# INTEGRATED MANAGEMENT OF RHIZOCTONIA LEAF BLIGHT OF AMARANTHUS (Amaranthus tricolor L.)

GIREESH (2014-11-199)

### DEPARTMENT OF PLANT PATHOLOGY

## **COLLEGE OF AGRICULTURE**

### VELLAYANI, THIRUVANANTHAPURAM-695522

### KERALA, INDIA

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# INTEGRATED MANAGEMENT OF RHIZOCTONIA LEAF BLIGHT OF AMARANTHUS (Amaranthus tricolor L.)

### GIREESH

(2014-11-199)

Thesis Submitted in partial fulfillment of the requirement for the degree of

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# Department of Plant Pathology COLLEGE OF AGRICULTURE VELLAYANI, THIRUVANANTHAPURAM- 695 522 KERALA, INDIA

**DECLARATION** 

I, hereby declare that this thesis entitled "Integrated management of Rhizoctonia leaf blight of Amaranthus (*Amaranthus tricolor* L.)" is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Vellayani Date : **Gireesh** (2014-11-199)

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### **CERTIFICATE**

Certified that this thesis entitled "Integrated management of Rhizoctonia leaf blight of Amaranthus (*Amaranthus tricolor* L.)" is a record of bonafide research work done independently by Mr. Gireesh (2014-11-199) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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# LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Per cent
μm	Micrometer
μl	Micro litre
-	
μg	Microgram
@	At the rate of
°C	Degree Celsius
CD	Critical difference
cm	Centimeter
mm	Millimeter
km	Kilometer
et al.	And other co workers
Fig.	Figure
g	Gram
>	Greater than
h.	Hours
i.e.	that is
1.	Litre
ml	Milli litre
DAI	Days After Inoculation
HAI	Hours After inoculation
DAT	Days After Transplanting
Qt	Quintal
Kg	Kilo gram
min	Minutes

N // 11'
Milli gram
Seconds
Species (Singular and plural)
Namely
Potato dextrose agar
Negative logarithm of hydrogen ions
Parts per million
Soluble Concentration
Registered product
Emulsifiable Concentration
Wettable granules
Number
Maximum
Minimum

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### **1. INTRODUCTION**

Amaranthus is the most important leafy vegetable consumed and cultivated in Southern India. Amaranthus (*Amaranthus tricolor* L.), widely known as 'poor man's spinach' is one of the cheapest, most accepted and commercially cultivated leafy vegetable in Kerala. It is a short duration crop that fits well with the crop rotation practiced in Kerala. The crop has been rediscovered as a promising food crop mainly due to its resistance to heat, drought, diseases and pests, and the high nutritional value of both seeds and leaves. The crop has excellent nutritional value because of their high content of essential micronutrients such as beta-carotene, iron, calcium, vitamin C and folic acid (Priya *et al.*, 2007).

*Rhizoctonia solani* Kühn (teleomorph: *Thanatephorus cucumeris* [Frank] Donk.) is a soil-borne fungus that causes disease on many economically important crop plants worldwide. The pathogen overwinters as soil-borne sclerotia and mycelium in plant debris and these constitute the primary inoculum. Control of the pathogen is difficult because of its ecological behavior, extremely broad host range and the high survival rate of sclerotia under various environmental conditions (Anderson, 1982; Ogoshi, 1987).

Among the different diseases affecting amaranthus, leaf blight disease caused by *R. solani* Kuhn is the most devastating, especially during the monsoon period. The disease is characterised by light cream coloured spots on the foliage which rapidly spread causing extensive damage leading to economic losses. On the under surface of the infected leaves, white powdery masses of basidiospores of the teleomorph of the causative fungus *T. cucumeris* (Frank) Donk are clearly visible (Nayar *et al.*, 1996). Susceptibility of popular cultivars and humid conditions in Kerala make the disease a serious constraint in amaranth cultivation. The pathogen infects more than 90 % of plants in the field and causes considerable economic loss owing to reduced marketability of the produce. Although chemical control of the disease through the use of fungicides can lessen the severity of this aerial blight disease (Gokulapalan et al., 1999), application of chemicals on a regular basis causes serious health hazards. In order to minimise pesticide residues in agricultural products, alternative methods for the management of pests and diseases using nonhazardous, eco-friendly agents have to be explored. Among biocontrol agents, Trichoderma harzianum and Pseudomonas fluorescens are more reliable and ecologically as well as economically sustainable. Plant activators, a category of novel chemicals, also induce the defense capabilities of plants. Soil solarization for a period of 4 to 6 weeks also helps in checking the disease by limiting the population of pathogenic propagules. Solarization initiates changes in the physical and chemical features of soil that improve the growth and development of plants. It is a simple, safe, and effective method that can be used with field, vegetable and flower crops. Research continues on field evaluation of new formulations of degradable, wavelength selective, and colored plastic mulches and on cropping systems to suit specific conditions. The use of plastic mulches for the production of vegetable crops continues to increase throughout the world.

Recognizing the potentiality of integrated disease management and the importance of leaf blight disease of amaranthus in Kerala, the present study was designed to study the effect of soil solarization, biocontrol agents, chemical activator, indigenous formulations, and new generation fungicides on growth, yield and severity of foliar blight of amaranthus in field condition. The main objectives of the study are:

To find out the efficacy of biocontrol agents, chemical activator, indigenous formulations and new generation fungicides in inhibiting the growth of *R*. *solani* under *in vitro* conditions. To evolve a management practice for the disease using soil solarization along with biocontrol agents, chemical activator, indigenous formulations and new generation fungicides under field conditions.

### 2. REVIEW OF LITERATURE

Foliar blight caused by *Rhizoctonia solani* is a serious disease of amaranthus (*Amaranthus tricolor* L.) and it was first reported in Kerala (Nayar *et al.*, 1996). They described the symptoms of disease as appearance of light cream coloured spots on the foliage which rapidly spread causing extensive damage leading to economic losses. On the under surface of the infected leaves, white powdery masses of basidiospores of the teleomorph of the causative fungus *Thanatephorus cucumeris* (Frank) Donk were observed. Gokulapalan *et al.* (2000) reported that the disease severity was found to be more during high humid conditions.

### 2.1. THE PATHOGEN

*R. solani*, the most widely recognized species of *Rhizoctonia* was originally described by Julius Kuhn on the potato in 1858. This fungus has long been called as a sterile fungus, as it was thought to be incapable of producing any type of spore. But now several species of *Rhizoctonia* have been reported to reproduce asexually as well as sexually. Thus, *R. solani* that produces sexual spores, the basidiospores, has now been put under the family Ceratobasidiaceae in the order Tulasinallales. The sexual fruiting structures and basidiospores (*i.e.* teleomorph) were first observed and described in detail by Prillieux and Delacroiz in 1891. Its perfect stage was named as *'T. cucumeris'*. *R. solani* was one of the most destructive species occurring globally and causing various maladies *viz.*, seed decay, damping off, wire stem, root and stem rots, canker, sheath blight and ear rot on more than 500 hosts (Crosier, 1943; Baker, 1947, 1970; Sherf and Mac Nab, 1986; Ogoshi, 1985, 1996; Agrios, 2005). Its infection occurred on roots, tubers and other plant parts, which develop below or above ground (Hedgecock, 1904; Bewley, 1923; Roth and Ricker, 1943; Parmeter, 1970; Haseeb, 1983; Agrios, 2005).

The species of *R. solani* are distinguished from one another primarily by cultural, hyphal morphology, size of sclerotia, monilioid cells and on the basis of pathogenicity. However, strains within the species may vary in host range, virulence, survival at various depths of soil and cultural characteristics (Lal, 1985). The characteristics of the genus Rhizoctonia are in vague and there has been some difference among researchers in the recognition of this genus. Progress in understanding the biology and pathology of Rhizoctonia can be traced to the realization that genetically diverse groups exist at several levels of organization (Curtis, 1939; Warcup and Talbot 1962; Anderson, 1982; Ogoshi, 1987; Currah *et al.*, 1987; Xiag *et al.*, 2008).

