STUDIES ON BACTERIAL WILT OF GINGER CAUSED BY *Ralstonia solanacearum* (Smith) Yabuuchi

By

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B.Sc. (AGRI.) (HONS.)

MASTER OF SCIENCE
(AGRICULTURE)
IN
PLANT PATHOLOGY

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DISSERTATION

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2015

CANDIDATE'S DECLARATION

I hereby declare that the thesis

or part thereof has not been

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me for a degree of

any University

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I also certify that the dissertation or part thereof has not been previously submitted by him for a degree of any university.

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CERTIFICATE-II

This is to certify that the dissertation entitled **"STUDIES ON BACTERIAL WILT OF GINGER CAUSED BY *Ralstonia solanacearum* (Smith) Yabuuchi "** submitted by **ROOP SINGH (Reg. No. 2013A/102M)** to the Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani in partial fulfillment of the requirement for the degree of **MASTER OF SCIENCE** (Agriculture) in the subject of **PLANT PATHOLOGY** has been approved by the student's advisory committee after oral examination in collaboration with the external examiner.

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CONTENTS

CHAPTER	TITLE	PAGE NO.
I	INTRODUCTION	1 - 4
II	REVIEW OF LITERATURE	5 - 42
III	MATERIALS AND METHODS	43 - 62
IV	RESULTS AND DISCUSSION	63 - 85
V	SUMMARY AND CONCLUSIONS	86 - 92
	LITERATURE CITED	I - XXIV
	APPENDIX	I - III
	ABSTRACT	

LIST OF TABLES

Table No.	Contents	In Between Page No.
1.	Effect of various culture media and cultural characteristics of *R. solanacearum*	67 - 68
2.	Biochemical characteristics of *R. solanacearum*	69 - 70
3.	*In vitro* evaluation of antibacterial chemicals against *R. solanacearum*	71 - 72
4.	*In vitro* evaluation of bioagents against *R. solanacearum*	72 - 73
5.	*In vitro* evaluation of aqueous phytoextracts against *R. solanacearum*	74 - 75
6.	*In vitro* evaluation of acetone phytoextracts against *R. solanacearum*	76 - 77
7.	Effect of organic amendment on pre-emergence rhizome rot and post emergence seedling mortality caused by *R. solanacearum* in ginger	79 - 80
8.	Effect of organic amendments application on growth parameters in ginger against *R. solanacearum*	80 - 81
9.	Effect of antibacterial chemicals, bioagents, botanicals and organic amendments on pre emergence rhizome rot and post emergence seedling mortality caused by *R. solanacearum* in ginger	83 - 84
10.	Effect of antibacterial chemicals, bioagents, botanicals and organic amendments on growth parameters in ginger against *R. solanacearum*	84 - 85

LIST OF PLATES

Plate No.	Plates	In Between Page No.
I	Symptoms of bacterial wilt of ginger on farmers field (A), wilted plant (B) and ooze test (C) of *R. solanacearum*	63 - 64
II	Culture of *R. solanacearum* on TZC medium (A), CPG medium (B), virulent colonies (C) and avirulent colonies (D)	64 - 65
III	Agar slant (A) and water stock (B) of *R. solanacearum*	64 - 65
IV	Pathogenicity test of *R. solanacearum* on ginger	64 - 65
V	Cultural characteristics of *R. solanacearum* on various culture media	67 - 68
VI	Number of colonies of *R. solanacearum* on various culture media	67 - 68
VII	Biochemical tests to characterize *R. solanacearum*	69 - 70
VIII	*In vitro* evaluation of antibacterial chemicals at 400 & 1500 ppm against *R. solanacearum*	71 - 72
IX	*In vitro* evaluation of antibacterial chemicals at 500 & 2000 ppm against *R. solanacearum*	71 - 72
X	*In vitro* evaluation of bioagents against *R. solanacearum*	72 - 73
XI	*In vitro* evaluation of aqueous phytoextracts at 5 % against *R. solanacearum*	74 - 75
XII	*In vitro* evaluation of aqueous phytoextracts at 10 % against *R. solanacearum*	74 - 75
XIII	*In vitro* evaluation of aqueous phytoextracts at	74 - 75

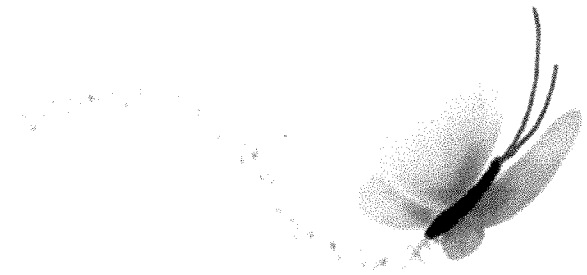
	20 % against *R. solanacearum*	
XIV	*In vitro* evaluation of acetone phytoextracts at 5 % against *R. solanacearum*	76 - 77
XV	*In vitro* evaluation of acetone phytoextracts at 10 % against *R. solanacearum*	76 - 77
XVI	*In vitro* evaluation of acetone phytoextracts at 20 % against *R. solanacearum*	76 - 77
XVII	Effect of organic amendments against *R. solanacearum*	79 - 80
XVIII	View of Pot culture experiment on evaluation of antibacterial chemicals, bioagents, botanicals and organic amendments against *R. solanacearum*	83 - 84

LIST OF FIGURES

Fig. No.	Title	In Between Page No.
1.	Effect of various culture media and cultural characteristics of *R. solanacearum*	67 - 68
2.	*In vitro* evaluation of antibacterial chemicals against *R. solanacearum*	71 - 72
3.	*In vitro* evaluation of bioagents against *R. solanacearum*	72 - 73
4.	*In vitro* evaluation of aqueous phytoextracts against *R. solanacearum*	74 - 75
5.	*In vitro* evaluation of acetone phytoextracts against *R. solanacearum*	76 - 77
6.	Effect of organic amendments on pre-emergence rhizome rot and post emergence seedling mortality caused by *R. solanacearum* in ginger	79 - 80
7.	Effect of organic amendments application on growth parameters in ginger against *R. solanacearum*	80 - 81
8.	Effect of antibacterial chemicals, bioagents, botanicals and organic amendments on pre emergence rhizome rot and post emergence seedling mortality caused by *R. solanacearum* in ginger	83 - 84
9.	Effect of antibacterial chemicals, bioagents, botanicals and organic amendments on growth parameters in ginger against *R. solanacearum*	84 - 85

ABBREVIATIONS

/	-	Per
%	-	Per cent
@	-	At the rate of
Av	-	Average
cfu	-	Colony forming unit
C.D.	-	Critical difference
mm	-	millimeter (s)
cm	-	Centimeter (s)
CRD	-	Completely Randomized Design
Conc.	-	concentration (s)
DAI	-	Day After Inoculation
Dia	-	Diameter
e.g.	-	Exampli Gratia (for Example)
et al.	-	and others
etc.	-	Etcetera
Fig.	-	Figure (s)
g	-	gram
hr	-	Hour (s)
i.e.	-	That is
kg	-	kilogram (s)
Max.	-	Maximum
mg	-	miligram
Min.	-	Minimum
ml	-	milliliter
mm	-	millimeter
No.	-	Number (s)
°C	-	degree celsius
TZC	-	Triphenyl Tetrazolium Chloride
CPG	-	Caseamino Acid Peptone Glucose
NA	-	Nutrient Agar
ppm	-	Part per million
MIC	-	Minimum inhibition concentration
S.E.	-	Standard Error
spp.	-	Species
Tr	-	Treatment
viz.,	-	videlicet (namely)



INTRODUCTION

CHAPTER I

INTRODUCTION

India is considered as a *"magical land of spices"*. No other country in the world has such a diverse variety of spice crops as India. Indian spices are known for their excellent aroma, flavor and pungency not easily matched by any other country. India has been a leading spice-producing, consuming and exporting country of the world.

Ginger, a very useful herb plant, is said to be originated from India, China and Java. It is grown throughout the tropical areas of the world and also commonly found in South East Asia especially in Indo Malaysia. Ginger is scientifically named as *Zingiber officinale* (Roscoe). In 1807, an English botanist, William Roscoe (1753-1831) named the plant as *Zingiber officinale* in his publication.

The name *Zingiber* is consequent from the Sanskrit word for "horn-shaped" and refers to the protuberances on the rhizome. *Zingiber officinale* belongs to the botanical family of the *Zingiberaceae.* Ginger is a monocotyledon, with a slender, perennial herblike habit but is usually grown as annual. It is 30 to 100 cm tall with a robust branched rhizome borne horizontally near the surface of soil and the stem is surrounded by the leaves.

In India, Spices is cultivated in an area of 3076 thousand hectares with annual production of 5744 thousand metric tonnes and productivity of 1.9 metric tonnes per hectares during 2012-13. However, during 2013-14 area under spices cultivation was increased with 3163 thousand hectares with annual production of 5908 thousand metric tonnes but productivity was 1.9 metric tonnes per hectares (Anonymous, 2014).

In India, ginger is cultivated in an area of 136.26 thousand hectares with annual production of 682.83 thousand metric tonnes and productivity of

and flu, catarrh, congestion, coughs, sinusitis, sores on the skin, sore throat, diarrhea, colic, cramps, chills and fever. Besides that, ginger oil is used for cooking, as a flavoring for cookies, biscuits and cake, and it is the main flavor in ginger ale, a sweet, carbonated, non-alcoholic beverage.

Ginger crop is affected by several diseases caused by fungi, bacteria, nematodes, and abiotic factors. Among the biotic causes bacteria are most important which cause the major diseases *viz.,* Bacterial wilt (*Ralstonia solanacearum*), Bacterial soft rot (*Erwinia* spp.). The important fungal diseases include, Rhizome rot/Soft rot (*Pythium aphanidermatum*), Pythium soft rot (*Pythium graminicolum*), Yellows disease (*Fusarium oxysporium* f. sp. *zingiberi*), Sclerotium rot (*Sclerotium rolfsii*), nematode diseases like root knot disease caused by *Meloidogyne* spp. and abiotic causes like Sunburn (due to high light intensity), Lime-induced chlorosis (due to excessive liming in soil).

Among the bacterial diseases infecting ginger crop, bacterial wilt caused by *Ralstonia solanacearum* (Smith) Yabuuchi, is one of the most destructive disease causing accountable qualitative and quantitative losses. Orian (1953) reported this disease for the first time from Mauritius. Later on, the disease was reported from Hawaii (Ishii and Aragaki, 1963), Malaya (Jamil, 1964) and Australia (Hayward *et al.,* 1967). In India the disease was first reported from Madras state (Thomas, 1941). Later, it was reported from Kerala (Sharma and Jain, 1978), Bihar (Ojha *et al.*, 1986) and Himachal Pradesh (Dohroo, 1991). Under favorable conditions, the disease cause linear streaks on the collar region of the pseudostem. Later leaves become flaccid with intense yellowish bronze color and droop. The leaves roll up and the whole plant dries. Pseudostems come off easily with a gentle pull. Milky bacterial exudate oozes out on pressing the rhizome gently (Sarma, 1994).

Ralstonia solanacearum is an aerobic non-spore forming, Gram negative, Plant pathogenic bacterium. *R. solanacearum* is motile with tuft of polar flagella. It colonizes the xylem, causing bacterial wilt in a very wide

range of potential host plants. *Ralstonia* synonymous to *Pseudomonas* with a similarity in most aspects except, it does not produce fluorescent pigment. It belongs to kingdom: Bacteria; Phylum: Proteobacteria; Class: Beta-Proteobacteria; Order: Burkholderiales; Family: *Ralstoniaceae* and Genus: *Ralstonia.*

Keeping in view, the economic importance of bacterial wilt disease, present investigations were planned and conducted on *Ralstonia solanacearum* with following objectives.

1. To isolate and prove pathogenicity of bacterium *Ralstonia solanacearum.*
2. To study the cultural and biochemical characteristics of *Ralstonia solanacearum.*
3. *In vitro* evaluation of antibacterial chemicals, biocontrol agents, botanicals and organic amendments against bacterial wilt of ginger.
4. Integrated management of bacterial wilt of ginger (pot culture).

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Ralstonia solanacearum is a highly heterogeneous bacterial pathogen that causes severe wilting of many important plants (Smith *et al.*, 1995). The literature available on *R. solanacearum* on the aspects *viz.*, occurrence, distribution, losses caused, symptomatology, isolation and pathogenicity, cultural and biochemical characteristics, *in vitro* evaluation of the antibacterial chemicals, bioagents, botanicals, organic amendments and integrated management have been collected and being presented herein this chapter.

2.1. Disease occurrence, distribution, and yield losses

2.1.1. Occurrence and distribution

Sharma and Jain (1978) identified that causal agent of bacterial wilt of ginger *is Ralstonia solanacearum.* (Smith) Yabuuchi, biotype III.

Samuel *et al.* (1986) reported that the bacterial wilt disease incidence increases when the nematodes are present in the ginger soil.

Mulya *et al.* (1990) found that in Indonesia, the race I of biovar III of *Pseudomonas solanacearum* is considered as the cause of ginger rhizome wilt.

Dohroo (1991) recorded the occurrence of bacterial wilt of ginger in Himachal Pradesh.

Hayward (1991) observed that the disease was widely distributed in tropical, subtropical and warm temperate regions of the world with a host range of 44 plant families.

Dake and Manoj (1995) reported that *Pseudomonas solanacearum* is reported for the first time causing bacterial wilt of Kaempferia (*Kaempferia galanga*) from Kerala, India.

Deberdt *et al.* (1999) reported that *R. solanacearum* and nematode populations usually coexists together in tropical and subtropical areas; the nematode feeding wounds produced on roots serve as entry for the bacteria thus the high correlation between the presence of root knot nematode and the level of bacterial infection. The presence of root knot nematodes increases more the disease compared to reniform nematode even in presence of tomato varieties that are normally resistant to bacterial wilt.

Poussier *et al.* (1999) affirmed that *R. solanacearum* is one of the most important diseases in tropical, subtropical and warm temperate regions worldwide and also stated the possibility for the disease to occur in cool temperate areas. This diverse species differs in 39 host range, geographical distribution, pathogenicity, epidemiological relationships, and physiological properties.

Denny (2000) reported that during infection, the bacteria become motile and can rapidly spread throughout the vascular system of the plant, presumably carried along by the transpirational flow.

Dokun *et al.* (2000) reported that bacterial wilt is found particularly on potato, tomato, eggplant, capsicum and other crops including ginger, bean and groundnut as well as ornamentals such as anthurium (*Anthurium andreanum* Grp. Sierra) in Mauritius.

Pradhanang *et al.* (2000) reported that the presence of the bacteria on 29 natural hosts different from potato and tomato and it was possible to artificially infect 28 other hosts.

Chris *et al.* (2001) reported that the bacterial wilt of potato occurs throughout Asia, Africa, Europe, Mexico, the Caribbean and South America. It has also been recorded in all states of Australia except Tasmania and Western Australia. The most serious reports have come from Queensland, New South Wales and Victoria. In these states there are areas where the disease is considered endemic. Bacterial wilt was introduced on one or more occasions through the planting of uncertified seed.

Horita and Suchiya (2001) reported that bacterial wilt disease caused by *R. solanacearum* has been reported mainly for solanaceous crops including tomato (*Lycopersicon esculentum* Mill), potato (*Solanum tuberosum* L.), tobacco (*Nicotiana tabacum* L.), eggplant (*S. melongena* L.), and sweet pepper (*Capsicum annuum* L.). To date, more than 34 species in 18 families of plants have been reported as hosts in Japan. Several workers have studied the classification of Japanese strains of *R. solanacearum*. However, the systematic relationship among strains is still poorly defined.

Stevenson *et al.* (2001) reported that *R. solanacearum* is highly heterogeneous species containing hundred of distinct strains differing in natural host range, geographic distribution, biochemical and genetic characteristics and is found worldwide (Grover *et al.*, 2006).

Kumar *et al.* (2004) studied that bacterial wilt caused by *Ralstonia solanacearum* is a disease widely distributed in tropical, sub-tropical and temperate regions worldwide. Bacterial wilt of ginger is reported from India, China, Japan, Indonesia, Hawaii and many other ginger growing countries. In India the disease is found in Kerala, Karnataka, Himachal Pradesh, Sikkim, West Bengal, Assam and other North Eastern States. The pathogen is primarily rhizome-borne and is believed to be transmitted to many ginger growing areas

through latently infected rhizomes. Secondary spread with the field and neighboring localities is through rain splashes and runoff water.

Kumar and Sarma (2004) studied that *R. solanacearum* incited wilt disease is one of the major constraints of ginger in small and marginal farming communities. The strain causing bacterial wilt of ginger in India belongs either to biovar 3 or 4; the former being the most virulent in India.

Kumar and Hayward (2005) reported that the bacterial wilt disease inflicts serious economic losses to small and marginal farmers who depend on this crop for their livelihood. Geographical distribution of the pathogen is expanding in recent years due to the unintentional transmission of the bacterium through infected rhizomes of ginger, which are the primary propagules.

Elphinstone (2005) observed that *R. solanacearum* Race 1 strains have been reported in at least 21 countries; biovar 2 Race 3 has been reported on potato in Burundi, Egypt, Ethiopia, Kenya, Libya, Reunion, Rwanda, South Africa, Tanzania and Uganda. Isolates collected from potato in Kenya, Nigeria and Cameroon were found to have the biovar 2T phenotype. An interesting question is whether this strain was introduced on potato seed from S. America or Europe. In that distribution, Race 1 is reported on many economically important hosts, Race 2 on banana and others *Musaceae* and Race 3 on potato and other *solanaceae* (Elphinstone, 2005), Race 4 is reported on ginger (Kumar *et al.*, 2004).

Agrios (2005) studied that the disease is favored by high temperatures and generally limited to areas without frozen soils, being particularly severe in the tropical and subtropical areas.

Kusumoto and Takikawa (2005) found that a sudden wilt of bellflower (*Campanula lactiflora*) was observed in Japan in 1997. A bacterium

that formed white fluidal and mucoid colonies resembling those of *Ralstonia solanacearum* was isolated from the infected plants. The bacterium was bacteriologically identified as biovar 3 of *R. solanacearum*. This is the first report of *R. solanacearum* affecting a plant species of the *Campanulaceae* family.

Agarwal *et al.* (2006) carried out an extensive survey of Himachal Pradesh, India to monitor the prevalence of bacterial wilt, caused by *R. solanacearum*, in solanaceous vegetables (tomato, brinjal and capsicum). Observed that maximum wilt incidence was recorded in Mandi district (45%), followed by Kangra (41.73%), Kullu (28%), Hamirpur (16%) and Solan (12.68%).

Sagar (2006) conducted a survey in major ginger growing areas of Karnataka and observed that the maximum average disease incidence (23.70 %) in Shimoga district and least incidence (5.86 %) were recorded in Haveri district.

Rahman *et al.* (2010) carried out a survey in some districts of Bangladesh reported that the wilt incidence was recorded maximum (22.52 %) in Rangpur district followed by Jessore (20.56 %) and Panchagarh (20.00 %) while the lowest wilt incidence was recorded in Jamalpur district (6.12 %).

Singh *et al.* (2010) conducted a survey to study the status of bacterial wilt of solanaceous crops caused by *R. solanacearum* in Northern and Eastern states of India such as Jammu & Kashmir, Himachal Pradesh, Uttrakhand, Jharkhand and West Bengal. They observed that bacterial wilt disease incidence in tomato and chilli was quite low (1 to 3 %) during summer season, whereas in rainy season, it was (4 to 60 %) in tomato and (3 to 40 %) in brinjal. Disease incidence in tomato crop was more as compared to other solanaceous crops like brinjal, chilli, capsicum, and potato.

Bochre and Papdiwal (2011) carried out a survey of bacterial diseases of vegetables was undertaken in Aurangabad district. In all 14 diseases were found on different species including wilt of brinjal caused by *R. solanacearum* (Smith) Yabuuchi *et al.*

Begum *et al.* (2012) reported that the distribution of bacterial wilt caused by *Ralstonia solanacearum* in different vegetable growing areas of Pakistan (during April-September 2008-09) revealed that sweet pepper was highly susceptible with overall incidence percentage of 21.9 % followed by hot pepper (16.6 %), tomato (13.3 %), potato (10.5 %) and brinjal (5.5 %). Aggregate incidence in the whole country was 13.8 %. Most of the isolates were obtained from soil (17.2 %) and plant samples (18.8 %). The disease was mainly distributed in Islamabad Capital Territory and Punjab with bacterial wilt incidence of 19.2 % and 13.9 % respectively.

Ahmed *et al.* (2013) carried out a survey in some selected potato growing districts of Bangladesh during (December to February 2011), observed that the highest wilt incidence was recorded in district Munshigonj (22.65 %), followed by Nilphamari (19.98 %) and the lowest incidence was recorded in Jamalpur (9.07 %).

Fanhong M. (2013) reported that the bacterium *Ralstonia solanacearum* is the causal agent for bacterial wilt on more than 200 plant species from 50 botanical families, including important crops such as potato, tomato, ginger, eggplant, pepper, tobacco and banana. *R. solanacearum* is considered a species complex, and is also a widely accepted model organism for the study of bacterial pathogenicity in plants.

Sagar *et al.* (2014) reported that bacterial wilt (brown rot) incited by *Ralstonia solanacearum* is a major constraint on potato production worldwide and in many potato growing regions of India.

Avinash and Umesha (2014) reported that *R. solanacearum* strains are isolated from different fields of Karnataka, India, and are characterized to determine the distribution and diversity of soil borne pathogen. The isolated strains of *R. solanacearum* showed maximum identity as biovar 2.

2.1.2. Yield losses

Sharma and Jain (1978) reported that bacterial wilt has been estimated to cause a loss as high as 90 % in Kerala.

Mathew *et al.* (1979) reported that the disease is endemic in majority of the ginger growing areas *viz.*, Kerala, Sikkim and many other northeastern regions of the country causes yield loss up to 100 per cent under conducive conditions (Dohroo, 1991).

Kisun R. (1987) conducted the studies on assessment of loss in yield of tomato cv. Pusa Ruby due to bacterial wilt were conducted by paired plot technique and also by creating disease at different stages (0-90 days) during summer, monsoon and winter seasons. Loss in number and weight of fruits from 31.47 to 91.06 %, respectively when yield was estimated by paired plot technique. Plant mortality and loss in yield ranged from 10 to 100 and 10.83 to 90.62 % respectively at different stages of inoculations. Plant mortality and per cent yield loss were pronounced when the plants were inoculated up to the age of 60 days. In both the methods maximum loss was recorded during summer season.

Walker and Collion (1998) reported that the bacterial wilt causes economic problems for about three million farm families in 80 countries causing a devastating annual loss exceeding $ 950 million.

Sumithra *et al.* (2000) conducted a field survey in the state of Karnataka. They were observed that bacterial wilt of brinjal caused by *Ralstonia solanacearum* is wide spread in the state, causing heavy losses.

Zhang *et al.* (2001) reported that disease incidence in the field usually ranges from 10 to 40 % but the disease is also known to destroy the crop completely.

Fortnum (2001) observed that economic impact of bacterial wilt in tobacco in USA has been increased for the last few years. Economic losses in Carolina state were estimated to exceed $ 40 million and yield losses ranging from (10 to 30 %) are incurred every year in Australia. In the southern part of Vietnam, yield losses reported on groundnut went up to (20 %) while in Uganda losses were up to 10 per cent.

Sambasivam and Girija (2005) reported host resistant and loss in ginger cultivation by *R. solanacearum* in Kerala. Many a times this important cash crop is subjected to premature wilting resulting in 100 % crop loss.

Allen *et al.* (2006) reported that the plant pathogenic bacterium, *Ralstonia solanacearum*, probably causes more economic damage to agricultural crop production than any other bacterial plant pathogen. It affects a wide range of plant species, and because it survives so well in the soil matrix, is difficult to avoid and control. *Ralstonia solanacearum* has always caused the greatest havoc in agricultural regions of the tropics and subtropics, and it still does.

Mepharishvili (2012) observed that in June 2010, a wilt disease affecting tomato seedlings in Chkhorotsku region of Western Georgia, causing up to 100 % plant loss.

2.2. Symptomatology

Gota (1992) reported that young plants were affected more and sudden wilting of foliage takes place. The symptoms occur as discoloration of the vascular system from pale yellow to dark.

Sarma (1994) studied that water-soaked patches or linear streaks on the collar region of the pseudostems. Later leaves become flaccid with intense yellowish bronze color and droop. The leaves roll up and the whole plant dries. Pseudostems come off easily with a gentle pull. Milky bacterial exudate oozes out on pressing the rhizome gently.

Rajan *et al.* (2002) found that the first symptoms of *R. solanacearum* infected plants were, downward curling of leaves and golden brown/rusty brown discoloration seen on older leaves.

Umesha *et al.* (2005) found that the bacterium *B. solanacearum*, which causes bacterial wilt disease in vegetables. Infected stem and root portions showed discoloration when cut open. Milky white bacterial ooze was observed when the plant material was placed in water. Healthy plant material did not show any discoloration or bacterial ooze.

Agrios (2005) described the symptoms as older plant leaves first shows wilting before the youngest leaves or one side wilting and stunting and finally the plant wilt permanently and dies.

Wang and Lin (2005) observed the disease symptoms under field conditions; the disease first appears in scattered patches. Wilting signs are first seen on younger leaves during hot weather. Occasionally wilted plants recover when temperature is lowered at the end of the day. Permanent wilting takes place after a few days and wilted leaves retain green color and do not fall off. Browning of vascular system occurs in lower parts of the stem. Some times rottening of roots is seen due to invasion of saprophytic microbes. Key test for the identification and distinction of this disease from other diseases (wilts caused by *Fusarium* spp. and *verticillium* spp.) is the appearance of slimy, whitish bacterial ooze when infected stem sections are placed in water.

Kumar and Abraham (2008) found that the bacterial wilt is characterized by bacterial entry into the host followed by its multiplication and movement through the xylem vessels of the host plant. In the process, they interfere with the translocation of water and nutrients which in turn results in drooping, wilting and death of the above ground parts of the plants. In the case of ginger, the first noticeable symptom of bacterial wilt is downward curling of leaves due to loss of turgidity and within 3 to 4 days the leaves dry up. The affected rhizome starts rotting and putrefying due to attack of saprophytic soil microorganisms. The rotted rhizomes emit foul smell and the affected plants die within 2 to 3 weeks.

Roy *et al.* (2008) were studied the symptomatology of bacterial wilt disease caused by *R. solanacearum* in Jati (*Nicotiana tabacum*) and Motihari (*N. rustica*) tobacco and hollow stalk in Motihari tobacco in terai zone of West Bengal in India. Symptoms show water-soaked patches or linear streaks on the collar region of the pseudostems. Later leaves become flaccid with intense yellowish bronze color and droop. The leaves roll up and the whole plant dries. Pseudostems come off easily with a gentle pull. Milky bacterial exudate oozes out on pressing the rhizome gently.

Champoiseau *et al.* (2009) reported that massive invasion of the cortex might have resulted in the appearance of water soaked lesions on the external surface of the stem. Infected stem exude tiny drops of dirty white or yellowish viscous ooze from several vascular bundles.

Monther and Kamaruzaman (2010) studied that *R. solanacearum* is characterized by sudden wilting of foliage. The bacterium multiplies rapidly in the vascular system; finally the xylem elements are filled with bacterial cells and slime.

Li *et al.* (2010) reported that the bacterial inoculated plant showed Symptoms of irregular, black necrotic lesions on the leaf margins with wilting symptoms.

Chandrashekara and Prasannakumar (2012) reported that the isolation of the bacterium was done from tomato (*S. lycopersicum*), brinjal (*S. melongena*), potato (*S. tuberosum*), bird of paradise (*Strelitzia reginae*), ginger (*Z. officinale*), chili (*C. annuum*), capsicum (*C. annuum*), davana (*A. pallens*) and coleus (*C. forskohlii*) plants showing typical symptoms of bacterial wilt. Such signs were: lower leaves turning pale yellow, loss of leaf turgidity followed by drooping of leaves and sudden wilt of the plants. The vascular bundles of the infected plants showed brown discoloration.

Nelson (2013) studied that "Green wilt," the diagnostic symptom of the disease. This occurs early in the disease cycle and precedes leaf yellowing. Infected green ginger leaves roll and curl due to water stress caused by bacteria blocking the water-conducting vascular system of the ginger stems, leaf yellowing and necrosis. Leaves of infected plants invariably turn yellow and then necrotic brown.

White *et al.* (2013) reported that the symptoms of bacterial wilt of ginger include leaf yellowing and curling followed by necrosis and lethal wilting of the plant. Since ginger is vegetatively propagated, it is important to plant seed pieces that are not infected with the bacterial wilt pathogen, because diseased seed pieces will develop into diseased plants and lead to crop losses.

Simly and Swain (2014) reported that the bacterial inoculated plants, deep water shocked spots initially appeared at the collar region which progressed both upward and downward. Mild drooping and curling of leaf margins of lower leaves were the conspicuous symptoms of bacterial invasion.

Kai *et al.* (2014) reported that the plant species infected with this pathogen usually show the typical symptoms of yellowing and wilting, which may be followed by necrosis and death. The bacteria invade the roots of diverse plant hosts from the soil via wounds or sites of secondary root emergence and disseminate into the xylem vessels.

Antony *et al.* (2014) reported that the bacterial wilt pathogen infects plants by entering through root wounds and at sites of secondary root emergence, then multiplies and colonizes the xylem vessels and moves quickly to aerial parts of the plant all the way through the vascular system. In xylem vessels, the bacterial population can reproduce comprehensively and rapidly attain very high levels.

2.3. Isolation and Pathogenicity

Winstead and Kelman (1952) confirmed that the pathogenicity test by Koch's postulation. A set of three seedlings were inoculated with sterile distilled water to serve as control and another three with inoculum. The plants were observed for the symptoms. The pathogen was isolated from the inoculated plants and cultured on TTC medium and cultural characteristics observed. It was reinoculated on healthy brinjal seedlings and observed for the same symptoms.

Schell M. A. (2000) reported that the bacterium *R. solanacearum* pathogenicity is distinctly regulated in early or late stages of infection in response to environmental conditions, such as the presence of host plant cells and bacterial population densities (Hikichi *et al.,* 2007).

Dhital *et al.* (2001) reported that all fifteen potato strains of *R. solanacearum* from Nepal produced fluidal and irregular colonies with pink or light red at centers on TZC medium at 30°C after 48 h of incubation.

Williamson *et al.* (2002) found that seven to ten days after inoculation, geraniums inoculated with BB-Feutz and 12-4-6 SD was wilted and

chlorotic, and 3 weeks after inoculation they were largely dead. One week after inoculation, tomato plants were fully wilted, and all inoculated plants were dead after 2 weeks. Bacteria re-isolated from wilted plants had cultural characteristics typical of *R. solanacearum*. No wilting was observed in the non-inoculated control plants.

Rajan *et al.* (2002) studied that the bacterial wilt symptoms developed after about 12 days of inoculation and wilting occurred after 15 days of inoculation.

Kumar and Sarma (2004) reported that wilting started after 6 days of inoculation at the concentration of 3.2×10^{8} and 3.2×10^{7} cfu ml^{-1} when the inoculums was placed in between bottom leaf sheath and pseudostem. At the concentration of 3.2×10^{8} cfu ml^{-1} the wilting started after 6 days and complete wilting was observed on 8^{th} day.

Umesha *et al.* (2005) reported that the plants inoculated with the pathogen for pathogenicity test showed wilting symptoms after 15 days, leaves turned yellow, with severe defoliation. Sterile distilled water dipped plants remain healthy

Kumar A. (2006) studied that the pseudostem inoculation resulted in wilting of plants in 5 to 7 days, followed by the soil inoculation method in 7 to10 days, rhizome inoculation method in 45 to 60 days and the *in vitro* method in 10 to 14 days.

Lemessa and Zeller (2007A) reported that eighty one isolates of *R. solanacearum* bacteria on Triphenyl Tetrazolium Chloride (TTC) medium were collected from different solanaceous crops i.e. potato, tomato and pepper plants and potato tubers at various sites in Ethiopia of these, 62 strains were identified as *R. solanacearum* based on their cultural characteristics on TTC medium.

Mathews *et al.* (2008) reported that the race 4 ginger strains affected nearly all ornamental ginger species tested. Symptoms ranged from flagging or wilting to plant death. Although the majority of the plants wilted within 10 DAI, the final pathogenicity assessment was recorded 21 DAI, at which time all affected plants were severely wilted or dead.

Zhu *et al.* (2010) found that the bacterium *Ralstonia solanacearum* is a nonsporulating gram-negative soil-borne pathogen that causes lethal wilt diseases of many plants around the world. Its growth and pathogenicity characteristics for entering long term stationary phase were investigated by a prolonged 20 day laboratory culture.

Chakravarty and Kalita (2011) studied the stem wilted plant showed milk white ooze consisting bacterial cells and their extra cellular polysaccharide in sterile distilled water, upon inoculation on TZC medium. The bacteria growth on medium dull white fluidal irregular round colonies with light pink centers was obtained.

Chaudhry and Rashid (2011) found that when fragments were cut from plant samples in which symptoms of disease were evident, fluidal pinkish red centered colonies, typical of *Ralstonia solanacearum* were observed on TTC medium. Typical isolated colonies were picked and purified for confirmation of bacterial wilt causing pathogen *Ralstonia solanacearum*. Bacterial cultures were stored in sterilize distilled water for pathogenicity and confirmatory tests. Virulence of an isolate can be determined on the basis of colony colour on this particular media. Virulent wild type colonies are usually large, elevated, fluidal and either entirely white or with a pale red center; avirulent mutant colonies were butyrous, deep-red often with a bluish border.

Artal *et al.* (2012) proved the pathogenicity by the soil drenching method, 5.0 ml of bacterial suspension was inoculated to each of the seedlings by

drenching the soil around the root zone with the help of micro pipette. Before inoculation, the roots were slightly severed by inserting a sharp knife 1.0 cm away from the stem. Root severing was done to ensure bacterial penetration through roots in all the three crops tomato, brinjal and chilli recorded significantly highest bacterial wilt incidence of 98.0, 95.0 and 90.0 per cent in tomato, brinjal and chilli, respectively.

