiveness of fungicides i.e. rovral (Iprodione). dithane M- 45, zineb, topsin M-7 and ridomil 5G against early blight disease of tomato. Among these, iprodione gave the best control and maximum yield was recorded with 5% iprodione treatment.

Sinha and Prasad (1991) tested 7 fungicides in the field over 3 seasons against early blight disease of tomato caused by *A. solani*. They found dithane M-45 (Mancozeb) @ 0.2% most effective among tested fungicides.

Smith and Littrell (1980) suggested the initiation of fungicidal spray programme at the first appearance of symptom or even before for reducing the epidemic.

Tofoli (2003) evaluated the effectiveness of various fungicides for managing early blight (*Alternaria solani*) as well as their effect on tomato fruit yield, following early blight severity in leaf lets and stems; percentage of leaf drop; incidence of healthy, infected and sun-damaged fruits; yield and the percentages of large, medium and small sized fruits were evaluated. The highest levels of disease control, quality and increase in fruit yields were obtained with pyraclostrobin + metiram, fenamidone + chlorothalonil, famoxadone + cymoxanil + Mancozeb, kresoxim-methyl, azoxystrobin, difenconazole, tebuconazole, pyrimethanil, cyprodinil, famoxadone + mancozeb followed by prochloraz, fluazinam, procymidone, iprodione, mancozeb and chlorothalonil.

Chapter - III

MATERIAL AND METHODS

Chapter-III

MATERIALS AND METHODS

The present investigations were carried out on disease intensity, symptomatology, epidemiological effect, *in-vitro* study about natural farm product, plant extract, fungicides, and management aspects. The materials used and methods followed are described below in details.

3.1. Materials:

3.1.1 Glass wares and other experimental equipment

Corning and Borosil made glassware's were used throughout the experimental study. Glassware's were cleaned by washing with detergent and finally rinsed with tap water after that drying glassware's were sterilized in hot air oven at 180°C for 2 hours. The metallic equipment's like forceps, needle and cork borer were sterilized by dipping in alcohol and heating to red hot over flame of a spirit lamp. The surface sterilization of Alternaria plant parts and diseased materials were done by dipping them in 0.1% mercuric chloride solution for one minute and washed in sterilized water for 3 times. The culture media was sterilized in autoclave at 15 lbs pressure per square inch (1.05 kg/cm^2) for 20 minutes. The soil and sand were sterilized at 30 lbs pressure per square inch (3.1 kg/cm^2) for two hours for two consecutive days.

3.1.2 Sterilization

A. Sterilization of glass wares

Corning and Borosil make glassware were used during the investigation. All the glassware were cleaned with detergent and rinsed with tap water 2-3 times. These were air-dried and then kept in hot air oven for sterilization at 180°C for at least 2 hrs. Plastic wares were sterilized by alcohol.

B. Sterilization of inoculating needles, forceps and cork–borer

Clean inoculation needle was sterilized by dipping the loop of needle in spirit and heating over the flame until red-hot. The process was repeated 2-3 times. Forceps and cork-borer were also sterilized in the way of needle.

C. Sterilization of laminar air flow

Prior to the day of inoculation of fungus sample, the laminar air flow was saturated with alcohol vapors. At the time of inoculation the laminar air flow chamber was wiped with 70% alcohol or general spirit. Then only required instruments were kept in the chamber and exposed to UV rays for 15-20 min. All the operation *viz.,* transfer, inoculation etc. were done over a gas burner flame.

D. Culture media

The media was sterilized in an autoclave at 15 lbs pressure (p.s.i) for 15 min. Liquid media sterilized at 10 lbs p.s.i. for 10 min. and process was repeated after 24 hrs. Potato dextrose agar and Potato dextrose broth medium were used in the course of investigation.

Medium	Ingredients	Quantity
Potato dextrose agar	Peeled and sliced potato	200.0 g
	Dextrose	20.0 g
	Agar-agar	20.0 g
	Distilled water	1000 ml
Potato dextrose broth	Peeled and sliced potato	200.0 g
	Dextrose	20.0 g
	Distilled water	1000 ml

E. Sterilization of plant parts

Surface sterilization of plant parts and diseased materials were done by dipping them in 1 per cent sodium hypochloride solution for one minute and washed in sterilized water for three times.

3.1.2. Seeds (tomato genotypes)

The seeds of tomato varieties were obtained from Department of Plant pathology, College of Agriculture, Rajmata Vijayaraje Scindia Krishi Vishwavidhyalaya Gwalior, (M.P.). Seeds of the tomato variety were used for

management against Alternaria blight of tomato.

3.1.3. Experimental site:

All the field experiments were conducted at the experimental field of Department of Plant Pathology, College of Agriculture; Gwalior (Madhya Pradesh) during *Rabi* season 2019-20 and the laboratory work was done in the Department of Plant Pathology, College of Agriculture, Gwalior. (Madhya Pradesh).Gwalior is situated in Northern part of Madhya Pradesh at an elevation of 211.52 meters from mean sea level and lies between latitude and longitude 26º14' North and 78º15' East, respectively. The topography of the experimental field was plain with good irrigation facilities.

3.1.4. Test organism

The pathogen *Alternaria solani* was isolated from Alternaria infected leaf of tomato genotype.

3.2. Methods:

3.2.1. Symptomatology:

Generally, infection and symptoms of *Alternaria solani* are appear on stem, fruits and leaves under different stages of plant growth under varying environmental conditions. The studies were carried out on progressively development of the symptoms under artificial inoculation.

3.2.2. Collection, Isolation, purification and identification of pathogen:

3.2.2.1. Collection

Tomato leaves showing typical Alternaria blight symptoms of dark brown spots with concentric rings surrounded by discoloured tissue were collected from infected field during survey. Diseased samples were brought to laboratory and examined under microscope for preliminary examination and were kept in humid chamber for observing the sporulation on naturally infected tomato leaves of diseased plants.

3.2.2.2. Isolation

For isolation of pathogen, small pieces of the leaves were cut from the diseased portion along with some healthy tissues and surface sterilized with 1%

sodium hypochlorite solution for one minute followed by three consecutive washings with sterilized distilled water. The surface sterilized pieces were transferred to Petri plates containing Potato Dextrose Agar (PDA) and incubated at 25±1ºC. After seven days of incubation, the fungal growth transferred aseptically to PDA slants and purified following Single Spore Technique (Tuite, 1969).

3.2.2.3 Purification and Identification of the pathogen

For purification, spores obtained from culture slants after 10 days incubation at 25±1ºC were suspended in sterilized water. The dilution of suspension was adjusted such that in one loopful 15-20 conidia (spores) could be counted under low power objective of the microscope. One such loopful was mixed with 20 ml of melted and cooled sterilized plain agar medium and poured in sterilized Petridish. After 12 hrs of incubation, the germinating spores were located and marked under microscope with the help of dummy objective and transferred to PDA slants. These were subsequently allowed to grow and sporulate. The culture was further maintained by periodically transfer on PDA slants. Identification of the fungus was made after examination of conida under microscope (under 10X) from mature pure culture stage and Ocular micrometer were used to measure the length, breadth and number septa of conidia.

3.3. Pathogenicity

Tomato plants were sown in sterilized pots, containing sterilized soil + FYM in 3:1 ratio. The pathogenicity of the pathogen was tested under pot conditions by spore suspension technique. Tomato plants were inoculated by spraying with spore suspension of *A. solani*. The spore cum mycelial suspension was prepared in sterilized distilled water and desired spore concentration (1×10^3 spores/ml) was obtained. 50-60 days old tomato plants were inoculated by spraying the spore cum mycelial suspension with the help of an atomizer. The control was also maintained by spraying the sterilized distilled water only. By covering the inoculated plants with polythene bags and spraying sterilized water, high humidity was maintained for 48 hrs. Observations were recorded for disease appearance after 15 days of inoculation. Pathogen was re-isolated from artificially inoculated plants and resulting culture was compared with original one to confirm the identity of the

pathogen. (Neergaard, 1945; Ellis and Gibson, 1975) and morphological characters.

3.4. To study the epidemiological aspects of the disease development.

An experiment was laid out at experimental farm on five different dates to determine the effect of dates of sowing on early blight of tomato .The sowing started from 10th of November, 17th, 24th 31st and dec 7th during 2019-20. The meteorological parameters data on temperature, relative humidity (RH) and rainfall were also recorded separately weekly interval during crop season in year 2019-2020 from the Meteorological Department of College of Agriculture Gwalior. The disease severity of tomato was recorded in the field. Selection-22 variety was planted in 5 different dates/treatments *i.e.* Nov 10, 17, 24, 31, and Dec 07, 2020 at the seven days intervals. Average intensity of the disease was recorded on 30 tagged plants (3 replication, 10 plants per replicate) throughout the season in the field. The disease severity and fruit yield of tomato were correlated with weather parameter amongst weather parameter with PDI were also looked out.

Experiment details

Design: RBD Replications: 3

Treatments: 05 Cultivar: Selection-22

3.5. In-vitro evaluation of fungicides against the *A. solani*

In laboratory experiment, nine fungicides and control were evaluated in laboratory against *Alternaria solani* by poisoned food techniques (Nene and Thapliyal, 1979). Nine fungicides *viz*., Hexaconazole (0.1%), Chlorothalonil (0.3%), Tebuconazole + Trifloxystrobin (0.1%) Propiconazole (0.1%), Azoxystrobin (0.1%), Difenoconazole (0.1%), Tricyclazole (0.1%), and Pyraclostrobin (0.1%), were taken with three replications (Table-4). The required quantity of the fungicides was added in PDA and sterilized in autoclave. Twenty ml of medium was poured in each pre-sterilized petri plate. A seven mm mycelium disc of seven days old culture of *A. solani* was placed on the medium and the plates were kept in upside down position to maintain the growth of pure culture. The medium without fungicides served as control. The radial growth of the fungal colony at 3, 5 and 7 days after inoculation was recorded along with inhibition percentage. Per

cent growth inhibition was calculated by Vincent's (1947) formula.

$$\text{Per cent inhibition} = \frac{C - T}{C} 100$$

Where,

C = diameter of the colony in check (average of both diagonals)

T = diameter of colony in treatment (average of both diagonals)

Experimental details

Design – CRD

Treatment - 10

Replication – 03

Table-1: List of fungicides.

S.No	Fungicides	Trade Name	Concentration (%)
1	Hexaconazole 5% EC	SITARA	0.1
2	Trifloxystrobin 50% + Tebuconazole 25% w/w WG (75 WG)	NATIVO	0.1
3	Chlorothalonil 75% WP	KAVACH	0.3
4	Propineb 70% WP	ANTRACOL	0.3
5	Azoxystrobin 25% SC	AMISTAR	0.1
6	Difenoconazole 25% EC	SCORE	0.1
7	Propiconazole 25% EC	TILT	0.1
8	Pyraclostrobin 20% WG	HEADER	0.1

3.6. *In-vitro* evaluation of botanicals against *A. solani*.