#### 2.1. SOIL SOLARIZATION

The global changes and constant increase in the erosion of the natural ecosystem emphasise the importance of soil solarization as a viable environmental integrated disease management (IDM) tool in agricultural production systems. Soil solarization has been used to control weeds and soil borne pathogens in a number of crops. Soil solarization is the hydrothermal process of raising temperature by the use of clear plastic sheeting (Reghenzani, 1988; Devay *et al.*, 1991). The effectiveness of soil solarization is influenced by several factors such as soil moisture, soil type, season, duration of solar heating, type of mulching materials and organic and inorganic matter content of soil (Vilasini, 1996). Soil under the plastic is then soaked with water via one or more hoses or pipe outlets inserted under one end of the tarp. The soil may be irrigated before laying the plastic, but the plastic should be applied immediately after irrigation to avoid water loss. The plastic should be left in place for 4 to 6 weeks to allow the soil to heat to the greatest depth possible. The principle mode of action is direct thermal inactivation of soil borne pathogens and pests (Schrader, 2000).

Katan *et al.* (1976) reported that transparent and white polyethylene should be used for solarization, because it transmits most of the solar radiation that heat the soil. The effectiveness of soil solarization is influenced by the type of polyethylene material used. According to Pullman *et al.* (1981), polyethylene sheets of 25  $\mu$ m thick were more effective in heating soils and in killing soil borne fungi than 100  $\mu$ m thick sheets. Soil solarization enhances plant growth by improving soil structure, temperature, moisture etc. Soil solarization improves germination and helps in the development of healthy seedlings, further it helps in reducing uses of herbicides (Schrader, 2000). The effectiveness of soil solarization as an established soil-borne pests control method is well demonstrated under various agro ecosystems, especially in regions with high levels of solar radiation, but also in cloudy weather (Peachey *et al.*, 2001).

Extensive studies on fungi by Stapleton and Devay (1982, 1984) on microbial changes in the soil during and after solarization reported that population of fungi was greatly reduced immediately following solarization. R. solani causing seed rot and seedling disease of many crops was also controlled by solarization. Soil solarization has also been successfully combined with fungal biocontrol agents, Trichoderma *harzianum* which was added to the soil or planting material (Osman and Sahab, 1983; Katan, 1987). Meron et al. (1989) and Gamliel and Katan (1991) also reported the increased count of pseudomonas in solarized soil. Chandran (1989) and Sainamol (1992) suggested that the fungal population was reduced by solarization. Kaewruang et al. (1989) noticed that solarization significantly increased the population of actinomycetes (1.2 fold) antagonistic to Fusarium oxysporum, F. solani and R. solani at 0-10 cm depth. The use of organic amendments (manure, crop residues) together with soil solarization (biofumigation) elevates the soil temperature by 1-3 °C, and improves pest control due to a generation and accumulation of toxic volatiles (Rubin et al., 2007). According to Shukla and Dwivedi (2011), soil solarization also helps to increases the productivity of soil both by suppressing the pathogens as well as by

increasing the metabolic rate of soil materials, which helps to enhance the fertility of the soil.

Many theories have been put forward to explain the increased plant growth response in solarized soil. Upon solarization, minerals are released and the nutritional status in soil is improved which results in increased yield. Other mechanisms for stimulation of plant growth are stimulation of beneficial organisms (Nair *et al.*, 1990), destruction of pathogens and nullification of toxins in soil (Katan, 1981) and production of beneficial chemicals like fulvic acid (Davis and Sorensen, 1986). Increased colonization of (183-631 %) of plant roots by plant growth promoting fluorescent pseudomonas from inoculated seed also occurred in solarized soil (Stapleton and Devay, 1984). Increased growth response is observed in plants cultivated in solarized soil. This is mainly evident as increase in plant height, number of leaves, better root formation and yield. Several soil borne pathogens can be controlled by solarization. This includes fungi like *Pythium*, *Phytophthora*, *Fusarium*, *Rhizoctonia* etc. (KAU, 2011).

### 2.2. BIOLOGICAL CONTROL OF Rhizoctonia solani

In recent years, efforts have been made to restrict the use of chemicals to protect the environmental degradation. The use of non-chemical means of management of plant pathogens was advocated much while developing integrated disease management (IDM) for combating diseases. In this regard the use of biocontrol agents proved to be one of the most important component of IDM, which has a bright future for sustainable agriculture. So far, more than 100 biocontrol agents belonging to fungi, bacteria, viruses, nematodes, protozoa, etc. have been described, characterized and tested to combat various diseases and to improve agricultural productivity (Baker and Cook, 1974; Kloepper *et al.*, 1980; Rodriguez-Kabana *et al.*, 1984; Jatala, 1986; Chet, 1987; Kerry, 1990: Stirling. 1991; Dickson *et al.*, 1994; Hoitink and Boehm, 1999; Harman *et al.* 2004). Among these,

*Trichoderma* spp. and *Pseudomonas* spp. have been used as biocontrol agents against several soil borne plant pathogens (Baker, 1968; Baker and Cook. 1974; Papavizas and Lumsden, 1980; Windham *et al.*, 1986; Chet, 1987; Weller, 1988; Kerry. 1990; Stirling, 1991; Cook, 1993; Harman *et al.*, 2004). In an experiment conducted for the management of leaf blight disease disease of amaranthus, foliar application of *Pseudomonas fluorescens* (2 %) and soil application of *Trichoderma harzianum* (2 %) gave better control of the disease (71.87 % over control) compared to cowdung slurry 10 % and turmeric baking soda combination in the ratio 5:1. All treatments significantly resulted in higher yield compared to untreated control (Sheela *et al.*, 2015).

### 2.2.1. Biocontrol by Trichoderma harzianum

Fungi belongs to the genus *Trichoderma* are important biocontrol agents (BCAs) of several soil borne phytopathogens (Benítez *et al.*, 2004). *Trichoderma* use different mechanisms for the control of phytopathogens which include mycoparasitism, competition for space and nutrients, secretion of antibiotics and fungal cell wall degrading enzymes (Kubicek *et al.*, 2001; Howell, 2003; Benítez *et al.*, 2004; Harman *et al.*, 2004). Prasad and kumar (2011) evaluated three isolates of *Trichoderma* spp. against sheath blight disease of rice in glasshouse condition. Among the three potential *Trichoderma* spp., TN3 was found highly effective against *R. solani* under *in vitro* conditions. In field condition it was found most effective in reducing disease incidence and increasing grain yield in rice.

Gokulapalan and Nair (1984) reported that *T. harzianum*, *T. viride*, *Aspergillus flavus* and *A. niger* exhibited inhibitory avction on *R. solani* infecting rice. Monga (1993) studied the effect of nine *Trichoderma* spp. against cotton root rot pathogens *R. solani* and *M. phaseolina* under *in vitro* conditions. They reported that one isolate of *T. harzianum* was effective against *R. solani* whereas, an isolate of *T. virens* showed antagonistic effect against *M. phaseolina*. According to the *in vitro*  experiments done by Naeimi *et al.* (2010) several strains belonging to *T. harzianum*, *T. virens* and *T. atroviride* showed excellent biocontrol against *R. solani*. These potential antagonist strains were further evaluated for their effectiveness in controlling sheath blight under glasshouse conditions. Among the 55 selected strains, seven significantly controlled the disease. *T. harzianum* AS12-2 was the most effective strain in controlling rice sheath blight, better even than propiconazole. Elzerjawi (2015) conducted antagonistic test and showed that *T. harzianum* isolate significantly reduced the mycelial diameter of *R. solani* with the per cent mycelial growth inhibition by 82.21 %.

Use of *T. harzianum* in solarized field infested with *R. solani* has been shown to improve disease control while delaying the buildup of inoculum (Chet *et al.*, 1982). Gandhi and Kumar (2006) studied the antagonistic effect of fungal biocontrol agent's *viz. T. harzianum*, *T. viride* and *G. virens* against *R. solani* on potato. They reported that all the biocontrol agents inhibited the growth *of R. solani* significantly. Among all the treatments, *T. viride* (60 %) was found highly effective in inhibiting the growth *of R. solani*.

Das and Hazarika (2000) claimed that, *T. harzianum* was found to be more effective than *T. viride* in reducing sheath infection of rice caused by *R. solani* and increase in yield. *Trichoderma* could have a stimulatory effect on plant growth as a result of modification of soil conditions (Naseby *et al.*, 2000). Smitha (2000) conducted several pot culture experiments and found that soil application followed by foliar spray with one per cent suspension of the talc based formulated product of *Trichoderma* was very effective in reducing the intensity of foliar blight caused by *R. solani* and was selected as the mode of delivery in the field. Matloob *et al.* (2013) concluded that the biocontrol agent *T. harzianium* decreased root rot disease incidence and increased plant resistance against infection with *R. solani* and improve plant growth and yield.