Rashmi *et al.* (2012) reported that the inoculation through soil drenching recorded significantly highest bacterial wilt incidence of 98.0, 95.0 and 90.0 per cent in tomato, brinjal and chilli, respectively, followed by inoculation through axil puncturing which recorded 78.0, 88.0 and 78.0 per cent wilt incidence. The leaf clipping method, however, recorded the lowest wilt incidence of 74.0, 48.0 and 40.0 per cent respectively.

Ahmed *et al.* (2013) isolated *R. solanacearum* from the potato tuber producing colonies with pink or light red colour with whitish margin on TTC medium indicated all groups of isolates were virulent.

Seleim *et al.* (2014) isolated the bacteria on triphenyl tetrazolium chloride medium and fifteen isolates shown typical morphological and cultural characteristics were confirmed as *Ralstonia solanacearum* biovar 2 race 1. Pathogenicity tests showed that all isolates proved to be pathogenic to tomato plants, varied from 52 to 97 % wilting. This is the first report of *R. solanacearum* biovar 2 race 1 causing bacterial wilt in tomato crop in Egypt.

Thomas and Upreti (2014) reported that the disease symptoms appeared earlier and with more severity in 2 weeks old seedlings followed by 3, 4 and 5 weeks old saplings recording 74 %, 68 %, 63 % and 49 % mortality, respectively, after four weeks of inoculation suggesting that older the seedlings, less the susceptibility to the pathogen. The growth characteristics of seedlings (shoot height, shoot and root weights) showed a significant increase with seedling

age (0.21, 0.54, 1.14 and 2.09 g gross weight/seedling at 2, 3, 4 and 5 weeks, respectively) indicating healthier saplings with delay in inoculation time.

Sagar *et al.* (2014) studied that the bacterial wilt infected potato stems were collected in *kharif* 2011 season from wilt affected areas of Hassan and Chikmagalur districts of Karnataka state. A total of 15 bacterial strains were recovered from wilt affected potato stems. On TZC agar medium, these strains yielded typical virulent type colonies, which were cream coloured, irregularly shaped , highly fluidal with pink pigmentation in the centre.

Zulperi *et al.* (2014) reported that the infected plants produced bacterial wilt symptoms as naturally infected samples while control plants remained uninfected. Basically, all strains expressed wilting 4 to 8 weeks after inoculation. All strain cultures were re-isolated and they resembled the morphological and biochemical characteristics of *R. solanacearum.*

2.4. Cultural and biochemical characteristics

Kelman (1954) reported that avirulent colony types of *R. solanacearum* could easily be differentiated by the pigmentation from the wild virulent types of *R. solanacearum.* The bacterium *R. solanacearum* developed two types of colonies on tetrazolium chloride (TZC) medium on which virulent colonies appeared white with pink centers and non-virulent colonies appeared dark red.

Schaad (1980) tested each medium size colony of *R. solanacearum* from TTC plates was mixed with a few microliters of water on a glass slide and gram stained. Staining results were observed under microscope for negative reddish pink staining or positive reaction violet blue staining and showed positive reaction for potassium hydroxide solubility test.

Suslow *et al.* (1982) tested the bacterial colonies of *R. solanacearum* were picked using wire loop and mixed rigorously with few drops of KOH solution (3 %) on a glass slide. The test was recorded as positive when bacterial solution got sticky forming slime threads with wire loop.

Denny and Hayward (2000) reported that the bacterium *R. solanacearum* is a gram-negative rod shaped, 0.5-0.7 x 1.5-2.0 micrometer and motile by one to four polar flagella. It showed positive reaction for catalase, starch hydrolysis, nitrate reduction and negative reaction for levan production, , indole production and hydrogen sulfide hydrolysis.

Dhital *et al.,* (2001) reported that all thirteen strains of *R. solanacearum* were arginine dihydrolase negative and oxidase, catalase and urease positive. All of them oxidized citrate within 4 to 5 days of inoculation by changing blue media into green. All the strains produced nitrate and ammonia after 2 to 3 days of inoculation and they showed positive reactions in levan production, motility, lipolytic and oxygen relation. These strains also hydrolyzed tween 80 and produced black color in potato slants.

Williamson *et al.* (2002) reported that the typically for *R. solanacearum*, the three geranium strains (BB-Feutz, BB-Esser, and 12-4-6 SD) were gram negative, oxidase positive, and catalase positive. On CPG medium, colonies were irregular, smooth, creamy white, and fluidal, and produced a brown pigment after 48 hrs. Colony morphology was similar on TZC medium, except that colonies were off-white with red centers.

Kumar and Sarma (2004) reported that the colonies were irregular, white and fluidal with incubation period of 48 to 72 hours on CPG and SMSA. Strains from ginger and other hosts could be differentiated on CPG medium on the basis of colony fluidity. Colonies of ginger strains were highly fluidal with

characteristic spiral pink centre whereas in the case of other strains fluidity and pink centre was less conspicuous.

Umesha *et al.* (2005) evaluated that on Kelman's medium plates, the bacterial colonies were pinkish white, mucoid, smooth, complete, convex after 24 hrs. The bacterial colonies on the nutrient agar were creamish white, mucoid, circular, convex with entire margins. *Ralstonia solanacearum* showed positive reaction for kovac's oxidase test, nitrate reduction test and negative reaction for gram staining, gelatin hydrolysis, arginine dihydrolase test.

Sambasivam and Girija (2006) evaluated that the bacteria isolated on triphenyl tetrazolium chloride (TZC) medium and they appeared as small creamy white colonies with pink centre. All the isolates showed positive reaction for solubility in KOH, nitrate reduction, production of catalase and oxidase enzymes and fermentation of glucose.

Lemessa and Zeller (2006) found that all the isolates of *R. solanacearum* from Ethiopia produce fluidal and irregular colonies with a red centre and whitish periphery on triphenyl tetrazolium chloride (TZC) medium after 48 hrs of incubation. However, when the strains lost their virulence upon storage, the colony becomes smaller and round with deep colour. On CPG medium Ethiopian isolates produce larger and whitish fluidal colonies which turn brown after 48 hrs of incubation.

Vanitha *et al.* (2009) found that *R. solanacearum* showed positive reaction for KOH solubility , kovacs' oxidase test, nitrate reduction, tobacco hypersensitive reaction, Pathogenicity test and negative reaction for gram's staining, levan formation and arginine dihydrolase.

Rahman *et al.* (2010) studied that gram's staining, potassium hydroxide solubility test and kovac's oxidse test revealed that all groups of *R. solanacearum* isolates are gram negative. The virulent isolates produce pink or

light red color colonies or colonies with characteristic red center and whitish margin and avirulent isolates produce smaller, off-white and non-fluidal or dry on TZC medium after 24 hours of incubation.

Chaudhry and Rashid (2011) found that *R. solanacearum* positive for, KOH test, kovacs oxidase test, catalase test, oxidation/fermentation of glucose, hydrolysis of tween 80 and negative for gram staining, arginine dihydrolase, levan production, salt tolerance, lecithinase detection, gelatin hydrolysis and production of fluorescent pigment.

Zhang *et al.* (2011) reported that *Ralstonia solanacearum* positive for motility, oxidase, strictly aerobic, PHB, production of acid with glucose, H_2S production, esterase (Tween 80) and negative for levan production, gram staining, methyl red test, VP test, indole test, l-valine, gelatin Liquefaction and arginine dihydrolase.

Marques *et al.* (2012) studied that seven isolates produced colonies that were white, smooth, fluid, irregularly round and opaque. In Kelman's medium the virulent colonies presented a reddish center and white borders, while the avirulent ones were completely red. Gram reaction was negative, there was no production of fluorescent pigment and they were catalase and oxidase positive. In the test of oxidation or fermentation of glucose, they were oxidative.

Narasimha and Srinivas (2012) reported that virulent isolates grown on TZC medium were highly fluidal, white colored with a light pink centre and round to irregular margin, 7.0-9.0 mm diameter. On the other hand, the avirulent colonies were round, deep red color with narrow bluish border. Microscopic studies revealed that bacterial isolates were gram-negative, rod-shaped, 0.5-0.7 x 1.5-2.0 µm in size non-capsulated and non-spore forming, strictly aerobic bacterium.

Kumar *et al.,* (2013) reported that *R. solanacearum* exhibit twitching motility. So, we also looked for twitching motility in F1C1. F1C1-streaked plates were observed after 24 hrs of incubation, under a compound microscope with 4X objective. Finger-like projections emerging out of the streaked edges were observed on the plates, suggesting F1C1 is capable of manifesting twitching motility. Twitching motility is basically due to the presence of type-IV pili on gram-negative bacterial cell envelope, and *R. solanacearum* demonstrates identical features (Liu *et al.,* 2001).

Javeria and Kumar (2014) reported that appearance of colonies of *R. solanacearum* were observed on TZC agar medium after 48 to 72 hrs of incubation at 28°C. Colonies of virulent or pathogenic strains of *R. solanacearum* appeared smooth, fluidal, irregular, colored with pink centers of growth and somewhat translucent under transmitted light. Virulent colonies after 48 hrs of growth could be differentiated from avirulent and non-pathogenic type colonies on the basis of colony texture, pink pigmentation and fluidity. Colonies size of virulent strains were non spore forming, rod shaped, gram-negative and their size measured between 4.5 to 9.5 mm. Non-pathogenic strains on selective TZC agar medium formed uniformly round, deep red colored colonies with a narrow colored margin, whereas pathogenic strains formed white colored irregular, fluidal, colonies with pink in center.

Maji and Chakrabartty (2014) reported that among biochemical test performed the isolates which showed positive results for loop formation, oxidase test, catalase test, production of white dense precipitate around bacterial colonies on Tween 80 and oxidation of glucose and showed negative results for gram reaction and levan production.

Seleim *et al.* (2014) reported that all tested isolates have a positive reaction for motility, oxidase test, catalase test, H_2S production, growth at 37°C

and NaCl tolerance (0.5, 1 and 1.5%) tests. In other hand, they have a negative reaction for gram stain, spore formation, gelatin hydrolysis, arginine dihydrolase, NaCl tolerance (2%), indole formation, aesculin hydrolysis, levan production and growth at 41°C tests.

Zulperi *et al.* (2014) reported that all isolates resembled morphological characteristics of *R. solanacearum* by producing fluidal colonies that were entirely white with pink centre after overnight incubation on Kelman's-TZC agar. They were also gram-negative with rod-shaped structure and gave positive results to potassium hydroxide, kovacs oxidase and catalase tests.

Shahbaz *et al.* (2015) isolated the *Ralstonia solanacearum* associated with chilli seeds from Pakistan. The bacteria produced fluidal pinkish red centered colonies, typical of *R. solanacearum* were observed on TTC medium. Among the biochemical test performed, isolates were positive for hypersensitive reaction, KOH loop test, catalase test, kovacs test and oxidation of glucose and isolates showed negative response in gram staining, Levan and Lipase production. The biochemical characterization concluded that 32 isolates out of 47 performed uniformly and those can be considered as *R. Solanacearum.*

2.5. Disease management strategies

2.5.1. *In vitro* efficacy of antibacterial chemicals

Hidaka and Murano (1956) studied *in vitro* effect of streptomycin on the behaviour of *R. solanacearum*, and they found that streptomycin at 0.3 µg/ml water inhibited and killed of *R. solanacearum.*

Dutta and Verma (1969) studied the efficacy of streptocycline in controlling bacterial wilt of eggplant and found that seedling treatment of variety Pusa purple long with the antibiotic (1g/l) for 30 minutes before planting gave best result.

Indersenan *et al.* (1981) reported that treatment of ginger rhizomes with plantomycin at 1000 ppm was found effective against the seed borne bacteria *R. solanacearum.*

Ojha *et al.* (1986) observed that rhizome treatment of ginger with organomercurials like Emission-6 (0.2%) plus plantomycin (0.05%) for 30 minutes in addition to three sprays, first at 30 days after planting followed by 15 days interval resulted in 100 per cent control of disease.

Khan *et al.* (1997) reported that Emison-6 at 1000 ppm produced maximum inhibition zone (33.3 mm) followed by streptomycin sulphate (26.66 mm) thousand fold reductions in the pathogen population was noticed in antibacterial chemical treated soil. Under field studies, at 0.10 per cent Emison -6 reduced the incidence of wilt by 78.01 per cent followed by bleaching powder application.

Singh *et al.* (2000) found that streptomycin and streptopenicillin were superior over other antibiotics against the pathogen under *in- vitro* and *in-vivo* conditions.

Devanath *et al.* (2002) assessed that two chemicals *in vitro* 200 ppm streptocycline and 0.25 % copper oxychloride was found effective against *R. solanacearum* in ginger.

Dubey (2005) conducted an experiment with tomato and revealed that seedling root dip treatment with streptocycline (200 ppm) found effective control of bacterial wilt caused by *R. solanacearum*

Venkatesh (2005) conducted *in vitro* evaluation of bactericides on the growth of *Ralstonia solanacearum,* eight compounds comprising of bactericides and antibiotics were tested out of them sulphamethaxazole and streptocycline were found effective at 500 and 1000 ppm respectively.

Sambasivam and Girija (2005) evaluated that all the isolates were found resistant to ampicillin and rifampicin at 100 µg/ml concentrations. They were highly sensitive to chloramphenicol at 150 µg/ml and kanamycin at 50 µg/ml.

Sunder *et al.* (2011) found that the average zone of inhibition of 13.06 ± 3.04 mm and 12.59 ± 2.93 mm were produced against RSN 6 and RSN 12, *R. solanacearum* isolates respectively. The best zone of inhibition was obtained with ciprofloxacin (33 mm) followed by ofloxacin (30 mm), Tetracycline (27 mm), Gentamycin (24 mm).

Sangoyomi *et al.* (2011) evaluated that ciprofloxacin resulted in the highest result with a zoning diameter of 13 mm followed by ofloxacin (10 mm) while pefloxacin, drovid and cotrimozazole produced zoning diameters between 8 and 9 mm. Norfloxacin and clindomycin had very low zoning diameters of 6 and 2.5 mm respectively.

Singh *et al.* (2012) evaluated that *in vitro,* population of *R. solanacearum* was reduced drastically by increasing the concentration of the chemicals significantly. Minimum population of *R. solanacearum* 5.89 log10 cfu/ml was found in bleaching powder (1.0 %) followed by calcium chloride (1.0 %) 5.91 log10 cfu/ml.

Pankaj *et al.* (2013) reported that chemical streptocycline, copper oxycloride and their combination formed inhibition zone at all the concentration.

Gupta and Razdan (2013) evaluated that among the antibiotics plantomycin (streptomycin sulphate + tetracycline) was most effective followed by pencillin. The least effective antibiotics tested were azithromycin, erythromycin and ampicillin.

Owoseni and Sangoyomi (2014) reported that the highest zone of inhibition was obtained with augmentin (14 mm) and this was followed by amoxycilin (12 mm) and gentamycin (11 mm).

2.5.2. *In vitro* efficacy of bioagents

Gallardo *et al.* (1989) reported that the inhibition of *R. solanacearum* under *in vitro* by using the antagonists Bc-8 strain of *P. fluorescens*. The growth of *R. solanacearum* was inhibited by *P. fluorescens* (str. Bc-8) or its culture extracts. It was found that extract contain rod shaped particles with the helical structure with 150 nm long and 25 nm diameter. These were interpreted as bacteriocin floucin Bc-8.

Ciampi *et al.* (1996) isolated siderophore like substances from *P. fluorescens* responsible for inhibiting the growth of *R. solanacearum* and reported that these substances are responsible for inhibiting the growth of the pathogen and this siderophore activity is more when iron in the medium is limited which chelate the iron compound required for the growth of *R. solanacearum*.

Guo *et al.* (2001) reported that the *Pseudomonas* species is most effective for control of bacterial wilt of ginger.

Li *et al.* (2003) evaluated 36 strains of antagonists under *in vitro* condition against *R. solanacearum* among them, strain B47 showed highest antagonistic activity and highest efficiency to disease in the green house and field condition.

El-Sayed *et al.* (2003) evaluated *Pseudomonas fluorescens*, *Streptomyces fumigatisclerotis*, *S. griseviridis*, *S. rochei*, *S. violaceusniger* and *Bacillus subtilis* against *R. solanacearum* in *in vitro* and *in vivo* conditions.

Pseudomonas fluorescens and *Streptomyces griseviridis* were the most effective in the reduction of disease severity.

Sun *et al.* (2004) reported that the *Bacillus* sp. and *Pseudomonas* sp. are the most popular biocontrol agents. Several living microbial products have been commercialized as biocontrol agents, such as a wettable powder of *Bacillus subtilis* (Cohn) Y1336, a water suspension of *Pseudomonas fluorescens* (Migula), a mixture of wettable powder and granule of *Paenibacillus polymyxa* (Ash, Priest and Collins).

Sood (2005) conducted an experiment in order to determine the potential of antagonistic rhizobacteria against *R. solanacearum*, 386 rhizobacterial isolates were screened for their activity by dual culture technique using cross streak, tooth pick and paper disc methods. Six isolates of *Pseudomonas fluorescens* and one isolate of *Bacillus cereus* showed maximum antagonistic activity against *R. solanacearum.*

Doan and Nguyen (2005) evaluated that two strains of *Pseudomonas fluorescens* (B16 and VK58) and one strain of *Bacillus subtilis* (B.16) were identified as promising for biological control of bacterial wilt.

Ramesh (2006) studied the effect of seedling dip treatment with biocontrol agents on brinjal for the management of *Ralstonia solanacearum.* During 2003, the least incidence of 32.30 per cent was observed in treatment with combination of *Trichoderma viride* and *Pseudomonas fluorescens.* The disease incidence in untreated control was 86 per cent

Henok *et al.* (2007) reported that out of the 50 fluorescent isolates screened against *P. solanacearum* on KB medium only three local isolates i.e., Pfwt 3 (Wondogenet), Pfw1 (Wolayta), Pfs1 (Shashamane) and Pfri (reference strain) were capable of inhibiting the growth of the pathogen while others did not produce any antibiosis. The diameter of inhibition zones ranged from 1.2 to 2.4

cm where the minimum and maximum inhibition caused by Pfri and Pfw1, respectively. The isolate Pfw1 from Wolayta and Pfs2 from Shashamane produced higher zones of inhibition than the rest including the reference strain from India.

Lemessa and Zeller (2007 B) studied that common control measures against bacterial wilt antagonistic rhizobacteria such as *Bacillus* spp.

Liza and Bora (2008) studied that the bacterial population in soil the highest reduction was observed in the treatment with *P. fluorescens* @15 g/kg of soil.

Vanitha *et al.* (2009) evaluated that bacterial wilt incidence was reduced significantly in all 20 tomato cultivars tested, in plants raised from seeds treated with *P. fluorescens* followed by challenge inoculation with *R. solanacearum*. The bacterial wilt incidence in the untreated control ranging from 12 to 85% was reduced to 2 to 30% in the treated cultivars.

Liza and Bora (2009) studied the compatibility of *Trichoderma harzianum* and *Pseudomonas fluorescens* against *Meloidogyne incognita* and *Ralstonia solanacearum* complex on brinjal. They studied the population dynamics of *Trichoderma harzianum* and *Pseudomonas fluorescens* from two different substrates *viz.*, vermicompost and wheat bran after different days of storage related to both the substrates, the population density of *Pseudomonas fluorescens* significantly increased up to 45 days of storage. The highest reduction of *Meloidogyne incognita* and *Ralstonia solanacearum* observed in combined application of *Trichoderma harzianum* and *Pseudomonas fluorescens.*

Maketon *et al.* (2010) evaluated seventy-eight bacterial isolates and two commercially available microorganisms for control of *Ralstonia solanacearum* race 1 biovar 4, a bacterium wilt disease pathogen of ginger in Thailand. Two bacteria, *Bacillus subtilis* K1 and *Pseudomonas fluorescence* PSI

2 and the commercially available fungus *Trichoderma harzianum* Ap-001 (Trisan^{TM}) provided the best disease control.

Nguyen and Ranamukhaarachchi (2010) found that three antagonists isolated from soil (*Bacillus megaterium, Enterobacter cloacae, Pichia guillermondii*) and *Candida ethanolica* showed high potential for disease suppression and also increased fruit weight, biomass and plant height. These results provide encouragement for continued research on biological control of bacterial wilt by antagonistic isolates from soil.

Li *et al.* (2010) reported that both *P. macerans* MB02-992 and *P. polymyxa* MB02-1007 showed strong inhibitory activities against seven strains of *R. solanacearum*, which were isolated from ginger, tomato, eggplant and mulberry, respectively, while the *in vitro* growth of strain E406 was unaffected by the two antagonistic bacteria.

Tomer and Sunaina (2010) reported that the *Bacillus* spp. (8B-7) antagonize *R. solanacearum in vitro* by siderophore mediated competition for iron, were as inhibition of pathogen growth by *Bacillus pantothenticus* (BM-11) is antibioses based. Correlations were also found between antagonistic activities of this *Bacillus* spp. These strains suppressed disease when mixed together with the pathogen through the soil or when seeds or seedlings were treated with the strains before transfer into soil infested with *Ralstonia solanacearum.*

Chaudhry and Rashid (2011) evaluated that all 10 strains of *P. fluorescens* tested showed antagonistic effects against *R. solanacearum*, with inhibition zone radii ranging from 3 to 29 mm *P. fluorescens* strain 5 was most potent inhibiting all test pathogen strains, followed by Pf 4, Pf 6, and Pf 9. However the activity of other *P. fluorescens* isolates varied considerably against different pathogen isolates. Among the *Trichoderma* spp. tested, against ten strains of *R. solanacearum*, *T. asperellum* showed most potent inhibiting all test

pathogen strains, followed by *T. viride, T. harzianum.* However the activity of other *Trichoderma* isolates varied considerably against different pathogen isolates.

Sivakumar *et al.* (2011) studied that *Bacillus megaterium* isolate NBAII 63 was highly inhibitory with 29.20 mm of inhibition zone against *R. solanacearum* followed by NBAII-7 with 17.40 mm, NBAII-33 with 15.30 mm.

Abdlwareth *et al.* (2012) reported that about 200 *Bacillus* isolates were isolated from tomato and potato rhizosphere and examined for their *in vitro* and *in vivo* antagonistic activities against *Ralstonia solanacearum* T-91, the causal agent of tomato bacterial wilt. Four isolates Aml, D16, D29 and H8 gave the highest means of inhibition diameter of 9.33, 8.33, 9.33 and 8.66 mm, respectively, while the other isolates offer a wide range of antagonistic activity from 5 to 7 mm in laboratory *in vitro* test.

Narasimha and Srinivas (2012) studied that *Trichoderma* spp. tested against ten strains of *Ralstonia solanacearum, T. asperellum* showed most potent inhibiting all test pathogen strain followed by *T. Viride* and *T. harzianum.* However activity of other *Trichoderma* isolates varied considerable against different pathogen isolates.

Narasimha *et al.* (2012) evaluated that the LAB I and LAB II were screened against *R. solanacearum* strains, of which Rsl, Rs 2, Rs 3, Rs 4 and Rs5 showed inhibition that ranged from an average of 22 to 24 and 23 to 25 mm radius zone respectively.

Khair *et al.* (2012) reported that *B. subtilis* as seed tuber treatment significantly reduced the bacterial wilt disease incidence in potato cultivars Diamante and Spounta, followed by *T. hamatum* and *T. album* compared with the controls. The percentage disease control was in the range 42.1 to 52.6%. The brown rot incidence (BRI) was in the range 21.2 to 46.3% as tuber treatment and

18.9 to 29.1 % as soil treatment in treated potato plants compared with a disease incidence of 60 % in the control plants.

Sreeja S. J. (2012) evaluated that maximum inhibition (29.25 %) of the pathogen was observed with Vellanikkara isolate (EAVK) which was on par with Ozhalapathy isolate (EAOP) (22.59 %) under *in vitro* conditions. The efficacy of culture filtrate of endophytic actinomycetes recorded maximum per cent inhibition (44.63 %) of the pathogen with Cherumkuzhy isolate (EACK) which was on par with Ozhalapathy isolate (EAOP) (42.59 %). It was concluded from the study that the Ozhalapathy isolate (EAOP) (*Streptomyces* spp.) was the most efficient among the endophytic actinomycetes isolates in the management of bacterial wilt pathogen under *in vitro* conditions.

Yang *et al.* (2012) reported that *Bacillus subtilis* strain 1JN2, *Myroides odoratimimus* 3YW8, *Bacillus amyloliquefaciens* 5YN8, and *Stenotrophomonas maltophilia* 2JW6 were showed biocontrol efficacies greater than 50 %. This is the first report of using *Myroides* spp. and *Stenotrophomonas* spp. as biocontrol agents against ginger wilt caused by *R. solanacearum,* and these strains show promise as commercial biocontrol agents.

Gupta and Razdan (2013) evaluated that two bacterial isolates of *Pseudomonas fluorescens* were effective than the fungal antagonists, against *Ralstonia solanacearum.* Among the fungal isolates, *T. viride* (Tv_4) was the most effective.

Makhlouf and Hamedo (2013) reported that the *Pseudomonas aeruginosa* and *Bacillus thuringiensis* were able to significantly reduction of the growth of pathogen and by far superior to others showed more inhibition zone 8.6 and 8.2 mm respectively in cross culture method and 4.6 and 4.3 mm respectively in filter paper disk method, while *P. stutzeri* and *P. syrinagae* showed the lowest

inhibition zone of pathogen 4.6 and 2.7 mm in cross culture method and 2.0 and 1.5 mm in filter paper disk method.

Liu *et al.* (2013) found that more than 100 efficient strains were isolated and they showed positive antagonistic effects against *R. solanacearum.* Two potential antagonistic strains, L-9 (*Brevibacillus brevis*) and L-25 (*Streptomyces rochei*) were selected for their strong and consistent inhibition of *R. solanacearum.*

Rahman *et al.* (2013) found that among 69 isolates of producer bacteria, five isolates viz. SS21, ST26, PE31, AC53 and IB55 showed antagonistic effect against three isolates of *R. solanacearum.* Distinct inhibition zones were developed around the colonies of antagonistic bacteria. Isolates SS21 (from *S, sysmbriifolium*) and isolate IB55 (from *Impatiens balsamina*) were antagonistic against all of the three isolates of *R. solanacearum.*

Raghu *et al.* (2013) evaluated that *P. fluorescens* resulted in maximum inhibition of *R. solanacearum* with an inhibition zone of 1.85 cm which was found significantly superior over other treatments followed by *B. subtilis* (1.0 cm) and *T. harzianum* (0.85 cm).

Singh *et al.* (2013) evaluated that out of 250 isolates of *Bacillus* species, 47 strains showed antagonistic ability against *R. solanacearum.* Maximum growth of *R. solanacearum* was inhibited by strain DTBS-5 to form inhibition zone of 5.5 cm *in vitro* and lowest wilt incidence of 14.3 and 7.6 % in Pusa Ruby and Arka Abha cultivars under glass house conditions, respectively. Plants treated with strain JTBS-9 had maximum fresh weight of 42.0 and 49.0 g and dry weight 6.1 and 6.6 g in tomato cultivars Pusa Ruby and Arka Abha after 45 days of transplanting, respectively.

2.5.3. *In vitro* efficacy of botanicals/phytoextracts

Hannudin and Djantnika (1989) studied the effect of some plant extracts on *R. solanacearum* growth in *in vitro.* Extracts from onion and garlic bulbs, roots and stems of crotalaria spp. and tagetus spp. were evaluated for suppression of *R. solanacearum.* They found that extracts from garlic bulbs inhibited bacterial growth.

Khan (1974) reported that garlic extract (*Allium sativum*) was inhibitory to the growth of *R. solanacearum* under *in vitro* conditions, whereas the onion extract had no effect.

Karuna and Khan (1993) reported that plant extracts obtained from *ocimum,* eucalyptus, citronella, neem, adathoda inhibited the growth of *R. solanacearum* under *in vitro* condition. Maximum inhibition zone of 43.9 mm was obtained in extract of eucalyptus followed by citronella (40.4 mm) and *ocimum* (37.2 mm) respectively.

Sasitorn (2003) evaluated that the extraction of fresh pomegranate fruit shell by 60 % ethyl alcohol of 10,000 ppm showed visible inhibition zone of average diameter of 0.8 cm and the inhibition zone became 1.28 cm when the concentration of the extract was 50,000 ppm. While, in 40 % ethyl alcohol, the extract of dry pomegranate fruit shell of 100,000 ppm caused the largest inhibition zone with average diameter of only 1.48 cm.

Khan *et al.* (2007) found that seven essential oils from aromatic plants were found effective at 1.00 mg concentration with significantly more inhibition zone in comparison to other concentrations of botanicals. Oil from *Mentha piperata* restricted the bacterial growth to 9.42 mm and was found statistically superior to others i.e. palmarosa (8.42 mm), geranium (8.00 mm), *M. arvensis* (7.83 mm), citronella (7.75 mm), *M. spicata* (7.50 mm) and lemon grass (7.17 mm) at the highest concentration of botanicals. Out of eleven methanol

extract of aromatic/medicinal plants only three (palmarosa, *M. arvensis, and M. longifolia)* were found effective. Maximum inhibition zone (38.00 mm) was recorded with palmarosa leaf extract followed by *M. longifolia* (13.50 mm) and *M. arvensis* (15.33 mm) at 1.00 mg botanical concentration.

Balestra *et al.* (2009) found that in *in vitro* tests with bacterial strains at a population density of 10^6 and 10^8 cfu ml_1, vegetal extracts from cloves of *A. sativum* and fruits of *F. carica* at concentrations of 1 and 30%, respectively showed best effects at 10^6 cfu ml_1 bacterial concentrations.

Elyousr and Asran (2009) reported that garlic exhibited the strongest antibacterial activity against bacterial wilt *in vitro* and *in vivo* followed by datura and nerium. Cold water extracts of these plant species were more effective than hot water extract in the development of the disease *in vivo.*

Paret *et al.* (2010) studied that *R. solanacearum* did not grow on plates amended with palmarosa oil at any concentration or lemongrass oil at 0.07 and 0.14 % at 48 hrs after incubation. At 72 hrs, growth was seen on plates amended with palmarosa oil at 0.04 %, but the populations were at ca. 2 log (10^2) CFU/ml, significantly lower than the control with ca. 8 log CFU/ml. At 48 hrs, *R. solanacearum* growth was significantly lower on plates amended with lemongrass oil at 0.04 % (ca. 3 log CFU/ml) than on controls (ca. 8 log CFU/ml).

Wagura *et al.* (2011) found that ethyl actate extracts of *Ipomoea batatas* at concentration of 0.4 mg ml^{-1} giving mean inhibition zone of 4.2 mm followed by ethyl actate extracts of brassica oleracea at concentration of 0.05 mg ml^{-1} that was 4.12 mm.

Narasimha *et al.* (2012) observed that among 12 tested plant extract against ten strains of *Ralstonia solanacerum*, Neem and pomegranate showed greater inhibition and guava, papaya, turmeric and gulancha tinospora showed

relatively higher level inhibition whereas Japanese mint and mustard showed relatively lower of inhibition activity.

Nezhad *et al.* (2012) evaluated that the most active essential oils against tested bacteria was thyme oil with the inhibition zone of 34.8 mm against *R. solanacearum* and the MIC of 1 µl/ml while this value was higher than streptomycin and erythromycin inhibition used as positive control. Essential oils of *Coriandrum sativum*, *Cuminum cyminum*, *Rosmarinus officinalis* and *Eucalyptus globulus* were in the next positions. The efficacy of essential oils from *E. globulus* was insignificant.

Deberdt *et al.* (2012) reported that *A. fistulosum* extract at concentrations of 50 and 100 % exhibited antimicrobial activity against *R. solanacearum* strain CFBP6783 at both bacterial concentrations (10^7 and 10^8 CFU/ml) used *in vitro*. The best inhibition effects of the extracts were observed at a bacterial concentration of 10^7 CFU/ml. Growth of *R. solanacearum* was significantly lower when the concentration of the *A. fistulosum* was 100 rather than 50 %, regardless of the bacterial concentration. At a concentration of 25 %, no effects of *A. fistulosum* was observed on growth of *R. solanacearum.*

Owoseni and Sangoyomi (2014) evaluated that the plant extracts from chloroform were the most active and this was followed by methanol and ethanol. The lowest activity was recorded from the hexane extracts. The chloroform extracts of *J. curcas* had the widest zone of inhibition of 15 mm followed by *O. gratissimum* (13 mm). All the solvent extracts of *A. sativum* were active except the hexane extract. The MIC of the active extracts were studied, the MIC of the *A. sativum* ethanolic extract was 0.25 mg/ml while it was 0.5 mg/ml for the *V. amygdalina* ethanol extract. The MIC of the *A. sativum* chloroform extract was 0.25 mg/ml; *J. curcas* chloroform extract MIC was 0.125

mg/ml, and the MIC for methanolic extract of both extracts were 0.5 mg/ml and 0.25 mg/ml respectively.

Alemu *et al.* (2014) evaluated that all aqueous extracts had significantly inhibited the growth of *R. solanacearum in vitro* compared with control. The antibacterial activity of aqueous extracts of five IAS extracts ranged from 8.56 to 26 mm. The highest diameter of inhibition zone was recorded from *E. crassipes* (26 mm) that was statistically different from all other treatment combinations and over the infected control.

2.5.4. *In vitro* efficacy of organic amendment

Ouedrago (1994) studied that increasing the doses of well-done compost to get a suppressive soil for *R. solanacearum* is another possibility to control the disease as experimented in Burkina Faso.

Sharma and Kumar (2000) reported that karanj cake effective in reducing the *Ralstonia* population maximum plant survival and increase in the yield.

Lemaga *et al.* (2001) demonstrated that soil amendments can help to reduce wilt incidence and increase the yield; but the best result came from the combination of organic and inorganic fertilizers, when potassium was added with an organic source of nitrogen.

Schonfeld *et al.* (2003) reported that soils with high organic matter content like those amended with cattle manure, sunhemp, household compost and crop residues are reported to release allellochemicals that suppress bacterial wilt populations.

Gorissen *et al.* (2004) demonstrated that the addition of pig slurry decreased significantly the population of *R. solanacearum* as well as reduced numbers of infected and diseased plants in the soil suppressiveness tests.

Sharma and Kumar (2004) reported that karanj cake act as organic amendment to reduce *Ralstonia solanacearum* population in soil and bleaching powder act as bactericide which was reduce the *Ralstonia* population in soil result good health and increased yield.

Bose *et al.* (2004) reported that soil amendment with soybean flour, rice husk+urea along with recommended fertilizer gives control of tomato bacterial wilt.