Eleven botanicals including control were evaluated in laboratory against *Alternaria solani* by poisoned food techniques (Nene and thapliyal, 1979). The details of the botanicals used in the present study are summarized in table-5.

Twenty per cent concentration was used for each botanical. Three replications were kept for each concentration. Required quantity of the botanicals was added in PDA and sterilized in autoclave. Twenty ml of medium was poured in each pre- sterilized petri plate. a seven mm mycelium disc of seven days old culture of *Alternaria solani* was placed on the medium and the plates were kept in upside down position to maintain. The medium without botanical served as control. The radial growth of the fungal colony at 3, 5 and 7 days after inoculation was recorded along with inhibition percentage. Per cent growth inhibition was calculated by Vincent's (1947) formula

$$\text{Per cent inhibition} = \frac{C - T}{C} 100$$

Experimental Details

Design –CRD

Treatment- 11

Replication -03

Table-2: List of Botanicals

S.No.	Botanical name	Plant part used	Concentration (%)
1.	*Ipomoea carnea*	leaves	20
2.	*Cynodon dactylon*	Leaves	20
3.	*Moringa oleifera*	Leaves	20
4.	*Curcuma longa*	Leaves	20
5.	*Annona reticulate*	Leaves	20
6.	*Turanta erecta*	Leaves	20
7.	*Aegle marmelos*	Leaves	20
8.	*Saraca asoca*	Leaves	20
9.	*Mentha*	Leaves	20
10.	*Allium sativum*	Bulb	20

3.6.1 Collection of botanicals:

All the plant products were collected from College of Agriculture, Gwalior Campus, *Ipomoea carnea*, *Cynodon dactylon*, Garlic, *Moringa oleifera*, *Turanta erecta*, *Saraca asoca*, *Aegle marmelos*, *Mentha*, Periwinkle and *Annona reticulate*.

3.6.2. Preparation of plant extract:

For the preparation of leaves crude extract, the botanicals leaves/rhizome /seeds were thoroughly washed in ordinary tap water .The leaves, rhizome and bulb were cut into small pieces then grinded and sieves (100 mesh) and collected into conical flask for further use of investigation. The crude was used @ 20 % solution of the respective botanical powder was incorporated into 80 ml Potato sucrose agar medium and pinch of streptomycin sulphate was mixed just before pouring.

3.7. *In-vitro* evaluation of natural farm product against *A. solani*.

Six natural farm products *viz.,* compost Tea, compost sat, Jiwamrit, Vermiwash, Cow urine and cow milk were evaluated @ 5 % concentration against *A. solani in-vitro.* The animal products were sterilized in conical flasks at 121^0 C and 15 lb pressure per square inch in autoclave for 20 minutes (Table-03). The required quantity of animal products was added to melted PDA medium, mixed thoroughly and poured into sterilized Petri plates and allowed to solidify. After solidification, each plate was inoculated with a 7 mm disc obtained from 10 days old actively growing culture of *A. solani*. These Petri dishes were incubated at 28 ± 2°C. The data was recorded after 7^{th} day of inoculation. The experiment was conducted in CRD with four replications. The inhibition percentage was calculated by measuring the mycelial growth in the animal product amended petri plate and in control. Per cent growth inhibition was calculated by Vincent's (1947) formula

$$\text{Per cent growth inhibition} = \frac{C - T}{C} 100$$

Experimental Details

Design –CRD

Treatment- 07

Replication -04

Table-3: List of natural farm products

S.No	Name of farm products	Method of preparation
1	Jivamrit	Mix all the ingredient (cow urine, cow dung, jaggary, pulse flour, fertile soil and water) in a drum with the help of a wooden stick. Shake the mixture 2-3 times per day regularly for 5-7 days for proper fermentation.
2	Vermivash	Put somedry grass and a 15-20 cm layer of 2-3 weeks old cow dung alongwith 100-200 earthworms in a pitcher. Put on the cow dung and again covered with dry grass. Allow the water to fall drop by drop into the pitcher. Collect the liquid coming from the pitcher with the help of a pipe.
3	Compost tea	A small gunny bag half filled with vermicompost is hanged over a water tub/bucket filled ¾ with water in a way that vermicompost remained submerged in water. The nutrients in the vermicompost get dissolved in water within 24 hours,thus making its colour like tea
4	Cow urine	Mix fresh cow urine and water, allow it to ferment for 15 days and then use.
5	Cow dung extract	Mix all the three ingredients (butter milk, water, cow urine) in a pot and allow to ferment for 15 days.

3.8. Field evaluation of selected fungicides and botanicals for the management of Alternaria blight.

The management of *Alternaria solani* through under natural field condition

after *in-vitro* studies the effective treatments including three botanical, three natural farm product and three chemicals were selected along with control for evaluation of their efficacy by foliar spray against Alternaria blight of tomato. Experiment was conducted in the field during *rabi* seasons of the year 2019-2020. The seeds of selection-22 cultivar were sown in plots and replicated thrice. The first spray was done given just after the appearance of the disease and subsequent two spray were given at an interval of 15 days. Standard agronomical practices were followed as per recommendations. Observations on disease intensity were recorded after 15 days of last spraying. Thirty five days after transplanting plants were sprayed with respective plant extracts and fungicides and second spray was applied at 50 days after transplanting. The per cent disease intensity recorded after 15 days of last spray of plant extracts and fungicides. The PDI of Alternaria blight was recorded using formula.

$$PDI = \frac{\text{Sum of all numerical rating}}{\text{Total no of observation x Maximum grade}} \times 100$$

Experiment details

Design: RBD Replications: 3 Treatments: 10

Treatments details

(1) *Allium sativum* @ 20%

(2) *Annona reticulate* @ 20%

(3) *Turanta erecta* @ 20%

(4) Cow urine @ 10%

(5) Compost tea @ 10%

(6) Jivamrit @ 10%

(7) Azoxystrobin @ 0.1%

(8) Hexaconazole @ 0.1%

(9) Difencozole @ 0.1%

(10) Control

Chapter - IV

RESULTS

Chapter-IV

RESULTS

Experiments were conducted according to the plan of work with suitable statistical design and standard methods. The observations were recorded as per time schedule and proper rating scale was followed for the research entitled “Studies on Early blight of tomato (*Lycopersicon esculentum* Mill.) incited by *Alternaria solani* (Ellis and Martin) Jones and Grout” Therefore, keeping this background in view the present studies are proposed with the following objectives:

1. Isolation, purification, identification and pathogenicity of pathogen.
2. Role of weather parameters on development of early blight.
3. *In-vitro* evaluation of botanicals against *Alternaria solani*.
4. *In-vitro* evaluation natural farm product against *Alternaria solani*.
5. *In-vitro* evaluation of fungicides against *Alternaria solani.*
6. *In-vivo* evaluation of botanicals, natural farm products and fungicides against early blight of tomato.

4.1. Symptomatology

Symptoms on affected plants started with yellowing and browning of the lower leaves, often developed from the leaf tips and along the margins of the leaf petiole. Circular or angular pale-brown spots appear on the leaves. Later on, lesions enlarged, coalesced and gave blighted appearance. Concentric rings with dark layers of spores were also observed on leaves and rotted fruits under moist conditions on blighted plants giving the typical “target board effect” or bull-eye” appearance. This is the most characteristic symptoms of early blight. In wet weather and severe infection, affected areas extended and form big rotting patches which get shriveled and covered petiole & stems also (Plate-1).

Initial symptoms

“Target board” symptoms

Symptoms on stem

Symptoms on fruit

Plate-1: Symptoms of early blight of tomato

4.2. Isolation, purification, identification and pathogenicity of pathogen

4.2.1. Isolation, purification, identification of the pathogen

Disease specimens were collected from fields of tomato. It is clear from the literature that PDA is most suitable medium to isolate and grow *Alterneria solani*, hence infected portion of leaf were taken from isolation under aseptic condition. The fungus emerged from infected bits on PDA medium and observed black mycelial growth at initially and later stage gradually converted into light black to dark black colour with fluffy growth. The pure culture of the fungus was prepared by single hyphal tip culture technique and maintained on PDA medium after incubation. The fungal growth Completely covered the petri dish with 7 days of incubation at 25 $\pm$ 1^0C temperature Identification of the fungus was carried out based on the morphological characters of the isolated fungus. The description of the fungus isolated is as follows. The conidiophores were formed singly or in groups, straight or flexuous brown to olivaceous brown. The conidia were solitary straight or muriform or ellipsoidal tapering to beak, pale or olivaceous brown, length 150-300 µm and 15-20 µm thick in the broadest part with 8- 10 transverse and 0-4 longitudinal septa. The beaks were flexuous, pale and sometimes branched. The description of this fungus agreed with the description given for *Alternaria solani* by Common Wealth Mycological institute, Kew, Surrey, England (Ellis, 1971). Thus, the pathogen causing early blight of tomato has been identified as *Alternaria solani* (Ellis and Martin) Jones and Grout. (Plate-2).

Identification of the fungus was carried out based on the morphological characters of the isolated fungus. The description of the fungus isolated is as follows. The conidiophores were formed singly or in groups, straight or flexuous brown to olivaceous brown. The conidia were solitary straight or muriform or ellipsoidal tapering to beak, pale or olivaceous brown, length 150-300 µm and 15-20 µm thick in the broadest part with 8- 10 transverse and 0-4 longitudinal septa. The beaks were flexuous, pale and sometimes branched. The description of this fungus agreed with the description given for *Alternaria solani* by Common Wealth Mycological institute, Kew, Surrey, England (Ellis,

1971). Thus, the pathogen causing early blight of tomato has been identified as *Alternaria solani* (Ellis and Martin) Jones and Grout. (Plate-2).

Plate-2: Pure culture and conidial structure of *Alternaria solani*.

4.2.2. Pathogenicity test

The pathogenicity test of the pathogen was tested under pot conditions by spore suspension technique. Tomato selection-22 variety plants were inoculated by spraying with spore suspension of isolated pathogen. The typical disease symptoms were observed and reisolation culture of *Alternaria solani.*

4.3. Role of weather parameters on development of early blight.

Early blight local susceptible cultivar was planted in five dates during *Rabi* 2019-20 and observation were recorded at weekly intervals, simultaneously the meteorological data were also recorded thereafter the correlation and regression studies were carried out in between the individual meteorological parameter and percent disease intensity of Alternaria blight. Three effective environmental factors *viz.,* temperature, relative humidity and rainfall are responsible for the development of Alternaria blight of tomato was studied in *rabi* 2019-20 (Table-4). In general, the cumulative per cent disease intensity was higher in the first date of sowing.