### 2.2.2. Biocontrol by Pseudomonas fluorescens

In recent past, the plant growth promoting rhizobacterium *P. fluorescens* has received considerable attention for their inherent quality to produce antibiotics, hydrogen cyanide and siderophores, which are involved in suppression of plant root pathogens (Kloepper *et al.*, 1980; O'Sullivan and O'Gara, 1992). The simple nutritional requirement and the ability to use many carbon sources that exude from roots and to compete with indigenous microflora may explain their ability to colonize the rhizosphere (Weller, 1988; Mazzola and Cook, 1991).

Howell and Stipanovic (1979) found that treating cotton seed with *Pseudomonas fluorescens* at the time of planting in *R. solani* infested soil increased seedling survival from 30 to 79 %. De Freitas and Germida (1991) reported that *P. fluorescens* strains have been found to suppress a wide range of plant diseases caused by microbial pathogens including foliar diseases caused by fungi such as *Gaeumannomyces graminis*, *Pythium* spp. and *R. solani* in green house as well as in field trials. Fifteen strains of Pseudomonads inhibited the growth of *R. solani* both *in vitro* and *in vivo* conditions (Dantre *et al.*, 2003). Suppression of brown patch disease on bent grass and the production of several secondary metabolites like 2,4-diacetylphoroglucinol (2,4-DAPG), hydrogen cyanide (HCN), siderophore and indole acetic acid (IAA) by *P. fluorescens* strain HP72 has been reported by Suzuki *et al.* (2003). The consortium of *P. fluorescens* and *T. viride* was found superior against pre-emergence damping off, powdery mildew, fruit rot, wilt/root rot of chilli crop in addition to higher yield (Naik *et al.*, 2013).

Smitha (2000) identified a flurescent pseudomonad ( $P_1$ ) as the best antagonist against R. solani causing foliar blight of amaranthus. Sudhakar *et al.* (2013) conducted a greenhouse study on evaluation of various *P. fluorescens* strains on

growth promotion, drought tolerance and yield enhancement in peanut cultivar "Narayani". Their results validated the efficacy of *P. fluorescens* strain in improving drought tolerant traits and higher biometric traits such as plant height, shelling per cent, pod yield and harvest index over control as well as 2 % urea spray under stress imposed field conditions.

*P. fluorescens* bacteria are common soil inhabitants that favour colonisation of plants, spreading over the root environment (rhizosphere) and above ground structures (phyllosphere) (Taylor *et al.*, 2013).

### 2.3. CHEMICAL ACTIVATOR

These are the chemicals which activate the defense genes in plants by providing signals via the signal transduction pathway mediated by salicylic acid. Plant activators do not have pesticidal activity and antibiotic activity and hence their adverse effects on human health and environment are minimal. In addition, since they do not interact directly with the pathogens, it is unlikely that plant pathogens will develop resistance to these chemicals. The success of defense inducers for plant disease control depends on our ability to manage their phytotoxicity either by chemical modification of the compound or by modifying their formulation. Since, plant activators would never be able to provide complete protection; they could be more suited as a component of integrated disease management (Huang and Hsu, 2003; Vidyashekaran, 2004; Sreeja, 2014).

According to Kuc (2001), systemic acquired resistance (SAR) is the phenomenon by which plant defense mechanisms are activated by a pathogen or their metabolites or by a diverse group of structurally unrelated organic and inorganic compounds. The SAR development is associated with the accumulation of salicylic acid, derived from enhanced phenyl propanoid biosynthesis (Gaffney *et al.*, 1993; Vernooji *et al.*, 1994; Maunch-Mani and Slusarenko, 1996). The mechanisms of

salicylic acid (SA) mediated SAR was reported by Dempsey *et al.* (1999). The mechanisms include alterations in the activity or synthesis of certain enzymes, the generation of free radicals and increased defense responses. Various kinds of chemicals can induce the same resistance spectrum and biological changes as in biological SAR induction on cucumber and tobacco (Metraux *et al.*, 1991; Kessman *et al.*, 1994).

#### 2.3.1. Acibenzolar-S-Methyl (ASM)

Plant activators, a category of novel chemicals, also induce the defense capabilities of plants. For example, acibenzolar-S-methyl (ASM), a structural analog of salicylic acid, has been reported to be effective against plant diseases caused by fungal, bacterial, and viral agents (Métrauxs, 2001). Acibenzolar-S-methyl is chemically benzo (1,2,3) thiadiazole-7-carbothioic acid-S-methyl ester (BTH). It was developed by Syngenta Crop Protection, Inc. in USA and is marketed as Bion® and Actigard®. It acts as a substitute for salicylic acid in SAR. (Ziadi *et al.*, 2001; Gent and Schwartz, 2005).

The chemical elicitor acibenzolar-S-methyl (ASM; Actigard 50 WG), which induces systemic acquired resistance (SAR), to determine the effect on bacterial wilt of tomato caused by *Ralstonia solanacearum* on moderately resistant cultivars under greenhouse and field conditions was investigated. In greenhouse experiments, ASM was applied as foliar spray and/or soil drench ( $3\mu g/ml$ ) before and as foliar spray ( $30\mu g/ml$ ) after transplanting. The chemical elicitor was ineffective in reducing bacterial wilt incidence on susceptible tomato cultivars Equinox and FL 47 when plants were inoculated with *R. solanacearum*. However, greenhouse studies indicated that ASM significantly enhanced resistance in cultivars with moderate resistance to bacterial wilt such as Neptune and BHN 466. It appeared that ASMmediated resistance was partially due to prevention of internal spread of *R. solanacearum* toward upper stem tissues of tomato plants (Pradhanang *et al.*, 2005). The leaves of pepper (*Capsicum anuum* L.) were inoculated with *Phytophthora capsici* 3 day after treatment with acibenzolar-S-methyl benzo [1, 2, 3] thiadiazole-7-carbothioic acid-S-methyl ester (ASM) and resistance to Phytophthora blight disease was evaluated. Results showed that *P. capsici* was significantly inhibited by ASM treatment by up to 45 % in field condition. The pepper plants responded to ASM treatments by rapid and transient induction of L-phenylalanine ammonia-lyase (PAL), increase in total phenol content and activities of chitinase and  $\beta$ -1, 3-glucanase. No significant increases in enzyme activities were observed in water-treated control plants compared with the ASM-treated plants. Therefore it may be suggested that ASM induces defense-related enzymes, PAL activity, PR proteins and phenol accumulation in ASM-treated plants and contribute to enhance resistance against *P. capsici* (Baysal *et al.*, 2005).

The influence of a chemical activator (Acibenzolar-S-Methyl) and four plant growth promoting rhizobacteria were evaluated on foliar blight (*R. solani* Kuhn) suppression of amaranthus. *In vitro* and *in vivo* experiments were conducted both under non-sterile soil and sterile soil conditions in which the PGPR and chemical activator were tried both individually and also in combination. Results of the experiments indicated that PGPR induced resistance against *R. solani* in 'Arun,' a susceptible amaranth variety. A native isolate of *P. fluorescens* PN026R was particularly effective in suppressing the leaf blight disease and promoting plant growth. Plants treated with PN026R showed minimum incidence of disease and disease severity by 67 and 35 % respectively, compared to 92 and 52 % for plants inoculated with pathogen alone. Combined application of PGPR and ASM recorded excellent control with respect to disease incidence and disease severity of 42 and 21 % respectively (Nair *et al.*, 2007; Nair and Anith, 2009).

### 2.4. INDEGENOUS ORGANIC FORMULATIONS

Plants are nature's chemical factories which provide the richest source of organic chemicals on earth. Plant derivatives possessing pesticidal properties are gaining worldwide importance as an alternative or supplement for the existing pesticides because of low cost, less environmental hazards and no risk of development of resistance by the pathogens. The pest control properties of plants can be utilized either directly by using plant tissues and/or dry powder, or crude derivative, such as organic extract, or if possible through industrial process after isolation and identification of the active compound/ compounds of plants (Grainge and Ahmad, 1988).

The possibility of controlling pathogenic fungi with organic formulations has long been considered and studied. The use of turmeric-baking soda combination for the management of soil borne disease in rice has been reported by Gangopadhyay (1998).