Islam and Toyota (2004) studied that bacterial wilt of tomato was suppressed in the poultry manure and FYM added soil.

Andriantsoa *et al.* (2006) tested different cruciferous plant as amendments to control *R. solanacearum* and found that 50 % of the total plant reduced the population of the bacteria.

Sharma and Kumar (2009) reported that karanj (*Pongamia*) cake resulted 30.5 and 33.2 % reduction in initial *Ralstonia* population at 10 and 20 kg/ha doses respectively.

Yadessa *et al.* (2010) found that effect on disease suppression and survival of the pathogen in the soil differed depending on amendment type and application rate. Higher disease severity was recorded in soil amended with 10% green compost compared to the control treatment. Complete suppression of *R. solanacearum* was observed in pots amended with 5 and 10 % farm yard manure (FYM), 1 % green compost and 10 % cocopeat.

Reddy *et al.* (2012) found that the best treatment for suppression of bacterial wilt in eggplant was seed treatment (1 hour) with 10 % aqueous extract

of vermicompost neem coupled with the application of vermicompost neem to the soil both during sowing as well as on transplantation. This treatment reduced the bacterial wilt by 100 %. The application of vermicompost alone was not enough to protect the plants against the disease but coupling it with aqueous seed treatment is important to achieve complete disease suppression and increase the yields.

Ashlesha and Paul (2012) reported that the unsterilized biodynamic compost tea showed 91.30 % inhibition at 2.0 % and 95.60 % inhibition at 4.0 % concentration in the bacterial growth whereas biosol (unsterilized) was also found effective in inhibiting bacterium with 92.30 %. Combination of biosol, cow urine, fermented butter milk, biodynamic compost tea, vermiwash and homa ash enhanced the survivability of seedlings upto 13.65 days at 1:10 ratio after 1 hr dip duration when seedlings were tested in Hoagland's solution. All organic inputs were evaluated under polyhouse conditions against bacterial wilt of *capsicum*. Drench with mixture of biosol, fermented butter milk, cow urine, vermiwash and homa ash before and after transplanting along with seedling dip in biosol for 10 min. before transplanting provided 86.64, 85.22 and 84.64 % disease control at Rajol, Palampur and Sungal areas, respectively.

2.6. Integrated management of bacterial wilt of ginger (pot culture).

Ojha *et al.* (1986) observed that treatment of seed rhizomes with Emisan 6 + plantomycin for 30 minutes followed by three sprayings, first at 30th day after planting and others at an interval of 15 days also gives good control of the disease.

Dubey (2005) reported that the interaction of karanj cake, bleaching powder and seedling root dip in streptocycline was found to be most effective

treatment as significantly minimum wilt incidence was recorded at 60, 90 and 130 days after transplanting with highest fruit yield.

Biswas *et al.* (2008) studied the effect of soil rectification for the control of bacterial wilt of tomato. They observed that soils treated with lime and lime + bleaching powder were found effective in minimizing bacterial wilt. Soil rectification with lime one month before transplantation and use of *Pseudomonas fluorescens* as biocontrol agent were effective to minimize the bacterial wilt incidence in field.

Hussain and Bora (2008) reported that integration of summer ploughing, half recommended dose each of carbofuran 3G, neem cake, streptocycline and full dose of *Trichoderma harzianum* were found superior treatments against *Meloidogyne incognita* and *Ralstonia solanacearum* complex in brinjal under field conditions. The treatment effectively improved all the plant growth parameters and yield of the crop with corresponding decrease in the nematode reproductive rate. The treatment also produced minimum final bacterial population in the soil along with less percent wilt incidence (PWI).

Sharma and Kumar (2009) reported that in alfisols of East India plateau soil application of karanj cake, bleaching powder, lime and seedling dip and spray of streptocycline. Resulted in reduction of initial population of *Ralstonia solanacearum* in soil in post monsoon seasons of 1998-99 to 2001-2002. Per cent reduction in initial population of *R. solanacearum* noted at 90 days after planting of tomato were 30.5 and 33.2 at 10 and 20 q karanj cake per ha. 31.9 and 35 at 30 and 45 kg bleaching powder per ha. 28.6 and 27.9 at seedling dip and spray of streptocycline (500 ppm) and mild reduction of 18.7 and 25 q lime per ha. Based on these results they recommended that bleaching powder and karanj cake are stronger bacteriostatic substance than lime and streptocycline.

Ravi and Suryanarayana (2011) studies that pre-sowing rhizome treated with combinations of 0.05 % streptocycline + 0.2 % copper oxycloride for 20 min and post sowing soil drench with 0.2 % bleaching powder and 0.1 % Metalaxyl MZ thrice at 20 days intervals from disease inception found very effective in reducing the PDI (2.79). The next best treatment was rhizome treatment with 0.05% streptocycline + 0.2 % copper oxychloride for 20 min. followed by soil drench with 0.2 % bleaching powder in reducing PDI (11.91). The treatment T_7 (rhizome treatment with 0.05 % streptocycline + 0.2 % copper oxychloride for 20 minutes and soil application with FYM enriched *Trichoderma harzianum* and *Pseudomonas fluorescens* of 1 kg each/ton of FYM stood next by recording PDI of 15.70. Untreated control showed PDI value (51.63) which exceeds more than 17 times the best treatment.

Sawant *et al.* (2014) reported that the highest reduction mortality was recorded with RDF + 66g Gypsum /kg soil + Copper oxychloride 2500 ppm +Streptocycline 300 ppm (75.23 %), followed by Neem seed kernel extract 20 % + Copper oxychloride + Streptocycline (73.84) and Noni fruit extract 20 % +Copper hydroxide + Streptocycline (72.68 %).

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

During the present investigations on bacterial wilt caused by *Ralstonia solanacearum* of ginger (*Zingiber officinale* Roscoe) various experiments were conducted at the Department of Plant Pathology, College of Agriculture, Vasantarao Naik Marathwada Krishi Vidyapeeth, Parbhani during 2014-15. The details of the materials used and methods followed for various experiments are described herein the following paragraphs.

3.1. Materials

3.1.1. Experimentation site

All the experiments (Plate and pot culture) were conducted at Department of Plant Pathology, College of Agriculture, VNMKV, Parbhani.

3.1.2. Disease samples

The plant and soil samples from wilt affected ginger plants showing typical symptoms of bacterial wilt were collected from farmer's field. The diseased samples were brought to the laboratory and subjected aseptically isolation of soil borne bacteria by serial dilution method and streak plate method for which TTC selective medium was used.

3.1.3. Culture media

Triphenyl tetrazolium chloride agar (TTC) medium was used as basal medium for isolation, sub culturing and multiplication of bacterial culture was carried out on casamino acid peptone glucose agar (CPG) medium and sterile water was used for the storage of pure culture of *R. solanacearum*. The synthetic

media and ingredients of non-synthetic media were obtained from the Department of Plant Pathology, College of Agriculture, Parbhani.

3.1.4. Chemicals

Standard chemicals, reagents, bactericides, culture media etc. required for the experimentation were obtained from the Department of Plant Pathology, College of Agriculture, Parbhani.

3.1.5. Glass-wares

The common glass-wares (Borosil, J-sil and Corning make) *viz.*, Petri-dishes, test tubes, conical flasks, volumetric flasks, measuring cylinder, glass rods, beakers, funnel, pipette, airtight plastic ependorf tubes etc. were obtained from the Department of Plant Pathology, College of Agriculture, Parbhani.

3.1.6. Equipments

The laboratory equipments *viz.*, Autoclave, Hot air oven, Laminar airflow cabinet, BOD Incubator, Refrigerator, Binocular Research Microscope, Electronic balance, etc. available at the Department of Plant Pathology, College of Agriculture, Parbhani were utilized, as and when required.

3.1.7. Rhizome seeds

The rhizomes of ginger were obtained from the local market and used for the experiments (pot culture).

3.1.8. Organic amendments

Organic amendments *viz.*, Vermicompost, FYM, Cotton seed cake, Karanj cake, Poultry manure, Sunflower cake, Neem seed cake etc. were obtained

from local market and Department of Plant Pathology, College of Agriculture, Parbhani and used as amendments for pot culture studies.

3.1.9. Antibacterial Chemicals

The following 6 antibiotics and 3 antibacterial fungicides were used for *in vitro* culture experiments conducted during present studies.

Sr. No.	Common name	Trade Name	Manufacture
1.	Streptocycline	Streptocycline	Hindustan Antibiotics Ltd. Mumbai
2.	Tetracycline	Tetracycline	Intervet India Pvt. Ltd. Pune
3.	Neomycin	Nebasulf	Pfizer Limited Bangalore
4.	Cephalexin	Lixen	Virbac Animal Health India Pvt. Ltd. Mumbai
5.	Dicrysticin	Dicrysticin-S	Cadila Healthcare Limited Vadodara
6.	Gentamycin	Genticyn	Nitin Lifesciences Ltd. Pune
7.	Copper oxychloride	Blitox	Syngenta Ltd., Mumbai
8.	Copper hydroxide	Kocide	Green crop International Ltd. Pune
9.	Azoxystrobin	Amistar	Syngenta Ltd. Mumbai

3.1.10. Plant extracts / botanicals

Plant species reported to exhibit antibacterial and therapeutic properties against bacterial pathogens and easily available were collected from the farms of College of Agriculture, Parbhani and adjoining fields. Following locally available 10 plant species / botanicals were used during present studies.

Sr.No.	Common Name	Botanical Name	Plant Part Used
1.	Garlic	*Allium sativum*	Clove
2.	Turmeric	*Curcuma longa*	Rhizome
3.	Tulsi	*Ocimum sanctum*	Leaves
4.	Neem	*Azadirachta indica*	Leaves
5.	Onion	*Allium cepa*	Bulb
6.	Parthenium	*Parthenium hysterophorus*	Leaves
7.	Mehandi	*Lawsonia innermis*	Leaves
8.	Karanj	*Pongamia pinnata*	Leaves
9.	Dhotra/Dhatura	*Datura stromonium*	Leaves
10.	Gokharu	*Tribulus terrestris*	Leaves

3.1.11. Biocontrol agents

Pure cultures and talc based formulations of biocontrol agents *viz., T. viride, T. harzianum, T. koningii, T. longibrachiatum, T. virens, Aspergillus niger, Bacillus subtilis* and *Pseudomonas fluorescens* were obtained from the Spawn Production-cum-Biocontrol Laboratory, Department of Plant Pathology, College of Agriculture, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani; maintained and multiplied on appropriate culture media and used for further studies.

3.1.12. Miscellaneous

Earthen pots (30 cm. dia.), plant protection appliances, inoculation needle, forceps, blotter paper, paper bags, polythene bags, spirit lamp, mercuric chloride, scales, sand, soil, FYM, screen house etc. were used during the course of present investigation.

3.2. Methods

3.2.1. Cleaning and sterilization of materials and reagents

All the glass-wares and plastic-wares were soaked overnight in potassium dichromate sulfuric acid solution. After 24 hours the washing solution was drained off and the materials were washed thoroughly in running tap water and rinsed with distilled water. The plastic-wares were air-dried and the glass-wares were dried in hot air oven. The cleaned and dried glass wares were wrapped in aluminum foil, covered with papers and autoclaved at 121°C with 15 psi for 20 minutes.

3.2.2. Sterilization of laminar air flow

All the experiments namely isolation, cultures studies and *in-vitro* evaluation of antibiotics, botanicals and biocontrol agents were conducted under aseptic condition in laminar air flow cabinet (Make: ACS Bangalore). Before working under the hood, the working surface was uniformly sterilized by sweeping with 70 per cent alcohol. Any material coming from outside was also sterilized with alcohol. The blades, forceps, inoculation loop etc. were sterilized by heating in the flame and before starting the experiments the hands were cleaned well with alcohol.

3.2.3. Isolation of *R. solanacearum* from bacterial wilt affected ginger plant and soil

The diseased plant and soil samples were collected from the farmer's field. The diseased plant samples were washed under tap water to remove the soil particle and air dried. The pseudostem of diseased plant of length 10 to 15 cm was first surface-disinfected with 70 % ethanol for 2 minutes and 1% sodium hypochloride for 5 minutes followed by repeated washing in sterile water for 5 minutes to remove traces of sodium hypochloride. The surface sterilized bits were suspended in the five-milliliter sterile distilled water taken in test tube for

ten minutes. After the water in test tube becomes turbid due to oozing of bacterial cells from cut ends of diseased tissue, the bacterial suspension was serially diluted in nine ml sterile water. One hundred microliter (1 ml) of the bacterial suspension was poured onto the surface of solidified Triphenyl tetrazolium chloride agar (TZC) medium (Kelmen, 1954) containing (g/L) peptone 10; casein hydrolysate 1; glucose 5; agar 20; and distilled water 1L ; pH 7.0 (1 % TZC will added to a final concentration of 5 ml/L after autoclaving) using spread plate technique. A loopful of bacterial suspension was streaked into TZC medium and incubated at 28±2°C for 48 hours.

To isolate the pathogen from soil, the soil samples were serially diluted and pathogen was isolated using TZC medium. At the end of incubation period, the plates were observed for the development of both the virulent and avirulent colonies of *R. solanacearum*. The virulent colonies were irregularly shaped, fluidal, dull white colonies with pink center. Whereas, avirulent colonies small, round, convex, butyrous with large red pigment and white fluidal colonies without pink center described by Kelman (1954).

3.2.4. Purification of pathogen

Typical virulent colonies of *R. solanacearum* were picked by inoculation loop, streaked separately on TZC medium in sterilized Petri-plates. The plates were incubated at 28°C for 48 hours. The well developed typical colonies *R. solanacearum* were picked up with sterile inoculated loop and suspended in sterile distilled water culture collection plastic ependorf tubes and stored at 4°C in refrigerator for further use as stock culture.

3.2.5 Pathogenicity test

Pathogenecity test was attempted to established host-pathogen interaction by pseudostem inoculation method (Kumar A. 2006). The ginger sprouts were raised by planting 30 g bits of seed rhizomes in steam sterilized

standard potting mixture with soil, sand, and FYM in 3:1:1 ratio. Fourty five days old plants were used for inoculation and a control treatment without inoculation was maintained.

The pathogenicity was conducted by preparing aqueous suspension of the bacterium grown on CPG or NA broth medium with a concentration of $5x10^8$ cfu/ml. Twenty micro liters of suspension was poured at the base of ginger plants by making injury to the pseudostem of ginger plants in pots. The pots were maintained at 25 per cent moisture holding capacity. The nutrients required for the plant growth were supplied through nutrient solution at an interval of fifteen days. The plants were watered regularly and observations on appearance of wilt symptoms were recorded. The plants expressing wilt symptoms were selected and bacterium was re-isolated as explained under above isolated pathogen showing typical characteristic of *R. solanacearum* so as to satisfy the Koch's postulate.

3.2.6. Cultural and biochemical characteristics of *Ralstonia solanacearum*

3.2.6.1. Cultural characteristics

A total of 8 culture media (2 synthetic, readymade, make: Hi media and 6 non-synthetic, prepared Appendix) were used to study their effect on colony count, colony color and shape of the colony on the culture media. All the 8 test media were sterilized in autoclave at 15 lbs / $inch^2$ pressure for 20 min and cooled media were poured (@ 20 ml/plate) in sterilized glass Petri plates (90 mm dia) and allowed to solidify at room temperature. The 0.1ml of 48 hours old bacterial culture taken with micropipette, placed at the center of surface solidified media, spread uniformly to obtain well separated bacterial colonies. The inoculated plates were incubated at 28±2°C for 72 hours. Observations were taken on the colony characters like color, number of colonies and shape of the colonies.

Experimental details

Design	:	CRD
Replications	:	Three
Treatments	:	Eight

Treatment details

T_1	:	Triphenyl tetrazolium chloride agar
T_2	:	Casamino acid glucose peptone agar
T_3	:	Nutrient agar
T_4	:	Yeast peptone media
T_5	:	Yeast extract milk agar
T_6	:	Yeast extract agar
T_7	:	Potato dextrose agar
T_8	:	Yeast extract glucose chalk agar

3.2.6.2. Biochemical characteristics

Biochemical characters of *R. solanacearum* were studied applying the biochemical tests *viz.,* Gram staining, Potassium hydroxidase, Catalase test, Starch hydrolysis, Motility test and Casein hydrolysis (Rahman *et al.*, 2010 ; Sambasivam and Girija 2006).

3.2.6.2.1. Gram staining

A loop full of the bacterium suspension was smeared on clean glass slide, air fixed by gentle heating on flame of the spirit lamp. Aqueous Crystal violet solution (0.5%) spread over this smear for 30 seconds and then washed with running tap water for a minute. This stained smear was later flooded with Grams iodine solution for one minute and rinsed in tap water. Later decolorized with 95% of ethanol until color runoff, washed with water and

treated with Safranin as counter stain about 10 seconds, washed with water, air /blot dried and observed under research microscope (make :- Olympus) at 100X using oil immersion technique.

3.2.6.2.2. Potassium hydroxide (KOH) test

A drop of 3 per cent potassium hydroxide was placed on clean glass slide and to this 48 hr old bacterial culture was mixed with clean inoculation loop and stirred for 10 sec and observed for slime threads. When raised the wire loop, if strands of viscid material seen, then the bacterium is gram-negative.

3.2.6.2.3. Catalase test

A loopful of 24 to 48 hrs old culture of the test bacterium was placed on a clean glass slide and to this a drop of 3% hydrogen peroxide (H_2O_2) was mixed and allowed to react for few minutes and observed for the production of gas bubbles.

3.2.6.2.4. Starch hydrolysis

The medium employed is referred to as starch broth and contains, peptone (10 g), beef extract (5 g), starch soluble (2 g), agar (20 g), water (1000 ml). Sterilized the medium by autoclaving and poured into sterilized Petri plates and on solidification of the medium, streaked pure culture of the test bacterium and incubated for 96 hrs at 28^0 C. Then flooded these plates with lugol's iodine and allow to react for few minutes. Reddish colored zones indicate negative reaction and appearance of yellowish, clear zones around the bacterial growth indicate positive reaction.

3.2.6.2.5. Motility test

The autoclaved and cooled motility agar medium (Appendix -I) was poured in glass Petri plates and allowed to solidify. On this solidified

medium loopful of pure culture (48 hrs old) of test bacterium streaked and incubated for 48 hrs. The motile bacteria forms spreading colony on the soft motility agar media.

3.2.6.2.6. Casein hydrolysis

Autoclaved and cooled skim milk agar medium was poured in glass Petri plates and allowed to solidify. On the solidified medium a loopful of culture (48hrs old) of the test bacterium was streaked and incubated for 48 hrs in an inverted position. A clear area /zone around the bacterial growth indicates positive reaction to casein hydrolysis, while absence of clear zone indicates negative reaction.

3.2.7. Disease management strategies

3.2.7.1. *In vitro* evaluation of antibacterial chemicals

Six antibiotics (each @400 and 500ppm), three fungicides (each @ 1500 and 2000 ppm) and two combinations of fungicide + antibiotic [(1000:500) and (1500:500)] by inhibiton zone assay method were evaluated *in vitro* against *R.solanacearum*. The mass multiplied broth culture of the test bacterium (2×10^8 cfu/ml) was seeded to autoclaved Nutrient agar medium, mixed thoroughly and poured into sterilized glass Petri plates allowed to solidify.

The solutions of the desired concentrations of the test antibiotics and fungicides were prepared separately. The filter paper discs (Whatman No.42) of 5mm in diameter were soaked separately in the respective chemical solutions for 5-10m minutes and transformed in center onto the solidified bacterium seeded NA medium in Petri plates. The inoculated plates were kept in the refrigerator at 4^0 C for 4 hours to allow diffusion of the chemical into medium. The untreated control plate containing with the test bacterium seeded NA and inoculated with filter paper disc soaked in distilled water was also maintained then the plates

were incubated at 28^0 C for 48 hrs. and observed for the production of inhibition zone around filter paper discs.

Experimental details

Design	:	CRD
Replications	:	Three
Treatments	:	Twelve

Treatment Details:

Treatment no.	Name of Antibiotics	Concentration (ppm)	
T_1	Sreptocycline	400	500
T_2	Cephalexin	400	500
T_3	Neomycin	400	500
T_4	Tetracycline	400	500
T_5	Dicrystacin	400	500
T_6	Gentamycin	400	500
	Antibacterial fungicides		
T_7	Blitox (Copper oxy chloride)	1500	2000
T_8	Kocide (Copper hydroxide)	1500	2000
T_9	Amistar	1500	2000
T_{10}	Blitox + Streptocycline	1000 + 500	1500 + 500
T_{11}	Blitox + Tetracycline	1000 + 500	1500 + 500
T_{12}	Control (Untreated)		

3.2.7.2. *In vitro* evaluation bioagents/antagonists

3.2.7.2.1. Bacterial antagonists

Two isolates of bacterial antagonist's *viz., Pseudomonas fluorescence* and *Bacillus subtilis* collected from Department of Plant Pathology

were tested for their efficacy in inhibiting the growth *Ralstonia solanacearum* by paper disc method. The virulent isolates *Ralstonia solanacerum* was multiplied on Nutrient broth. The 48 hours old culture of *R. solanacerum* containing 2×10^8 cfu/ml was mixed with molten (50°C) Nutrient agar, so as to get a thick lawn of bacteria on the surface of agar medium. The seeded medium poured into sterilized Petri-plates and allowed to solidify. Previously sterilized filter paper (Whatman No. 42) measuring 5 mm in diameter were soaked in different antagonist broth for 10 minutes and placed in the Petri-plates. The excess solution from the filter paper disc was removed by touching slide of the paper discs to the lid of Petri dishes containing broth of the same organism. Then the filter disc was placed in a marked position on the surface of the seeded agar medium. The inoculated plates were incubated at 28±2°C for 48 hours. The observations for the production of inhibition zone around the filter paper discs were recorded at 24, 48 and 72 hours of incubation respectively. The obtained results were analyzed statistically. Filter paper discs dipped in sterile water served as check.

3.2.7.2.2. Fungal antagonists

Six fungal isolates collected from the Department of plant pathology were tested for their inhibitory effect on *R. solanacearum in vitro* by inhibition zone assay method. All the fungal isolates were grown separately on Potato Dextrose Agar. The virulent isolate of *Ralstonia solanacearum* was multiplied on Nutrient broth. The 48 hours old culture of *R. solanacearum* containing 2×10^8 cfu/ml was mixed with molten (50°C) sterilized PDA (20 ml), then seeded PDA poured in sterilized Petri-plates and allowed solidify. Fungal discs of 5 diameters from margin of actively growing four days old culture removed and placed in the center of the plates containing PDA. The plates were incubated at 28±2°C for 4 days. The observation on the zone of inhibition around the mycelial disc against *Ralstonia solanacearum* was recorded after the incubation period.

Experimental details

Design : CRD

Replications : Three

Treatments : Nine

Treatment Details

Treatments No.	Biocontrol Agents
T_1	*Trichoderma viride*
T_2	*T. harzianum*
T_3	*T. virens*
T_4	*T. koningii*
T_5	*T. longibrachiatum*
T_6	*Pseudomonas fluorescence*
T_7	*Bacillus subtilis*
T_8	*Aspergillus niger*
T_9	Control (untreated)

3.2.7.3. *In vitro* evaluation of botanicals/phytoextracts

The present investigation aimed at screening some plant extracts for their antibacterial properties against *Ralstonia solonacearum.*

3.2.7.3.1. Aqueous extracts botanicals (Phytoextracts)

Fresh plant materials were collected and washed first in tap water and then in distilled water; 100 g of fresh sample was chopped and macerated in a surface sterilized pestle and mortar by adding 100 ml of sterile water (1:1 w/v). The extract was filtered through two layers of muslin cloth, filtrate thus used as a

stock solution. To study the mechanism of plant extracts, inhibition zone assay method was followed. A heavy suspension 42 hours old culture (2×10^8 cfu/ml) of *Ralstonia solonacearum*, multiplied in nutrient broth (20 ml) was mixed with molten (50°C) nutrient agar medium (1000 ml) contained in an Erleyenmayer's flask, the bacterial suspension was seeded to the lukewarm nutrient agar medium (1000 ml). The seeded medium was poured into the sterilized Petri plates and plates were allowed to solidify. Five, Ten and Twenty per cent each of plant extract was prepared by mixing 5, 10 and 20 ml of stock solution with 95, 90 and 85 ml of sterilized distilled water, respectively.

The filter paper discs (Whatman No. 42) measuring 5 mm in diameter were soaked in the respective concentrations for 5 min and transferred onto the surface of seeded medium in the Petri-plates. The inoculated plates were kept in refrigerator at 5°C for 4 hours to allow the diffusion of chemicals into the medium. The plates were then incubated at 28±2°C for 72 hours. The observations for the production of inhibition zone around the filter paper discs were recorded at 24, 48 and 72 hours of incubation respectively.

At the end of incubation period, observations were recorded for the production of inhibition zone representing the efficacy of plant extracts in inhibiting the growth of pathogen. The inhibition zone in each plate was measured in terms of millimeter in diameter and data obtained was analyzed statistically.

3.2.7.3.2. Acetone extracts botanicals (Phytoextracts)

Fresh plant materials were collected and washed first in tap water and then in distilled water; 100 grams of fresh sample was chopped and macerated in a surface sterilized pestle and mortar by adding 100 ml of acetone (1:1 w/v). The extract was filtered through two layers of muslin cloth, filtrate thus used as a stock solution. To study the mechanism of plant extracts inhibition zone

assay method was followed. A heavy suspension 42 hours old culture (2×10^8 cfu/ml) of *Ralstonia solanacearum*, multiplied in nutrient broth (20 ml) was mixed with molten (50°C) nutrient agar medium (1000 ml) contained in an Erleyenmayer's flask, the bacterial suspension was then seeded to the lukewarm nutrient agar medium (1000 ml). The seeded medium was poured into the sterilized Petri-plates and plates were allowed to solidify. Five, Ten and Twenty percent each of plant extract was prepared by mixing 5,10 and 20 ml of stock solution with 95, 90 and 85 ml of acetone, respectively.

The filter paper disc (Whatman No. 42) measuring 5 mm in diameter was soaked in the respective concentrations for 5 min and transferred onto the surface of seeded medium in the Petri-plates. The inoculated plates were kept in refrigerator at 5°C for 4 hours to allow the diffusion of chemicals into the medium. The plates were then incubated at 28±2°C for 72 hours. The observations for the production of inhibition zone around the filter paper discs were recorded at 24, 48 and 72 hours of incubation respectively.

At the end of incubation period, observations were recorded for the production of inhibition zone representing the efficacy of plant extracts in inhibiting the growth of pathogen. The inhibition zone in each plate was measured in terms of millimeter in diameter and data obtained was analyzed statistically.

Experimental details

Design	:	CRD
Replications	:	Three
Treatments	:	Twelve

Treatment Details:

Tr. No.	Common Name	Botanical Name	Plant Part Used	Concentration (%)		
T_1	Garlic	*Allium sativum*	Clove	5	10	20
T_2	Turmeric	*Curcuma longa*	Rhizome	5	10	20
T_3	Tulsi	*Ocimum sanctum*	Leaves	5	10	20
T_4	Neem	*Azadirachta indica*	Leaves	5	10	20
T_5	Onion	*Allium sepa*	Bulb	5	10	20
T_6	Parthenium	*P.hysterophorus*	Leaves	5	10	20
T_7	Mehandi	*Lawsonia innermis*	Leaves	5	10	20
T_8	Karanj	*Pongamia pinnata*	Leaves	5	10	20
T_9	Dhotra/Dhatura	*Datura stromonium*	Leaf	5	10	20
T_{10}	Gokhru	*Tribulus terrestris*	Leaves	5	10	20
T_{11}	Garlic+Turmeric	-	-	5	10	20
T_{12}	Control (untreated)					

3.2.7.4. Evaluation of organic amendment

A total of 10 organic amendments were evaluated against *R. solanacearum* by sick soil method in pot culture under screen house conditions. Except vermicompost, all the test amendments were crushed physically to rough powder and used for soil application.

The earthen pots (30 cm dia.) disinfected with 5 per cent solution of Copper sulphate were filled with autoclaved potting mixture of soil: sand: FYM (2:1:1). The mass multiplied (48 hr old nutrient broth culture: 2 $x10^8$ cfu/ml) of *R solanacearum* was drenched (@ 50 ml/ kg potting mixture) evenly to the potting

mixture in pots, these pots were incubated for 96 hrs in screen house to proliferate the bacterium and make the soil / potting mixture sick.

The coarse ground test amendments were applied (@ 50 g / kg mixture) in the earthen pots containing test bacterium sick soil/ potting mixture, mixed thoroughly, watered regularly and maintained in screen house. After 72 hrs of amendments application, surface sterilized (0.1 % $HgCl_2$) healthy rhizome of ginger were sown (10 rhizomes/pot), watered regularly and maintained in the screen house. Three pots / treatment / replication were maintained. The earthen pots containing *R. solanacearum* sick soil and sown with surface sterilized healthy rhizome of ginger, without amendment were maintained as untreated control.

The percentage seed germination, pre-emergence seed rot and post-emergence seedling mortality were calculated by following formulae.

$$\text{Germination (\%)} = \frac{\text{No. of rhizomes germinated}}{\text{Total no. of rhizomes sown}} \times 100$$

$$\text{PESR (\%)} = \frac{\text{No. of rhizomes ungerminated}}{\text{Total no. of rhizomes sown}} \times 100$$

$$\text{PESM (\%)} = \frac{\text{No. of seedlings died}}{\text{Total no. of seedlings}} \times 100$$

$$\text{Reduction (\%) in PESR \& PESM} = \frac{C-T}{C} \times 100$$

Where,

C= Per cent rot/mortality in control
T= Per cent rot/mortality in treatment

Vigour Index = [Shoot length (cm) + Root length (cm)] x Germination (%)

Experimental details

Design	:	CRD
Replications	:	Three
Treatments	:	Eleven

Treatment Details

T_1 : FYM

T_2 : Poultry manure

T_3 : Neem seed cake

T_4 : Sunflower seed cake

T_5 : Vermicompost

T_6 : Cotton seed cake

T_7 : Karanj cake

T_8 : Goat manure

T_9 : Groundnut cake

T_{10} : Safflower seed cake

T_{11} : Control (Untreated)

3.2.7.5. Integrated disease management (Pot culture)

Those antibacterial chemicals, botanicals, bioagents and organic amendments found effective against *Ralstonia solanacearum*, during present *in vitro* (plate and pot culture) studies were selected for integrated management of bacterial wilt of ginger (pot culture). The earthen pots (30 cm dia.) disinfected with 5 per cent of copper sulphate solution were filled with the autoclaved potting mixture of soil: sand: FYM (2:1:1). The virulent isolate of *Ralstonia solanacearum* was multiplied on Nutrient broth. The 48 hours old culture of *R. solanacearum* containing 2×10^8cfu/ml was inoculated (@ 50 ml / kg potting mixture) separately to the potting mixture in pots, mixed thoroughly, watered

adequately and incubated for 96 hrs in the screen house, to proliferate the pathogen and make the soil / potting mixture sick.

Surface sterilized (0.1 % $HgCl_2$) healthy rhizome seeds of ginger were sown (10 seeds/pot) in the earthen pots containing *R solanacearum* sick soil/potting mixture and maintained as untreated control. All these pots (treated and untreated) were watered regularly and maintained in the screen house for further observations. The details of the experiment were as given below.

Experimental details

Design : CRD

Replications : Three

Treatments : Eleven

Treatment Details

T_1 : Streptocycline (Rhizome treatment) @ 0.05 %

T_2 : Gentamycin (Rhizome treatment) @ 0.05 %

T_3 : *Pseudomonas fluorescence* (SA) @ 10g/kg soil

T_4 : *Trichoderma viride* (SA) @ 10g/kg soil

T_5 : Karanj cake (SA) @ 50 g/kg soil

T_6 : *A. sativum* aq. bulb extract 20% (SD) @ 20 ml/kg soil

T_7 : T_1 (ST) + T_3 (SA)

T_8 : T_1 (ST) + T_4 (SA)

T_9 : T_1 (ST) + T_5 (SA)

T_{10} : T_5 (SA) + T_6 (SD)

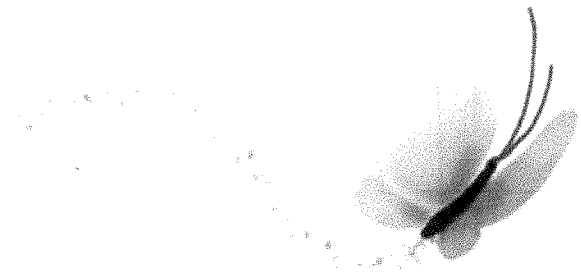
T_{11} : Control (Untreated)

Observations on rhizome germination and pre-emergence mortality (PEM) were recorded at fifteen days after sowing and that of wilting were recorded at 45 and 60 DAS. The percentage rhizome germination, pre-emergence

rhizome rot and post-emergence seedling mortality were calculated by the formulae as detailed in this chapter under 3.2.7.4.

3.2.8. Statistical analysis

The data obtained in all the experiments were statistically analyzed. The percentage values were transformed into arc sine values. The standard error (SE) and critical difference (C.D.) at level P=0.01 were worked out and results obtained were compared statistically.



RESULTS AND DISCUSSION

CHAPTER IV

RESULTS AND DISCUSSION

Present studies on the bacterial wilt [*Ralstonia solanacearum* (Smith) Yabuuchi] of ginger (*Zingiber officinale* Roscoe) were undertaken during 2014-15 on the aspects *viz.*, symptomatology, isolation and pathogenicity, cultural and biochemical characteristics, *in vitro* efficacy of the antibacterial chemicals, botanicals, bioagents, organic amendments and integrated management have been collected and being presented herein this chapter. The results obtained on all these aspects are being presented in the following paragraphs.