Correlation analysis of cumulative per cent disease intensity with weather parameters was indicated that maximum temperature (higher than 21.7 ^{0}C) and minimum temperature (less than 6.60^{0}C) and had positive correlation (0.6380*), relative humidity had negative and significant correlation and rainfall had no correlation during *rabi* 2019-20.

The analysis of all the five independent variables individually and in combinations best revealed that each weather factor played an important role in disease development in addition to other unknown factors.

Table-4. Influence of meteorological parameters on percent disease intensity of Alternaria blight of tomato during *Rabi* 2019-20.

Meteorological weeks	Duration	2019-20										
		Temperature (^{0}C)		Relative Humidity (%)		Rainfall (mm)	Per cent Disease intensity at different dates of sowing					
		Max.	Min.	Max.	Min.		10 Nov	17 Nov	24 Nov	31 Nov	07 Dec	Mean
45th	Nov.5-11	30.8	14.0	88.9	37.3	000.0	0.00	0.00	0.00	0.00	0.00	0.0
46th	Nov.12-18	29.7	14.7	84.4	43.1	000.0	0.00	0.00	0.00	0.00	0.00	0.0
47th	Nov. 19-25	28.5	11.0	93.3	41.7	000.0	4.37	0.00	0.00	0.00	0.00	0.87
48th	Nov-Dec.26-2	27.1	12.4	92.0	52.0	000.0	6.33	3.68	0.00	0.00	0.00	2.00
49th	Dec.3-9	24.0	6.7	94.7	43.3	000.0	10.45	7.85	3.50	0.00	0.00	4.36
50th	Dec.10-16	21.3	9.5	95.9	62.3	002.1	19.67	13.23	6.78	3.26	0.0	8.59
51th	Dec.17-23	19.2	4.9	94.4	54.7	000.0	29.38	21.29	10.53	6.27	2.97	14.09
52th	Dec.24-31	13.5	2.3	96.6	75.9	000.0	38.73	23.97	18.76	10.88	4.69	19.41
1th	January 1-7	21.7	6.6	96.3	59.0	000.0	53.56	49.33	23.33	20.77	18.57	33.11
2nd	January 8-14	20.4	5.6	91.3	72.6	011.2	56.21	52.11	43.87	40.69	35.53	45.68
3th	January 15-21	18.3	7.6	96.1	70.7	040.4	49.53	38.66	48.33	46.43	48.47	46.28
4th	January 22-28	22.8	5.2	88.4	45.6	000.0	38.47	33.5	48.23	49.31	45.37	42.98

Table-05: Correlation coefficient of disease intensity with major environmental factors.

Weather variables		Correlation coefficient (r)
Temperature	Maximum	0.6380
	Minimum	0.5412
Relative humidity	Maximum	0.2037
	Minimum	0.3301
Rainfall		0.1262

4.4. In-vitro evaluation of botanicals against *Alternaria solani.*

The botanicals *viz*., *Ipomoea carnea*, *Cynodon dactylon*, Garlic, *Moringa oleifera*, *Turanta erecta*, *Saraca asoca*, *Aegle marmelos*, *Mentha*, and *Annona reticulate* were evaluated in the form of crude extract @ 20% against *A.solani* under *in–vitro* condition. The fungal growth was recorded at 3, 5 and 7 days after inoculation and the data is summarized in table-6, plate-3 and Fig- 2 & 3**.** The data indicate that all the treatments were significantly superior over control.

At 3 days after inoculation, all the treatments were found significantly superior over control. Minimum mycelial growth was recored in treatment *Allium sativum* (18.33 mm), followed by *Curcuma longa* (23.33 mm), *Annona reticulate* (23.67 mm), *Turanta erecta* (26.33 mm), *Moringa oleifera* (29.67 mm), *Ipomoea carnea* (31.33 mm), *Mentha* (33.33 mm), *Saraca asoca* (48.33 mm), *Aegle marmelos* (52.67 mm), and *Cynodon dactylon* (60.67 mm), while maximum mycelial growth was recored in treatment control (65.67 mm).

At 5 days after inoculation, all the treatments were found significantly superior to control. Significantly less mycelial growth was recorded in *Allium sativum* (23.33 mm) followed by *Annona reticulate* (30.33 mm), *Curcuma longa* (32.67 mm), *Turanta erecta* (34.67 mm), *Ipomoea carnea* (37.67 mm), *Moringa oleifera* (38.67 mm), *Mentha* (43.33 mm), *Saraca asoca* (61.33 mm), *Aegle*

marmelos (63.33 mm), and *Cynodon dactylon* (73.67 mm), while maximum mycelial growth was recored in treatment control (79.00 mm).

At 7 days after inoculation *Allium sativum* (35.67 mm) was most effective followed by *Curcuma longa* (39.67 mm), *Annona reticulate* (41.67 mm), *Turanta erecta* (43.67 mm), *Ipomoea carnea* (49.67 mm), *Moringa oleifera* (52.33 mm), *Mentha* (53.33 mm), *Saraca asoca* (70.33 mm), *Aegle marmelos* (78.33 mm), and *Cynodon dactylon* (80.33 mm), while maximum mycelial growth was recored in treatment control (90.00 mm).

Table -6: Effect of different botanical extracts on mycelial growth of *A.solani.*

Botanical extracts	Conc. (%)	Radial mycelial growth (mm)		
		3 DAI	5 DAI	7 DAI
Ipomoea carnea	20	31.33	37.67	49.67
Cynodon dactylon	20	60.67	73.67	80.33
Moringa oleifera	20	29.67	38.67	52.33
Curcuma longa	20	23.33	32.67	39.67
Annona reticulate	20	23.67	30.33	41.67
Turanta erecta	20	26.33	34.67	43.67
Aegle marmelos	20	52.67	63.33	78.33
Saraca asoca	20	48.33	61.33	70.33
Mentha	20	33.33	43.33	53.33
Allium sativum	20	18.33	23.33	35.67
Control	-	65.67	79.00	90.00
SEm+		**1.86**	**1.54**	**1.63**
CD @ 5 %		**5.50**	**4.56**	**4.82**

*Mean of three replication

4.4.1. Percent reduction through botanicals in inhibiting the mycelial growth of *A.solani* under *in-vitro* condition.

At 3 days after inoculation, all the treatments were found significantly superior over control. Maximum inhibition of mycelial growth was recored in treatment *Allium sativum* (72.08 %), followed by *Curcuma longa* (64.47%), *Annona reticulate* (63.95 %), *Turanta erecta* (59.90%), *Moringa oleifera* (54.81%), *Ipomoea carnea* (52.26 %), *Mentha* (49.24 %), *Saraca asoca* (26.40 %), *Aegle marmelos* (19.79 %), and *Cynodon dactylon* (7.61 %), while minimum percent inhibition was recored in treatment control (0.00%).

At 5 days after inoculation, all the treatments were found significantly superior over control. Maximum inhibition of mycelial growth was recored in treatment *Allium sativum* (70.46 %) followed by *Annona reticulate* (61.60 %), *Turanta erecta* (59.90%), *Curcuma longa* (58.64 %), *Ipomoea carnea* (52.31 %), *Moringa oleifera* (51.05 %), , *Mentha* (45.15 %), *Saraca asoca* (22.36 %), *Aegle marmelos* (19.83 %), and *Cynodon dactylon* (6.74 %), while minimum percent inhibition was recored in treatment control (0.00%).

At 7 days after inoculation maximum percent inhibition *Allium sativum* (60.36 %) was recorded and its was most effective followed by *Curcuma longa* (55.92 %), *Annona reticulate* (53.70 %), *Turanta erecta* (51.47 %), *Ipomoea carnea* (44.81 %), *Moringa oleifera* (41.85 %), *Mentha* (40.74 %), *Saraca asoca* (21.85 %), *Aegle marmelos* (12.96 %), and *Cynodon dactylon* (10.74 %), while minimum percent inhibition was recored in treatment control (0.00%).

Table -7: Effect of different botanical extracts on inhibition of *A. solani.*

Botanical extracts	Conc. (%)	Inhibition percent		
		3 DAI	5 DAI	7 DAI
Ipomoea carnea	20	52.26	52.31	44.81
Cynodon dactylon	20	7.61	6.74	10.74
Moringa oleifera	20	52.82	51.05	41.85
Curcuma longa	20	64.47	58.64	55.92
Annona reticulate	20	63.95	61.60	53.70
Turanta erecta	20	59.90	56.11	51.47
Aegle marmelos	20	19.79	19.83	12.96
Saraca asoca	20	26.40	22.36	21.85
Mentha	20	49.24	45.15	40.74
Allium sativum	20	72.08	70.46	60.36
Control	-			