Safaa *et al.* (2013) studied that the antioxidant successfully protected cucumber seedlings from pre-emergence damping-off and post-emergence seedling mortality caused by a virulent isolate of *R. solani* even though this antioxidant did not reduce colony growth of *R. solani in vitro*. Therefore, it was suggested that some inducible defense mechanisms in cucumber seedling tissues, rather than a direct antifungal action against *R. solani*. Results clearly showed that mixture of antioxidant with micronutrients have the potential to induce systemic resistance in cucumber plants. They were applied as seed treatment at fairly low concentrations. The spraying of fish amino acid at weekly interval could reduce leaf spot disease and leaf feeder attack in amaranthus in farmers field (KAU, 2014). Foliar application of turmeric and sodium corbonate mixture in the ratio 5:1 significantly suppressed the leaf blight pathogen of amaranthus by 68.38 % over untreated control and also resulted in higher yield compared to untreated control (Sheela *et al.*, 2015).

### 2.5. NEW GENERATION FUNGICIDES

Mancozeb, copper oxychloride and carbendazim occupy most of the fungicide market in India. For the past more than 20 years, quite a good number of groups of systemic fungicides, such as benzimidazoles, thiophanates, carboxanilides, organophosphates, triazoies, morphalines, pridines, phenylalamides, alkylphosphates, tricyclazole are being used in India for controlling various crop diseases. However, their frequent and indiscriminate use often leads to atmospheric pollution and development of pesticide resistance in pathogens (Delp, 1980; Brent and Hollomon, 1998; Thind and Chahal, 2002). In this context, innovative approaches with limited use of chemicals which are ecology conscious and environment friendly are coming up as alternative strategies for disease management (Chet, 1987; Weller *et al.*, 2002).

#### 2.5.1. Azoxystrobin

Barry *et al.* (2003) in their nine years of trials reported that application of azoxystrobin (Quadris, Amistar) against the Rhizoctonia crown and root rot of sugar beet before infection has provided good to excellent control with yield improvement. Mocion *et al.* (2003) found that on mature turfgrass, maintained under fairway conditions, azoxystrobin, trifoxystrobin and tebuconazole were very effective against brown patch caused by *R. solani*. Using environmental parameters which were most conducive to disease development, azoxystrobin, prothioconazole, pyraclostrobin, difenoconazole or propiconazole, flutolanil, polyoxin D, and a water control were evaluated for their ability to suppress disease development by *R. solani* 17 days after planting. Azoxystrobin, Flutolanil and Polyoxin-D, provided the highest level of disease suppression (Bolton *et al.*, 2010).

Biswas (2004) observed that azoxystrobin 25 SC @ 1 ml/lit effectively controlled the disease with lowest disease severity 16.4 % and improved grain yield. Sundravadana *et al.* (2007) investigated the effect of azoxystrobin on growth of *R*.

*solani* under *in vitro* condition and its efficacy against rice sheath blight under field conditions. The results revealed that azoxystrobin at 1, 2, and 4 ppm, completely inhibited mycelial growth of the pathogen. The field study showed that foliar spray of azoxystrobin at 125, 250, and 500 g/ha significantly suppressed the development of sheath blight and enhanced yield level. According to Bag *et al.* (2016) strobilurin based molecules like azoxystrobin, trifloxystrobin, metominostrobin managed the sheath blight pathogen effectively and eco-friendly way than other commercially available fungicides.

### 2.5.2. Copper hydroxide

Common fungicides recommended earlier against sheath blight were copper, organomercury and organo-aresenic compounds (Ou, 1985). According to Joslin and Taber, (2003) copper fungicide not only controlled foliar diseases in tomato but also provided for yields 60 % more than the other treatments. In an evaluation, Champion WP (active ingredient- copper hydroxide) significantly reduced disease severity of foliar diseases in tomatoes (Seaman *et al.*, 2004). Similarly, (Wszelaki *et al.*, 2003) observed a significant reduction in disease severity with Champion WP. Dubey (2000) recommended the combined use of copper hydroxide and bio control agents for the management of web blight caused by *R. solani* in groundnut. The highest suppression of leaf blight disease of amaranthus in field condition was observed in copper hydroxide 1.5g / 1 (80.76 % over control) compared to other fungicide treatments and biocontrol treatment (Sheela *et al.*, 2015).

### 2.5.3. Tebuconazole

Applications of tebuconazole at 0.23 kg/ha have shown excellent efficacy against southern stem rot caused by *Sclerotium rolfsii* Sacco and moderate suppression of Rhizoctonia limb rot (Brenneman *et al.*, 1991; Besler *et al.*, 1996). According to Grichar and Jaks, (1995) tebuconazole reduced Rhizoctonia pod rot

from 17 to 70 % over the untreated check, but control of Rhizoctonia limb rot can be inconsistent. Six new fungicides were evaluated against R. solani causing sheath blight disease in rice at regional agricultural research station (RARS), Pattambi and rice research station (RRS), Moncompu from 2012 to 2015. Over all pooled analysis of the data showed that tebuconazole, trifloxistrobin + tebuconazole, fluzilazole and pencycuron were significantly superior over control in reducing the sheath blight disease severity. The yield was also increased on an average of 20-30 percent by the application of these fungicides (Sheela et al., 2015). Raju et al., (2008) reported the maximum efficacy of tebuconazole 250 EC (1.5 ml/ lit) for the management of sheath blight disease in rice. Krishnam et al. (2008) reported the efficacy of tebuconazole, propiconazole and hexaconazole 250 EC @ 1.5 g/lit against sheath blight disease of rice. The two years experiment was carried out by Hegde (2015), and the results of experiments revealed that, tebuconazole @ 0.2 % has significantly reduced the blast (17.72 %) and sheath blight (10.24%) incidence in rice and correspondingly increased the yield levels. Field studies were conducted to evaluate these fungicides against sheath blight tebuconazole 250 EC (1.5ml /lit) was effective in reducing the disease severity of rice sheath blight disease and improving the yield (Raji *et al.*, 2016).

#### 2.5.4. Mancozeb

Thangasamy and Rangaswamy (1989) studied the efficacy of mancozeb and carbendazim in the control of sheath blight disease of rice by applying them at different stages of crop growth like panicle initiation (65 days of sowing) or 80 days of sowing and found them effective in controlling the disease severity. Chakrabarty (1993) reported that the captafol, mancozeb, chloramphenicol, streptocycline and boric acid were found effective against curd-rot disease complex associated pathogens *Botrytis cinerea*, *Fusarium equiseti*, *Pythium aphanidermatum*. *R. solani* and a bacterium, *Erwinia caratovora* on cauliflower. Gupta *et al.* (2000) studied the effect of various fungicides as seed treatment against root rot of french bean caused

by *R. solani*. They reported that seed treatment with Dithane M-45, Bavistin and Alert was effective in controlling the disease 13.8, 16.6 and 18.2 %, respectively as compared to untreated control (28.3 %). Sarkar and Saxena (2007) studied the management of seed mycoflora viz., *Aspergillus niger, A. fluvus, Fusarium* spp., *Alternuria* spp., *R. solani, Macrophomina. phaseolina and Colletotrichum sp.* of sunhemp by using Bavistin, Thiram, Metalaxyl, Dividend and Dithane M-45 as seed treatment (each @ 2 g/kg seed) under *in vivo* conditions.

Rauf *et al*, (2007) reported that mancozeb (Dithane M 45) was effective at 500 ppm against black scurf of potato and based on their result they have registered it for the management of the disease. The field experiment was carried out for the evaluation of fungicides and biocontrol agents against the leaf blight disease of amaranthus. Results of the experiment revealed that mancozeb @ 3 g/lit was effective in suppressing the disease (76.75 % over control) compared to biocontrol treatments (Sheela *et al.*, 2015).

### 2.6. Integrated disease management (IDM)

According to Chet *et al.* (1982), use of *T. harzianum* in solarized field infested with *R. solani* has been shown to improve disease control while delaying the buildup of inoculum.

Chattopadhyay and Sen (1996) reported that the subsequent application of compatible fungicides has been found to support the growth of antagonists and also prevented the plant form the attack of soil borne pathogens.

Integration of bio-control agents with fungicides gave significantly higher disease control in several crops than that obtained either by bio-control agent or by fungicide alone (Sawant and Mukhopadyay 1990; Dubey, 1997).