4.1. Symptomatology

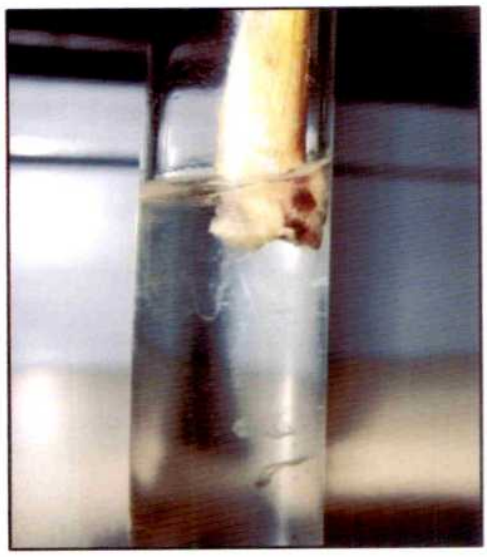
During field survey and pathogenicity test the typical symptoms induced by *Ralstonia solanacearum* on ginger plants were observed critically. Infected plants were, downward curling of leaves and golden brown/rusty brown discoloration seen on older leaves (PLATE I). Loss of leaf turgidity, older leaves were affected before the youngest leaves and plant wilted suddenly. Tentative diagnosis of the disease was made by ooze test (PLATE I). The ooze test was conducted by placing longitudinal section of diseased pseudostem from the identified plant in glass beaker containing clean water, within a few minutes; fine milky exude clouded the water streaming out from the margin of the cut end which revealed the presence of bacteria in the discolored Pseudostem.

Similar symptoms of ginger bacterial wilt were also reported earlier by many workers. Downward curling of leaves, loss of turgidity, older leaves turning pale yellow then necrotic brown and sudden wilting of plant due to *R. solanacearum* were reported (Sarma, 1994; Rajan *et al.*, 2002; Agrios, 2005; Kumar and Abraham, 2008; Roy *et al.*, 2008; Monther and Kamaruzaman, 2010; Chandrashekara and Prasannakumar, 2012; White *et al.*, 2013; Kai *et al.*, (2014). Leaves roll and curl due to water stress caused by bacteria blocking the water-conducting vascular system of the ginger stems, leaf

PLATE I

(A)

(B)



(C)

(A) Bacterial wilt of ginger on farmer's field
(B) Wilted plant
(C) Bacterial ooze from pseudostem of wilted plant

yellowing and necrosis were reported (Nelson, 2013). Milky white bacterial ooze was observed when the infected plant material was placed in water was reported (Sarma, 1994; Umesha *et al.*, 2005; Wang and Lin, 2005; Roy *et al.*, 2008; Champoiseau *et al.*, 2009).

4.2. Isolation of the pathogen

Isolation was made from the soil samples and bacterial ooze obtained from the infected discolored pseudostem of the plants by serially diluting the bacterial suspension in sterile distilled water and planting on TZC media (Kelman, 1954). Typical virulent colonies of *R. solanacearum* developed within 48 hours. The virulent colonies appeared well-separated, irregular fluidal, dull white colored with slight pink centre and non-virulent colonies appeared dark red on TZC media (PLATE II). The Colonies were irregular fluidal, creamy white color on Casamino acid peptone glucose agar (CPG) media. The well separated colonies were picked up and purified further by single colony isolation technique and then suspended in sterile distilled water in sterile plastic ependorf tubes and stored at room temperature this served as stock culture for further use (PLATE III).

Similar results were also reported (Lemessa and Zeller, 2007 A; Chakravarty and Kalita, 2011; Chaudhry and Rashid, 2011; Sagar *et al.*, 2014).

4.3. Pathogenicity test

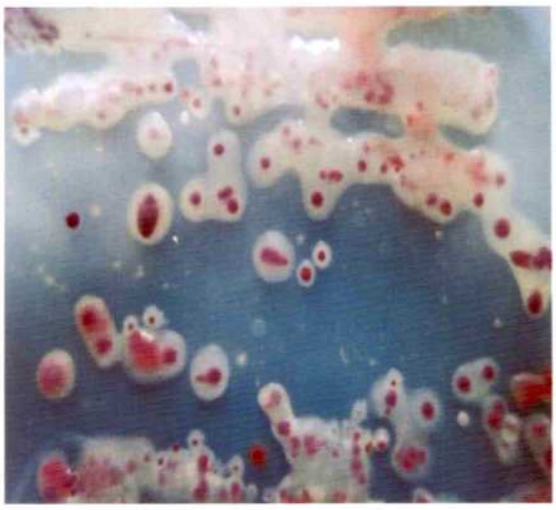
The bacterium was inoculated to the host plant (Ginger) under artificial condition by pseudostem inoculation method in screen house. The inoculated plant showed wilting symptoms 15 days after the inoculation. The isolate was found to be pathogenic to host plant, expressing wilt symptoms. The inoculated plant lost turgidity; leaves started dropping and plant wilted suddenly (PLATE IV).

Pathogenicity of *R. solanacearum* causing bacterial wilt was proved earlier by several workers (Winstead and Kelman, 1952; Schell M. A., 2000; Williamson *et al.*, 2002; Rajan *et al.*, 2002; Kumar and Sarma, 2004; Umesha *et al.*, 2005; Hikichi, 2007; Rashmi *et al.*, 2012; Artal, 2013; Thomas

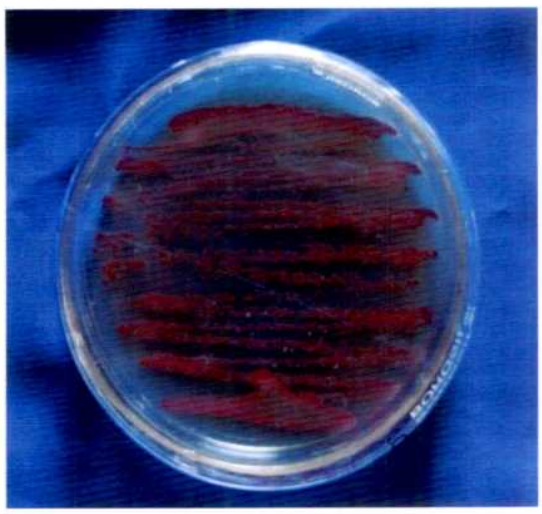
PLATE II

(A)

(B)



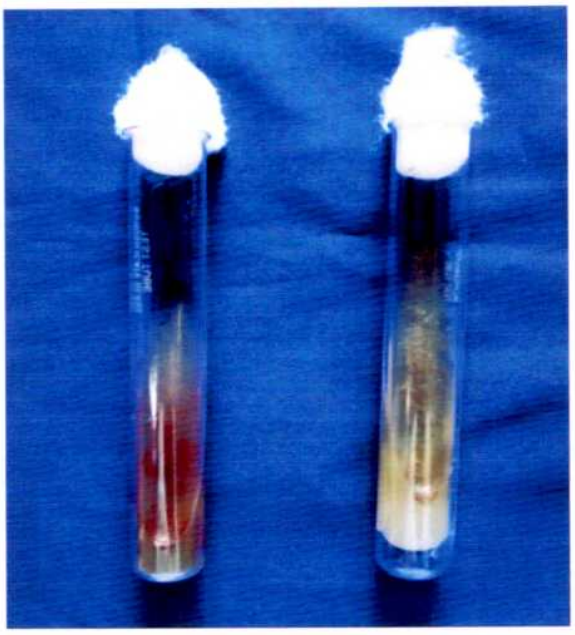
(C)



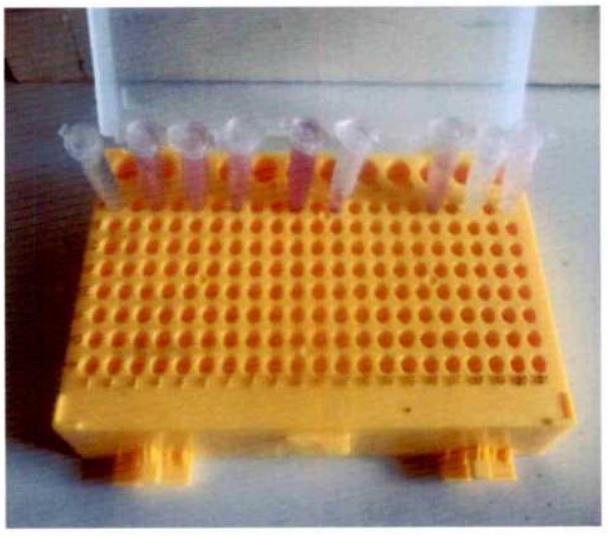
(D)

A. Culture of *Ralstonia solanacearum* on TZC medium
B. Culture of *Ralstonia solanacearum* on CPG medium
C. Virulent colonies of *R. solanacearum*
D. Avirulent colonies of *R. solanacearum*

PLATE III



Agar slant of *Ralstonia solanacearum*



Water stock of *Ralstonia solanacearum*

PLATE IV

Pathogenicity test of *R. solanacearum* on ginger

and Upreti, 2014; Zulperi *et al.*, 2014). Kumar A. (2006) proved the pathogenicity of *R. solanacearum* using susceptible ginger cultivar '*Himachal*'. Mathews *et al.* (2008) also proved the pathogenicity of *R. solanacearum* using ornamental ginger species, the final pathogenicity assessment was recorded 21 DAI.

4.4. Re-isolation

The test pathogen (*R. solanacearum*) was reisolated from the artificially diseased pseudostem of ginger on TZC plates and after 24 to 48 hours of incubation cultural characteristics observed and those compared was found similar to those characteristics of the pathogen isolated from naturally diseased plant as well as from artificially diseased plant of ginger in screen house.

Thus, on the basis of the characteristic symptoms of bacterial wilt disease exhibited on naturally and artificially diseased foliage of ginger, ooze test and cultural characteristics of the test pathogen isolated from naturally and artificially diseased foliage of ginger were found exactly similar. Hence, pathogenicity of *R. solanacearum* was proved by applying Koch's postulates.

4.5. Identification

Based on typical symptoms of bacterial wilt on foliage, cultural, morphological and biochemical characteristics, ooze test and pathogenicity test and on no comparison of these characters with the characters reported for *R. solanacearum* by Kelman (1954), Kumar and Sarma (2004), Sambasivam and Girija (2006) the test pathogen was identified as *R. solanacearum.*

4.6. Cultural characteristics

Cultural characteristics *viz.*, colony count, colony colour and colony shape of *R. solanacearum* on different period of time were studied *in vitro* using eight culture media and the results obtained are presented in Table 1 and depicted in PLATE V,VI and Fig. 1.

4.6.1. Colony count

The results (PLATE VI, Table 1) revealed that all the culture media tested encouraged better growth and variable colony count of *R. solanacearum*. At 24 hrs the mean colony count recorded with all the test media was ranged from 23.00 (Yeast extract chalk agar) to 61.33 (TZC). However, significantly highest mean colony count (61.33) was recorded on Triphenyl Tetrazolium Chloride Agar. This was followed by Potato dextrose agar (50.67), Casamino peptone glucose agar (48.00), Yeast extract peptone agar and Yeast extract milk agar (each 44.00), Nutrient agar (31.00) all four media with at par and Yeast extract agar (30.67). Yeast extract chalk agar was found least suitable with minimum mean colony count (23.00) of the test pathogen.

At 48 hrs the mean colony count recorded with all the test media was ranged from 31.00 (Yeast extract chalk agar) to 68.00 (TZC). However, significantly highest mean colony count (68.00) was recorded on Triphenyl Tetrazolium Chloride Agar. This was followed by Casamino peptone glucose agar (56.67), Potato dextrose agar (55.00), Yeast extract milk agar (46.67), Yeast extract peptone agar (45.67), Yeast extract agar (34.33) with at par and Nutrient agar (33.67). Yeast extract chalk agar was found least suitable with minimum mean colony count (31.00) of the test pathogen.

At 72 hrs the mean colony count recorded with all the test media was ranged from 37.67 (Yeast extract chalk agar) to 71.33 (TZC). However, significantly highest mean colony count (71.33) was recorded on Triphenyl Tetrazolium Chloride Agar. This was followed by Casamino peptone glucose agar (64.33), Potato dextrose agar (58.00), Yeast extract peptone agar (49.33), Yeast extract milk agar (48.00), Yeast extract agar (41.00) with at par and Nutrient agar (39.00). Yeast extract chalk agar was found least suitable with minimum mean colony count (37.67) of the test pathogen.

At 96 hrs the mean colony count recorded with all the test media was ranged from 40.33 (Yeast extract chalk agar) to 73.33 (TZC). However, significantly highest mean colony count (73.33) was recorded on Triphenyl Tetrazolium Chloride Agar. This was followed by Casamino peptone glucose

agar (68.33), Potato dextrose agar (56.33), Yeast extract peptone agar (50.67), Yeast extract milk agar (49.67), Yeast extract agar (43.33) with at par and Nutrient agar (42.00). Yeast extract chalk agar was found least suitable with minimum mean colony count (40.33) of the test pathogen.

Average colony count recorded with all the test media was ranged from 33.00 (Yeast extract chalk agar) to 68.50 (TZC). However, significantly highest average colony count (68.50) was recorded on Triphenyl Tetrazolium Chloride Agar. This was followed by Casamino peptone glucose agar (59.33), Potato dextrose agar (55.00), Yeast extract peptone agar (47.42), Yeast extract milk agar (47.09), Yeast extract agar (37.33) with at par and Nutrient agar (36.42). Yeast extract chalk agar was found least suitable with minimum average colony count (33.00) of the test pathogen.

4.6.2. Colony colour

The results (PLATE V, VI and Table 1) revealed that the white fluidal colonies with spiral pink centre were found on Triphenyl tetrazolium chloride agar. Cream or off-white color colonies were found on Casamino peptone glucose agar, Yeast extract agar and Potato dextrose agar. Cream white and dull white color colonies were found on Nutrient agar and Yeast extract milk agar. Yellow color colonies were found in Yeast extract peptone agar and Yeast extract chalk agar.

4.6.3. Colony Shape

The results (PLATE VI and Table 1) revealed that the Irregular, smooth, highly fluidal colonies were found on Triphenyl tetrazolium chloride agar, Casamino peptone glucose agar, Potato dextrose agar and Yeast extract chalk agar. Round small colonies were found on Nutrient agar, Yeast extract milk agar, Yeast extract agar and Yeast extract peptone agar.

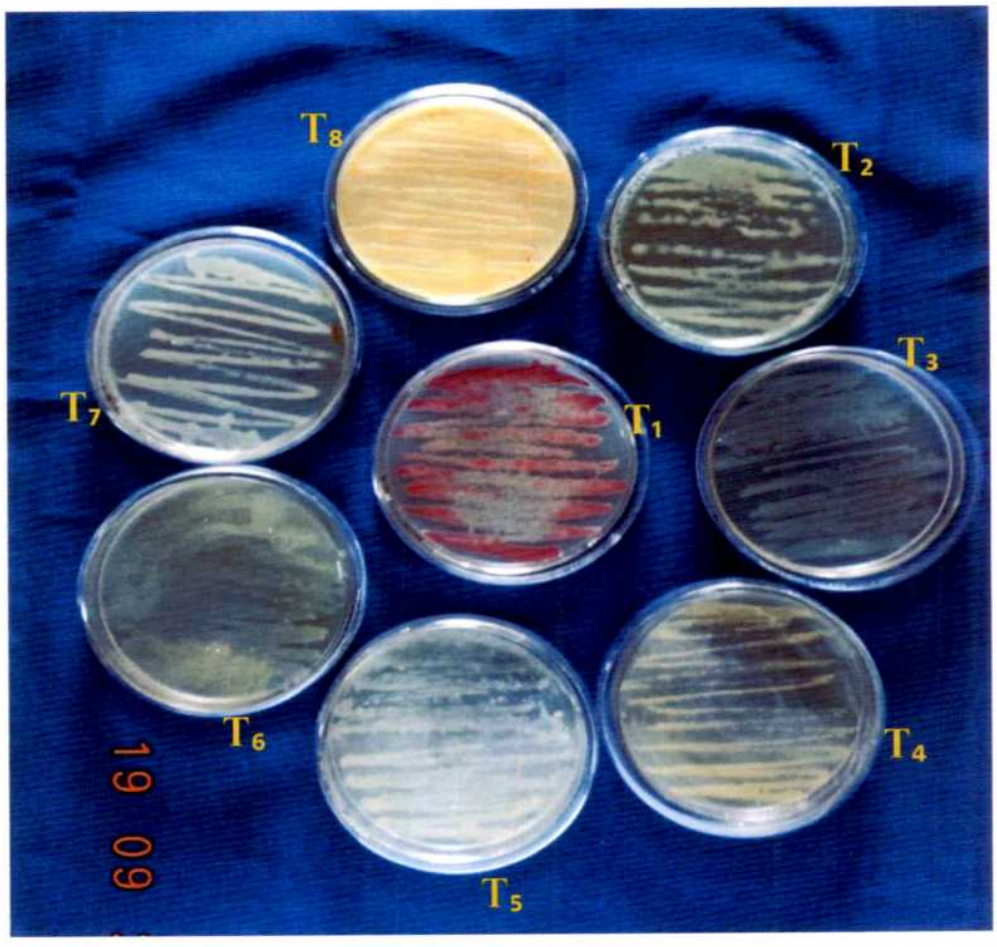
Similar results were also reported earlier by many workers. Virulent colonies appeared highly fluidal white with pink centers and non-virulent colonies appeared dark red reported (Kelman, 1954; Kumar and sarma, 2004; Sambasivam and Girija, 2006; Rahman *et al.,* 2010; Narasimbha and

Table 1. Effect of various culture media and cultural characteristics of *R. solanacearum*

Tr. No.	Media	Colony count*/ plate at					Colony Color	Colony Shape
		24 hrs	48 hrs	72 hrs	96 hrs	Av.		
T_1	Triphenyl Tetrazolium Chloride Agar	61.33	68.00	71.33	73.33	68.50	White fluidal with pink centre	Irregular, smooth, highly fluidal
T_2	Casamino Peptone Glucose Agar	48.00	56.67	64.33	68.33	59.33	Cream or off-white color	Irregular, smooth, highly fluidal
T_3	Nutrient Agar	31.00	33.67	39.00	42.00	36.42	Creamy white	Round, small
T_4	Yeast Extract Peptone Agar	44.00	45.67	49.33	50.67	47.42	Yellow	Round, small
T_5	Yeast Extract Milk Agar	44.00	46.67	48.00	49.67	47.09	Dull white	Round, small
T_6	Yeast Extract Agar	30.67	34.33	41.00	43.33	37.33	Cream or off-white color	Round, small
T_7	Potato Dextrose Agar	50.67	55.00	58.00	56.33	55.00	Cream or off-white color	Irregular, smooth, highly fluidal
T_8	Yeast Extract Chalk Agar	23.00	31.00	37.67	40.33	33.00	Yellow fluidal	Irregular, smooth
	S.E. ±	**0.87**	**0.91**	**1.03**	**1.36**	-		
	CD (P 0.01)	**2.60**	**2.71**	**3.08**	**4.07**	-		

*: Mean of three replications

PLATE V



Cultural characteristics of *R. solanacearum* on various culture media

T_1 : Triphenyl tetrazolium chloride agar

T_2 : Casamino acid glucose peptone agar

T_3 : Nutrient agar

T_4 : Yeast extract peptone agar

T_5 : Yeast extract milk agar

T_6 : Yeast extract agar

T_7 : Potato dextrose agar

T_8 : Yeast extract glucose chalk agar

PLATE VI

Number of colonies of *R. solanacearum* on various culture media

T_1 : Triphenyl tetrazolium chloride agar

T_2 : Casamino acid glucose peptone agar

T_3 : Nutrient agar

T_4 : Yeast peptone agar

T_5 : Yeast extract milk agar

T_6 : Yeast extract agar

T_7 : Potato dextrose agar

T_8 : Yeast extract glucose chalk agar

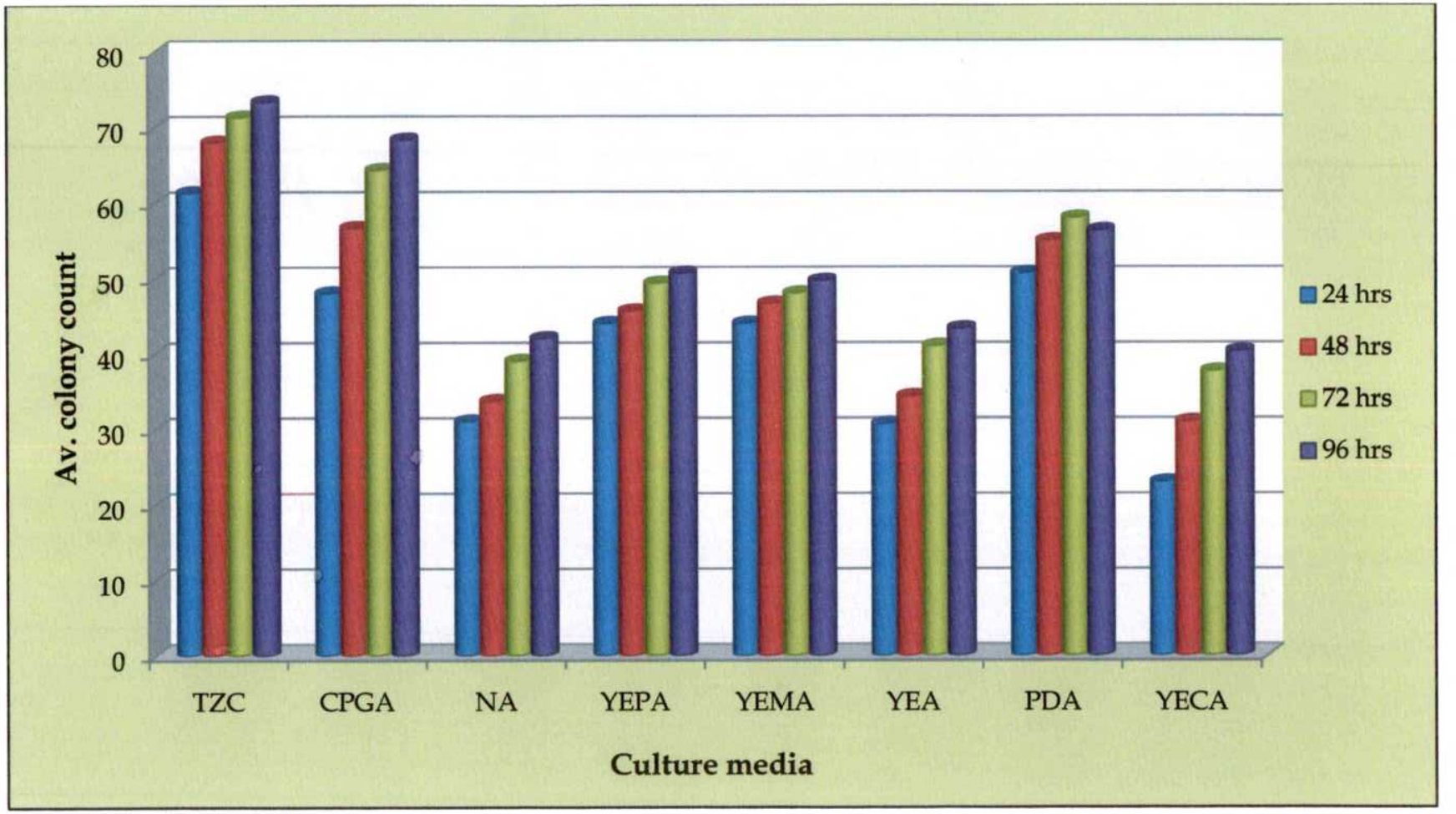


Fig.1. Effect of various culture media and cultural characteristics of *R. solanacearum*

Srinivas, 2012; Javeria and Kumar, 2014, Zulperi *et al.,* 2014; Shahbaz *et al.,* 2015). Irregular, smooth, creamy white and fluidal colonies appeared on CPG medium reported (Williamson *et al.,* 2002; Lemessa and Zeller, 2006) and creamish white, mucoid, circular colonies appeared on NA medium reported (Umesha *et al.,* 2005).

4.7. Biochemical characteristics

Different biochemical tests *viz.* Gram staining, Potassium hydroxides, Catalase test, Starch hydrolysis, Motility test and Casein hydrolysis were performed for confirmation of *Ralstonia solanacearum* and the results were presented in Table 2 and Plate VII.

4.7.1 Gram staining

Microscopic examination of Grams stained *R. solanacearum* mount elucidated that the test bacterium did not retained violet color of the primary stain (Crystal violet) but cells appeared pink colored due to counter staining with the stain safarin. Hence the test bacterium was gram negative, straight or curved rods, which is the characteristic feature of the plant pathogenic bacteria.

Similar results were also reported earlier by (Schaad, 1980; Denny and Hayward, 2000; Williamson *et al.,* 2002; Umesha *et al.,* 2005; Vanitha *et al.,* 2009; Rahman *et al.,* 2010; Chaudhry and Rashid, 2011; Zhang *et al.,* 2011; Narsimbha and Srinivas, 2012; Marques *et al.,* 2012; Javeria and Kumar, 2014; Maji and Chakrabarthy, 2014; Seleim *et al.,* 2014; Zulperi *et al.,* 2014; Shahbaz *et al.,* 2015).

4.7.2. Potassium hydroxide (KOH) test

Two drops of 3% potassium hydroxide (KOH) is placed on the fresh bacterium culture (24 to 48 hours old) and mixed using a laboratory loop or a wooden toothpick for 10 seconds. Formation of slime threads or loop is positive test and is indication of being gram-negative because gram negative bacteria have relatively fragile cell walls which are bounded by an outer

membrane. This outer membrane is readily disrupted by exposure to 3 % KOH releasing the viscous DNA. So those slime threads are actually DNA so this test is lytic release of DNA. But gram- positive bacteria by contrast possess a thicker, more rigid cell wall which resists the disruptive effect of KOH (Chaudhry and Rashid, 2011). Similar results were also reported earlier by (Suslow *et al.,* 1982; Sambasivam and Girija, 2006; Vanitha *et al.,* 2009; Rahman *et al.,* 2010; Chaudhry and Rashid, 2011; Maji and Chakrabarthy, 2014; Zulperi *et al.,* 2014; Shahbaz *et al.,* 2015).

4.7.3. Catalase test

A few drops of 3 % hydrogen peroxide (H_2O_2) were placed at the centre of sterile glass slide and loopful of bacterial inoculums was agitated in the solution. Formation of air bubbles indicating the positive reaction for the test. Therefore, the test bacterium is strictly aerobic.

Similar results were also reported earlier by (Dhital *et al.*,2001; Williamson *et al.,* 2002; Sambasivam and Girija, 2006; Chaudhry and Rashid, 2011; Zhang *et al.,* 2011; Marques *et al.,* 2012; Narsimbha and Srinivas, 2012; Maji and Chakrabarthy, 2014; Seleim *et al.*, 2014; Zulperi *et al.,* 2014; Shahbaz *et al.,* 2015).

4.7.4. Starch hydrolysis

In this test, observed that colorless zone was formed around the bacterial growth when flooded the agar surface with lugol's iodine solution. Formation of colorless zone indicating the positive reaction for the test. Therefore, the test bacterium is hydrolyzing starch by exoenzyme amylase reveals clear zone around the bacterial growth. This zone indicates starch was broken down to dextrins, maltose, and glucose/alpha-amylase. Similar results were also reported earlier by (Denny and Hayward, 2000).

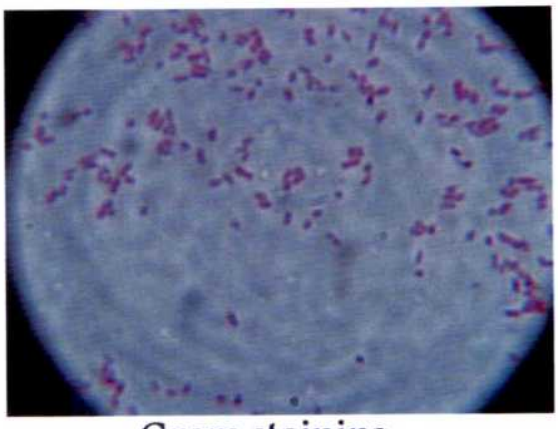
4.7.5. Motility tests

In this test, observed that test bacterium was formed spreading colony on the soft motility agar media. It's indicating the positive reaction for

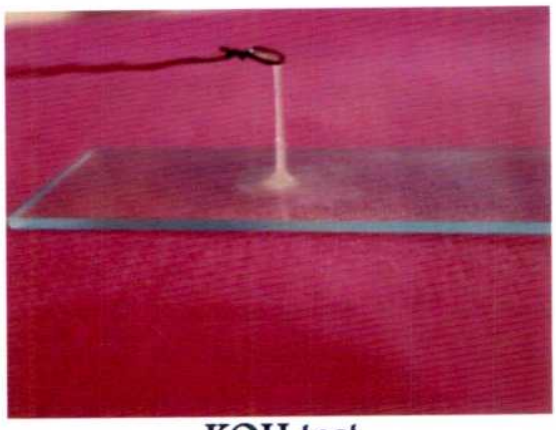
Table 2. Biochemical characteristics of *R. solanacearum*.

Sr. No.	Test	Inference
1.	Gram reaction	Gram negative
2.	KOH test	Positive
3.	Catalase test	Positive
4.	Starch hydrolysis	Positive
5.	Motility test	Positive
6.	Casein hydrolysis	Positive

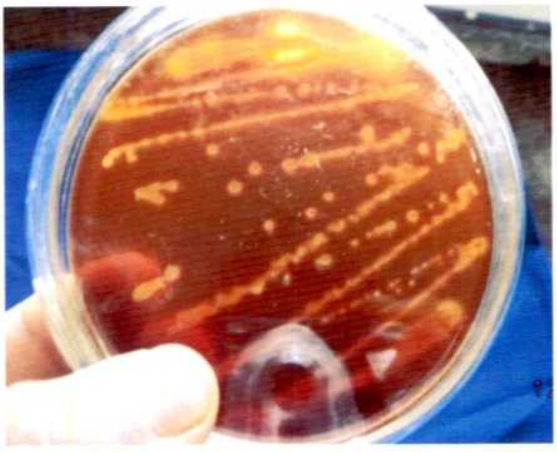
PLATE VII



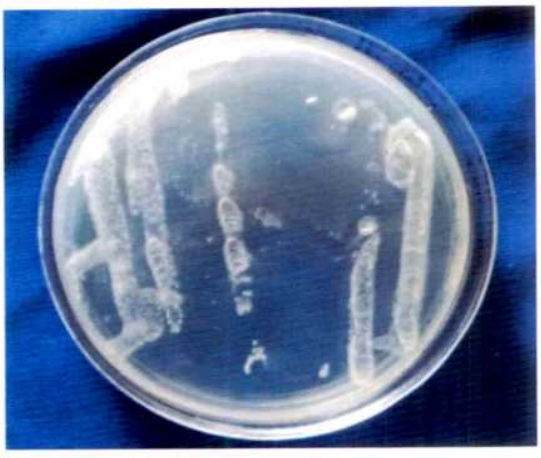
Gram staining



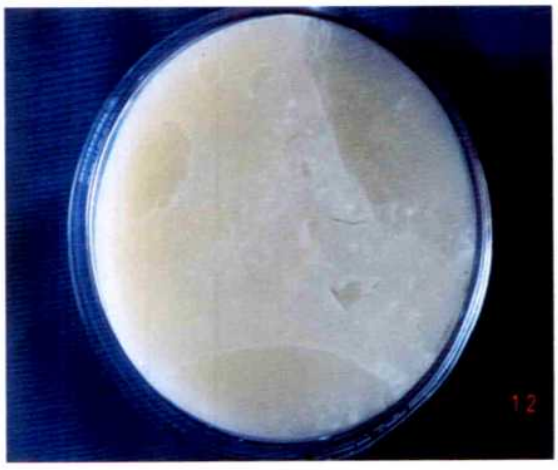
KOH test



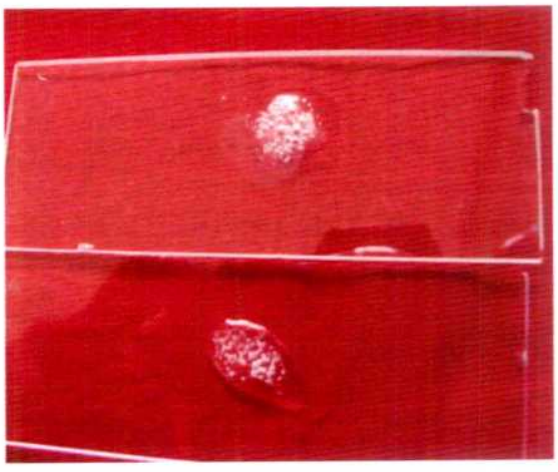
Starch hydrolysis



Motility test



Casein hydrolysis



Catalase test

Biochemical tests to characterize *R. solanacearum*

the test. Therefore, the test bacterium is motile. Kumar *et al.* (2013) reported that finger-like projections emerging out of the streaked edges were observed on the plates, suggesting F1C1 is capable of manifesting twitching motility. Twitching motility is basically due to the presence of type-IV pili on gram-negative bacterial cell. Similar results were also reported earlier by (Denny and Hayward, 2000; Dhital *et al.*, 2001; Liu *et al.*, 2001; Zhang *et al.*, 2011; Seleim *et al.*, 2014).

4.7.6. Casein hydrolysis

The test bacterium showed positive reaction for casein hydrolysis test. The casein hydrolysis colonies developed clear zone around the bacterial colony. The bacterium *R. solanacearum* secreted the proteolytic exoenzyme casease hydrolyze milk protein thus creating a zone of clearing around the bacterial growth.

4.8. *In vitro* evaluation of antibacterial chemicals, biocontrol agent, botanicals and organic amendments against bacterial wilt of ginger.

4.8.1. *In vitro* evaluation of antibacterial chemicals against *R. solanacearum*

Present investigation was carried out to evaluate antibacterial chemicals to find out their effectiveness against the growth of *Ralstonia solanacearum* (Smith E. F.) Yabuuchi *et al.* under *in vitro* condition and the results were presented in Table 3, Fig. 2 and Plate VIII, IX.

Total six antibiotics *viz.*, Sreptocycline, Cephalexin, Neomycin, Tetracycline, Dicrystacin, Gentamycin, and three antibacterial fungicides *viz.*, Blitox (Copper oxy chloride) Kocide (Copper hydroxide) and Amistar individually and in combination Blitox + Streptocycline, Blitox + Tetracycline were evaluated *in vitro* by inhibition zone assay method against *R. solanacearum.*

4.8.1.1. Antibiotics

At 400 ppm, inhibition was ranged from 6.9 mm (Dicrystacin) to 18.4 mm (Streptocycline). However, significantly highest inhibition was recorded in the antibiotic Streptocycline (18.4 mm). This was followed by the antibiotics *viz.,* Gentamycin (15.4 mm), Tetracycline (14.2 mm) and Neomycin (8.1 mm). Antibiotics, Cephalexin and Dicrystacin were found less effective with 7.5 and 6.9 mm inhibition, respectively.