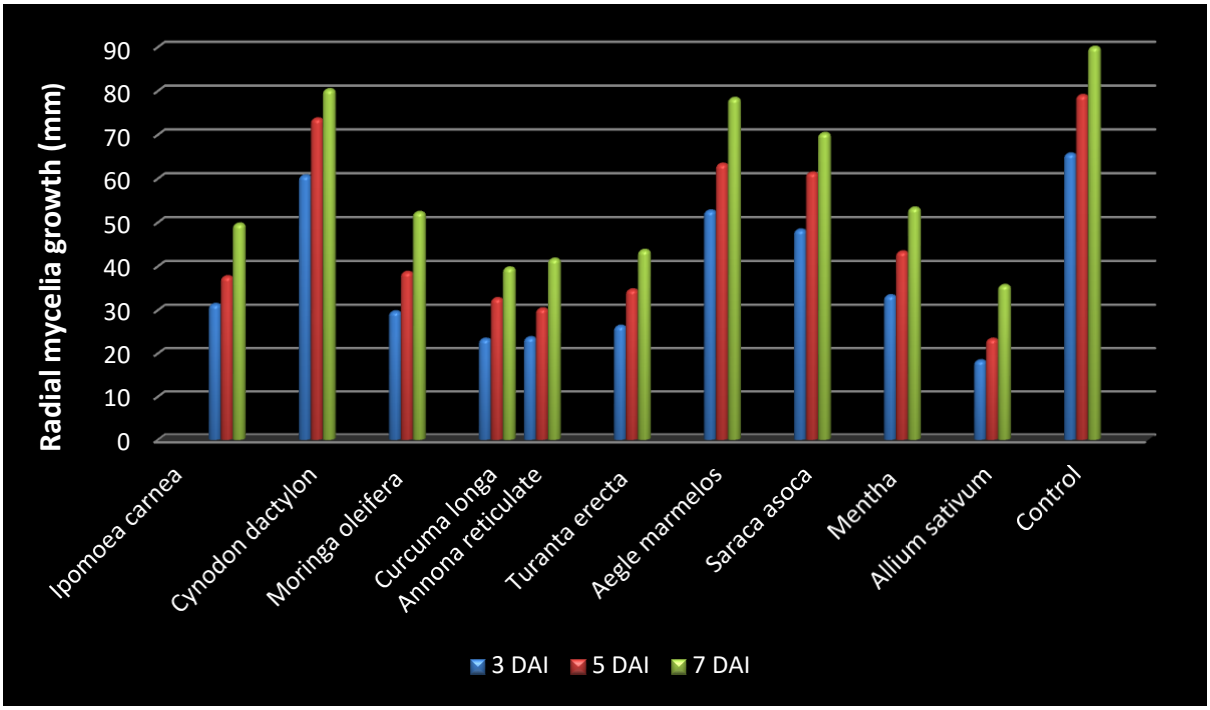

Fig- 1. *In-vitro* evaluation of botanicals on mycelial growth of *A. solani.*

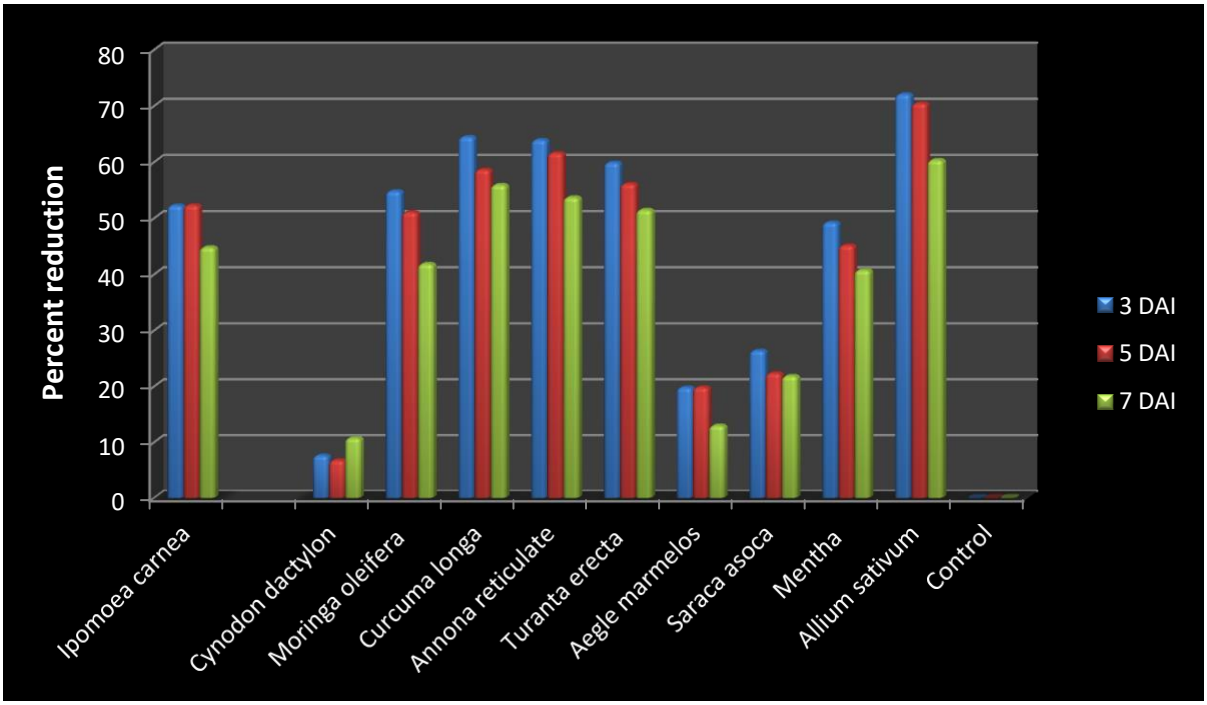

Fig-2. *In-vitro* percent reduction of mycelium growth over control

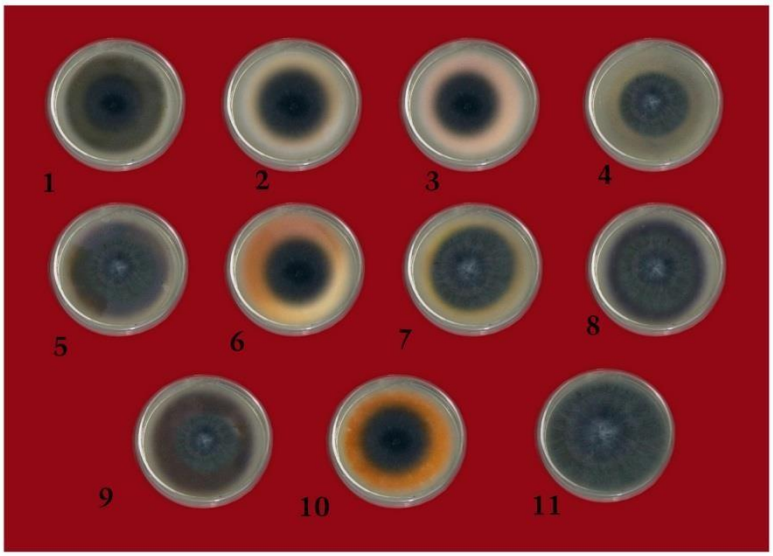

Plate.3-*In-vitro* evaluation of botanicals aganist *A.solani.*

1. Ipomoea carnea
2. Cynodon dactylon
3. Moringa oleifera
4. Curuma longa
5. Annona reticulate
6. Turanta erecta
7. Aegle marmelos
8. Saraca asoca
9. Mentha arvensis
10. Allium sativum
11. Control

4.5. *In-vitro* evaluation natural farm product against *Alternaria solani*.

Six natural farm product *viz*., Compost tea, Compost sat, Jivaamrit, Vermi wash, Cow urine, and Cow milk were evaluated in the form of crude extract @ 10% against *A. solani* under *in–vitro* condition. The fungal growth was recorded at 3, 5 and 7 days after inoculation and the data is summarized in table-8, plate-4 and Fig- 4 & 5**.** The data indicate that all the treatments were significantly superior over control.

At 3 DAI (days after inoculation), all the treatments were found significantly superior to control. Minimum mycelium growth was recorded in treatment cow urine (15.25 mm), followed by Compost sat (18.50 mm), Compost tea (19.00 mm), Jivaamrit (20.50 mm), Cow milk (21.50 mm) and Vermiwash (27.50 mm), while maximum mycelium growth was recorded in control (53.00 mm).

At 5 DAI (days after inoculation), all the treatments were found significantly superior to control. Minimum mycelium growth was recorded in treatment cow urine (25.00 mm), followed by Compost tea (31.50 mm), Compost sat (32.00 mm), Cow milk (33.25 mm), Jivaamrit (38.50 mm), and Vermiwash (41.25 mm), while maximum mycelium growth was recorded in control (75.50 mm).

At 7 DAI (days after inoculation), all the treatments were found significantly superior to control. Minimum mycelium growth was recorded in treatment cow urine (35.00 mm) was most effective followed by Compost tea (42.00 mm), Jivaamrit (45.25 mm), Compost sat (49.25 mm), Cow milk (51.00 mm), and Vermiwash (52.25 mm), while maximum mycelium growth was recorded in control (90.00 mm).

Table -8: Effect of different natural farm product on radial growth of *A. solani in-vitro.*

Natural farm product	Fungal mycelia growth in (mm)		
	3 DAI	**5 DAI**	**7 DAI**
Compost tea	19.00	31.50	42.00
Compost sat	18.50	32.00	49.25
Jivaamrit	20.50	38.50	45.25
Vermiwash	27.50	41.25	52.25
Cow urine	15.25	25.00	35.00
Cow milk	21.50	33.25	51.00
Control	53.00	75.50	90.00
SeM±	**1.84**	**1.38**	**1.35**
C.D. @ 5 %	**5.47**	**4.09**	**4.01**

*Mean of four replication

4.5.1. Percent reduction through natural farm product in inhibiting the mycelial growth of *A.solani* under *in-vitro* condition:

At 3 DAI (days after inoculation), all the treatments were found significantly superior to control. Maximum percent inhibition was recorded in treatment cow urine (71.22 %), followed by Compost sat (65.09 %), Compost tea (64.15 %), Jivaamrit (61.32 %), Cow milk (59.43 %) and Vermiwash (48.11 %), while minimum percent inhibition was recorded in control (0.00 mm).

At 5 DAI (days after inoculation), all the treatments were found significantly superior to control. Maximum percent inhibition was recorded in treatment cow urine (67.33 %), followed by %), Compost tea (58.27 %),

Compost sat (57.61 %), Cow milk (55.96 %), Jivaamrit (49.00 %), and Vermiwash (45.36 %), while minimum percent inhibition was recoderd in control (0.00 mm).

At 7 DAI (days after inoculation), all the treatments were found significantly superior to control. Maximum percent inhibition was recorded in treatment cow urine (61.11 %), followed by Compost tea (53.33 %), Jivaamrit (49.72 %), Compost sat (45.27 %), Cow milk (43.00 %), and Vermiwash (41.94 %), while minimum percent inhibition was recoderd in control (0.00 mm).