The seed and seedling treatment with captan, metalaxyl and carboxin found to eradicate the wilt causing pathogens or other soil microflora thereby less competition for BCA to colonize the seed and root surface and proliferate (Ram *et al.*, 2000).

The pot culture experiments conducted by Priyadarsini (2003) revealed that the *T. harzianum*, *P. fluorescens*, turmeric powder-baking soda 10:1 combination were found to be effective in improving the plant growth and reducing the disease incidence in amaranthus. Results also revealed that the control check of spraying 0.40 per cent mancozeb in cow dung supernatant was effective in reducing the disease intensity but the reduction was less when compared to the efficient biocontrol agents.

Gour and Sharma (2010) also reported superior results when *T. viride* -1 or *T. harzianum* (TG -1) were integrated with metalaxyl and cymoxanil 8 % + mancozeb 64 % to control root rot in cotton.

Effect of various disease management tools on seedling emergence, wilt incidence and yield of bell pepper *Capsicum annuum* L. Var. California Wonder was studied as part of integrated management strategies under both glass house and field conditions. Results of glass house and field studies revealed that integration of Captan + Metalaxyl with *Trichoderma harzianum* and *T. virens* proved superior control of wilt compared to individual treatments (Tariq *et al.*, 2012).

Jayashree *et al.* (2014) reported that selection of healthy rhizomes, soil solarization and incorporation of *Trichoderma*, seed treatment and soil application of biocontrol agents like *Trichoderma*, PGPR, well rotten cow dung done at the time of sowing and at regular intervals which was found to control the rhizome rot disease of ginger in field condition.

According to Acharya *et al.* (2015), among several chemicals, botanicals and bio-pesticides tested against rhizome rot disease of ginger, seed treatment with hot

water (50  $^{0}$ C for 10 min) and *Trichoderma* (10 gm per lit) and soil application of *Trichoderma* (1kg per 20 kg FYM) have been found effective in controlling the disease severity of rhizome rot in ginger.

Shresti (2005) studied the management of collar-rot complex caused by *Rhizoctonia bataticola, Fusarium chlamydosporum* and *Meloidogyne incognita* in *Coleus forskohlii* using bioagents, organic amendments and chemicals in different combinations. Combined application of an organic amendment (Neemto) with a bioagent (T. *viride*) was found to be most effective in reducing the wilt incidence, nematode population, number of galls and colony forming unit of R. *bataticola* and *F. chlamydosporum* in coleus.

### **3. MATERIALS AND METHODS**

The present study entitled "Integrated management of Rhizoctonia leaf blight of Amaranthus (*Amaranthus tricolor* L.)" was carried out in the Department of Plant Pathology, College of Agriculture, Vellayani and Coconut Research Station, Balaramapuram during the year 2014-2016. Details of materials used and the methods followed for the study are presented below.

3.1. ISOLATION OF AMARANTHUS LEAF BLIGHT PATHOGEN AND PURIFICATION OF THE ISOLATES

#### **3.1.1. Isolation of the pathogen**

Field samples of Amaranthus showing leaf blight symptoms were collected from Vellayani, Kalliyoor, Venganoor and Kakkamoola and isolation was carried out. For the isolation of the pathogen, leaves showing typical leaf blight symptoms were cut into small bits along with some healthy portions. The leaf bits were surface sterilized with 0.1 per cent mercuric chloride and then washed in three changes of sterile water. The bits were then placed on potato dextrose agar (PDA) (Appendix I) in sterile petri dishes and incubated at room temperature ( $25 \pm 2^0$  C). The fungal growth was noticed 24 h after inoculation. The growth was purified by hyphal tip method and transferred to PDA slants. This culture was used for further studies.

#### **3.1.2 Maintenance of the Culture**

The isolates were subcultured on PDA slants and allowed to grow for five days at room temperature  $(25\pm 2^{0} \text{ C})$ . Then the slants were kept in refrigerator at  $4^{0} \text{ C}$ , sub culturing was done once in a month. This culture was maintained and used throughout the study.

# **3.2. IDENTIFICATION OF THE PATHOGEN**

#### **3.2.1.** Morphological characters

The fungal morphology of *R*. *solani* was observed with regard to the growth rate and morphological character of the colony.

#### **3.2.1.1.** Colony characters

Five mm disc taken from all the fungal isolates were inoculated separately at the centre of a 90 mm sterile PDA medium and incubated at room temperature  $(25\pm2^0 \text{ C})$ . Observations on the radial growth and growth rate of mycelia were recorded at 24, 48 and 72 hours after inoculation in petri plates.

# 3.2.1.2. Hyphal Characters

Microscopic observations were taken for the shape of the hyphae and monilioid cells of young sclerotia.

# 3.2.1.3. Characters of perfect stage

On the undersurface of the infected leaves powdery coating made up of the hymenial layer of teleomorph of *R. solani, Thanatephorus cucumeris* was observed under the microscope. Basidia and basidiospores were observed for 10 X and 40 X magnifications under the compound microscope.

# 3.3. PATHOGENICITY TEST

#### **3.3.1.** Proving the Pathogenicity

Koch's postulates were proved for confirming the pathogenicity of the different isolates of *R. solani*. The thirty days old amaranthus seedlings were raised

in pots and leaves were mildly injured by giving pin pricks on the upper surface. The upper sides of the leaves were then inoculated with mycelial bits of seven days old fungal culture of the different isolates grown on PDA. A thin layer of moistened cotton was placed over the inoculated portion in order to provide humidity. Inoculated plants were covered using polythene bags to maintain humid condition. A uninoculated plants with pin pricks was maintained as control for the study. Reisolation of the pathogen was done from the leaves showing typical blight symptoms and the identity of the pathogen was established. The isolates were observed for time taken for symptom development and size of the lesions they produced. From among four isolates, the isolate which produced maximum disease symptoms was used for further studies.

#### **3.3.2.** Symptomatology

Symptoms of the disease caused by *R. solani* were studied by observing the naturally infected amaranthus leaves and also following the course of the development of the disease under artificial inoculation.

#### 3.4. IN VITRO STUDIES ON PATHOGEN SUPPRESSION

The *in vitro* experiments were carried out using complete randomized design (CRD) with three replications.

#### 3.4.1. In vitro evaluation of biocontrol agents against Rhizoctonia solani

#### 3.4.1.2. Trichoderma harzianum

The KAU isolate of *T. harzianum* was used in the study. Modified dual plate method by Skidmore and Dickinson (1976) was followed to test the effect of these antagonists on *R. solani*. Five mm diameter agar blocks of seven day old actively growing mycelial growth of *R. solani* and the *T. harzianum* placed five cm apart on PDA in a petri dish and incubated at room temperature  $(25\pm2^{\circ}C)$ . Three replications

were maintained. Plates inoculated with *R. solani* alone served as control. The nature of reaction of the fungal antagonists on *R. solani* was grouped as:

- A. Homogenous- Free intermingling between pairing organisms
- B. Over growth- The pathogen overgrown by the test organism
- C. Cessation-Cessation of the growth at the line of contact of cultures
- D. Aversion- A clear zone of inhibition between two organisms.

Inhibition of mycelial growth of the pathogen by each antagonist was studied using formula:

$$I = \frac{100 (C-T)}{C}$$

- I Inhibition of mycelial growth of the pathogen
- C Radial growth of pathogen in control plates (cm)
- T Radial growth of pathogen in treated plates (cm)

#### 3.4.1.3. Pseudomonas fluorescens

The KAU isolate of *P. fluorescens* was used in the study. The bacterial antagonist tested for antagonism to *R. solani* by dual culture technique (Utkhede and Rahe, 1983). The PDA medium was melted and poured into sterile petri plates. After solidification, culture bits of 5 mm size of the pathogen was placed at the centre of each dish. The bacterial isolate was then streaked 2.5 cm away on the both sides perpendicular to the pathogen placed at the centre. The percentage inhibition was calculated using the formula given in 3.4.1.2.

### 3.4.2. In vitro evaluation of chemical activator against Rhizoctonia solani

Actigard 50 WG containing the active ingredient acibenzolar -S- methyl (ASM). Using poison food technique the effect of ASM on the mycelial growth of *R*.