At 500 ppm, inhibition was ranged from 7.0 mm (Dicrystacin) to 21.7 mm (Streptocycline). However, significantly highest inhibition was recorded in the antibiotic Streptocycline (21.7 mm). This was followed by the antibiotics *viz.,* Gentamycin (19.6 mm), Tetracycline (18.8 mm) and Neomycin (8.3 mm). Antibiotics, Cephalexin and Dicrystacin were found less effective with 7.6 and 7.0 mm inhibition, respectively.

Average inhibition was ranged from 6.95 mm (Dicrystacin) to 20.05 mm (Streptocycline). However, significantly highest average inhibition was recorded in the antibiotic Streptocycline (20.05 mm). This was followed by the antibiotics *viz.,* Gentamycin (17.5 mm), Tetracycline (16.5 mm) and Neomycin (8.2 mm). Antibiotics, Cephalexin and Dicrystacin were found less effective with 7.55 and 6.95 mm inhibition, respectively.

4.8.1.2. Antibacterial fungicides

At 1500 ppm, inhibition was ranged from 6.1 mm (Copper hydroxide) to 11.9 mm (Copper oxychloride + Streptocycline). However, significantly highest inhibition was recorded in the combination of antibacterial fungicide and antibiotic Copper oxychloride + Streptocycline (11.9 mm). This was followed by the antibacterial fungicides *viz.,* Copper oxychloride (10.6 mm), Copper oxychloride + Tetracycline (10.5 mm). Antibacterial fungicides, Amistar and Copper hydroxide were found less effective with 7.2 and 6.1 mm inhibition, respectively.

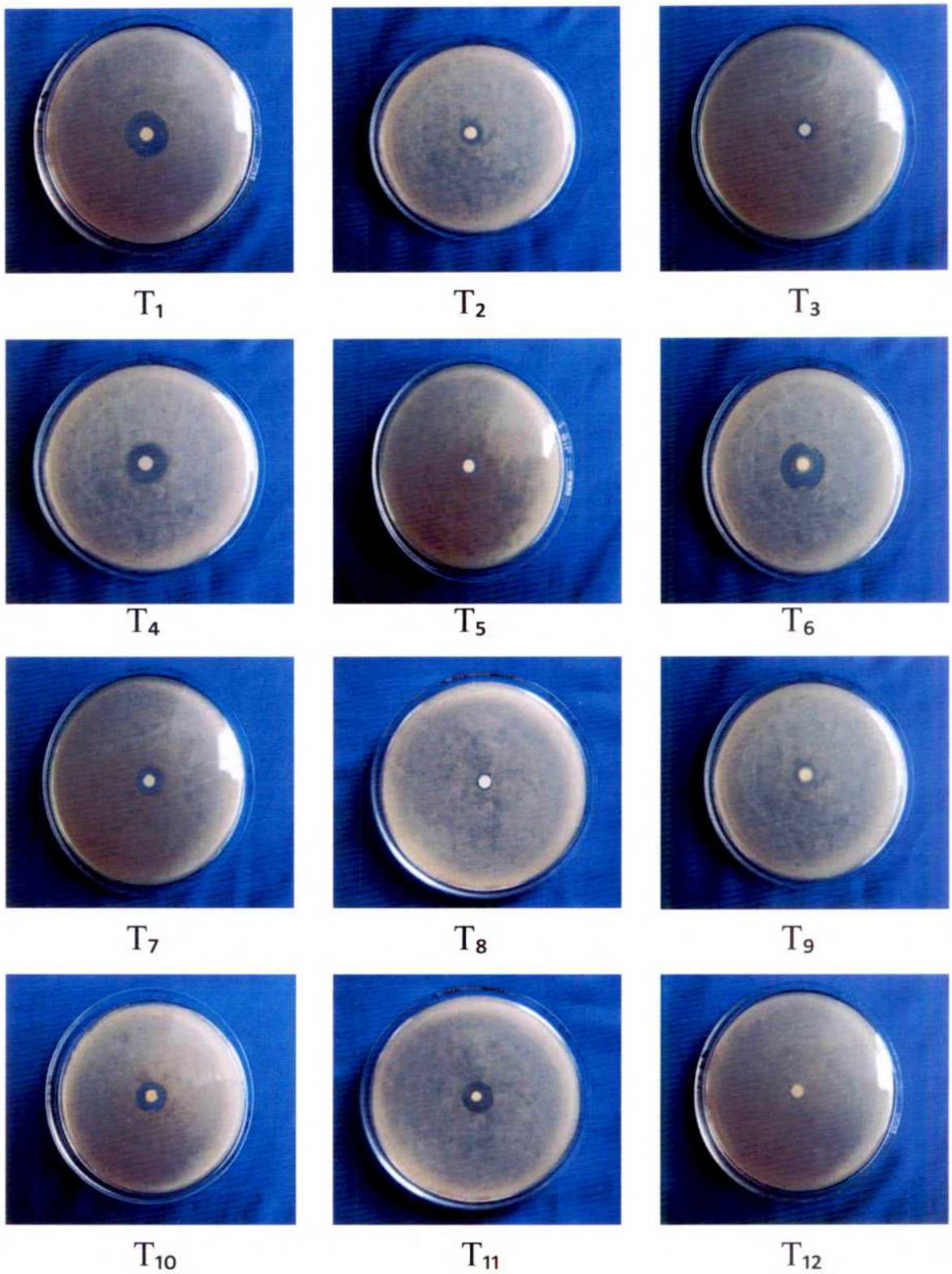
At 2000 ppm, inhibition was ranged from 6.3 mm (Copper hydroxide) to 12.0 mm (Copper oxychloride + Streptocycline). However,

Table 3. *In vitro* evaluation of antibacterial chemicals against *R. solanacearum*.

Tr. No.	Antibiotics	Inhibition zone (mm)* Concentration at		
		400 ppm	500 ppm	Av. (mm)
T_1	Sreptocycline	18.4	21.7	20.05
T_2	Cephalexin	7.5	7.6	7.55
T_3	Neomycin	8.1	8.3	8.2
T_4	Tetracycline	14.2	18.8	16.5
T_5	Dicrystacin	6.9	7.0	6.95
T_6	Gentamycin	15.4	19.6	17.5
	Antibacterial fungicides	**Inhibition zone (mm)* Concentration at**		
		1500 ppm	**2000 ppm**	**Av. (mm)**
T_7	Blitox (Copper oxy chloride)	10.6	11.6	11.1
T_8	Kocide (Copper hydroxide)	6.1	6.3	6.2
T_9	Amistar (Azoxystrobin)	7.2	7.3	7.25
T_{10}	Blitox + Streptocycline	11.9	12.0	11.95
T_{11}	Blitox + Tetracycline	10.5	10.7	10.6
T_{12}	Control (Untreated)	0.0	0.0	0.00
S.E. ±		**0.27**	**0.31**	-
CD (P 0.01)		**0.80**	**0.92**	-

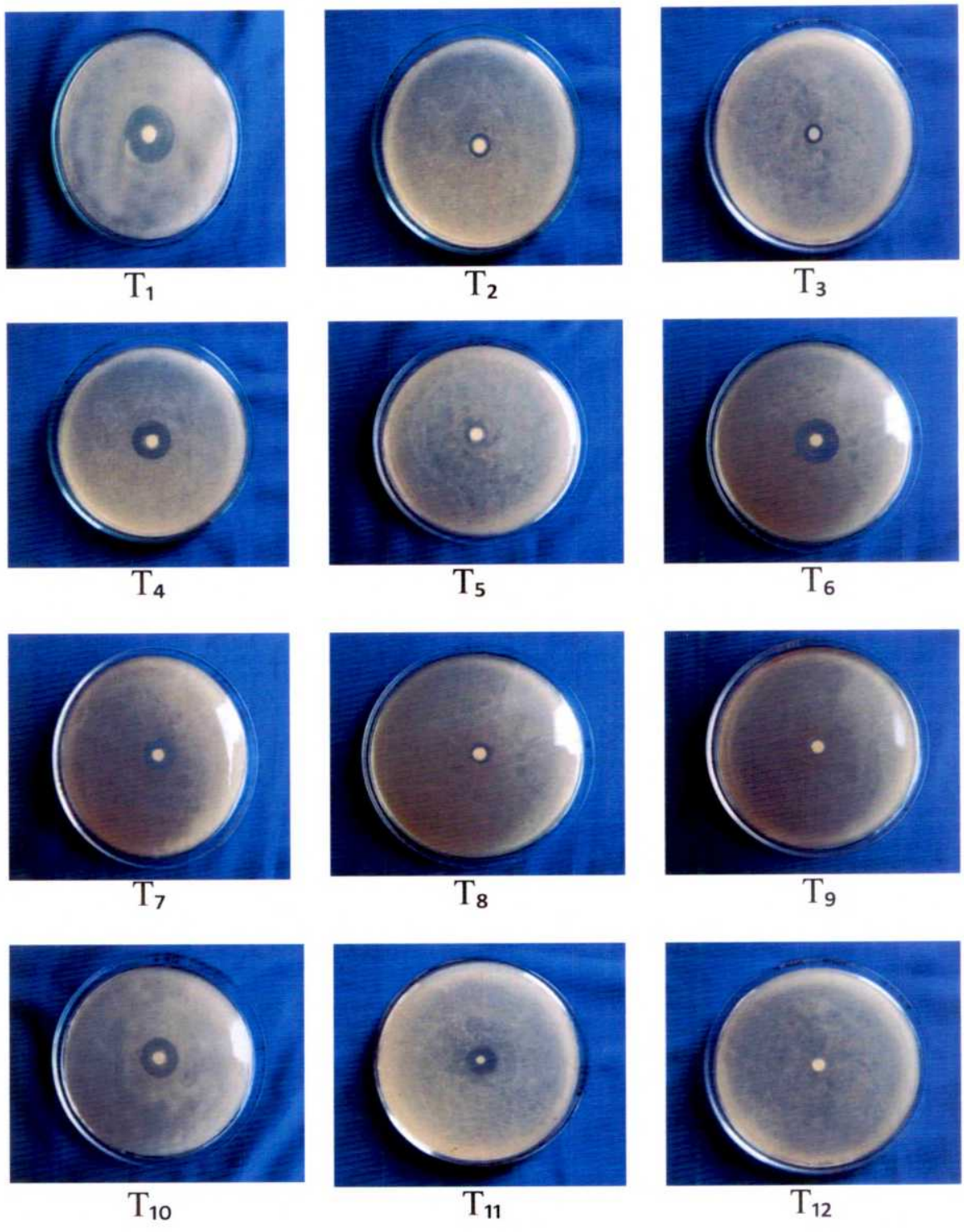
*-Mean of three replications

PLATE VIII



In vitro* evaluation of antibacterial chemical at 400 and 1500 ppm against *R. solanacearum

PLATE IX



In vitro evaluation of antibacterial chemical at 500 and 2000 ppm against *R. solanacearum*

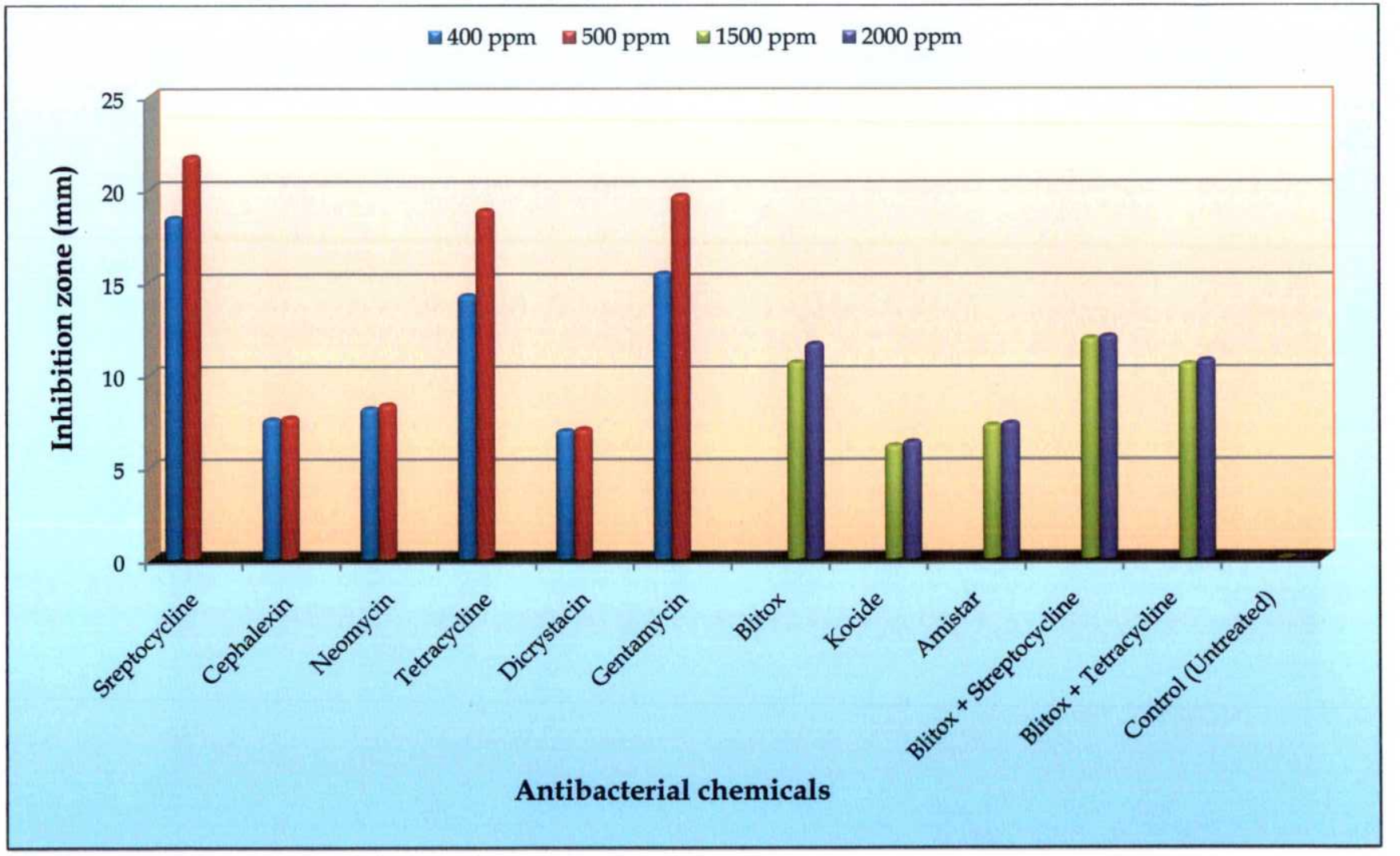


Fig.2. *In vitro* evaluation of antibacterial chemicals against *R. solanacearum*

significantly highest inhibition was recorded in the combination of antibacterial fungicide and antibiotic Copper oxychloride + Streptocycline (12.0 mm). This was followed by the antibacterial fungicides *viz.,* Copper oxychloride (11.6 mm), Copper oxychloride + Tetracycline (10.7 mm). Antibacterial fungicides, Amistar and Copper hydroxide were found less effective with 7.3 and 6.3 mm inhibition, respectively.

Average inhibition was ranged from 6.2 mm (Copper hydroxide) to 11.95 mm (Copper oxychloride + Streptocycline). However, significantly highest average inhibition was recorded in the combination of antibacterial fungicide and antibiotic Copper oxychloride + Streptocycline (11.95 mm). This was followed by the antibacterial fungicides *viz.,* Copper oxychloride (11.1 mm), Copper oxychloride + Tetracycline (10.6 mm). Antibacterial fungicides, Amistar and Copper hydroxide were found less effective with 7.25 and 6.2 mm inhibition, respectively.

Thus, all the antibiotics/antibacterial chemicals tested were found effective against *R. solanacearum*. However, antibacterial chemicals found most effective in the order of merit were Streptocycline, Gentamycin, Tetracycline, Copper oxychloride + Streptocycline, Copper oxychloride , Copper oxychloride + Tetracycline, Neomycin, Cephalexin, Amistar and Dicrystacin.

These results are in conformity with the findings of those reported earlier by several workers (Hidaka and Murano, 1956; Dutta and Verma, 1969; Indersenan *et al.,* 1981; Khan *et al.,* 1997; Singh *et al.,* 2000; Devnath *et al.,* 2002; Dubey, 2005; Venkatesh, 2005; Sunder *et al.,* 2011; Gupta and Razdan, 2013; Pankaj *et al.,* 2013; Owoseni and Sangoyomi, 2014).

4.8.2. *In vitro* evaluation of bioagents/antagonists against *R. solanacearum.*

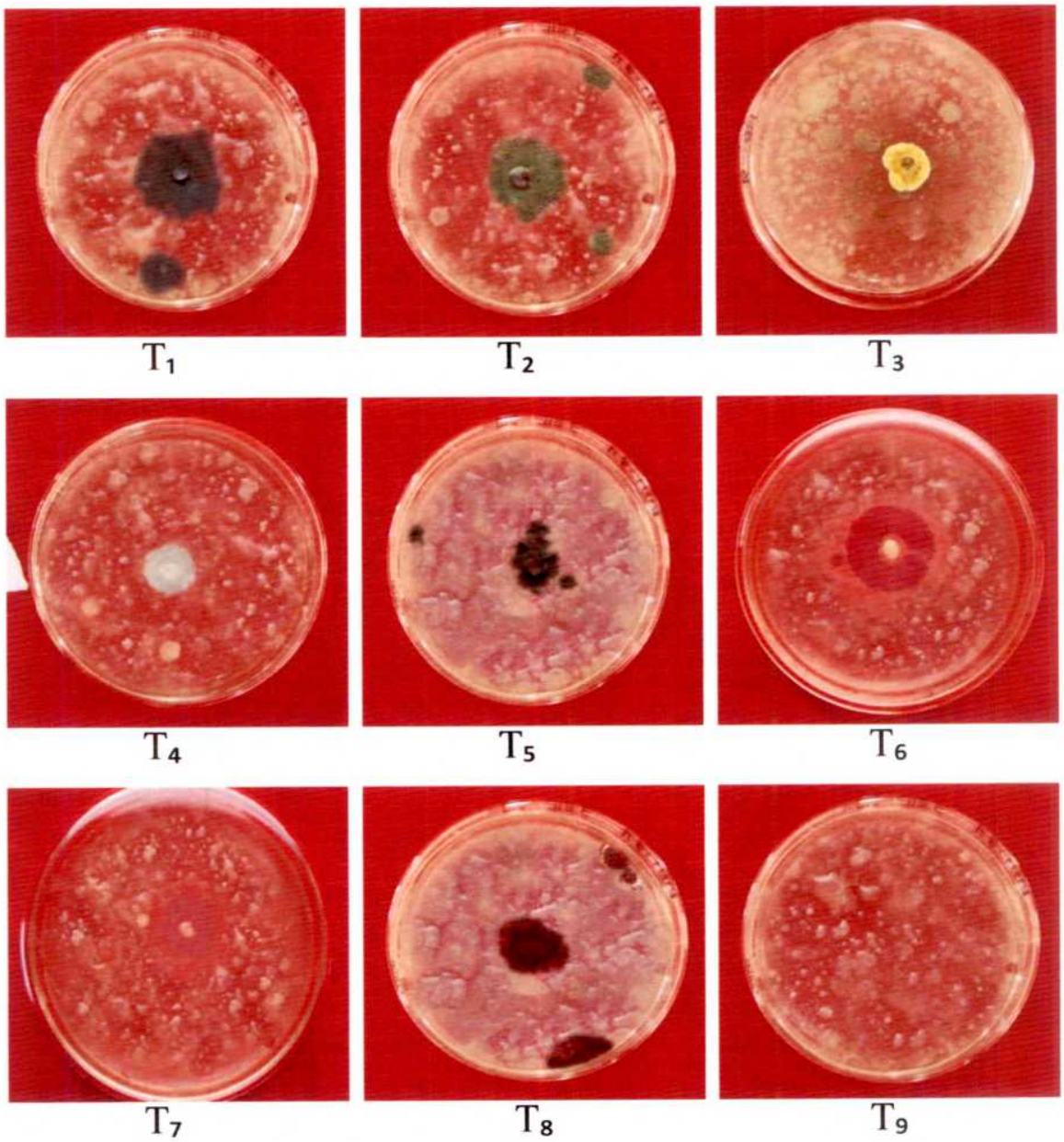
The six fungal antagonistic microorganism's *viz., Trichoderma harzianum, Trichoderma viride, Trichoderma koningii, Trichoderma virens, Trichoderma longibrachiatum, Aspergillus niger* and two bacterial antagonistic microorganisms *viz., Pseudomonas fluorescens* and *Bacillus subtilis* were

Table 4. *In vitro* evaluation of biocontrol agents against *R. solanacearum*

Tr. No.	Treatments	Inhibition zone (mm) *
T_1	*Trichoderma viride*	21.17
T_2	*Trichoderma harzianum*	16.33
T_3	*Gliocladium virens*	9.67
T_4	*Trichoderma koningii*	8.33
T_5	*Trichoderma longibrachiatum*	7.67
T_6	*Pseudomonas fluorescence*	24.33
T_7	*Bacillus subtilis*	19.33
T_8	*Aspergillus niger*	10.33
T_9	Control (untreated)	0.00
S.E. ±		**0.58**
CD (P 0.01)		**1.73**

*-Mean of three replications

PLATE X



In vitro* evaluation of bioagents against *R. solanacearum

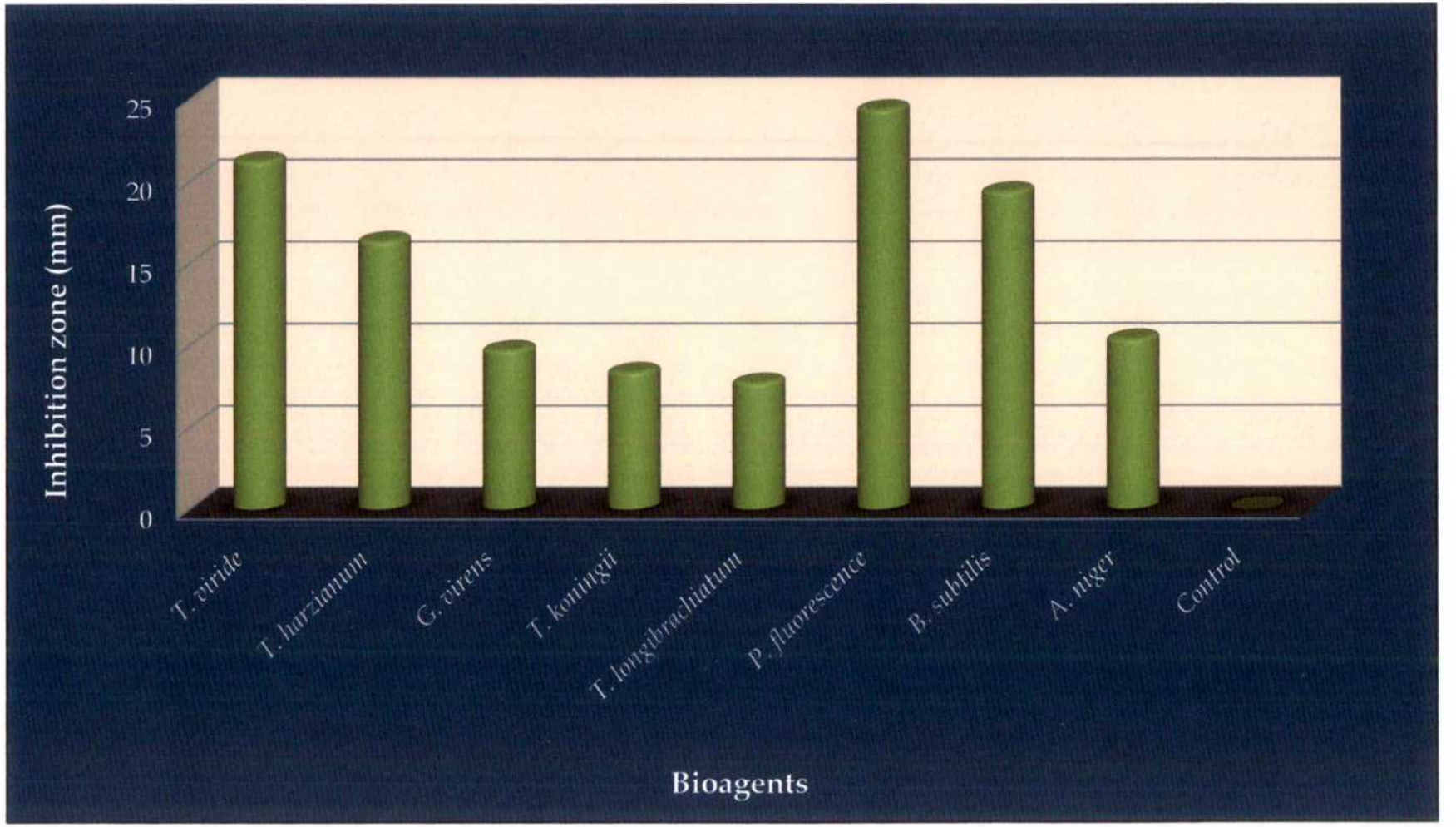


Fig.3. *In vitro* evaluation of bioagents against *R. solanacearum*

evaluated against *R. solanacearum* under *in vitro* condition by inhibition zone method as explained in the material and methods.

The results obtained on inhibition zone produced across the antagonistic microorganisms are presented in Table 4, Fig.3 and PLATE X.

Results (Table 4, Fig. 3) revealed that all the bioagents evaluated exhibited antibacterial activity against *R. solanacearum*. The results indicated that the antagonistic microorganism *P. fluorescens* resulted in maximum inhibition of the *Ralstonia solanacearum* with an inhibition zone of 24.33 mm which was found significantly superior over other treatments. The second and third best antagonists found were *T. viride* and *Bacillus subtilis* with an inhibition zone of 21.17 mm and 19.33 mm, respectively. This was followed by *T. harzianum* (16.33 mm) and *A. niger* (10.33). Whereas, the antagonists like, *T. virens*, *T. koningii* and *T. longibrachiatum* were moderately effective with slight inhibition zone of 9.67 mm, 8.33 mm and 7.67 mm, respectively.

Bioagents *viz., P. fluorescens* and *Bacillus subtilis* were reported efficient antagonists against *R. solanacearum* earlier by many workers (Gallardo *et al.,* 1989; Ciampi *et al.,* 1996; Guo *et al.,* 2001; El-Sayed *et al.,* 2003; Sun *et al.,* 2004; Sood *et al.,* 2005; Doan and Nguyen, 2005; Lemessa and Zeller, 2007 B; Henok *et al.,* 2007; Liza and Bora, 2008; Vanita *et al.,* 2009; Liza and Bora, 2009; Maketon *et al.,* 2010; Choudhry and Rashid, 2011; Khair *et al.,* 2012; Yang *et al.,* 2012; Gupta and Razdan, 2013; Raghu *et al.,* 2013). The species of *Trichoderma viz., viride* and *harzianum* were reported as efficient antagonists against *R. solanacearum* (Ramesh, 2006; Liza and Bora, 2009; Chaudhry and Rashid, 2011, Narsimbha and Srinivas, 2012; Gupta and Razdan, 2013; Raghu *et al.,* 2013).

4.8.3. *In vitro* evaluation of botanicals against *R. solanacearum.*

4.8.3.1. Aqueous extract botanicals (Phytoextracts)

Aqueous extracts (leaf, rhizome and bulb) of 10 botanicals were evaluated *in vitro* (each @ 5, 10 and 20 %) against *R. solanacearum* and the

results obtained on its inhibition are presented in the Table 5, Fig. 4 and PLATE XI, XII, XIII.

Results (Table 5) revealed that all the 10 botanicals evaluated were found effective against *R. solanacearum* and recorded inhibition zone of the test pathogen over untreated control. The inhibition zone was increased with increase in concentrations of the botanicals tested.

Inhibition

At 5 per cent concentration, inhibition was ranged from 5 mm (*T. terrestris* and *L. innermis*) to 7.4 mm (*A. sativum*). However, significantly highest inhibition was recorded with the botanical *A. sativum* (7.4 mm). This was followed by the botanicals *viz.*, *A. cepa* (6.9 mm), *A. sativum* + *C. longa* (6.2 mm), *A. indica* and *C. longa* (6.00 mm), *D. stromonium* (5.9 mm), all of which were at par to each other. Botanicals *P. hysterophorus, P. pinnata, T. terrestris* and *L. innermis* were found less effective with significantly least inhibition of below 5.5 mm respectively.

At 10 per cent concentration, inhibition ranged from 6 mm (*T. terrestris*) to 9.7 mm (*A. sativum*). However, significantly highest inhibition was recorded with the botanical *A. sativum* (9.7 mm). This was followed by the botanicals *viz.*, *A. cepa* (9.1 mm), *A. sativum* + *C. longa* (8.1 mm), *C. longa* (7.8 mm), *A. indica* (7.3 mm), *D. stromonium* (7.2 mm), all of which were at par to each other. Botanicals, *P. hysterophorus, O. sanctum, P. pinnata, L. innermis, T. terrestris* were found less effective with significantly least inhibition of below 6.6 mm respectively.