Table -9: Effect of different natural farm product on percent inhibition of *A. solani in-vitro.*

Natural farm product	Fungal mycelia growth in (mm)		
	3 DAI	**5 DAI**	**7 DAI**
Compost tea	64.15	58.27	53.33
Compost sat	65.09	57.61	45.27
Jivaamrit	61.32	49.00	49.72
Vermiwash	48.11	45.36	41.94
Cow urine	71.22	67.33	61.11
Cow milk	59.43	55.96	43.33
Control	-	-	-

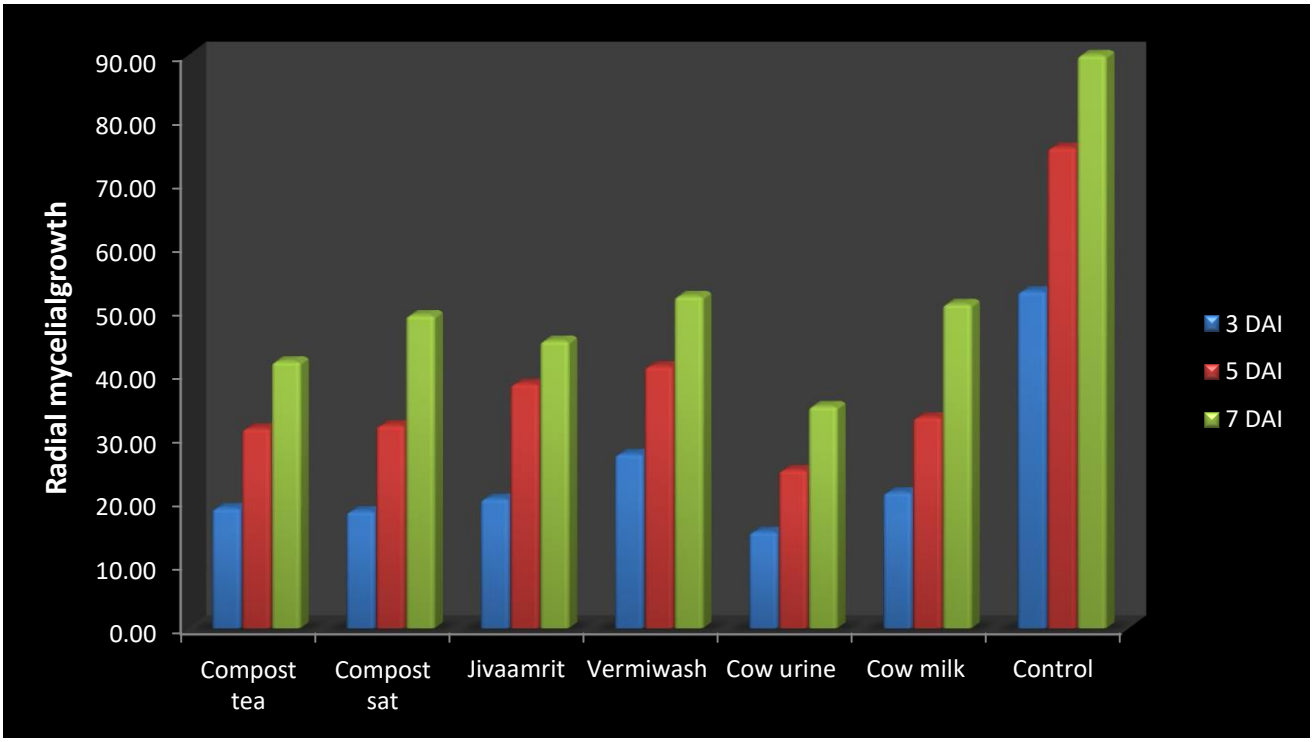

Fig-3. *In-vitro* evaluation of natural farm product on mycelial growth of *A. solani.*

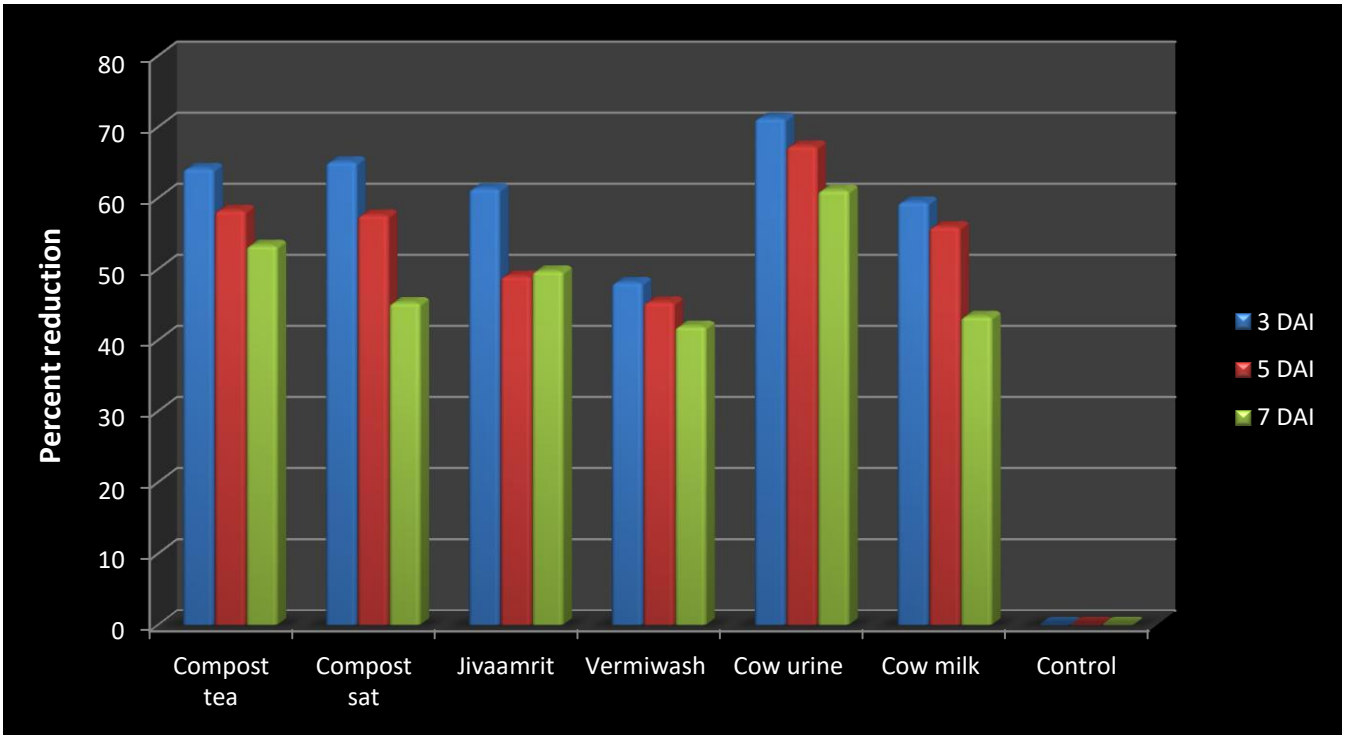

Fig-4. *In-vitro* percent reduction of mycelium growth over control

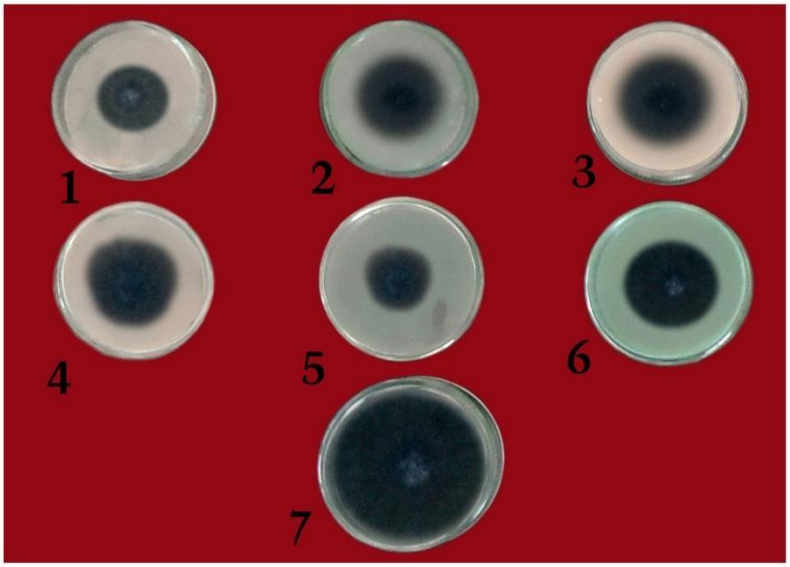

Plate.4- *In-vitro* evalution of natural farm product on mycelial growth of *A. solani*

1. **Compost tea**
2. **Compost sat**
3. **Jivaamrit**
4. **Vermiwash**
5. **Cow urine**
6. **Cow milk**
7. **Control**

4.6. In-vitro evaluation of fungicides against *A. solani.*

Eight fungicides were evaluated against *Alternaria solani* under laboratory condition by following poison food technique. The data on mycelial growth of test fungus against the respective treatments were summarized in table-10, plate-5, fig- 6 & 7

At 3 DAI (days after inoculation), all the treatments were found significantly superior to control. In treatment Hexaconazole, Difenoconazole, Tebuconazole + Trifloxystrobin and azoxystrobin no mycelium growth were found. Minimum mycelial growth was recorded in Chlorothalonil (6.67 mm), followed by Propineb (14.33 mm), Propiconazole (16.67 mm), Pyraclostrobin (26.67 mm), while maximum mycelail growth (56.00 mm) was observed with control.

At 5 DAI, all the treatments were found significantly superior to control. No growth was observed in Hexaconazole, Difenoconazole and Azoxystrobin. Minimum mycelia growth was recorded in Tebuconazole + Trifloxystrobin (5.67 mm) followed by Chlrothalonil (12.67 mm), Propineb (23.67 mm), Propiconazole (29.33 mm) and Pyraclostrobin (36.33mm), whereas maximum mycelail growth (78.00 mm) was observed with control.

At 7 DAI, all the treatments were found significantly superior over control. No growth was observed in Hexaconazole and Azoxystrobin. Minimum mycelial growth was recorded in Difenoconazole (5.67 mm) followed by Trifloxystrobin 50% + Tebuconazole (6.33 mm), Chlorothalonil (26.00 mm), Propineb (38.00 mm), Propiconazole (37.33), Pyraclostrobin (54.67 mm), while maximum mycelail growth was recorded in treatment control (90.00 mm).

Table -10: Effect of different fungicides on radial growth and inhibition of *A. solani in-vitro*

Fungicides	Conc (%)	Mycelia growth in (mm)		
		3 DAI	5 DAI	7 DAI
Hexaconazole 5% EC	0.1	0.00	0.00	0.00
Trifloxystrobin 50% + Tebuconazole 25%	0.1	0.00	5.67	6.33
Chlorothalonil 75% WP	0.3	6.67	12.67	26.00
Propineb 70% WP	0.3	14.33	23.67	38.00
Azoxystrobin 25% SC	0.1	0.00	0.00	0.00
Difenoconazole 25% EC	0.1	0.00	0.00	5.67
Propiconazole 25% EC	0.1	16.67	29.33	37.33
Pyraclostrobin 20% WG	0.1	26.67	36.33	54.67
Control	-	56.00	78.00	90.00
SEM±		**1.96**	**1.14**	**1.41**
C.D. @ 5 %		**5.87**	**3.14**	**4.23**

*Mean of three replication

4.6.1. Percent reduction through fungicides in inhibiting the mycelial growth of *A.solani* under *in-vitro* condition:

At 3 DAI all the treatments were found significantly superior to control. In treatment hexaconazole, Difenoconazole, Tebuconazole + Trifloxystrobin and Azoxystrobin no mycelium growth were found or maximum percent inhibition was recorded (100.00%) followed by Chlorothalonil (88.08%), Propineb (74.41 %), Propiconazole (70.23 %), Pyraclostrobin (52.97 %), while minimum percent inhibition (0.00 %) was observed with control.

At 5 DAI, all the treatments were found significantly superior to control. Maximum percent inhibition was observed in Hexaconazole, Difenoconazole and

Azoxystrobin followed by Tebuconazole + Trifloxystrobin (92.73%), chlrothalonil (83.75 %), Propineb (69.65 %), Propiconazole (62.39 %) and Pyraclostrobin (53.42 %), while minimum percent inhibition was observed with control.