*solani* was tested. 200 mg Actigard 50 WG was dissolved in one litre of sterile water to get a concentration of 100 ppm of ASM. To 100 ml each of sterile molten PDA medium in 250 ml conical flasks, appropriate quantity of the stock solution was added aseptically to get varying concentration of ASM. These media with different concentrations of ASM was poured into sterile petridishes. Fungal mycelial discs of five mm diameter were cut out from seven days old culture of *R. solani* grown on PDA medium using a cork borer and placed at the centre of each dish. Inoculated plates were incubated at room temperature, three replications were maintained for each combination. Plates without ASM inoculated with *R. solani* at the centre served as control. Observation on the growth of the pathogen was taken at regular intervals. The percentage inhibition was calculated using the formula given in 3.4.1.2.

# 3.4.3. In vitro evaluation of indigenous organic formulations against Rhizoctonia solani

#### 3.4.3.1. Fish amino acid

Fish amino acid was prepared by following the steps described Weinert *et al.*, (2014), mixing one kg of sardine fish (*Sardina pilchardus*) with one kg of jaggery in a plastic can and keeping the plastic can under shade condition. The mouth of the can was covered with paper and tied with string and kept undisturbed for 25 days. After 25 days, the content was filtered through muslin cloth and stored in the same can. The filtered content was used in the *in vitro* and *in vivo* studies.

#### 3.4.3.2. Turmeric powder baking soda

As per the quantities mentioned below, three ratios of turmeric powderbaking soda combination were added to 200 ml Potato dextrose agar taken in 250 ml conical flasks before autoclaving. Media containing the different concentrations of turmeric – baking soda were poured into sterile petri dishes after sterilization. From the edges of seven day old mycellial growth of *R. solani* five mm discs were cut out with a cork borer of 0.5 cm diameter. The discs were then placed in the centre of each petriplate. For each combination, three replications were maintained. Plates containing PDA with *R. solani* inoculated at the centre served as control. Observation on the mycelial growth of the pathogen was recorded.

Table1. Different ratios of turmeric powder- baking soda combination tested against *R. solani (in vitro)*.

Ratios of turmeric powder-	Quantity of turmeric-	Quantity of baking soda in
baking soda	powder in 200 ml PDA	200 ml PDA
6:4	0.48g	0.32g
8:2	0.64g	0.16
10:1	0.727g	0.073g

Different concentrations of the fish amino acid and turmeric powder-baking soda combinations tested against *R. solani* is given in Table 2.

# 3.4.4. In vitro evaluation of new generation fungicides against Rhizoctonia solani

The *in vitro* suppression of the *R. solani* using fungicides was done using poisoned food technique described by Nene and Thaliyal (1993). Four commercially available fungicides were used for evaluation. The desired concentration of the fungicide (Table 3) was weighed out. Three conical flasks containing 50 ml of distilled water and another containing 50 ml of double strength PDA were taken and were sterilized by autoclaving at 1.1 kg/ cm<sup>2</sup> for 20 min. The fungicide was added into the distilled water and was shaken thoroughly. This was then added into 50 ml of molten double strength PDA to get the desired concentrations. The amended medium was then poured into sterile petri plates under aseptic conditions and was allowed to solidify. The same procedure was repeated for all the fungicides. Each

# Table 2. Different concentrations of the indigenous formulations tested against R. solani (in vitro).

Name	Concentrations used
Fish aminoacid	2.5 %, 5%, 10%
Turmeric powder and baking soda combination	6:4,10:1,8:2

Table 3. Different concentrations of the fungicides tested against *R. solani (in vitro)*.

Chemical name	Trade name	Concentrations used (%)
Azoxystrobin	Mirador	0.10, 0.15, 0.20
Copper hydroxide	Kocide	0.10, 0.15, 0.20
Tebuconazole	Folicur	0.05, 0.10, 0.15
Mancozeb	Indofil M - 45	0.20, 0.40, 0.60

plate was inoculated in the centre with 5 mm mycelial disc cut out from seven days old *R. solani* under aseptic condition. The plates were sealed using parafilm and were incubated at room temperature  $(25 \pm 2^{0} \text{ C})$ .

Unamended medium with the pathogen at the centre served as the control. Observations were taken when the mycelium of the pathogen completes full growth in the control petri plates. The percentage inhibition was calculated using the formula given in 3.4.1.2.

# 3.5. FIELD STUDIES ON PATHOGEN SUPPRESSION AND PLANT GROWTH PROMOTION

Two experiments have been carried out, one in solarized plots and the other in non-solarized plots. The details of experiment are as follows; Design - RBD, Treatments – 11, Replication - 3, Variety - Arun, Plot size - 2 x 2 m, Location-Coconut Research Station, Balaramapuram.

#### **3.5.1. Land preparation**

The field study has been conducted at CRS, Balaramapuram as an intercrop in 53 year old coconut plantation (Plate 1, 2). The experimental site was incorporated with required quantity of organic manure in soil. Fertilizer application and other cultural operations have been done as per Package of Practices Recommendations of crops, KAU (2011).

### **3.5.2.** Soil solarization

Soil solarization was carried out at Coconut Research Station, Balaramapuram, in the manually leveled plots arranged in randomized block design with three replications, for a period of five weeks from first week of January till the second week of February, 2016 (Appendix II). The experimental site was incorporated with required quantity of organic manure in soil, irrigated, ploughed and leveled prior to imposing solarization treatment. Later, the plots were covered with transparent polythene sheets of 150 gauge thickness. The edges of sheets were sealed with soil to keep it in position and also to maintain the temperature and moisture inside the polyethene mulch. Adequate care was taken to keep the sheet in close contact with the surface of soil to prevent the formation of air pockets between the soil and the polyethene sheet. After the period of solarization, the sheet was removed and transplanting was done (Katan, 1981).

#### **3.5.3. Raising of Amaranthus**

Seeds of amaranthus, variety Arun were procured from the Instructional Farm, College of Agriculture, Vellayani. The seeds were broadcasted in nursery beds, 21 days old seedlings were transplanted into each plot. Each plot of 2 x 2 m were prepared in flat beds of both solarized and non solarized plots with row spacing of 30 cm and plant to plant spacing is 20 cm. The number of plants were maintained 100 plants per plot and the crop was irrigated by using hose pipe. The crop was raised during the period from February 2016 to April 2016.

#### **3.5.4.** Disease assessment

Treatments application was given to the plants after first appearance of leaf blight disease and further treatment application was done at 20, 35 and 50 days after transplanting. Biometric observations such as plant height, number of leaves were recorded at 30 DAT and plant height, no. of leaves, root length, root weight, fresh weight and dry weight were recorded at the time of harvest of the crop.

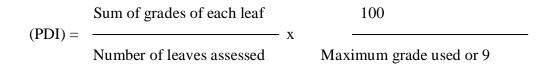
Disease incidence was recorded 20 days after transplanting and disease severity were recorded at 30, 40, 50 and 60 DAT. For assessing disease incidence, the number of infected and total number of plants in each treatment was recorded and disease incidence was calculated using the formula given below.

Number of plants diseased x 100

Disease incidence (%) =

Total number of plants assessed

Disease severity from each treatment was rated on ten randomly selected leaves (three from top, four from middle and three from bottom) from each of ten randomly selected plants. Each leaf was scored using a 0-9 scale (KAU, 1996), where 0=no disease; 1=1 to 10% infected leaf area; 3=>10 to 25% infected leaf area; 5=>25 to 50% infected leaf area; 7=>50-75% infected leaf area; 9=>75% leaf area infected. In addition, % disease severity or Percentage Disease Index (PDI) was calculated using the formula suggested by Wheeler (1969).



# 3.5.5. Treatments

Two separate experiments have been carried out, one in solarized plots and the other in non-solarized plots, both consisting of 11 treatments as described below.

- 1. *Pseudomonas fluorescens* talc formulated product 2% foliar spray (KAU formulation)
- 2. *Trichoderma harzianum* talc formulated product 2% foliar spray (KAU formulation)
- 3. Fish aminoacid 5%
- 4. Acibenzolar-S-Methyl (ASM) soil application 75 ppm
- 5. Acibenzolar-S-Methyl (ASM) foliar spray- 100 ppm

- 6. Turmeric powder-baking soda combination 10:1
- 7. Azoxystrobin 23% SC (125 g ai /500 l) 0.15 %
- 8. Copper hydroxide @ 1.5 g/l
- 9. Tebuconazole @ 0.1%
- 10. Mancozeb in cowdung supernatant @ 0.4%
- 11. Absolute control.