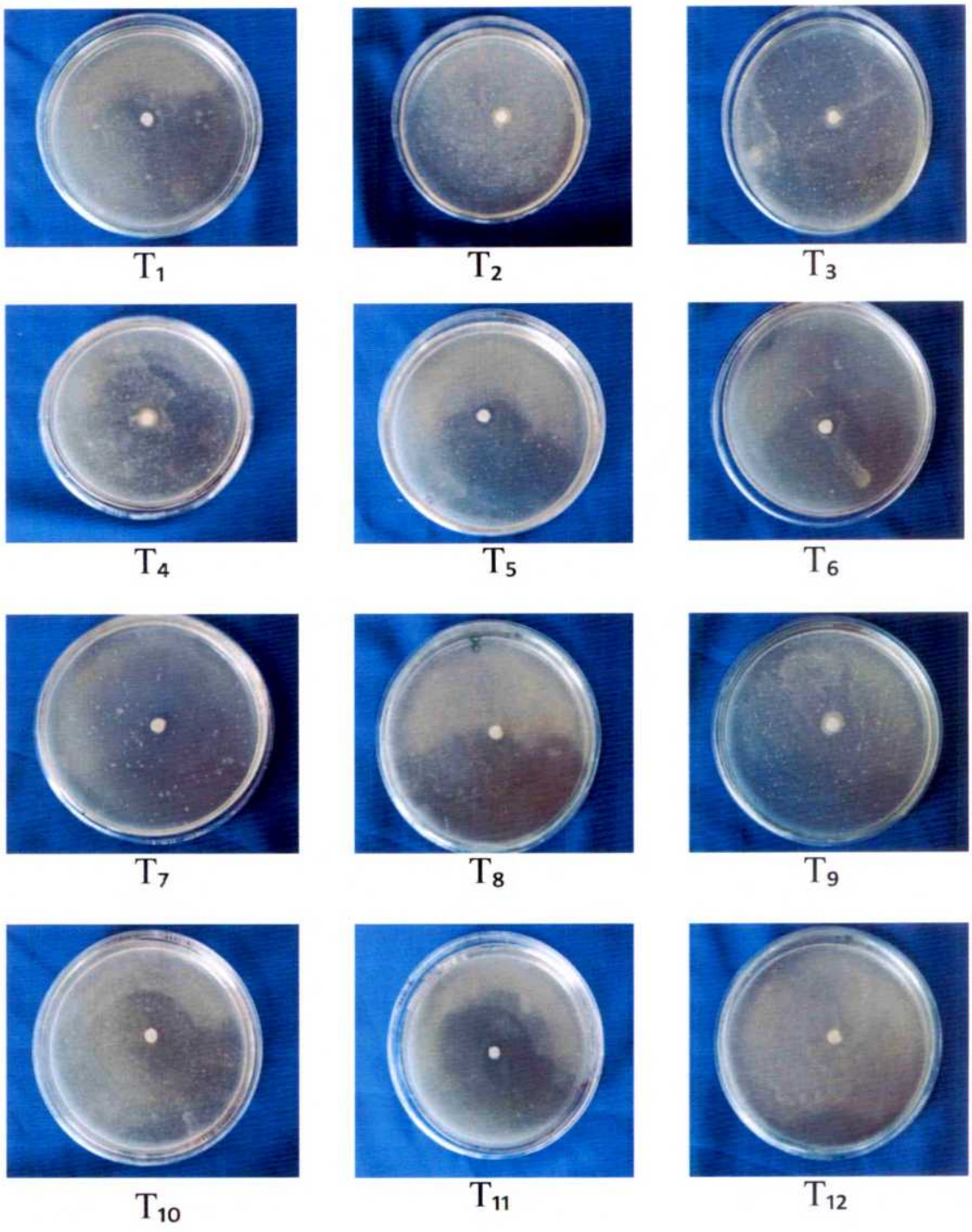
At 20 per cent concentration, inhibition was ranged from 7.9 mm (*L. innermis*) to 12.3 mm (*A. sativum*). However, significantly highest inhibition was recorded with the botanical *A. sativum* (12.3 mm). This was followed by the botanicals *viz.*, *A. cepa* (11.1 mm), *A. sativum* + *C. longa* (10.8 mm), *A. indica* (10.5 mm), *D. stromonium* (10.2 mm), *T. terrestris* (9.5 mm), *C. longa* (9.3 mm), all of which were at par to each other. Botanicals *O. sanctum, P.*

Table 5. *In vitro* evaluation of aqueous phytoextracts against *R. solanacearum*

Tr. No.	Treatments	Inhibition zone (mm)* Concentration at			
		5 %	10%	20 %	Av. (mm)
T_1	Garlic (*A.sativum*)	7.4	9.7	12.3	9.8
T_2	Turmeric (*C. longa*)	6.0	7.8	9.3	7.7
T_3	Tulsi (*O. sanctum*)	5.3	6.3	8.9	6.8
T_4	Neem (*A. indica*)	6.0	7.3	10.5	7.9
T_5	Onion (*A. cepa*)	6.9	9.1	11.1	9.0
T_6	Parthenium(*P. hysterophorus*)	5.5	6.6	8.8	7.0
T_7	Mehandi (*L. innermis*)	5.0	6.1	7.9	6.3
T_8	Karanj (*P. pinnata*)	5.1	6.1	8.8	6.7
T_9	Dhotra (*D. stromonium*)	5.9	7.2	10.2	7.8
T_{10}	Gokhru (*T. terrestris*)	5.0	6.0	9.5	6.8
T_{11}	Garlic +Turmeric	6.2	8.1	10.8	8.4
T_{12}	Control (untreated)	0.0	0.0	0.0	0.0
S.E.±		**0.15**	**0.22**	**0.36**	-
CD(P=0.01)		**0.45**	**0.64**	**1.05**	-

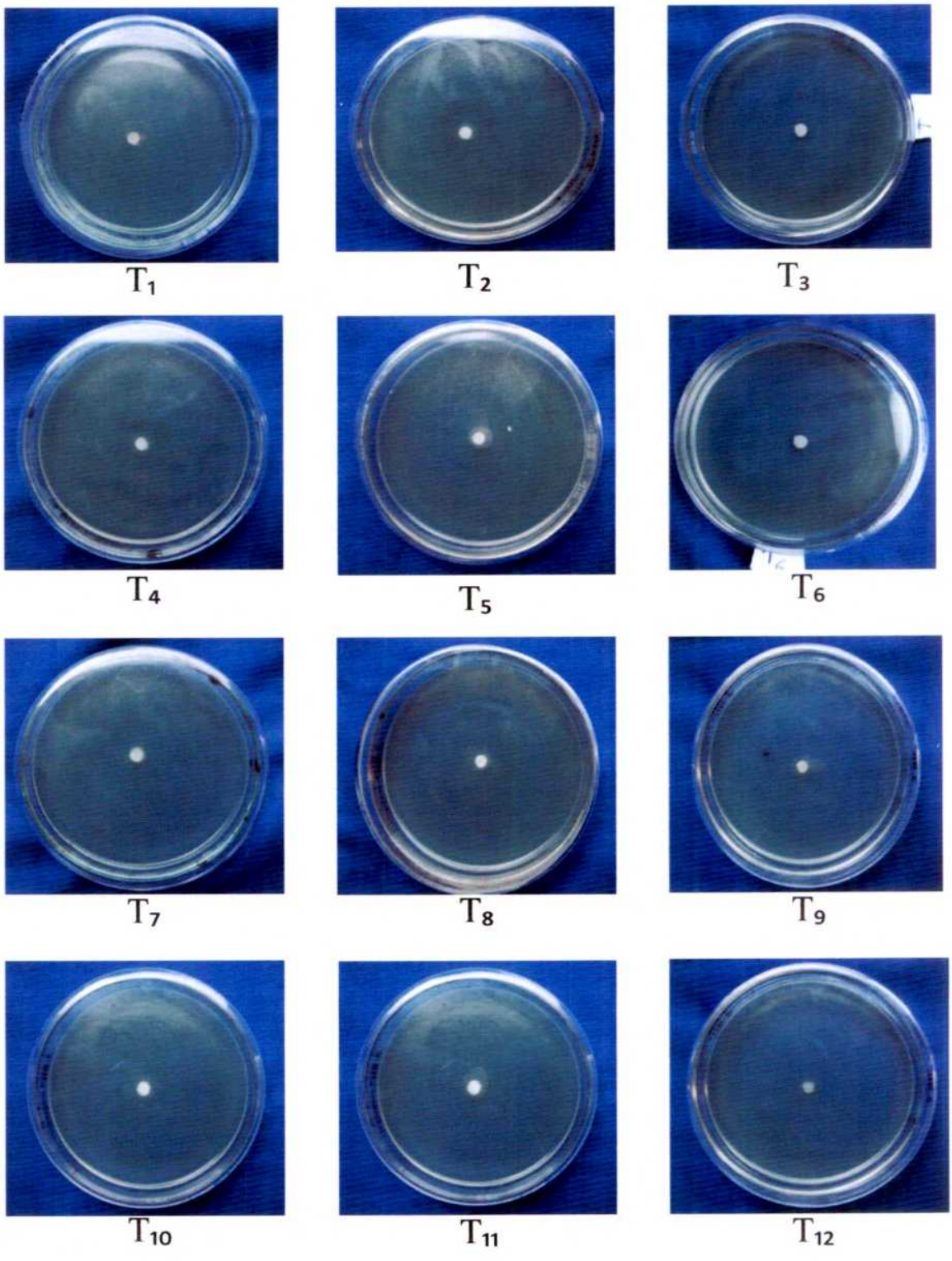
*-Mean of three replications

PLATE XI



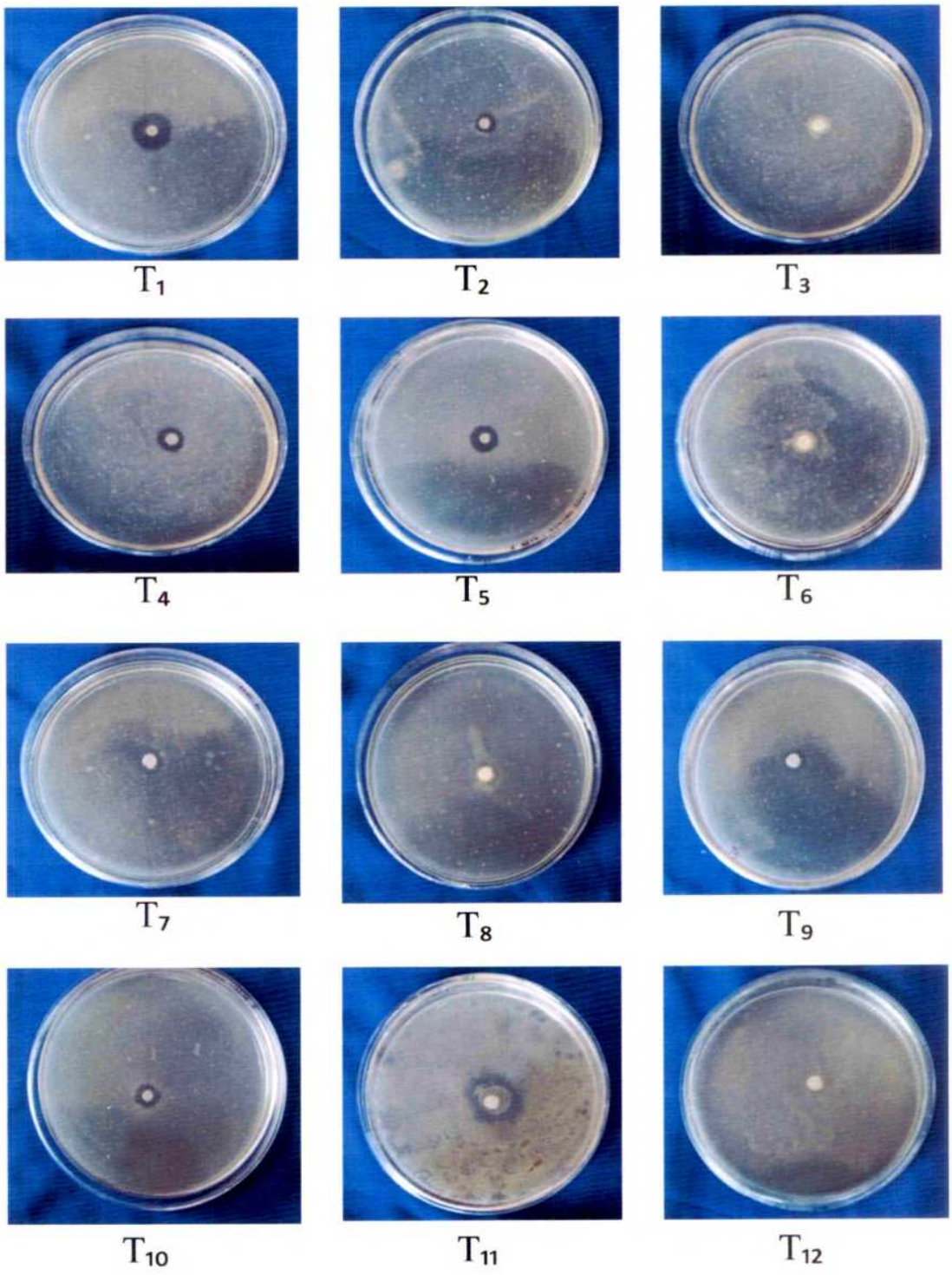
In vitro evaluation of phytoextracts aqueous at 5 % against *R. solanacearum*

PLATE XII



In vitro* evaluation of phytoextracts acqueous at 10 % against *R. solanacearum

PLATE XIII



In vitro evaluation of phytoextracts acqueous at 20 % against *R. solanacearum*

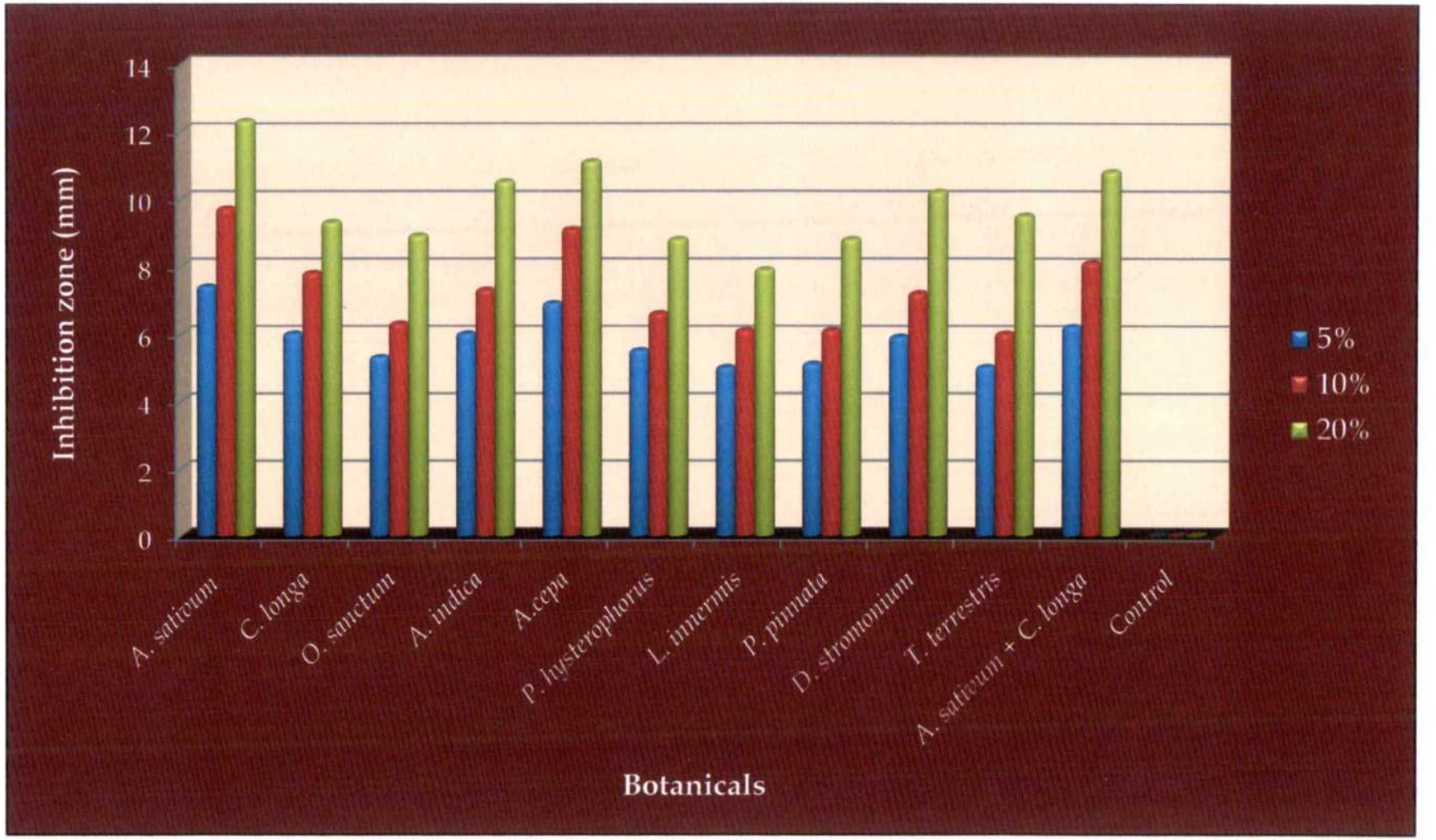


Fig.4. *In vitro* evaluation of aqueous phytoextracts against *R. solanacearum*

hysterophorus, P. pinnata, L. innermis were found less effective with significantly least inhibition of below 8.9 mm respectively.

Average inhibition was ranged from 6.3 mm (*L. innermis*) to 9.8 mm (*A. sativum*). However, significantly highest average inhibition was recorded with the botanical *A. sativum* (9.8 mm). This was followed by the botanicals *viz.*, *A. cepa* (9.0 mm), *A. sativum* + *C. longa* (8.4 mm), *A. indica* (7.9 mm), *D. stromonium* (7.8 mm), *C. longa* (7.7 mm), *P. hysterophorus* (7.0 mm), all of which were at par to each other. Botanicals *O. sanctum, T. terrestris, P. pinnata, L. innermis* were found less effective with significantly average least inhibition of below 6.8 mm respectively.

4.8.3.2. Acetone extracts botanicals (Phytoextracts)

Acetone extracts (leaf, rhizome and bulb) of 10 botanicals were evaluated *in vitro* (each @ 5, 10 and 20 %) against *R. solanacearum* and the results obtained on its inhibition are presented in the Table 6, Fig. 5 and PLATE XIV, XV, XVI.

Inhibition

At 5 per cent concentration, inhibition was ranged from 5.3 mm (*L. innermis*) to 7.2mm (*A. sativum*). However, significantly highest inhibition was recorded with the botanical *A. sativum* (7.2 mm). This was followed by the botanicals *viz.*, *D. stromonium* (6.9 mm), *A. cepa* (6.5 mm), *A. sativum* + *C. longa* (6.4 mm), *P. hysterophorus* (6.1 mm) all of which were at par to each other. Botanicals, *A. indica, P. pinnata, T. terrestris, O. sanctum, L. innermis* were found less effective with significantly least inhibition of below 5.9mm respectively.

At 10 per cent concentration, inhibition was ranged from 6.1 mm (*P. pinnata*) to 10.8 mm (*A. sativum*). However, significantly highest inhibition was recorded with the botanical *A. sativum* (10.8 mm). This was followed by the botanicals *viz.*, *D. stromonium* (10.3 mm), *A. cepa* (9.6 mm), *A. sativum* + *C. longa* (8.8 mm), *P. hysterophorus* and *C. longa* (7.8 mm) all of which were at par to each other. Botanicals, *T. terrestris, A. indica, O. sanctum, L. innermis, P.*

pinnata were found less effective with significantly least inhibition of below 7.4 mm respectively.

At 20 per cent concentration, inhibition was ranged from 6.4 mm (*P. pinnata*) to 13.1 mm (*A. sativum*). However, significantly highest inhibition was recorded with the botanical *A. sativum* (13.1 mm). This was followed by the botanicals *viz.*, *D. stromonium* (12.2 mm), *A. cepa* (10.8 mm), *A. sativum* + *C. longa* (10.5 mm), *C. longa* (8.7 mm) all of which were at par to each other. Botanicals, *P. hysterophorus, A. indica, T. terrestris, O. sanctum, L. innermis, P. pinnata* were found less effective with significantly least inhibition of below 8.4 mm respectively.

Average inhibition was ranged from 6.1 mm (*L. innermis* and *P. pinnata*) to 10.4 mm (*A. sativum*). However, significantly highest average inhibition was recorded with the botanical *A. sativum* (10.4 mm). This was followed by the botanicals *viz.*, *D. stromonium* (9.8 mm), *A. cepa* (9.0 mm), *A. sativum* + *C. longa* (8.6 mm), *C. longa* (7.5 mm), *P. hysterophorus* (7.4 mm), *A. indica* (7.1 mm), all of which were at par to each other. Botanicals *T. terrestris, O. sanctum, P. pinnata, L. innermis* were found less effective with significantly average least inhibition of below 6.8 mm respectively.

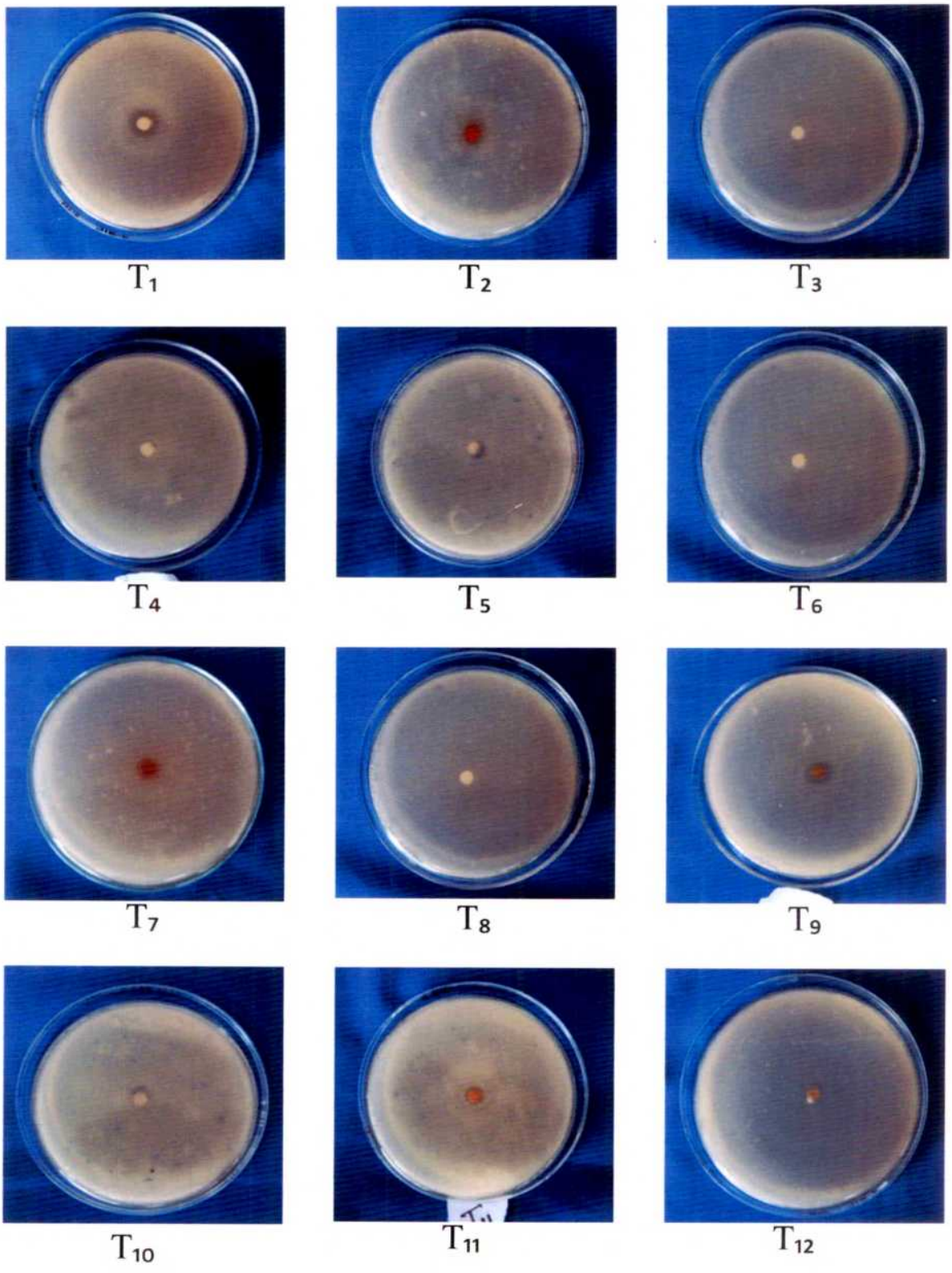
Similar effects of the test botanicals/phytoextracts against R. solanacearum were reported earlier by several workers. Hannudin and Djantnika (1989) reported that extracts from garlic bulbs inhibited bacterial growth under *in vitro* conditions. Khan (1974) reported that garlic extract (*Allium sativum*) was inhibitory to the growth of *R. solanacearum* under *in vitro* conditions. Balestra *et al.* (2009) reported that extracts from cloves of *A. sativum* and fruits of *F. carica* (@ 10 and 30%), respectively, showed best effects at 10^6 cfu ml_1 bacterial concentrations under *in vitro* condition. Elyousr and Asran (2009) reported that garlic exhibited the strongest antibacterial activity against bacterial wilt *in vitro* and *in vivo* followed by datura and then nerium. Cold water extracts of these plant species were more effective than hot water extract in the development of the disease *in vivo*. Owoseni and Sangoyomi (2014) reported that the plant extracts from chloroform were the most active and this was followed by

Table 6. *In vitro* evaluation of acetone phytoextracts against *R. solanacearum*.

Tr. No.	Treatments	Inhibition zone (mm) * Concentration at			
		5 %	10%	20 %	Av. (mm)
T_1	Garlic (*A. sativum*)	7.2	10.8	13.1	10.4
T_2	Turmeric (*C. longa*)	6.0	7.8	8.7	7.5
T_3	Tulsi (*O. sanctum*)	5.4	6.7	7.2	6.4
T_4	Neem (*A. indica*)	5.9	7.2	8.3	7.1
T_5	Onion (*A. cepa*)	6.5	9.6	10.8	9.0
T_6	Parthenium (*P.hysterophorus*)	6.1	7.8	8.4	7.4
T_7	Mehandi (*L. innermis*)	5.3	6.3	6.7	6.1
T_8	Karanj (*P. pinnata*)	5.8	6.1	6.4	6.1
T_9	Dhotra (*D. stromonium*)	6.9	10.3	12.2	9.8
T_{10}	Gokhru (*T. terrestris*)	5.6	7.4	7.4	6.8
T_{11}	Garlic + Turmeric	6.4	8.8	10.5	8.6
T_{12}	Control (untreated)	0.0	0.0	0.0	0.0
S.E.±		**0.18**	**0.20**	**0.32**	-
CD(P=0.01)		**0.54**	**0.59**	**0.94**	-

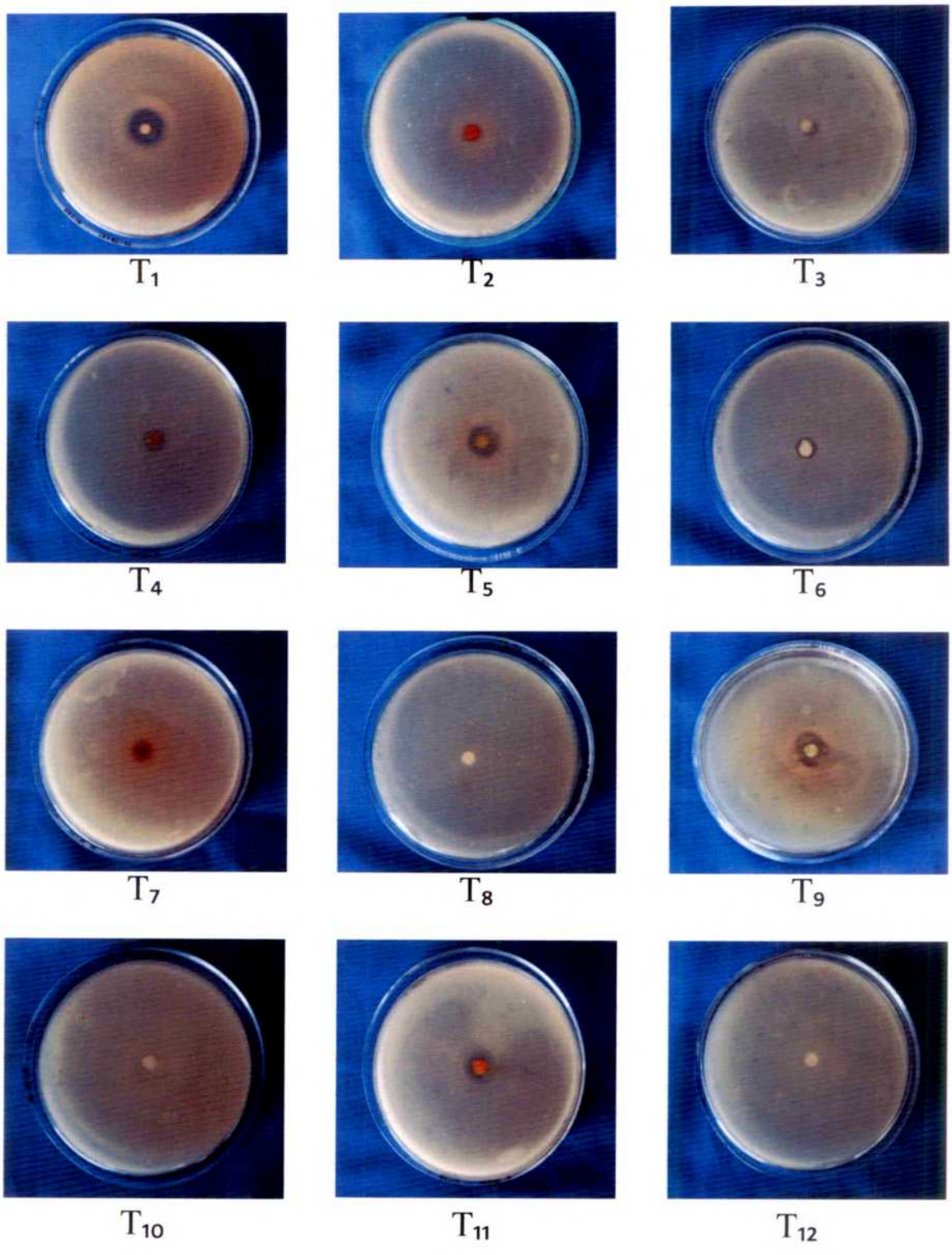
*-Mean of three replications,

PLATE XIV



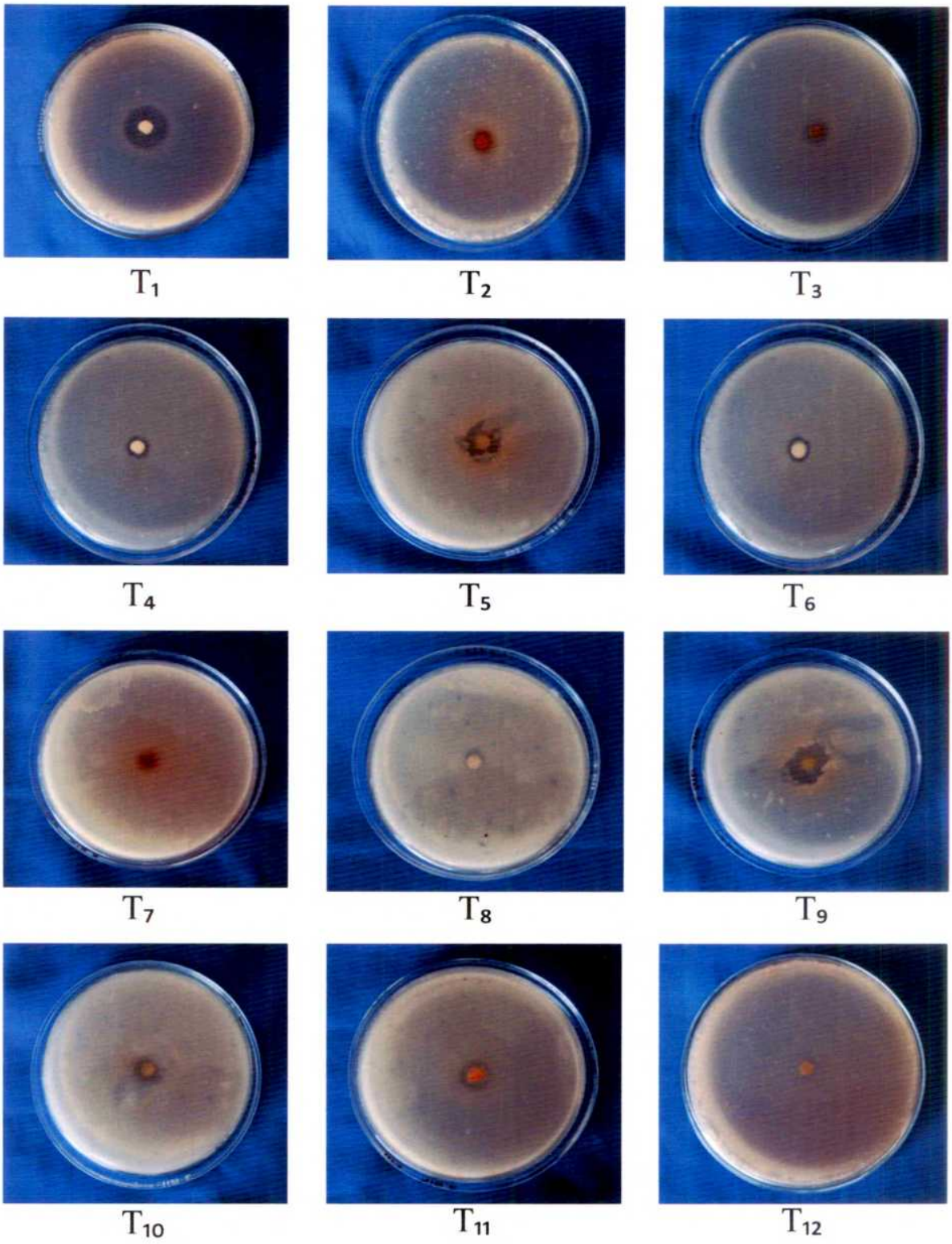
In vitro* evaluation of acetone phytoextracts at 5 % against *R. solanacearum

PLATE XV



In vitro evaluation of acetone phytoextracts at 10 % against *R. solanacearum*

PLATE XVI



In vitro evaluation of acetone phytoextracts at 20 % against *R. solanacearum*

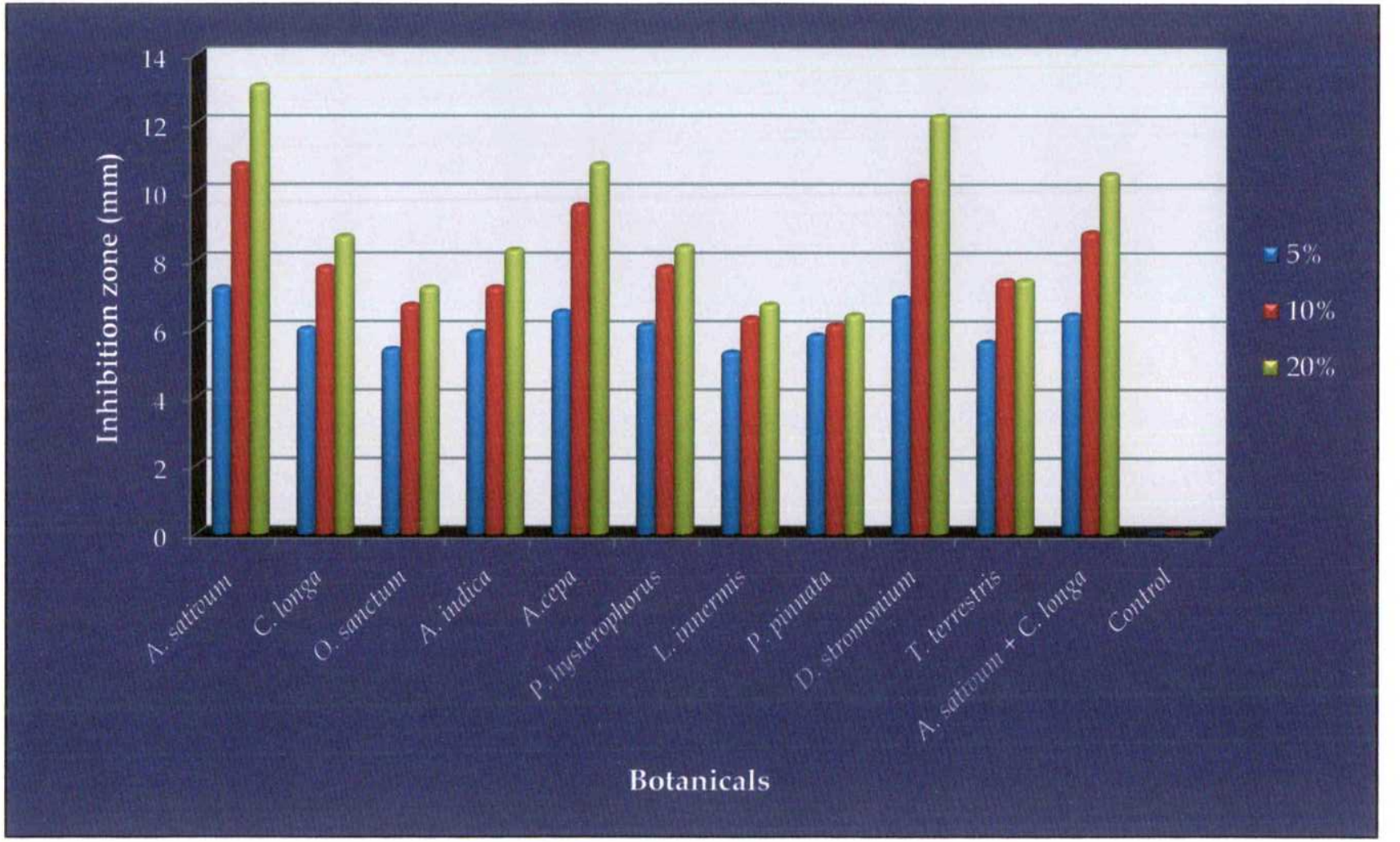


Fig.5. *In vitro* evaluation of acetone phytoextracts against *R. solanacearum*

methanol and ethanol, the lowest activity was recorded from the hexane extracts. All the solvent extracts of *A. sativum* were active except the hexane extract.

4.8.4. Evaluation of organic amendments against *R. solanacearum* (Pot culture)

A total of 10 amendments were evaluated as pre-sowing soil application to assess their efficacy against *R. solanacearum*, employing sick soil technique and sowing susceptible ginger local variety in pot culture under screen house conditions. The results obtained on rhizome germination, pre-emergence rhizome rot (PESR) and post- emergence seedling mortality (PESM) are presented in the Table 7, Fig.6, PLATE XVII and that of on growth parameters in Table 8, Fig.7.