At 7 DAI, all the treatments were found significantly superior over control. Maximum percent inhibition was observed in Hexaconazole and Azoxystrobin followed by Difenoconazole (93.70 %), Trifloxystrobin 50% + Tebuconazole (92.96 %), Chlorothalonil (71.11 %), Propineb (57.77 %), Propiconazole (58.52 %), Pyraclostrobin (39.25 %), while minimum percent inhibition was observed with control (0.00%).

Hexaconazole and Azoxystrobin were found most effective treatment against management of *Alternaria solani* these treatment were completely inhibited mycelium growth of test pathogen and these treatment are further tested in field experiment against alternaria blight of tomato.

Table -11: Effect of different fungicides on percent inhibition of *A. solani.*

Fungicides	**Conc (%)**	**Fungal mycelia growth in (mm)**		
		3 DAI	**5 DAI**	**7 DAI**
Hexaconazole 5% EC	0.1	100	100	100
Trifloxystrobin 50% + Tebuconazole 25%	0.1	100	92.73	92.96
Chlorothalonil 75% WP	0.3	88.08	83.75	71.11
Propineb 70% WP	0.3	74.41	69.65	57.77
Azoxystrobin 25% SC	0.1	100	100	100
Difenoconazole 25% EC	0.1	100	100	93.70
Propiconazole 25% EC	0.1	70.23	62.39	58.52
Pyraclostrobin 20% WG	0.1	52.97	53.42	39.25
Control	-	-	-	-

*Mean of three replication

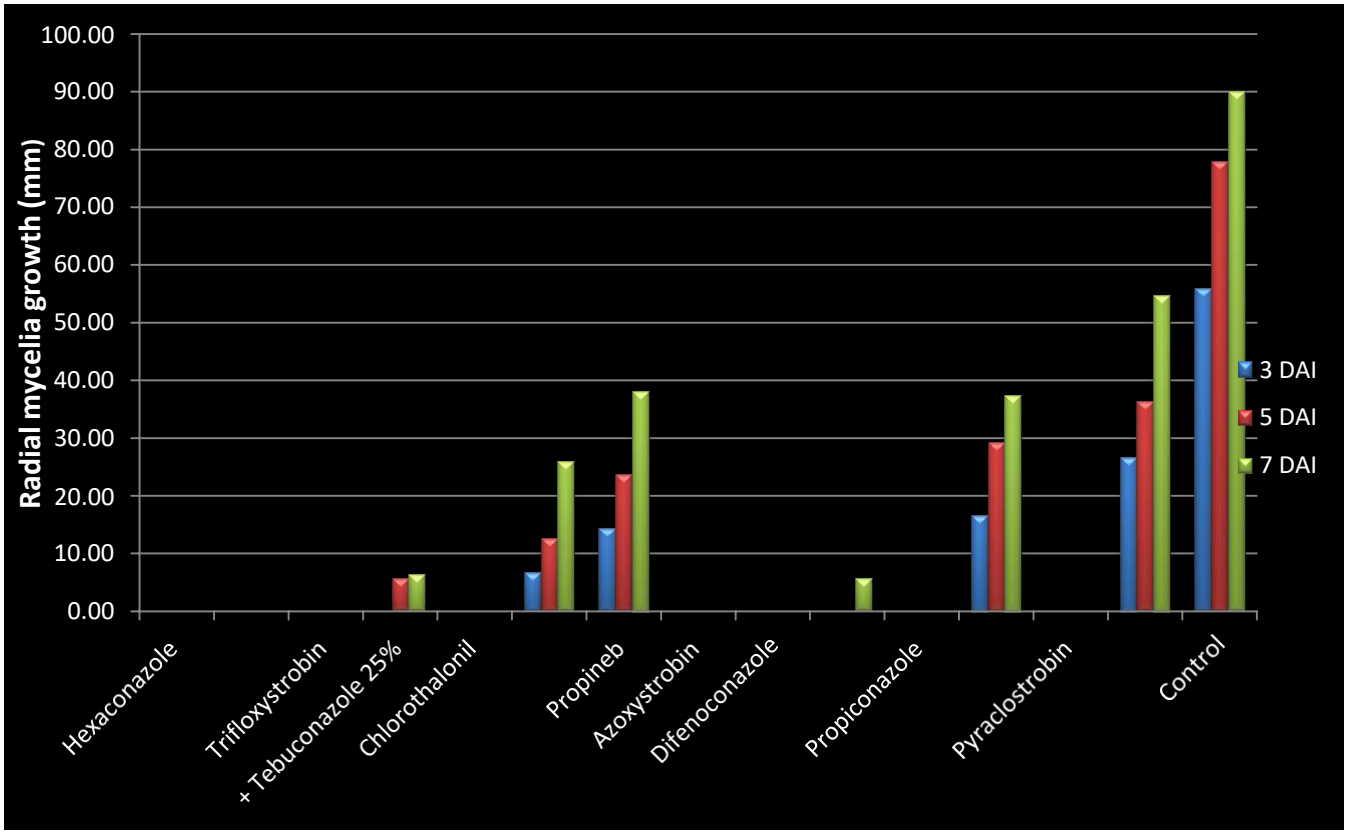

Fig-5. *In-vitro* evaluation of fungicide on mycelial growth of *A. solani.*

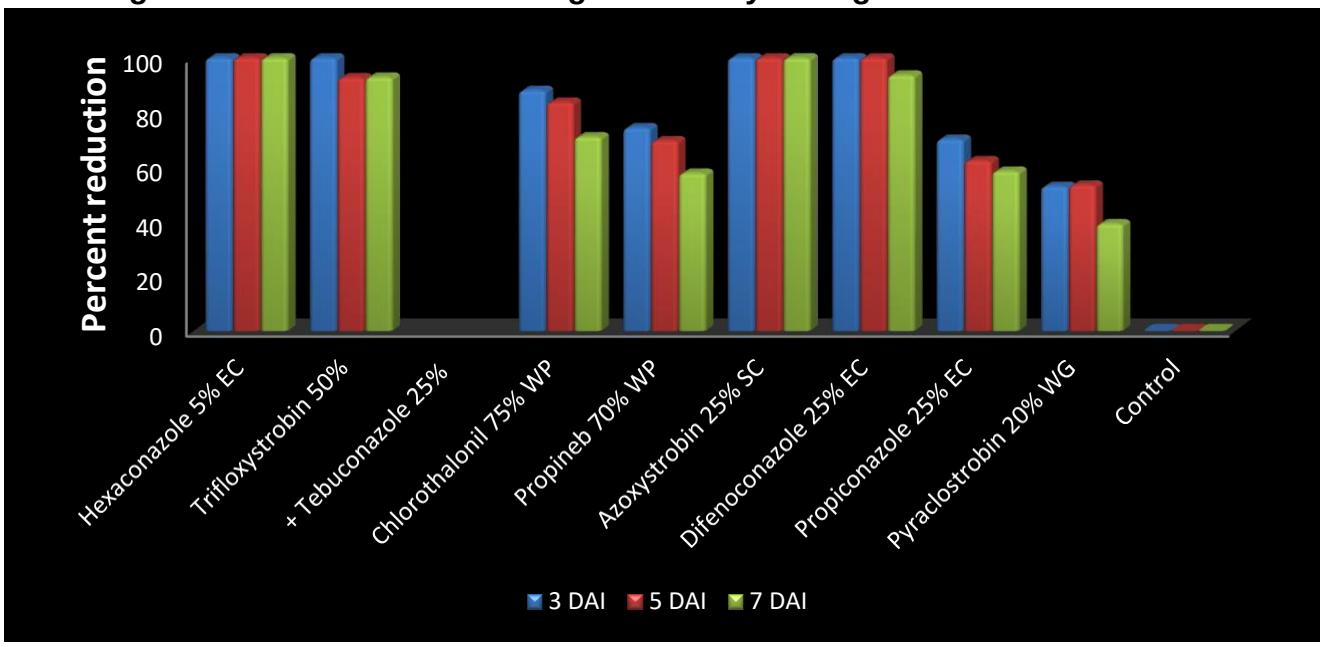

Fig-6. *In-vitro* percent reduction of mycelium growth over control

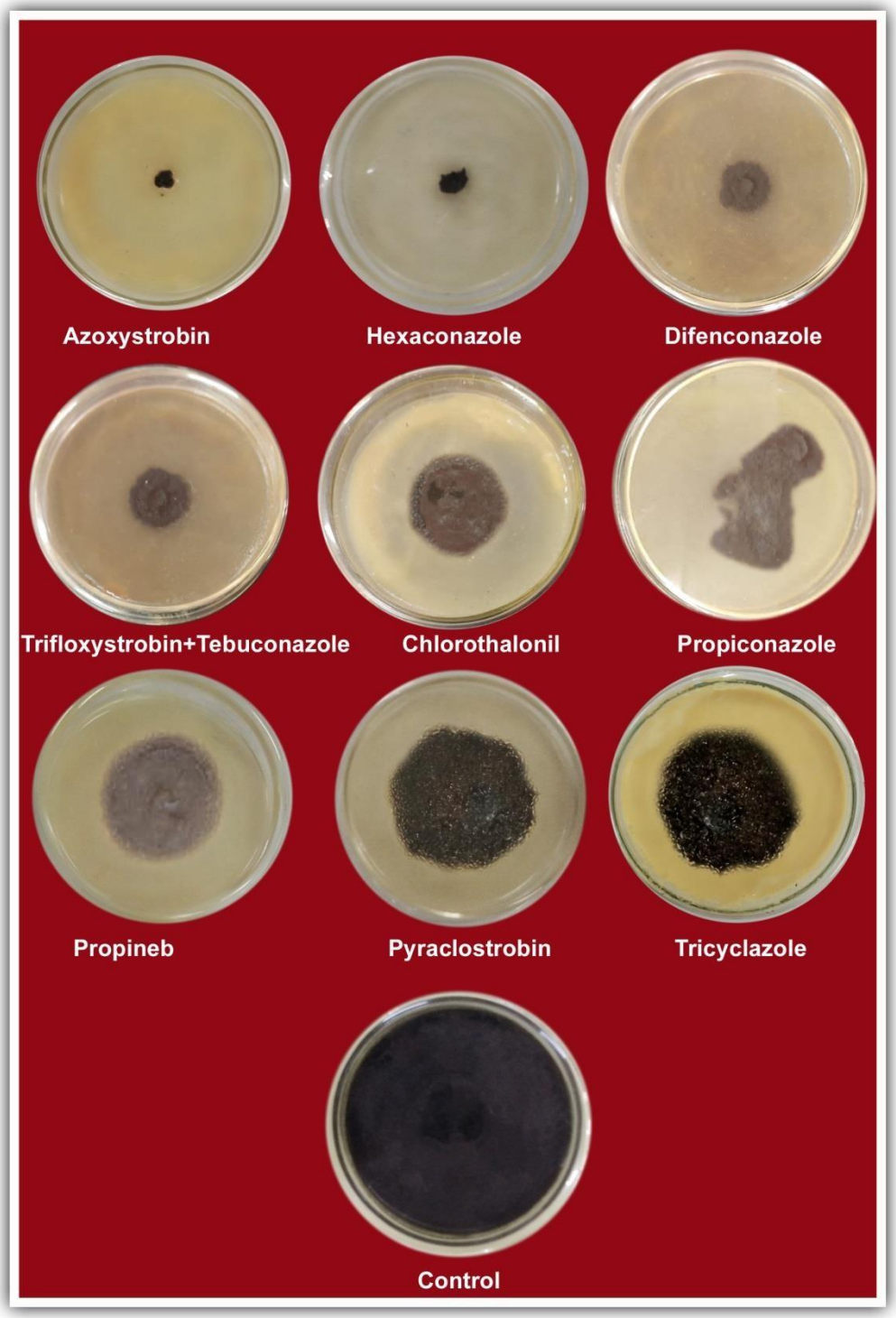

Plate.5- In- vitro evalution of fungicides aganist *Alternaria solani* after 7th day of incubation.