Design - RBD Treatments - 11 Replication - 3 Variety - Arun Plot size - 2 x 2 m Location- Coconut Research Station, Balaramapuram

# **3.5.6.** Observations

Observations of amaranthus plants such as height of the plant and number of leaves were taken at 30 DAT and finally at harvest of crop and all other observations were taken at harvest of the crop.

# 3.5.6.1. Number of days for the appearance of the leaf blight disease

After transplanting, number of days taken for the first appearance of the disease in each plot was observed and recorded.

# 3.5.6.2. Disease incidence

The incidence of disease under natural epiphytotic condition was recorded as

Number of plants diseased x 100

Disease incidence (%) =

Total number of plants assessed

# 3.5.6.3. Disease severity

Based on the extent of damage caused by the disease, disease severity was assessed by scoring at 10 days interval using 0-9 grade scale as described below (Plate 3).

Disease severity was graded using a 0-9 scale (KAU, 1996)

Grade	Description
0	No infection
1	1-10 per cent of leaf area infected
3	11-25 per cent of leaf area infected
5	26-50 per cent of leaf area infected
7	51-75 per cent of leaf area infected
9	> 76 per cent of leaf area infected

# 3.5.6.4. Days to flowering

Number of days taken for flowering of plants was recorded.

# **3.5.6.5.** *Biometric characteristics*

# 3.5.6.5.1. Height of the plant (cm)

The length of the shoot from the ground level to the growing tip of 10 plants from each plot was recorded.

# 3.5.6.5.2. Number of leaves

The number of leaves was estimated by counting the number of leaves per plant of 10 plants in each plot.

# 3.5.6.5.3. *Root length* (*cm*)

The length of longest root of 10 plants of each plot was measured after uprooting of plants.

# 3.5.6.5.4. *Root weight (kg)*

The weight of roots of 10 plants was recorded by using electronic balance and measured in grams.

# 3.5.6.6. Yield

## 3.5.6.6.1. Fresh weight (kg)

The fresh yield was recorded after the harvest.

# 3.5.6.6.2. Dry weight (kg)

The dry yield was taken after sun drying of the harvested plants separately for 2 weeks.

# 3.5.6.7. Incidence of other diseases and pests

The infestation by the other pest and diseases were periodically observed and per cent incidence was recorded.

# 3.6. STATISTICAL ANALYSIS

Data generated from the experiment were subjected to statistical analysis applying ANOVA technique was tested by 'F' test. In the cases where the effects were found to be significant, critical difference values were calculated for each observation using table 't' values at 5 per cent level of significance. Then, the significance of treatments were compared with critical difference values.

# **4. RESULTS**

The experiments were conducted under laboratory and field conditions during the 2014-2016 at Department of Plant Pathology, College of Agriculture, Vellayani and Coconut Rsearch Station, Balaramapuram. The results obtained from laboratory and field studies are summarized below.

#### 4.1. ISOLATION OF THE PATHOGEN

Field samples of amaranthus showing leaf blight symptoms were collected from Vellayani, Kalliyoor, Venganoor and Kakkamoola locations (Plate 4). Isolates were purified and mentained on PDA slants after the isolation of different isolates separately. The fungal isolates were numbered as RS1, RS2, RS3 and RS4 for Vellayani, Kalliyoor, Venganoor and Kakkamoola isolates respectively (Plate 5).

#### 4.2. IDENTIFICATION OF THE PATHOGEN

Initially the mycelial growth of *R. solani* in petri plate was creamish in colour later colony colour in all the isolates was light hyaline-brown, with aerial mycelium. All isolates produced alternating dark and light concentric rings in the growth. The young branches arose at approximately right angles to the main hyphae. Mature hyphae showed right angle branching and cross septa were found close to the branching point (Plate 6). Young white sclerotial pinheads were formed from masses of monilioid cells in 5-day-old culture (Plate 7). The sclerotia turned brown after 24-48 h. Mature sclerotia were dark brown in colour with no definite form and most of them were scattered all over the petri dish. The pathogen isolated was identified as *R. solani* based on their typical colony characteristics. Identification of the pathogen was confirmed as *R. solani* at Agharkar Research Institute, Pune (Accession No. 3/426/2016/Myc/1574). There were differences in growth rates among the isolates on PDA medium at 24, 48 and 72 h after inoculation (HAI). Significantly lower growth rate was observed for isolates RS2 and RS3 at 24, 48 and 72 HAI and higher growth rate was observed in RS1 and followed by RS4 at 24, 48 and 72 HAI (Table 4). The different isolates of *R. solani* varied in their time of sclerotial formation when grown on PDA. The isolates RS1 and RS4 produced sclerotia six days after inoculation, whereas isolate RS2 took 8 days for sclerotial formation followed by RS3 which took 9 days.

### 4.3. PATHOGENICITY TEST

### 4.3.1. Symptomatology

Symptomatology was studied by observing and collecting the infected field samples from four different locations near to the College of Agriculture, Vellayani. Symptoms of the disease began as small irregular whitish creamy spots on the leaves which enlarge under high humidity. The spots gradually became translucent with irregular brown coloured margins. Severely infected leaves showed shot-hole symptoms leading to defoliation. On the undersurface of the infected leaves, powdery coating consisting of the hymenial layer of teleomorph of *R. solani*, *T. cucumeris* was observed (Plate 8).

#### **4.3.1.** Proving the Pathogenicity

Koch's postulates was proved for the pathogenicity of different isolates of *R*. *solani*. Artificial inoculation was carried out on healthy leaves of amaranthus plant with 1cm diameter mycelial discs cut from seven days old culture of *R*. *solani* grown on PDA medium after giving definite number of pin pricks with the help of a sterile stainless steel needle. All the four isolates have taken three days for the first development of symptoms. Development and progression of the lesions size after 24, 48, 72 and 92 hours after first development of symptoms is given in Table 5 (Plate 9, 10, 11, 12). Symptoms progression of isolate RS1 was very fast compared

Isolatas	Colon	y diameter	(cm)*	Colony	Time of sclerotial
Isolates	24 HAI	48 HAI	72HAI	colour	formation (DAI)
RS1	$2.88^{a}$	$4.48^{a}$	8.03 <sup>a</sup>	Hyaline	6
KSI	2.00	4.40	8.03	brown	0
RS2	1.43 <sup>c</sup>	3.08 <sup>c</sup>	6.55 <sup>°</sup>	Hyaline	8
N32	1.45	5.00	0.55	brown	0
RS3	$1.40^{c}$	$3.28^{\circ}$	6.45 <sup>c</sup>	Hyaline	9
105	1.40	5.20	0.45	brown	,
RS4	1.73 <sup>b</sup>	$3.68^{b}$	$7.25^{b}$	Hyaline	6
K34	1.73	5.08	brown	0	
CD (0.05)	0.163	0.283	0.489		

Table 4. Growth rate of Rhizoctonia solani on PDA medium.

\*Mean of three replications, digits followed by same letter in a column do not differ significantly.

HAI- Hours After Inoculation, DAI- Days After Inoculation

Table 5. Lesion development on amaranthus leaves by <i>R. solani</i> isolates	Table 5. Lesion develo	pment on amaranthus	leaves by R.	. solani isolates.
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Isolate of	Time of first development of symptoms (Days after	Size of the		fter first deve ns (cm x cm)	lopment of
R. solani	inoculation)**	24 h	48 h	72 h	96 h
RS1	3	1.5 x 1.1	2.5 x 2.0	4.0 x 3.5*	5.5 x 3.7*
RS2	3	0.5 x 0.5	1.5 x 0.8	2.5 x 2.0	4.5 x 3.6*
RS3	3	1.0 x 1.7	1.6 x 1.5	4.0 x 3.2	5.2 x 3.8*
RS4	3	0.5 x 0.3	2 x 1.5	2.5 x 2.3	3.8 x 3.0*

\*\* Mean of three replications, \*Defoliation of severely infected leaf.

to all other isolates and the inoculated leaves of RS1 defoliated 72 h after first symptom development where as RS2, RS3 and RS4 isolates inoculated leaves defoliated 96 h after first symptom development. Re-isolation of the pathogen from the artificially inoculated amaranthus leaves yielded *R. solani* identical to the original culture. Among the four isolates, RS1 therefore selected as the most virulent isolate for use in further studies.