4.8.4.1. Effect on rhizome germination

Results (Table 7) revealed that all the test amendments recorded significantly improved rhizome germination, over untreated control and it was ranged from 30.00 to 73.33 per cent, as against 20.00 per cent in untreated control. However, significantly highest rhizome germination was recorded with karanj cake (73.33). This was followed by the amendments *viz.*, neem seed cake (60.00%), farm yard manure (50.00 %), vermicompost (46.67 %), poultry manure (43.33%), sunflower seed cake and safflower seed cake (each 36.67 %). Whereas, cotton seed cake, goat manure (each 33.33 %) and groundnut cake (30.00%) were found least effective with comparatively minimum rhizome germination. Further, all the test amendments recorded significant increase in rhizome germination, over control and it was ranged from 33.33 (groundnut cake) to 72.72 (karanj cake) per cent. However, highest increase in rhizome germination was recorded with karanj cake (72.72 %). This was followed by the amendments *viz.*, neem seed cake (66.66 %), farm yard manure (60.00 %), vermicompost (57.14 %), poultry manure (53.84 %), sunflower seed cake and safflower seed cake (each 45.45 %). Whereas, cotton seed cake, goat manure

(each 39.99 %) and groundnut cake (33.33 %) were found less effective increase in rhizome germination.

4.8.4.2. Pre-emergence rhizome rot

Results (Table 7) revealed that all the organic amendments applied in sick soil (*R. solanacearum*) were found effective against *R. solanacearum* and recorded PESR in the range of 26.67 to 70.00 per cent as against 80.00 per cent in untreated control. However, significantly least pre-emergence seed rot was recorded with the amendment karanj cake (26.67 %). This was followed by the amendment *viz.*, neem seed cake (40.00 %), farm yard manure (50.00 %), vermicompost (53.33 %), poultry manure (56.66 %), sunflower seed cake and safflower seed cake (each 63.33 %). Whereas, cotton seed cake, goat manure (each 66.66 %) and groundnut cake (70.00 %) were found less effective.

4.8.4.3. Post- emergence seedling mortality (PESM)

The results (Table 7) indicated that all the organic amendments tested were found effective against *R. solanacearum* and recorded PESM in the range of 31.66 to 70.66 per cent as against 86.66 per cent in untreated control. However, significantly least pre-emergence seedling mortality was recorded with the amendment karanj cake (31.66 %). This was followed by neem seed cake (53.66 %), farm yard manure (54.33 %), poultry manure (55.67 %), sunflower seed cake (58.00 %) and vermicompost (58.33 %). Whereas, safflower seed cake (67.33 %) goat manure (67.50 %), cotton seed cake (68.66%), groundnut cake (70.66 %), were found less effective.

The average mortality (PESR + PESM) recorded with all the treatments were ranged from 29.16 to 70.33 per cent as against 83.33 per cent in untreated control. However, significantly least average mortality was recorded with the amendment karanj cake (29.16 %). This was followed by neem seed cake (46.83 %), farm yard manure (52.16 %), vermicompost (55.83 %), poultry manure (56.16 %) and sunflower cake (60.66 %). Whereas, safflower seed cake (65.33 %), goat manure (67.08 %), cotton seed cake (67.66 %) and groundnut cake (70.33 %) were found less effective.

4.8.4.4. Reduction in mortality

Result (Table 7 and Fig. 6) revealed that both PESR and PESM were significantly reduced with the application of all the organic amendments tested. The percentage reduction in PESR recorded with test organic amendments were ranged from 12.50 to 66.66 per cent as against 0.00 per cent in untreated control. However, significantly highest reduction in PESR was recorded with karanj cake (66.66 %). This was followed by the organic amendments *viz.*, neem seed cake (50.00 %), farm yard manure (37.50 %), vermicompost (33.33 %), poultry manure (29.16 %), sunflower seed cake and safflower seed cake (each 20.83 %). Whereas, goat manure, cotton seed cake (each 16.66 %) and groundnut cake (12.50 %) were found less effective.

The percentage reduction in PESM recorded with test organic amendments were ranged from 18.28 to 63.28 per cent as against 0.00 per cent in untreated control. However, significantly highest reduction in PESM was recorded with karanj cake (63.28 %). This was followed by the organic amendments *viz.*, neem seed cake (37.91 %), farm yard manure (37.22 %), poultry manure (35.32 %), sunflower seed cake (32.91 %) and vermicompost (32.58%). Whereas, safflower seed cake (21.98 %), goat manure (21.91 %), cotton seed cake (20.55 %) and groundnut cake (18.28 %) were found less effective.

The average (PESR +PESM) reduction in mortality recorded with all the organic amendments tested were ranged from 15.39 to 64.97 per cent as against 0.00 per cent in untreated control. However, significantly highest reduction in average mortality was recorded with karanj cake (64.97 %). This was followed by the organic amendments *viz.*, neem seed cake (43.95 %), farm yard manure (37.36 %), vermicompost (32.95 %), poultry manure (32.24 %) and sunflower seed cake (26.87 %). Whereas, safflower seed cake (21.40 %), goat manure (19.28 %), cotton seed cake (18.60 %) and groundnut cake (15.39 %) were found less effective.

Thus, all the organic amendments applied in sick soil (*R. solanacearum*) were found effective in reducing the pre-emergence rhizome rot

Table. 7 Effect of organic amendment on pre-emergence rhizome rot and post emergence seedling mortality caused by *R. solanacearum* in ginger

Tr. No.	Treatments	Germination (%)	Rot/ mortality (%)*		Average mortality (%)	Reduction over control (%)		Average reduction (%)
			PESR	PESM		PESR	PESM	
T_1	FYM	50.00 (45.00)	50.00 (45.00)	54.33 (47.48)	52.16 (46.23)	37.50 (37.76)	37.22 (37.59)	37.36 (37.67)
T_2	Poultry manure	43.33 (41.16)	56.66 (48.82)	55.67 (48.25)	56.16 (48.53)	29.16 (32.68)	35.32 (36.46)	32.24 (34.59)
T_3	Neem seed cake	60.00 (50.76)	40.00 (39.23)	53.66 (47.09)	46.83 (43.18)	50.00 (45.00)	37.91 (38.00)	43.95 (41.52)
T_4	Sunflower cake	36.67 (37.26)	63.33 (52.73)	58.00 (49.60)	60.66 (51.15)	20.83 (27.15)	32.91 (35.00)	26.87 (31.22)
T_5	Vermicompost	46.67 (43.09)	53.33 (46.90)	58.33 (49.79)	55.83 (48.340	33.33 (35.26)	32.58 (34.80)	32.95 (35.03)
T_6	Cotton seed cake	33.33 (35.26)	66.66 (54.73)	68.66 (55.95)	67.66 (55.34)	16.66 (24.08)	20.55 (26.95)	18.60 (25.54)
T_7	Karanj cake	73.33 (58.90)	26.67 (31.09)	31.66 (34.24)	29.16 (32.68)	66.66 (54.73)	63.28 (52.70)	64.97 (53.71)
T_8	Goat manure	33.33 (35.26)	66.67 (54.73)	67.50 (55.24)	67.08 (54.98)	16.66 (24.08)	21.91 (27.90)	19.28 (26.04)
T_9	Groundnut cake	30.00 (33.21)	70.00 (56.78)	70.66 (57.20)	70.33 (56.99)	12.50 (20.70)	18.28 (25.31)	15.39 (23.09)
T_{10}	Safflower cake	36.67 (37.26)	63.33 (52.73)	67.33 (55.13)	65.33 (53.92)	20.83 (27.15	21.98 (27.95)	21.40 (27.55)
T_{11}	Control (Untreated)	20.00 (26.56)	80.00 (63.43)	86.66 (68.57)	83.33 (65.90)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
SE ±		**2.66**	**3.18**	**2.44**	**2.81**	**3.35**	**3.11**	**3.23**
CD (P=0.01)		**7.80**	**9.32**	**7.14**	**8.23**	**9.83**	**9.12**	**9.47**

*-Mean of three replications,

Figures in parentheses are angular transformed values

PLATE XVII

Effect of organic amendments against *R. solanacearum*

Treatments details

T_1:	FYM	T_7:	Karanj cake
T_2:	Poultry manure	T_8:	Goat manure
T_3:	Neem seed cake	T_9:	Groundnut cake
T_4:	Sunflower seed cake	T_{10}:	Safflower seed cake
T_5:	Vermicompost	T_{11}:	Control (Untreated)
T_6:	Cotton seed cake		

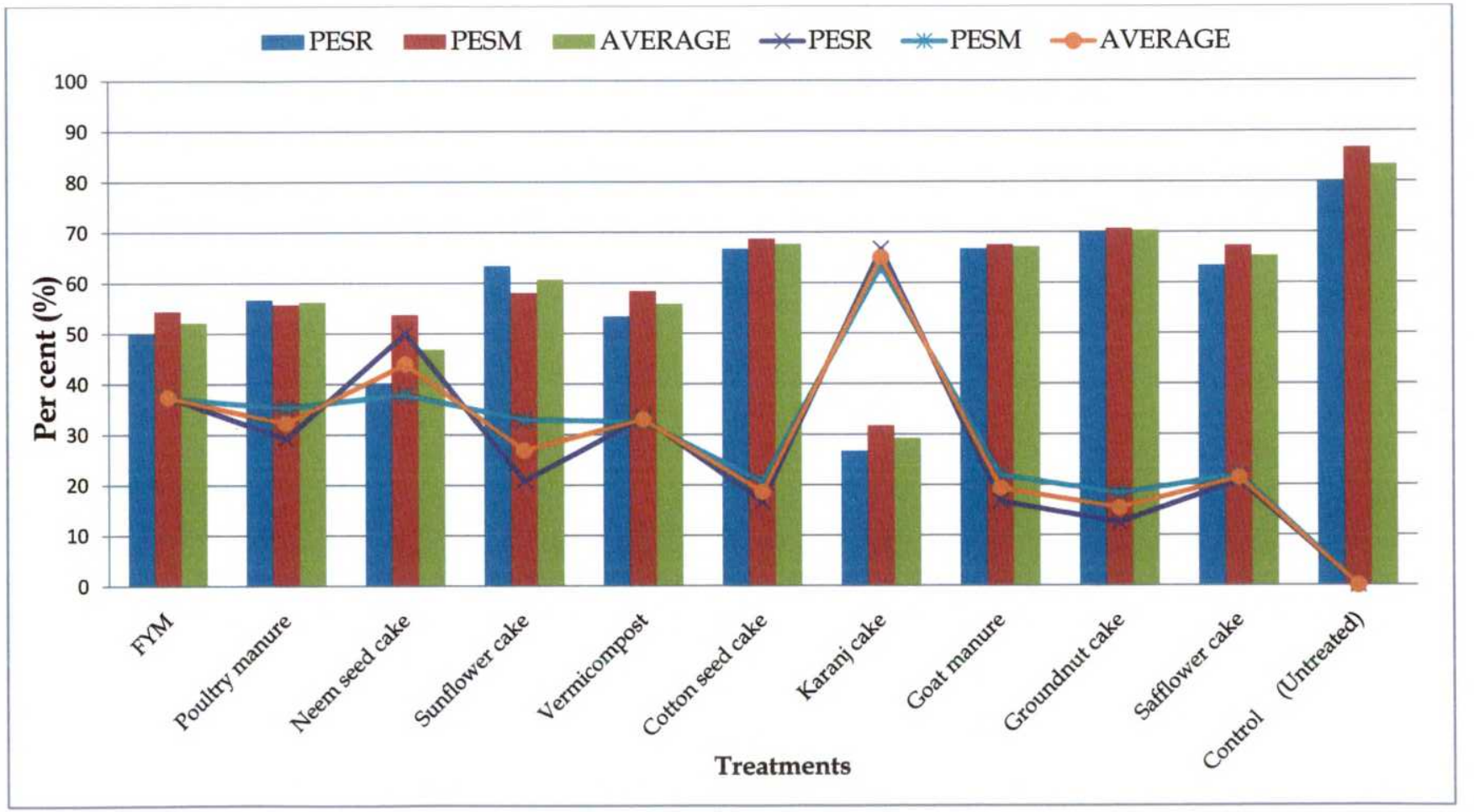


Fig. 6. Effect of organic amendments on pre-emergence rhizome rot and post emergence seedling mortality caused by *R. solanacearum* in ginger

as well as post-emergence seedling mortality in ginger. However, karanj cake was found most effective with highest average reduction in mortality. In the order of merit of effectiveness in reducing mortality, the other organic amendments found effective were neem seed cake, farm yard manure, vermicompost, poultry manure, sunflower seed cake, safflower seed cake, goat manure, cotton seed cake and groundnut cake.

4.8.4.5. Effect on growth parameters

Results (Table 8 and Fig. 7) revealed that all the amendments tested for management of *R. solanacearum* (pot culture) also influenced the growth characteristics in ginger.

All the test amendments improve root length, shoot length and vigour index in ginger. Of the amendments tested, significantly highest root length (6.16 cm), shoot length (12.56 cm) and vigour index (1372.73) were recorded with Karanj cake. The second best amendment found was neem seed cake (5.26 cm, 10.26 cm and 931.20) respectively. This was followed by the amendments *viz.,* farm yard manure (4.76 cm, 8.83 cm and 679.50), vermicompost (4.25 cm, 8.23 cm and 582.44), poultry manure (4.23 cm, 6.56 cm and 467.53) respectively, sunflower seed cake (4.08 cm, 6.13 cm and 374.40), safflower seed cake (3.66 cm, 5.96 cm and 352.76) of root length, shoot length and vigour index respectively.

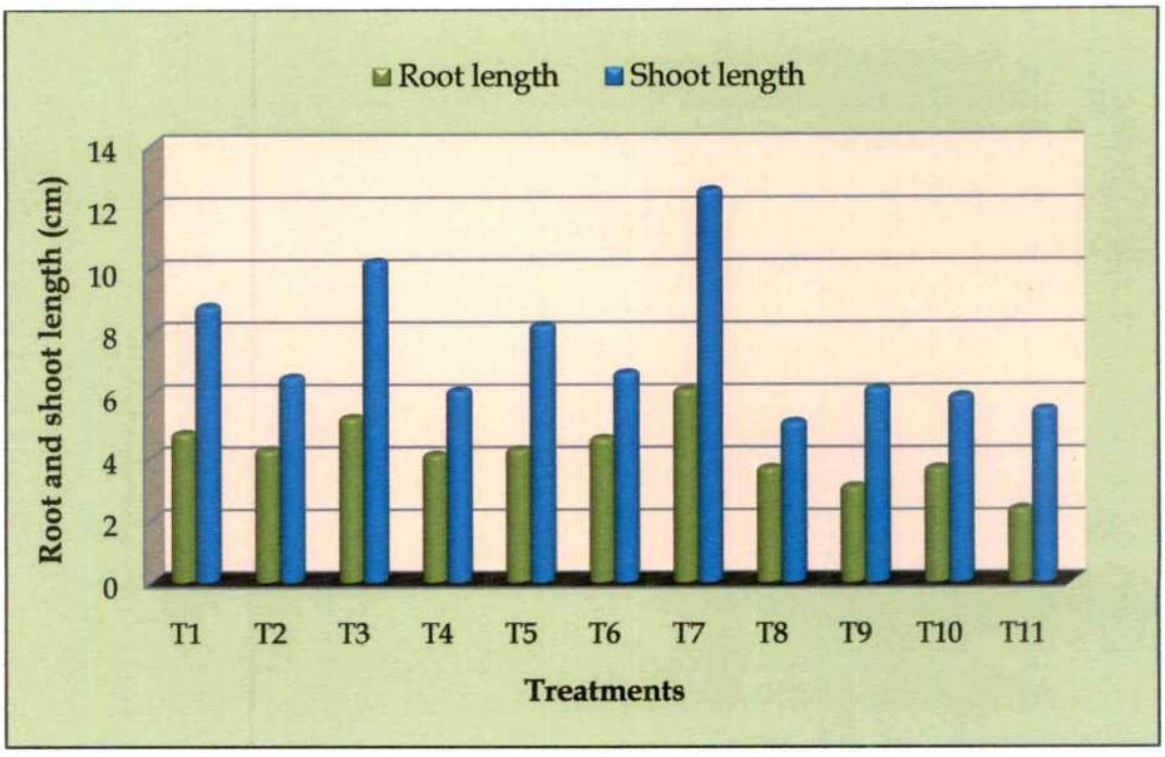
Rest of the amendments except cotton seed cake, goat manure and groundnut cake were found at par to each other and recorded root length 4.60, 3.66, 3.06 cm, shoot length 6.70, 5.13, 6.20 cm and vigour index 376.62, 282.97, 277.80, respectively, as against significantly least root length (2.36 cm) , shoot length (5.53 cm) and vigour index (157.80) in untreated control.

Results of the present study obtained for the organic amendments *viz.*, karanj cake, neem seed cake, farm yard manure, vermicompost, poultry manure, sunflower seed cake, safflower seed cake, goat manure, cotton seed cake and groundnut cake against *R. solanacearum* are in conformity with those reported earlier by several workers (Sharma and Kumar, 2000; Lemaga *et al.,*

Table. 8 Effect of organic amendments application on growth parameters in ginger against *R. solanacearum*

Tr. No.	Treatments	Germination (%)*	Root Length (cm)*	Shoot Length (cm)*	Vigour Index*
T_1	FYM	50.00 (45.00)	4.76	8.83	679.50
T_2	Poultry manure	43.33 (41.16)	4.23	6.56	467.53
T_3	Neem seed cake	60.00 (50.76)	5.26	10.26	931.20
T_4	Sunflower cake	36.67 (37.26)	4.08	6.13	374.40
T_5	Vermicompost	46.67 (43.09)	4.25	8.23	582.44
T_6	Cotton seed cake	33.33 (35.26)	4.60	6.70	376.62
T_7	Karanj cake	73.33 (58.90)	6.16	12.56	1372.73
T_8	Goat manure	33.33 (35.26)	3.66	5.13	282.97
T_9	Groundnut cake	30.00 (33.21)	3.06	6.20	277.80
T_{10}	Safflower cake	36.67 (37.26)	3.66	5.96	352.76
T_{11}	Control (Untreated)	20.00 (26.56)	2.36	5.53	157.80
	SE ±	**2.66**	**0.25**	**0.53**	-
	CD (P=0.01)	**7.8**	**0.73**	**1.55**	-

*: Mean of three replications
Figures in parentheses are angular transformed values



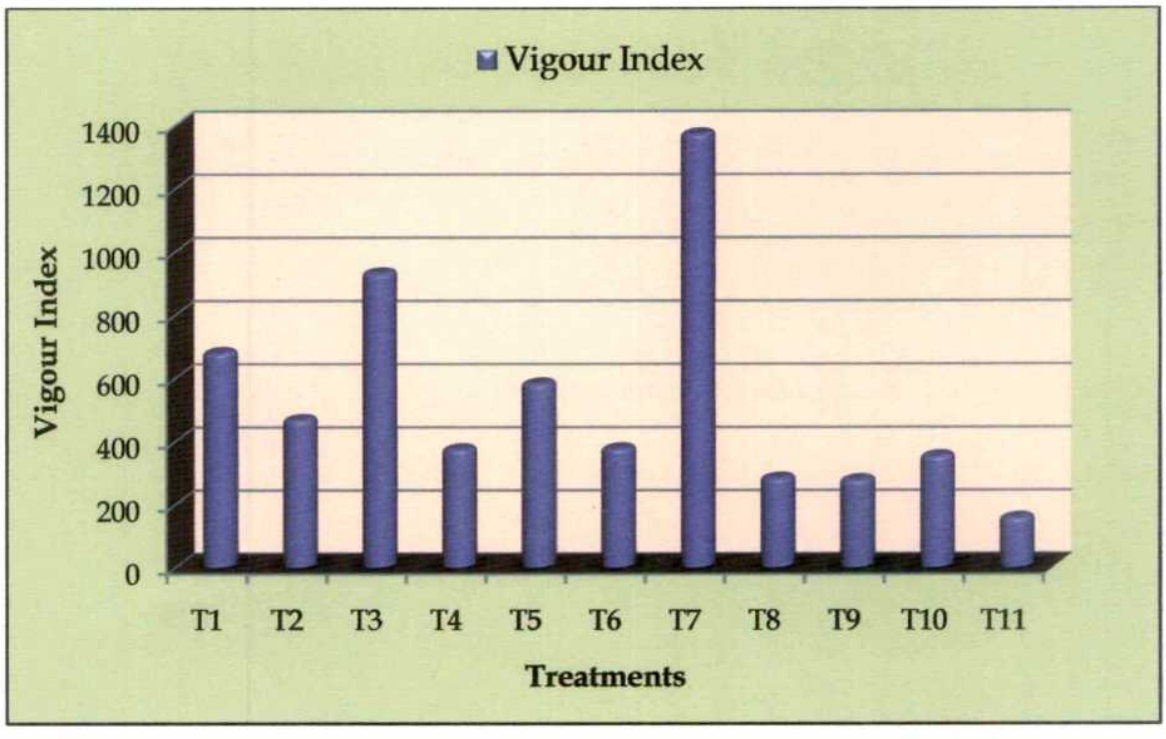


Fig.7. Effect of organic amendments application on growth parameters in ginger against *R. solanacearum*

2001; Schonfeld *et al.*, 2003; Sharma and Kumar, 2004; Islam and Toyota, 2004; Sharma and Kumar, 2009; Yadessa *et al.*, 2010; Reddy *et al.*, 2012)

4.9. Integrated disease management

The efficacy of antibacterial chemicals (2), bioagents (2), organic amendments (1), aqueous extracts of the botanicals (1) were evaluated (alone and in combination) for the integrated management of bacterial wilt of ginger (*R. solanacearum*) in pot culture under green house conditions. The results obtained on percentage rhizome germination, pre emergence rhizome rot (PESR), post emergence seedling mortality (PESM), their reductions and effect on growth parameters are presented in the Table 9, 10 and depicted in the Plate XVIII and Fig. 8 and 9.

4.9.1. Effect on rhizome germination

Results (PLATE XVIII, Table 9 and Fig. 8) revealed that all the treatments exhibited improved rhizome germination, over untreated control and it was ranged from 43.33 to 86.67 per cent, as against 33.33 in untreated control. However, significantly highest rhizome germination was recorded with streptocycline + *P. fluorescens* (86.67 %). This was followed by the treatments *viz.*, streptocycline + karanj cake (80.00 %), streptocycline + *T. viride* (78.33%), streptocycline (73.33 %), karanj cake + *A. sativum* (66.66 %), *P. fluorescens* (64.66 %), karanj cake (60.00 %). Whereas, *T. viride* (51.67 %), *A. sativum* (48.33 %) and gentamycin (43.33 %) were found least effective with comparatively minimum rhizome germination. Further, all the test amendments recorded significant increase in rhizome germination, over control and it was ranged from 23.07 (gentamycin) to 61.54 (streptocycline + *P. fluorescens*) per cent. However, highest increase in rhizome germination was recorded with streptocycline + *P. fluorescens* (61.54 %). This was followed by the treatments *viz.*, streptocycline + karanj cake (58.33 %), streptocycline + *T. viride* (57.44 %), streptocycline (54.54 %), karanj cake + *A. sativum* (50.00 %), *P. fluorescens* (48.45 %), karanj cake (44.45 %). Whereas, *T. viride* (35.49 %), *A. sativum*

(31.03 %) and gentamycin (23.07 %) were found less effective increase in rhizome germination.

4.9.2. Pre-emergence rhizome rot

Results (Table 9) indicated that all the treatments significantly influenced Pre-emergence seed rot over untreated control. The pre emergence rhizome rot (PESR) recorded in all the treatments was ranged from 13.33 to 56.67 per cent, as against 66.66 per cent in untreated control. However, significantly least pre-emergence rhizome rot was recorded with the treatment streptocycline + *P. fluorescens* (13.33 %). This was followed by the treatments *viz.,* streptocycline + karanj cake (20.00 %), streptocycline + *T. viride* (21.67 %), streptocycline (26.67 %), karanj cake + *A. sativum* (33.33 %), *P. fluorescens* (35.33 %), karanj cake (40.00 %). The treatments *viz., T. viride, A. sativum,* gentamycin were found comparatively less effective with maximum PESR, 48.33, 51.67 and 56.67 per cent respectively.

4.9.3. Post- emergence seedling mortality (PESM)

The results (Table 9) indicated that all the treatments tested were found effective against *R. solanacearum* and recorded PESM in the range of 13.67 to 57.67 per cent as against 70.67 per cent in untreated control. However, significantly least pre-emergence seedling mortality was recorded with the treatment streptocycline + *P. fluorescens* (13.67 %). This was followed by the treatments *viz.,* streptocycline + karanj cake (17.83 %), streptocycline + *T. viride* (20.00 %), streptocycline (30.00 %), karanj cake + *A. sativum* (37.33 %), *P. fluorescens* (40.00 %), karanj cake (46.57 %). The treatments *viz., T. viride, A. sativum,* gentamycin were found comparatively less effective with maximum PESM, 51.67, 57.00 and 57.67 per cent respectively.

The average mortality (PESR + PESM) recorded with all the treatments were ranged from 13.49 to 57.16 per cent as against 65.16 per cent in untreated control. However, significantly least average mortality was recorded with the treatment streptocycline + *P. fluorescens* (13.49 %). This was followed

by the treatments *viz.*, streptocycline + karanj cake (18.91 %), streptocycline + *T. viride* (19.99 %), streptocycline (28.33 %), karanj cake + *A. sativum* (35.33 %), *P. fluorescens* (37.66 %), karanj cake (43.33 %). The treatments *viz., T. viride, A. sativum*, gentamycin were found comparatively less effective with maximum PESM, 49.99, 54.33 and 57.16 per cent respectively.

4.9.4. Reduction in mortality

Result (Table 9 and Fig. 8) revealed that both PESR and PESM were significantly reduced with the application of all the treatments tested. The percentage reduction in PESR recorded with test treatments were ranged from 14.67 to 80.15 per cent as against 0.00 per cent in untreated control. However, significantly highest reduction in PESR was recorded with streptocycline + *P. fluorescens* (80.15 %). This was followed by the treatments *viz.*, streptocycline + karanj cake (69.83 %), streptocycline + *T. viride* (67.45 %), streptocycline (59.91 %), karanj cake + *A. sativum* (49.99 %), *P. fluorescens* (46.50 %), karanj cake (39.67 %). Whereas, the treatments *viz., T. viride, A. sativum* and gentamycin were found least effective with comparatively minimum reduction in PESR, 26.98, 22.61 and 14.67 per cent.

The percentage reduction in PESM recorded with test treatments were ranged from 18.34 to 80.63 per cent as against 0.00 per cent in untreated control. However, significantly highest reduction in PESM was recorded with streptocycline + *P. fluorescens* (80.63 %). This was followed by the treatments *viz.*, streptocycline + karanj cake (74.79%), streptocycline + *T. viride* (71.66 %), streptocycline (57.45 %), karanj cake + *A. sativum* (46.99 %), *P. fluorescens* (43.38 %), karanj cake (33.92 %). Whereas, the treatments *viz., T. viride, A. sativum* and gentamycin were found least effective with comparatively minimum reduction in PESR, 26.91, 19.28 and 18.34 per cent.

The average (PESR +PESM) reduction in mortality recorded with all the organic amendments tested were ranged from 16.50 to 80.39 per cent as against 0.00 per cent in untreated control. However, significantly highest reduction in average mortality was recorded with streptocycline + *P. fluorescens*

Table. 9 Effect of antibacterial chemicals, bioagents, botanicals and organic amendments on pre emergence rhizome rot and post emergence seedling mortality caused by *R. solanacearum* in ginger

Tr. No.	Treatments	Rate of application	Germination (%)*	Rot/ mortality (%)*		Average mortality (%)	Reduction over control (%)		Average reduction (%)
				PESR	PESM		PESR	PESM	
T_1	Streptocycline	(ST) @ 0.05 %	73.33 (58.90)	26.67 (31.09)	30.00 (33.21)	28.33 (32.15)	59.91 (50.71)	57.45 (49.28)	58.68 (49.99)
T_2	Gentamycin	(ST) @ 0.05 %	43.33 (41.16)	56.67 (48.83)	57.67 (49.41)	57.16 (49.11)	14.67 (22.52)	18.34 (25.35)	16.50 (23.96)
T_3	*Pseudomonas fluorescence*	SA @10g/kg soil	64.66 (53.52)	35.33 (36.46)	40.00 (39.23)	37.66 (37.85)	46.50 (42.99)	43.38 (41.19)	44.94 (42.09)
T_4	*Trichoderma viride*	SA @ 10g/kg soil	51.67 (45.95)	48.33 (44.04)	51.67 (45.95)	49.99 (44.99)	26.98 (31.29)	26.91 (31.24)	26.94 (31.26)
T_5	Karanj cake	SA @ 50 g/kg soil	60.00 (50.76)	40.00 (39.23)	46.67 (43.09)	43.33 (41.16)	39.67 (39.03)	33.92 (35.62)	36.79 (37.34)
T_6	*A. sativum* aq. 20 % Bulb extract	SD 20% @ 20 ml/kg soil	48.33 (44.04)	51.67 (45.95)	57.00 (49.02)	54.33 (47.48)	22.61 (28.39)	19.28 (26.04)	20.94 (27.23)
T_7	$T_1 + T_3$	ST @ 0.05 % + SA @10g/kg soil	86.67 (68.58)	13.33 (21.41)	13.67 (21.69)	13.49 (21.54)	80.15 (63.54)	80.63 (63.88)	80.39 (63.71)
T_8	$T_1 + T_4$	ST @ 0.05 % + SA @10g/kg soil	78.33 (62.25)	21.67 (27.74)	20.00 (26.56)	19.99 (26.55)	67.45 (55.21)	71.66 (57.83)	69.55 (56.50)
T_9	$T_1 + T_5$	ST @ 0.05 % + SA @50g/kg soil	80.00 (63.43)	20.00 (26.56)	17.83 (24.97)	18.91 (25.77)	69.83 (56.68)	74.79 (59.86)	72.31 (58.25)
T_{10}	$T_5 + T_6$	SA @ 50g/kg + SD @20 ml/kg soil	66.66 (54.73)	33.33 (35.26)	37.33 (37.66)	35.33 (36.46)	49.99 (44.99)	46.99 (43.27)	48.49 (44.13)
T_{11}	Control	Untreated	33.33 (35.26)	66.66 (54.73)	70.67 (57.20)	65.16 (53.82)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
SE ±			2.54	2.39	2.41	2.40	2.59	2.51	2.55
CD (P=0.01)			7.45	7.00	7.07	7.03	7.59	7.35	7.47

*:Mean of three replications, Figures in parentheses are angular transformed values

PLATE XVIII

View of Pot culture experiment on evaluation of antibacterial chemicals, bioagents, botanicals and organic amendments against *R. solanacearum*

Treatments details

T_1 :	Streptocycline	T_6 :	Garlic aq. extract
T_2 :	Gentamycin	T_7 :	$T_1 + T_3$
T_3 :	*P. fluorescence*	T_8 :	$T_1 + T_4$
T_4 :	*Trichoderma viride*	T_9 :	$T_1 + T_5$
T_5 :	Karanj cake	T_{10} :	$T_5 + T_6$
		T_{11} :	Control (Untreated)

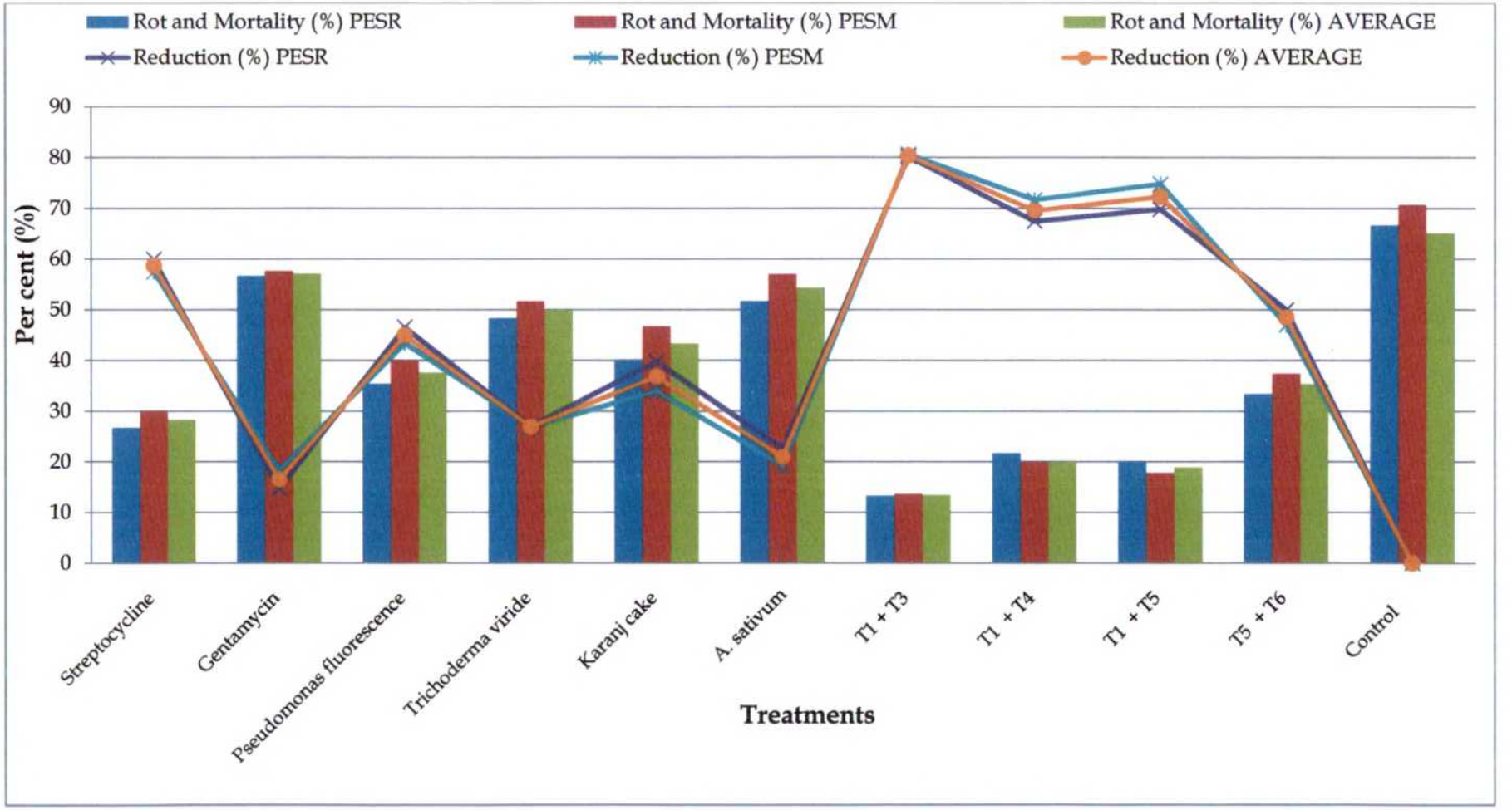


Fig. 8. Effect of antibacterial chemicals, bioagents, botanicals and organic amendments on pre-emergence rhizome rot and post emergence seedling mortality caused by *R. solanacearum* in ginger

(80.39 %). This was followed by the treatments *viz.*, streptocycline + karanj cake (72.31%), streptocycline + *T. viride* (69.55 %), streptocycline (58.68 %), karanj cake + *A. sativum* (48.49 %), *P. fluorescens* (44.94 %), karanj cake (36.79 %). Whereas, the treatments *viz., T. viride, A. sativum* and gentamycin were found least effective with comparatively minimum reduction in average mortality, 26.94, 20.94 and 16.50 per cent.