4.7. *In-vivo* evaluation of botanicals, natural farm product and fungicides against early blight of tomato.

Nine product of botanicals, natural farm product and fungicides were evaluated against Alternaria blight of tomato under natural field conditions table-plate-6 and fig-8). The data summizered in table reveals that all the treatments were found effective against alternaria blight of tomato over control. The minimum percent disease intensity was recorded in Hexaconazole @ 0.1% (13.33 %) followed by Azoxystrobin (22.00 %), Difenoconazole (25.00%), Cow urine (26.00 %), Compost tea (29.00 %), *Allium sativum* (33.67 %), *Annona reticulate* (39.00%), Jivamrit (39.33%) and *Turanta erecta* (49.00 %), while maximum intensity was recorded in control (61.00%). Significantly less percent disease intensity (13.33 %) was recorded in Hexaconazole @ 0.1%.

Table -12: Efficacy of botanicals, natural farm product and fungicides for the management of Alternaria blight disease of tomato under natural field conditions.

Treatments	Conc. (%)	Percent disease intensity*
Allium sativum	20	33.67
Annona reticulate	20	39.00
Turanta erecta	20	49.00
Cowurine	10	26.00
Compost tea	10	29.00
Jivamrit	10	39.33
Azoxystrobin	0.1	22.00

Hexaconazole	0.1	13.33
Difenacozole	0.1	25.00
Control	-	61.00
SEm±		**1.27**
CD (p=0.05)		**3.80**

* Average of three replications **

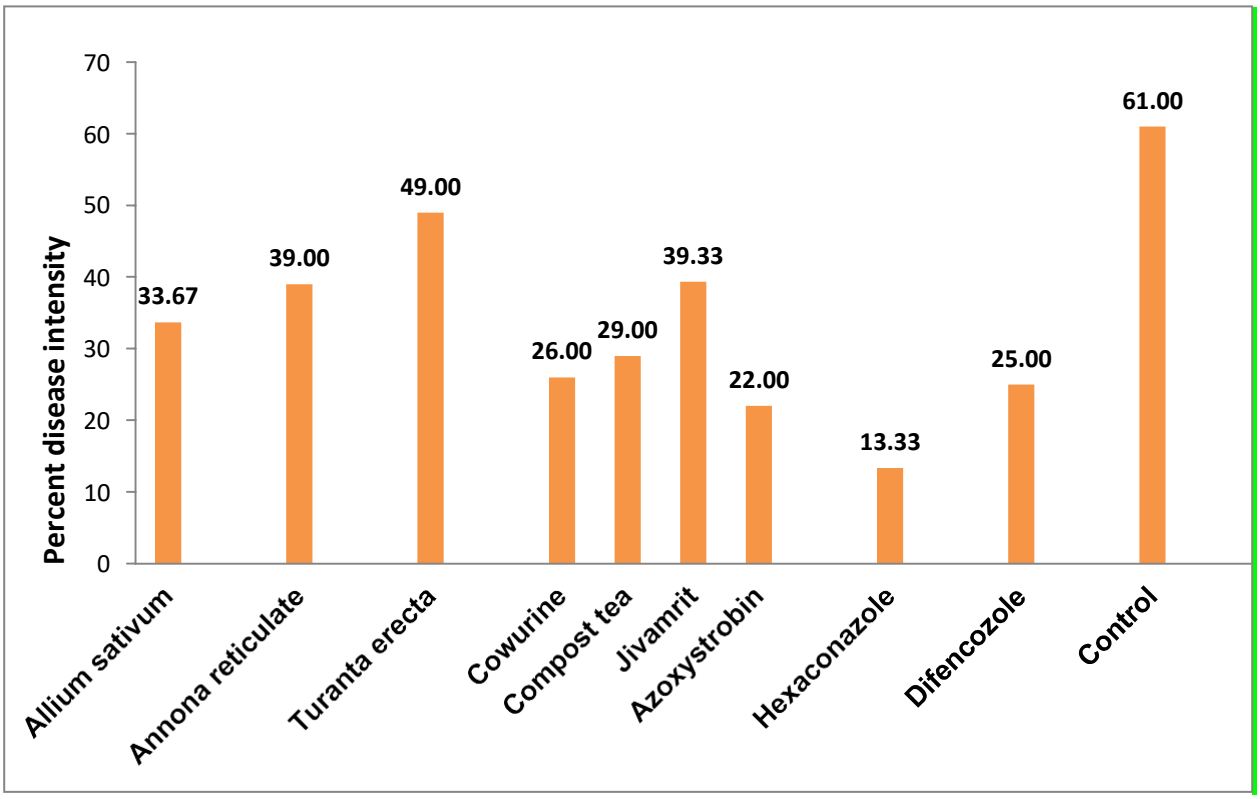

Fig-7. Effect of botanicals, natural farm product and fungicides on disease intensity of Alternaria blight of tomato

View of different treatment applied for the management of early blight of tomato

View of plant under Hexaconazole treatment

View of plant under control treatment

Plate-6. Field evaluation of selective natural farm product, plants extract and fungicides for the management of early blight of tomato

Chapter - V

DISCUSSION

Chapter -V

DISCUSSION

"Studies on Early blight of tomato (*Lycopersicon esculentum* Mill.) incited by *Alternaria solani* (Ellis and Martin) Jones and Grout", Role of weather parameters on development of early blight*, In-vitro* evaluation of botanicals *viz., Ipomoea carnea*, *Cynodon dactylon*, Garlic, *Moringa oleifera*, *Turanta erecta*, *Saraca asoca*, *Aegle marmelos*, *Mentha*, and *Annona reticulate* against *Alternaria solani, In-vitro* evaluation natural farm product *viz.,* Compost tea, Compost sat, Jivaamrit, Vermi wash, Cow urine, and Cow milk against *Alternaria solani*. *In-vitro* evaluation of fungicides *viz*., Hexaconazole, Difenoconazole, Azoxystrobin, Tebuconazole + Trifloxystrobin, Chlrothalonil, Propineb, Propiconazole and Pyraclostrobin, against *Alternaria solani. In-vivo* evaluation of botanicals, *viz., Allium sativum*, *Annona reticulate*, *Turanta erecta* natural farm products viz., cow urine, Compost tea and Jivamrit and fungicides *viz.,* Hexaconazole, Azoxystrobin, Difenoconazole were selected against early blight of tomato.

Results obtained on these aspects have been discussed in the following paragraphs.

5.1. Isolation, purification, identification of the pathogen

Disease specimens were collected from fields of tomato. The pathogen was isolated from infected leaves under ascetic conditions on PDA medium. The pure culture of the fungus was prepared by single hyphal tip culture technique. Identification of the fungus was carried out based on the morphological characters of the isolated fungus. The conidiophores were formed singly or in groups, straight or flexuous brown to olivaceous brown. The conidia were solitary straight or muriform or ellipsoidal tapering to beak, pale or olivaceous brown, length 150-300 µm and 15-20 µm thick in the broadest part with 8- 10 transverse and 0-4 longitudinal septa. The beaks were flexuous, pale and sometimes branched (Plate-2) The description of this fungus agreed with the description given for *Alternaria solani* by Common Wealth Mycological institute, Kew, Surrey, England (Ellis, 1971). Thus, the pathogen causing

early blight of tomato has been identified as *Alternaria solani* (Ellis and Martin) Jones and Grout. The diseased samples showing characteristic symptoms were collected from College Research Farm, College of Agriculture, Gwalior. The pathogenicity of isolated pathogen was confirmed on the basis of Koch's postulates. The reisolated fungus was identified as *Alternaria solani* on the basis of morphological characteristics. Simillarly Arunakumara (2006) isolates *Alternaria solani* from tomato leaves and observed that conidia were solitary straight or slightly flexuous, oblong or ellipsoidal tapering to a beak, smooth, 150-300 um in length, 13-20 µm thick in the broadest part with 8-10 transverse and 1-4 longitudinal septa.

5.2. Role of weather parameters on development of early blight

In epidemiological studies, results of *rabi* 2019-20 cropping seasons with respect to weather parameters revealed that Alternaria blight of tomato the cumulative per cent disease intensity was higher in the first date of sowing.

Correlation analysis of cumulative per cent disease intensity with weather parameters was indicated that maximum temperature (higher than 21.7 ^{0}C) and minimum temperature (less than 6.60^{0}C) and had positive correlation (0.6380*), relative humidity had negative and significant correlation and rainfall had no correlation during *rabi* 2019-20. The findings are in the agreement with the results obtained by earlier workers. Rajivkumar and Singh (1996) studied the influence of weather factors on development of leaf spot of sunflower caused by *A. helianthi* under field conditions during *kharif* 1990 and 1991. The Most important weather factors favoring disease development were the temperature and relative humidity ranging from 27°C – 29°C and 78-80 per cent, respectively, whereas rainfall did not affect the disease development because it was erratic and abnormal distribution during both the years. The disease intensity was highest in last week of August in both the years. There after there was a gradual decline in disease severity. Champawat and Sharma (2009) studied the influence of environmental factors such as temperature, relative humidity and rainfall on the development of Alternaria blight of tomato from Rajasthan. The multiple regression analysis revealed that weather parameters contribute 77 per cent towards disease incidence. Maximum and

minimum temperature has positive while maximum and minimum RH have negative significant correlations with appearance of Alternaria blight of tomato. The linear regression coefficients for temperature were positively significant while the both RH was negatively significant.