Thus, all the treatments were found effective in reducing the pre-emergence rhizome rot as well as post-emergence seedling mortality in ginger. However, streptocycline + *P. fluorescens* was found most effective with highest average reduction in mortality. In the order of merit of effectiveness in reducing mortality, the other treatments found effective were streptocycline + karanj cake, streptocycline + *T. viride*, streptocycline, karanj cake + *A. sativum*, *P. fluorescens*, karanj cake, *T. viride, A. sativum* and gentamycin.

4.9.5. Effect on growth parameters

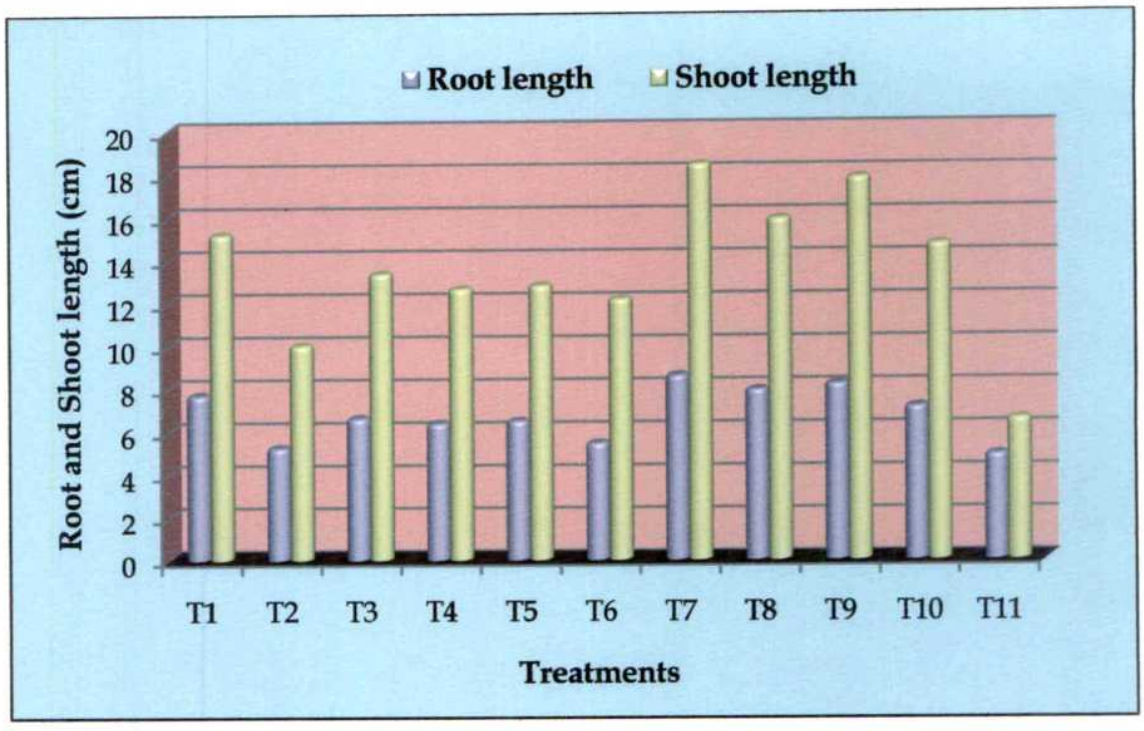
Results (Table 10, Fig. 9) revealed that all the treatments tested for integrated management of *R. solanacearum* (pot culture) also influenced the growth characteristics in ginger.

All the test amendments improve root length, shoot length and vigour index in ginger. Of the amendments tested, significantly highest root length (8.60 cm), shoot length (18.43 cm) and vigour index (2342.69) were recorded with streptocycline + *P. fluorescens*. The second best treatment found was streptocycline + karanj cake (8.20 cm, 17.80 cm and 2080.00) respectively. This was followed by the treatments *viz.*, streptocycline + *T. viride* (7.93 cm, 15.90 cm and 1866.60), streptocycline (7.70 cm, 15.20 cm and 1679.25), karanj cake + *A. sativum* (7.13 cm, 14.70 cm and 1455.18), *P. fluorescens* (6.63 cm, 13.36 cm and 1292.55) and karanj cake (6.50 cm, 12.80 cm and 1158.00) respectively. Rest of the treatments except gentamycin were found at par to each other and recorded average root length of 5.26 cm, shoot length of 10.03 cm and

Table. 10 Effect of antibacterial chemicals, bioagents, botanicals and organic amendments on growth parameters in ginger against *R. solanacearum*

Tr. No.	Treatments	Germination (%)*	Root Length (cm)*	Shoot Length (cm)*	Vigour Index*
T_1	Streptocycline (ST) @ 0.05 %	73.33 (58.90)	7.70	15.20	1679.25
T_2	Gentamycin (ST) @ 0.05 %	43.33 (41.16)	5.26	10.03	662.51
T_3	*Pseudomonas fluorescence* (SA) @ 10g/kg soil	64.66 (53.52)	6.63	13.36	1292.55
T_4	*Trichoderma viride* (SA) @ 10g/kg soil	51.67 (45.95)	6.38	12.65	983.28
T_5	Karanj cake (SA) @ 50 g/kg soil	60.00 (50.76)	6.50	12.80	1158.00
T_6	*A. sativum* aq.ex. 20% (SD) @ 20 ml/kg soil	48.33 (44.04)	5.46	12.16	851.57
T_7	T_1 (ST) + T_3 (SA)	86.67 (68.58)	8.60	18.43	2342.69
T_8	T_1 (ST) + T_4 (SA)	78.33 (62.25)	7.93	15.90	1866.60
T_9	T_1 (ST) + T_5 (SA)	80.00 (63.43)	8.20	17.80	2080.00
T_{10}	T_5 (SA) + T_6 (SD)	66.66 (54.73)	7.13	14.70	1455.18
T_{11}	Control (Untreated)	33.33 (35.26)	4.90	6.56	381.96
	SE ±	**2.54**	**0.18**	**0.32**	-
	CD (P=0.01)	**7.45**	**0.53**	**0.94**	-

*: Mean of three replications, Figures in parentheses are angular transformed values



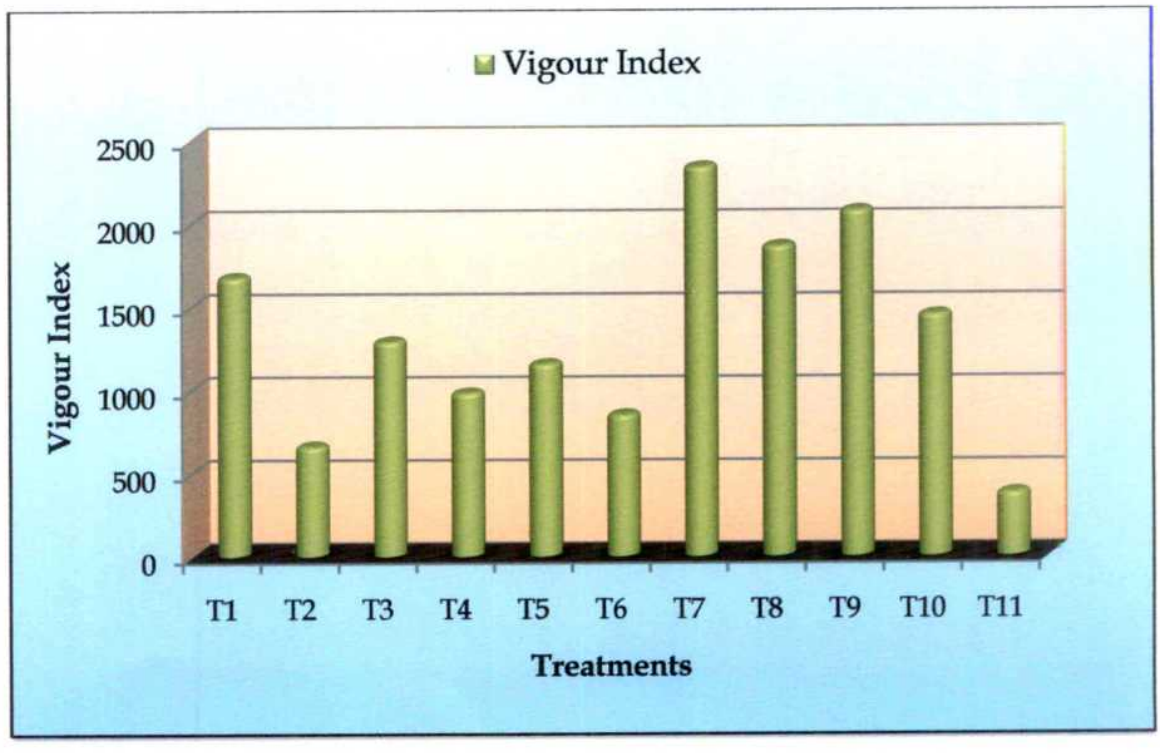
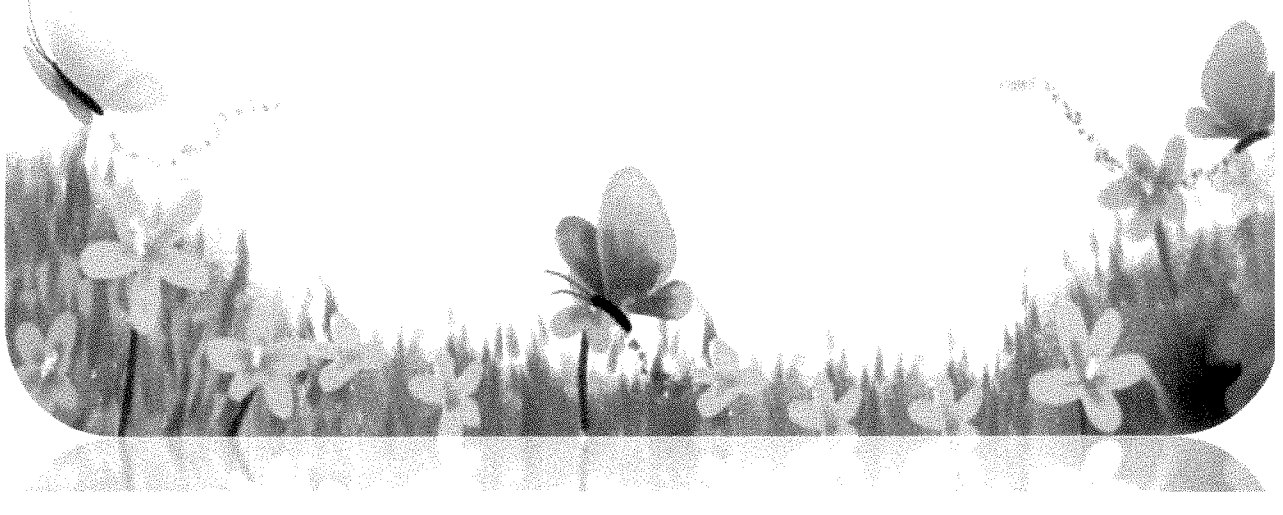


Fig.9. Effect of antibacterial chemicals, bioagents, botanicals and organic amendments on growth parameters in ginger against *R. solanacearum*

vigour index, 662.51 respectively as against significantly least root length (4.90 cm) , shoot length (6.56 cm) and vigour index (381.96) in untreated control.

Results of the present study obtained on the integrated bioefficacy of the antibacterial chemicals, botanicals, bioagents and the amendments against bacterial wilt of ginger are in conformity with those reported earlier by several workers (Ojha *et al.,* 1986; Biswas *et al.,* 2008; Hussain and Bora, 2008; Sharma and Kumar, 2009; Ravi and Suryanarayana, 2011; Sawant *et al.,* 2014).

SUMMARY AND CONCLUSION



CHAPTER VI

SUMMARY AND CONCLUSIONS

Ginger (*Zingiber officinale* Roscoe) is one of the important commercial spice crop grown throughout the tropical areas of the world. Ginger contains an Oleoresin (6.5 %) commercially known as "Gingeberene" which is obtained by solvent extraction from ginger rhizomes. It also contain many nutrients, vitamins, alcohol, protein. Hence, it is consumed by most of the people of the world. There are several reasons for low productivity of ginger, of which the diseases caused by a large number of fungal, bacterial and viral pathogens are the major constraints. During last few years bacterial wilt disease incited by *Ralstonia solanacearum* (Smith) Yabuuchi, has attained its severe proportion, thereby inflicting heavy quantitative as well as qualitative losses in ginger crop. The disease has been reported to cause yield losses of about 40 to 100 per cent (Sharma and Jain, 1978; Mathew *et al.,* 1979; Dohroo, 1991; Zhang *et al.,* 2001; Sambasivam and Girija, 2005).

Although, satisfactory control of the disease with various chemicals has been documented in the literature; however, rapid and extensive use of the agrochemicals to control plant diseases may pose several problems like disturbance of the ecological balance, toxicity to non target organisms, development of resistance among the pathogens population, environmental pollution and increased health risks. Therefore, several alternative methods *viz.,* use of biocontrol agents, phytoextracts, organic amendments etc are gaining importance and it is imperative to exploit these alternatives for eco-friendly disease management, which will sustain the agriculture with food safety and better tomorrow for the mankind.

Therefore, Present investigations on bacterial wilt of ginger were undertaken with the defined objectives and the findings thereof are being summarized in the following paragraphs.

During field survey, pathogenicity test, and management studies, typical symptoms of bacterial wilt disease observed were: downward curling of leaves and golden brown/rusty brown discoloration seen on older leaves. Losses of leaf turgidity, older leaves were affected before the youngest leaves. Permanent wilting takes place after a few days. Tentative diagnosis of the disease was made by ooze test, the appearance of slimy, milky bacterial ooze when infected pseudostem sections are placed in water.

The pathogen (*R. solanacearum*) was isolated from the soil samples and bacterial ooze obtained from the infected discolored pseudostem of the plants by serially diluting the bacterial suspension in sterile distilled water and planting on TZC media (Kelman, 1954). The separated colonies were purified and suspended in sterile distilled water in sterile polypropylene tubes this served as stock culture for further use.

Pathogenicity of the bacterium was proved by inoculating a bacterial suspension ($5x10^6$cfu/ml) to the pot grown ginger plants of 45 days old. The bacterial suspension of about 50 ml was poured to the base of the plants. The inoculated plant showed wilting symptoms 15 days after the inoculation. The symptoms observed on artificially diseased ginger were found similar to those observed on naturally diseased ginger crop under field conditions. Applying the Koch's postulates pathogenicity of *R. solanacearum* was proved successfully.

Based on symptomatology (naturally and artificially diseased ginger plants), ooze test, cultural and biochemical characteristics, microscopic observations and the pathogenicity test proved; the test pathogen was identified as *Ralstonia solanacearum* (Smith) Yabuuchi.

All of the 8 culture media tested encouraged better growth and variable colony count, colony color and colony shape of *R. solanacearum*. Average colony count recorded with all the test media was ranged from 33.00 (Yeast extract chalk agar) to 68.50 (TZC). However, significantly highest average colony count (68.50) was recorded on Triphenyl Tetrazolium Chloride Agar. This was followed by Casamino peptone glucose agar (59.33), Potato

dextrose agar (55.00), Yeast extract peptone agar (47.42), Yeast extract milk agar (47.09), Yeast extract agar (37.33) with at par and Nutrient agar (36.42). Yeast extract chalk agar was found least suitable with minimum average colony count (33.00) of the test pathogen. Irregular, highly fluidal colonies with pink centre were found in Triphenyl Tetrazolium Chloride Agar; irregular, smooth, highly fluidal Cream or off-white color colonies in Casamino peptone glucose agar, Yeast extract agar and Potato dextrose agar. Round small cream white and dull white color colonies were found on Nutrient agar and Yeast extract milk agar and round small yellow color colonies were found in Yeast extract peptone agar and Yeast extract chalk agar.

The studies on biochemical characteristics of *R. solanacearum* which showed positive reactions for potassium hydroxide solubility test, catalase test, starch hydrolysis test, motility test and casein hydrolysis test and showed negative reaction for gram staining.

All the six antibiotics, three antibacterial fungicides and two combi antibacterial chemical tested were found effective against *R. solanacearum.* However, antibiotic Streptocycline (20.05 mm) recorded significantly highest average inhibition zone. This was followed by the antibiotics *viz.,* Gentamycin (17.5 mm), Tetracycline (16.5 mm) and antibacterial fungicide, significantly highest average inhibition was recorded in the combination of antibacterial fungicide and antibiotic Copper oxychloride + Streptocycline (11.95 mm). This was followed by the antibacterial fungicides *viz.,* Copper oxychloride (11.1 mm), Copper oxychloride + Tetracycline (10.6 mm).

All the six fungal and two bacterial antagonists tested, exhibited significant inhibition of *R. solanacearum.* However, *P. fluorescens* resulted in maximum inhibition of the *Ralstonia solanacearum* with an inhibition zone of 24.33 mm which was found significantly superior over other treatments. The second and third best antagonists found were *T. viride* and *Bacillus subtilis* with an inhibition zone of 21.17 mm and 19.33 mm, respectively. Rest of the

bioagents tested were moderately effective with slight inhibition of the test pathogen.

Aqueous extracts of 10 botanicals evaluated *in vitro* (each @ 5, 10 and 20 %) were found bacteriostatic/antibacterial to *R. solanacearum.* However, significantly average highest inhibition was recorded with the botanical *A. sativum* (9.8 mm). This was followed by the botanicals *viz.*, *A. cepa* (9.0 mm), *A. sativum* + *C. longa* (8.4 mm), *A. indica* (7.9 mm). In acetone extracts of botanicals, significantly average highest inhibition was recorded with the botanical *A. sativum* (10.4 mm). This was followed by the botanicals *viz.*, *D. stromonium* (9.8 mm), *A. cepa* (9.0 mm), *A. sativum* + *C. longa* (8.6 mm), *C. longa* (7.5 mm).

A total of 10 amendments evaluated (pot culture) as pre- sowing soil application were found effective against *R. solanacearum.* However, Karaj cake and neem seed cake were found most effective with significantly highest rhizome germination (73.33 and 60.00 %) respectively. This was followed by the amendments *viz.*, farm yard manure (50.00 %) and vermicompost (46.67 %). All the test amendments recorded significant reduction in average mortality (PESR and PESM) over untreated control. However, significantly highest reduction in average mortality was recorded with karanj cake (64.97 %). This was followed by the organic amendments *viz.*, neem seed cake (43.95 %), farm yard manure (37.36 %) and vermicompost (32.95 %). All the test amendments tested improved the growth parameters *viz.*, root length, shoot length and vigour index in ginger. However, karanj cake gave significantly highest root length (6.16 cm), shoot length (12.56 cm) and vigour index (1372.73). The second and third best amendments found were neem seed cake (5.26 cm, 10.26 cm and 931.20) and farm yard manure (4.76 cm, 8.83 cm and 679.50) respectively.

The most effective antibacterial chemicals, bioagents, botanicals and organic amendments selected and evaluated (pot culture, sick soil) for integrated management *R. solanacearum* were found to encourage seed germination, reduce pre- and post- emergence mortalities along and improved

the growth parameters in ginger. However, significantly highest seed germination was recorded with streptocycline + *P. fluorescens* (86.67 %), with significantly least average mortality (13.49 %) and its highest average reduction (80.39 %) The second and third highest seed germination was recorded with the treatments *viz.*, streptocycline + karanj cake (80.00 %) and streptocycline + *T. viride* (78.33 %), with comparatively minimum average mortality, respectively of 18.91 and 19.99 per cent and average mortality reduction respectively of 72.31 and 69.55 per cent. All the test treatments tested improved the growth parameters *viz.*, root length, shoot length and vigour index in ginger. However, streptocycline + *P. fluorescens* gave significantly highest root length (8.60 cm), shoot length (18.43 cm) and vigour index (2342.69). The second and third best treatments found were streptocycline + karanj cake (8.20 cm, 17.80 cm and 2080.00) and farm yard manure (7.93 cm, 15.90 cm and 1866.60) respectively.

CONCLUSIONS

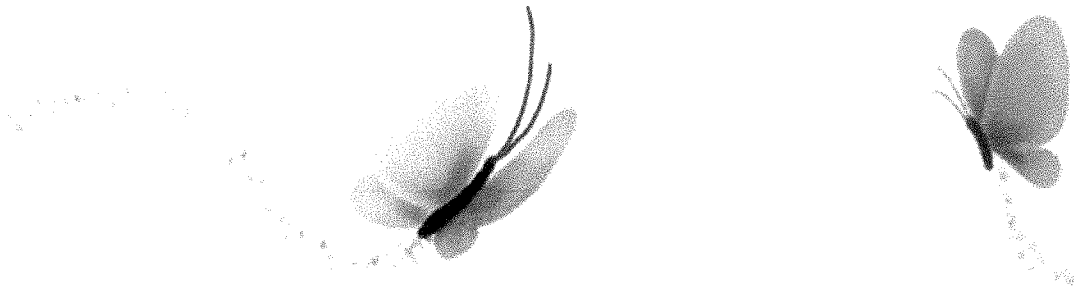
Thus, from the results obtained on various aspects during present investigations on bacterial wilt of ginger (*R. solanacearum*) disease, following conclusions are being drawn:

- *Ralstonia solanacearum* (Smith) Yabuuchi, is one of the major constraints in the production of ginger, causing heavy quantitative as well as qualitative losses.
- The pathogen (*R. solanacearum*) was isolated from the soil samples and bacterial ooze obtained from the infected discolored pseudostem of the plants by serially diluting the bacterial suspension in sterile distilled water and planting on TZC media. The bacterial culture were purified and maintained for further use.

- Pathogenicity of *R. solanacearum* was successfully proved on ginger under controlled conditions of screen house.
- The pathogen, *R. solanacearum* grew better colony count on a wide range of culture media tested; however, Triphenyl Tetrazolium Chloride Agar, Casamino peptone glucose agar Potato dextrose agar Agar were found most suitable with maximum colony count and irregular, smooth highly, fluidal white colony with pink centre and irregular, smooth, highly fluidal Cream or off-white color colonies .
- In biochemical characteristics of *R. solanacearum* which showed positive reactions for potassium hydroxide solubility test, catalase test, starch hydrolysis test, motility test and casein hydrolysis test and showed negative response for gram staining.
- All the test antibacterial chemicals, evaluated *in vitro* found bacteriostatic/antibacterial to *R. solanacearum.* However, the antibacterial chemicals *viz.*, Streptocycline, Gentamycin and Tetracycline were most effective against *R. solanacearum.*
- All the eight antagonists tested *in vitro* proved potential antagonists against *R. solanacearum.* However, *P. fluorescens*, *T. viride* and *Bacillus subtilis* were most effective against *R. solanacearum.*
- Aqueous extracts of all the 10 botanicals evaluated *in vitro* found bacteriostatic / antibacterial to *R. solanacearum*. However, *A. sativum* , *A. cepa A. sativum* + *C. longa*, *A. indica* were most effective against *R. solanacearum.* In acetone extracts of botanicals, However, *A. sativum*, *D. metal* , *A. cepa, A. sativum* + *C. longa* and *C. longa* were most effective against *R. solanacearum.*
- All the 10 amendments evaluated (pot culture, sick soil) as pre-sowing soil application were found to improve seed germination and growth parameters, reduce pre- and post emergence mortality as well as average mortality caused by *R. solanacearum* in ginger. However, karanj cake ,

followed by neem seed cake, farm yard manure and vermicompost, were found most promising against *R. solanacearum.*

- The studies on integrated management of bacterial wilt (*R. solanacearum*) disease under pot culture conditions indicated that the treatments *viz.*, streptocycline, bioagent *P. fluorescens, T. viride,* organic amendment, karanj cake and botanical *A. sativum* were most effective and economical for the management of bacterial wilt of ginger.



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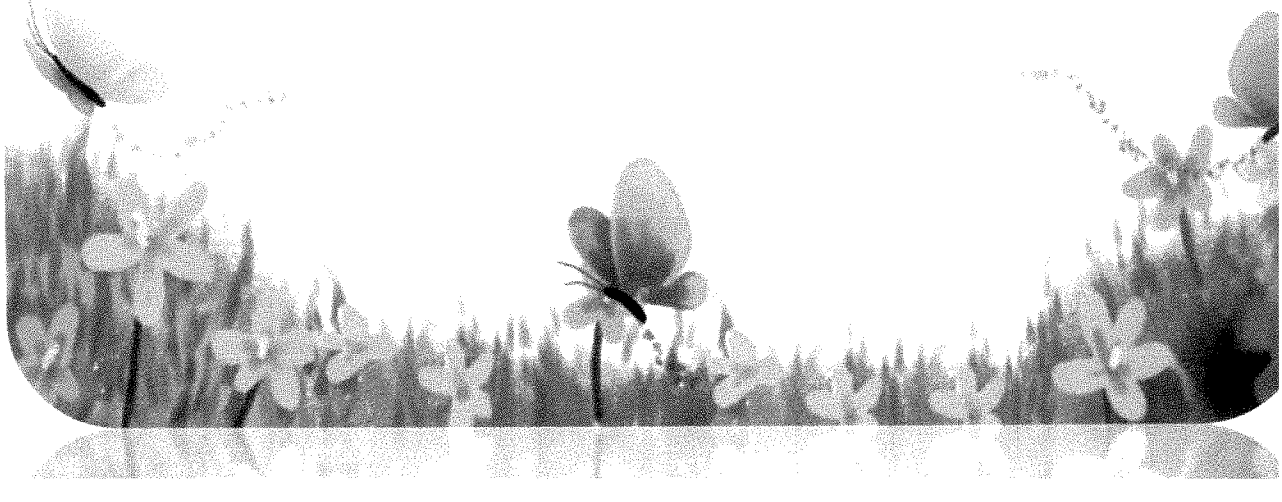
APPENDIX



APPENDIX

Composition of culture media used in the experiment

1. Triphenyl Tetrazolium Chloride (TZC) Agar

Casein hydrolysate	:	1 g
Peptone	:	10 g
Glucose	:	5 g
2, 3, 5-TTC	:	0.005% or 5ml (1% stock solution)
Agar-agar	:	20 g
Distilled water	:	1000ml

2. Casamino Peptone Glucose (CPG) Agar

Casein hydrolysate	:	1 g
Peptone	:	10 g
Glucose	:	5 g
Agar-agar	:	20 g
Distilled water	:	1000ml

3. Casamino peptone Glucose (CPG) Broth

Casein hydrolysate	:	1 g
Peptone	:	10 g
Glucose	:	5 g
Distilled water	:	1000ml

4. Nutreint Agar (NA)

Peptone	:	5 g
Beef extract	:	3 g
Agar-agar	:	20 g
Distilled water	:	1000ml

5. Nutreint Broth

Peptone	:	5 g
Beef extract	:	3 g
Distilled water	:	1000ml

6. Yeast Extract Glucose Chalk Agar (YGCA)

Yeast extract	:	10 g
Glucose	:	3 g
Chalk	:	20 g
Agar –agar	:	20 g
Distilled water	:	1000 ml

7. Yeast Extract Agar (YEA)

Yeast extract	:	3 g
Peptone	:	5 g
Agar–agar	:	20 g
Distilled water	:	1000 ml

8. Yeast Peptone Media (YPM)

Yeast extract	:	5 g
Peptone	:	10 g
Agar –agar	:	20 g
Distilled water	:	1000 ml

9. Yeast Extract Milk Agar (YEMA)

Yeast extract	:	3 g
Peptone	:	5 g
Milk	:	5 ml
Agar–agar	:	20 g
Distilled water	:	1000 ml

10. Potato Dextrose Agar (PDA)

Peeled Potato	:	200 g
Dextrose	:	10 g
Agar –agar	:	20 g
Distilled water	:	1000ml

Slice potato and boil. Take the infusion and make the volume to one liter.

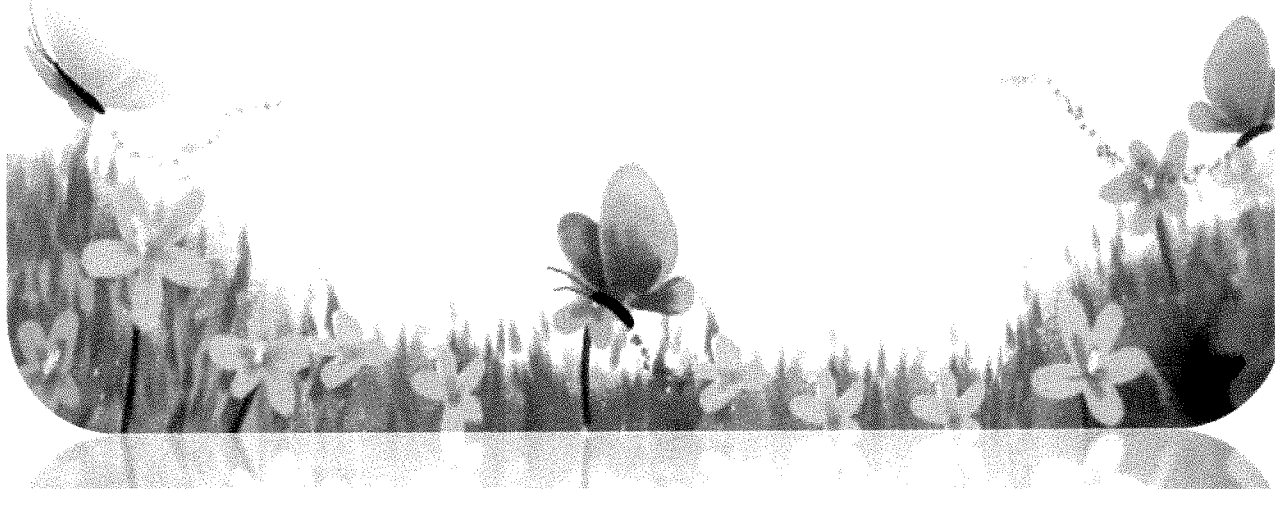
11. Soft Motility Agar

Peptone	:	1 g
Sodium chloride	:	5 g
Agar-agar	:	4 g
Distilled Water	:	1000 ml

12. Milk Agar

Peptone	:	4 g
Beef extracts	:	5 g
Agar-agar	:	20 g
Distilled water	:	1000 ml
Milk (Casein)	:	5 ml

ABSTRACT



STUDIES ON BACTERIAL WILT OF GINGER CAUSED BY *Ralstonia solanacearum* (Smith) Yabuuchi

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ABSTRACT

Bacterial wilt caused by *R. solanacearum* (Smith) Yabuuchi, is one of the most widely spread and destructive disease of ginger (*Zingiber officinale* Roscoe), thereby causes yield losses 40-100 per cent. Therefore, present investigations on *R. solanacearum* were undertaken with the objectives *viz.*, symptomatology, pathogenicity, cultural and biochemical characteristics, *in vitro* evaluation of antibacterial chemicals, bioagents, botanicals and organic amendments and integrated disease management strategies.

The pathogen was isolated successfully from the naturally diseased ginger plant specimens and soil samples collected from farmer's field, purified and maintained in water stock for further studies. The typical symptoms of bacterial wilt observed during, pathogenicity test and pot culture experiments were downward curling of leaves and golden brown/rusty brown discoloration seen on older leaves. Losses of leaf turgidity, older leaves were affected before the youngest leaves. Permanent wilting takes place after a few days. Tentative diagnosis of the disease was made by ooze test, the appearance of slimy, milky bacterial ooze when infected pseudostem sections are placed in water.

Pathogenicity of the test pathogen was proved applying Koch's postulates and using ginger local variety under controlled conditions of the screen house. Based on symptomatology, cultural and biochemical characteristics and pathogenicity test, the test pathogen was identified as *R. solanacearum* (Smith) Yabuuchi.

The test pathogen encouraged better growth and variable colony count, color and shape on the culture media tested *viz.*, Triphenyl Tetrazolium Chloride Agar (68.50 mm) with smooth irregular white colonies with pink centre. Casamino peptone glucose agar (59.33) and Potato dextrose agar (55.00) each with irregular cream and off-white color colonies.

The bacterium showed positive reactions for potassium hydroxide solubility test, catalase test, starch hydrolysis test, motility test and casein hydrolysis test and showed negative reaction for gram staining.

Studies on *in vitro* evaluation of the antibacterial chemicals, bioagents, botanicals and organic amendments revealed that all the treatments significantly inhibited growth of the test pathogen over untreated control. However, antibacterial chemicals *viz.*, Streptocycline, Gentamycin, Tetracycline, Copper oxychloride; bioagents *viz., P. fluorescence, T. viride, B. subtilis* and *T. harzianum* and botanicals *viz., A. sativum, A. cepa, D. stromonium, A. sativum + C. longa and A. indica*; organic amendments viz., karanj cake, neem seed cake, FYM, vermicompost were most prominent one. The integrated disease management studies indicated that the antibiotics *viz.*, streptocycline the bioagent *P. fluorescence T. viride* the botanical *A. sativum* and the organic amendment karanj cake were most effective in combination for the management of bacterial wilt disease.