5.3. In-vitro evaluation of botanicals against *Alternaria solani*

Ten plant extracts were evaluated against *A. solani* under laboratory condition by following poison food technique. *Allium sativum* (35.67 mm) was most effective followed by *Curcuma longa* (39.67 mm), *Annona reticulate* (41.67 mm), *Turanta erecta* (43.67 mm), *Ipomoea carnea* (49.67 mm), *Moringa oleifera* (52.33 mm), *Mentha* (53.33 mm), *Saraca asoca* (70.33 mm), *Aegle marmelos* (78.33 mm), and *Cynodon dactylon* (80.33 mm), while maximum mycelial growth was recored in treatment control (90.00 mm). Similar results were found by Patni *et al.,* (2005). They evaluated extracts of six plants *i.e*. *Azadirachta indica*, *Parthenium hysterophorus*, *Calotropis procera*, *Datura alba*, *Eucalyptus globulus* and *Polyalthia longifolia* against Alternaria blight disease of Indian mustard incited by *A. brassicae*. Among these, *Eucalyptus globulus*, *Polyalthia longifolia* and *Calotropis procera* extracts, in that order, were promising in limiting the growth and sporulation of the pathogen. Sahu *et al.,* (2014) evaluated antifungal activities of 9 plant extracts against *Alternaria solani* causing early blight of tomato using radial growth technique. They found the leaf extract of Ashwagandha (*W. somnifera*) was most effective in inhibiting the mycelial growth of A. solani (62.56%). Extract of *D. stramonium* (34.65%) and *A. indica* (25.27%) exhibited moderate activity with average mycelial inhibition of 34.65 and 25.27% respectively. *Allium crispum (*jungli pyaj), *Mentha requienii* (pudina), *Lepidium sativum* (chandrasur), *Ocimum tenuiflorum* (Tulsi) and *Calotropis gigantia* (Aak) also inhibited mycelial growth of Alternaria blight.

5.4. *In-vitro* evaluation natural farm product against *Alternaria solani*.

Seven natural farm product were evaluated all the treatments were found significantly superior to control. Minimum mycelium growth was recorded in treatment cow urine (35.00 mm) was most effective followed by Compost tea (42.00 mm),

Jivaamrit (45.25 mm), Compost sat (49.25 mm), Cow milk (51.00 mm), and Vermiwash (52.25 mm), while maximum mycelium growth was recorded in control (90.00 mm). Similar results were found by khatib *et.al.,* (2021) they evaluated eight natural farm product *in-vitro* study. The maximum inhibition percent was recored in compost tea. Garg (2020) reported 10% cow urine are very much effective against *A.solani* and also recorded minimum mycelial growth and maximum inhibition percent was recorded.

5.5. In-vitro evaluation of fungicides against *A. solani.*

Nine fungicides were evaluated against *A. solani* under laboratory condition by following poison food technique. No mycelial growth was observed in hexaconazole and azoxystrobin at 3, 5 and 7 days after inoculation. Similar results were found by Ginoya and Gohel (2015) and Kumar and Singh (2017). Ginoya and Gohel (2015) found hexaconazole and azoxystrobin (18.2%) + difenconazole (11.4%) completely inhibited the mycelial growth of *A. alternata*. Wheras Kumar and Singh (2017) was found Hexaconazole 5% EC exhibited 100.00 percent inhibition in mycelium growth of *A. solani in-vitro.*

5.6. *In-vivo* evaluation of botanicals, natural farm product and fungicides against early blight of tomato

Effect of natural farm product, fungicides and botanicals were studied against Alternaria blight of tomato under natural field conditions. Results revealed that all the treatments were found effective against Alternaria disease of tomato over control. The minimum percent disease intensity was recorded in Hexaconazole @ 0.1% (13.33 %) followed by Azoxystrobin (22.00 %), among three natural farm product the minimum percent disease intensity was recorded in treatment Cow urine (26.00 %), Compost tea (29.00 %), and Jivamrit (39.33%), whereas three botanicals minimum percent disease intensity was recorded in *Allium sativum* (33.67 %) followed by *Annona reticulate* (39.00) and *Turanta erecta* (49.00 %), while maximum intensity was recorded in control (61.00%). Significantly less percent disease intensity (13.33 %) was recorded in Hexaconazole @ 0.1%.Our results are agreement with Kumar *et al*. (2007) and Sallam and Kamal (2012). Kumar *et al*. (2007) reported that hexaconazole @ 0.05% and

azoxytrobin @ 0.2% was very effective in managing early blight of tomato. Whereas Sallam and Kamal (2012) reported the efficacy of botanical extracts of *Ocimum basilicum* @ 5%, *Azadirachta indica* @ 5%, *Eucalyptus chamadulonsis* @ 5 %, *Datura stramonium* @ 5%, *Nerium oleander* @ 5% and *Allium sativum* @ 5% against early blight disease of Tomato. They recorded all botanicals reducing the disease severity (25.1- 45.2 %) and increased the yield (33.33-76.2 %) of tomato as compared to control. khatib *et.al.,* (2021) reported compost tea are used against *Alternaria helinthi* in sunflower crop they find out that minimum percent diseases intensity were recorded in compost tea, however Garg (2020) reported minimum percent diseases intensity were recorded in cow urine (28.70%).

Chapter - VI

SUMMARY, CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK

Chapter – VI

SUMMARY CONCLUSION AND SUGGESTIONS FOR FURTHER WORK

6.1. Summary

The present investigation was undertaken with a view to generate information on **"**Studies on Epidemiology and management of early blight of tomato caused by *Alternaria solani*" to find out the study about epidemiological aspects of the disease development, *in-vitro* study for natural farm product, Botanicals, fungicides and management of disease through fungicide, botanicals and natural farm product sources. Findings on these studies are beings summarized here in symptoms on affected plants started with yellowing and browning of the lower leaves, often developed from the leaf tips and along the margins of the leaf petiole. Circular or angular pale-brown spots appear on the leaves. Later on, lesions enlarged, coalesced and gave blighted appearance. Concentric rings with dark layers of spores were also observed on leaves and rotted fruits under moist conditions on blighted plants giving the typical "target board effect" or bull-eye" appearance. This is the most characteristic symptoms of early blight. In wet weather and severe infection, affected areas extended and form big rotting patches which get shriveled and covered petiole & stems also.

The pathogenicity test of the pathogen was tested under pot conditions by spore suspension technique. Tomato selection-22 variety plants were inoculated by spraying with spore suspension of isolated pathogen. The typical disease symptoms were observed and reisolation culture of *Alternaria solani.*

In present studies, Correlation analysis of cumulative per cent disease intensity with weather parameters was indicated that maximum temperature (higher than 21.7 ^{0}C) and minimum temperature (less than 6.60^{0}C) and

had positive correlation (0.6380*), relative humidity had negative and significant correlation and rainfall had no correlation during *rabi* 2019-20. The analysis of all the five independent variables individually and in combinations best revealed that each weather factor played an important role in disease development in addition to other unknown factors.

Among the ten botanicals were evaluated against *A. solani* under *in-vitro* condition. At 7 days after inoculation the mycelium growth was minimum recored in treatment *Allium sativum*, while maximum mycelial growth was recored in treatment control. Percent inhibition At 7 days after inoculation maximum percent inhibition was recorded *Allium sativum* (360.36 %), while minimum percent inhibition was recored in treatment control (0.00%).

Among six natural farm products were evaluated against *A. solani* under *in-vitro* condition. Minimum mycelium growth was recorded in treatment cow urine (35.00 mm), while maximum mycelium growth was recorded in control (90.00 mm). Percent inhibition At 7 DAI (days after inoculation), Maximum percent inhibition was recorded in treatment cow urine (61.11 %), while minimum percent inhibition was recoderd in control (0.00 mm).

Among of ten fungicides were evaluated against *A. solani* under *in-vitro* condition. No growth was observed in hexaconazole and azoxystrobin. Minimum mycelial growth was recorded in difeconazole (5.67 mm), while maximum mycelia growth was recorded in treatment control (90.00 mm). Maximum percent inhibition was observed in hexaconazole and azoxystrobin.

Nine product of botanicals, natural farm product and fungicides were evaluated against Alternaria blight of tomato under natural field conditions The minimum percent disease intensity was recorded in Hexaconazole @ 0.1% (13.33 %) while maximum intensity was recorded in control (61.00%). Significantly less percent disease intensity (13.33 %) was recorded in Hexaconazole @ 0.1%.

6.2. CONCLUSION

Correlation analysis of cumulative per cent disease intensity with weather parameters was indicated that maximum temperature (higher than 21.7 ^{0}C) and minimum temperature (less than 6.60^{0}C) and had positive correlation (0.6380*), relative humidity had negative and significant correlation and rainfall had no correlation during *rabi* 2019-20.

Among ten botanicals minimum mycelium growth was recored in *Allium sativum*, while maximum mycelial growth was recored in control. Maximum percent inhibition was recorded *Allium sativum*, while minimum in treatment control.

Among six natural farm products minimum mycelium growth cow urine, while maximum in control (90.00 mm). Maximum percent inhibition cow urine, while minimum in control.

Among ten fungicides were evaluated against *A. solani* under *in-vitro* condition. No growth was observed in hexaconazole and azoxystrobin. Maximum percent inhibition was observed in hexaconazole and azoxystrobin.

Nine product of botanicals, natural farm product and fungicides. The minimum percent disease intensity was recorded in Hexaconazole @ 0.1% (13.33 %) while maximum intensity was recorded in control (61.00%).

6.3. Suggestions for further work

- The resistant varieties are cheapest source to control this disease so that more entries should be screened out to find out resistant or immune lines of tomato.
- Use of different ITK'S will be undertaken so that the use of fungicides can be minimized.
- To study different isolates of pathogen to understand the plant pathogen interaction for the better management practices.

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VITA

VITA

Name of author	:	**Rashmi Singh**
Father's name	:	Ramkrapal Singh
Date of Birth	:	31/03/1988
Address	:	Gram Beldara, post pahadi District -Satna (M.P.)
Mobile Number	:	+91 7067678749
Email Id	:	rashmisinghchoudary29@gmail.com

Academic qualifications:

Name of examination	Board/university	Year	Subject	Division /marks
10th	M.P. board Bhopal	2009	All Sub.	59.00%
12th	M.P. board Bhopal	2011	PCMB	66.00%
B.Sc.	RVSKVV, Gwalior	2017	Agriculture	67.10 %
M.Sc. (Ag)	RVSKVV, Gwalior	2022	Plant Pathology	74.00 %

I have submitted my thesis in 2022, by the course work in partial fulfillment of the requirement for the degree of Master of Science (Plant Pathology).

(**Rashmi Singh)**