#### 4.4. IN VITRO STUDIES ON PATHOGEN SUPPRESSION

## 4.4.1. In vitro evaluation of biocontrol agents against Rhizoctonia solani

# 4.4.1.1. Trichoderma harzianum

The KAU isolate of *T. harzianum* was tested for its antagonism against *R. solani*. *T. harzianum* showed 49.56 per cent inhibition under *in vitro* condition. Regarding the nature of reaction, *T. harzianum* found to overgrow the pathogen when subjected to dual culture (Plate 14). Though initially a clear zone of inhibition between the paired cultures was observed after six days the isolate was found to overgrow the pathogen (Table 6).

#### **4.4.1.2.** *Pseudomonas fluorescens*

The KAU isolate of *P. fluorescens* was used in the study to test its antagonism against *R. solani* (Plate 10). *P. fluorescens* caused 28.3% inhibition of the pathogen under *in vitro* condition (Plate 13) (Table 6).

#### 4.4.2. In vitro evaluation of chemical activator against Rhizoctonia solani

Effect of different concentration of actigard (ASM) on the mycelial inhibition of the pathogen is shown in Table 7. (Plate 15). Progressive decrease in mycelial growth of the pathogen was observed with increase in concentration of actigard compared to control. Actigard at 100 ppm concentration shown minimum diameter

Table 6. Effect of biocontrol agents on mycelial	suppression of <i>R. solani</i> .
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Biocontrol agent	Percentage mycelial inhibition*	Nature of reaction	
	5 days old	4 days old	7 days old
Pseudomonas fluorescens	28.30	Aversion	Over grown by pathogen
Trichoderma harzianum	49.56	Overgrowth	Over growth of the pathogen

Mean of three replications\*

Table 7. Effect of chemical activator on mycelial growth of *R. solani*.

Concentration of ASM in the medium	Diameter of mycelial growth (cm)	Percentage mycelial inhibition
5 ppm	6.98	22.30 (28.17)e
25 ppm	6.50	27.70 (31.75)d
50 ppm	5.88	34.33 (35.87)c
75 ppm	2.68	69.67 (56.66)b
100 ppm	2.15	75.67 (60.45)a
No ASM	9.00	0.00 (0.67)f
CD (0.05)		3.318

Mean of three replications\*, values in the parenthesis are arc-transformed\*\* Treatments with same alphabets in the superscript, do not differ significantly. of mycelia growth of 2.15 cm cm followed by 75 ppm concentration (2.68 cm), 50 ppm concentration (5.88 cm), 25 ppm concentration (6.50 cm) and 5 ppm concentration (6.98 cm).

# 4.4.3. In vitro evaluation of indigenous organic formulations against Rhizoctonia solani

Organic preparations such as fish amino acid and turmeric powder baking soda combination were evaluated for its efficiency to suppress the pathogen *R. solani*. The treatments were tested at recommended dose, at lower dose and at high dose with filtration. The results of effect of treatments on growth of the pathogen under *in vitro* condition is described in Table 8 (Plate 16, 17). Effect of turmeric powder baking soda combination was significantly superior than fish amino acid at recommended dose and inhibited the growth of the *R. solani* by 64.40 %. Fish amino acid caused less suppression of the pathogen (29.00 %).

#### 4.4.4. In vitro evaluation of new generation fungicides against Rhizoctonia solani

The results of the *in vitro* evaluation of four fungicides by poisoned food technique (Table 9) revealed that two chemicals *viz.*, tebuconazole and mancozeb gave 100 % inhibition to the growth of the pathogen under *in vitro* conditions at recommended dose and was significantly different from the effect of other chemicals (Plate.21, 19) However, copper hydroxide gave 71.33 % inhibition of *R. solani* and was significantly superior to the azoxystrobin which gave inhibition of 46.07 % and azoxystrobin was less effective than other chemicals used for the study under *in vitro* conditions (Plate 18, 20).

Treatments	Percentage mycelial inhibition			
(Concentration)**	Lower dose*	Recommended dose*	Higher dose*	
Fish amino acid	15.13	29.00	35.33	
(2.5 %, 5 %, 10 %)	(22.89)b	(32.55)b	(36.46)b	
Turmeric powder and	57.00	64.40	57.73	
baking soda combination (6:4, 10:1, 8:2)	(49.05)a	(53.38)a	(49.46)a	
Control	00.00	00.00	00.00	
Control	(0.96)	(0.96)	(0.96)	
CD (0.05)	4.655	2.828	2.452	

 Table 8. Effect of indigenous organic preparations on mycelial suppression of *R. solani*.

\*Mean of three replications, \*\*values in the parenthesis are arc-transformed

Treatments with same alphabets in the superscript in a column do not differ significantly.

Concentrations are given in the order low dose, recommended dose and high dose.

		Percentage mycelial inhibition*			
Chemical name (concentration)**	Trade name	Lower dose	Recommended dose	Higher dose	
Azoxystrobin (0.10, 0.15, 0.20 %)	Mirador	34.77 (36.06) <sup>c</sup>	46.07 (42.74) <sup>c</sup>	46.07 (42.75) <sup>c</sup>	
Copper hydroxide (0.10, 0.15, 0.20 %)	Kocide	68.00 (55.62) <sup>b</sup>	71.33 (57.66) <sup>b</sup>	73.87 (59.49) <sup>b</sup>	
Tebuconazole (0.05, 0.10, 0.15 %)	Folicur	100.00 (89.26) <sup>a</sup>	100.00 (89.26) <sup>a</sup>	100.00 (89.26) <sup>a</sup>	
Mancozeb in cow dung supernatant (0.20, 0.40, 0.60 %)	Indofil M - 45	100.00 (89.26) <sup>a</sup>	100.00 (89.26) <sup>a</sup>	100.00 (89.26) <sup>a</sup>	
Control		$\begin{array}{c} 00.00 \\ \left( 0.74  ight)^{ m d} \end{array}$	$\begin{array}{c} 00.00 \\ (0.74)^{ m d} \end{array}$	$\begin{array}{c} 00.00 \\ (0.74)^{d} \end{array}$	
CD (0.05)		5.986	3.660	6.363	

Table 9. Efficacy of fungicides on the *in vitro* suppression of *R. solani*.

Mean of three replications\*, values in the parenthesis are arc-transformed\*\*

Treatments with same alphabets in the superscript in a column do not differ significantly.

Concentrations are given in the order low dose, recommended dose and high dose.

# 4.5. FIELD STUDIES ON PATHOGEN SUPPRESSION AND PLANT GROWTH PROMOTION

#### 4.5.1. Integrated management of Rhizoctonia leaf blight of amaranthus

The effect of soil solarization along with biocontrol agents, chemical activator, indigenous organic formulations and new generation fungicides on the suppression of Rhizoctonia leaf blight of amaranthus was studied by field experiment with 11 treatments (Plate 22). The treatments were given to the plants after the natural infection by the pathogen.

### 4.5.1.1. Number of days for the appearance of the leaf blight disease

There was no significant difference observed for the number of days taken for first appearance of leaf blight disease of amaranthus in all the soil solarized plots (Table 10). The number of days taken for first appearance of Rhizoctonia leaf blight ranged from 15 to 17 days (Plate 23).

# 4.5.1.2. Disease incidence

The data on the effect of soil solarization along with different treatments on disease incidence found to be statistically non significant as shown in Table 11. Among all the treatments, soil application of acibenzolar-S-methyl (75 ppm) and foliar application of acibenzolar-S-methyl (100 ppm) recorded the less disease incidence of 30.41 % and 30.42 % respectively. Treatments with ASM application gave stunting effect and early flowering in amaranthus at later stages of growth (Plate 24). However, soil solarization along with foliar spray of copper hydroxide (0.15%) and absolute control gave maximum disease incidence of 36.65 % and 37.66 % respectively.

Sl.No	Treatments	Number of days for first appearance of leaf blight disease*
1	Pseudomonas fluorescens	17.00
2	Trichoderma harzianum	17.00
3	Fish aminoacid	15.33
4	(ASM) soil application	15.33
5	(ASM) foliar spray	15.00
6	Turmeric powder-baking soda combination	15.33
7	Azoxystrobin	15.67
8	Copper hydroxide	15.33
9	Tebuconazole	16.00
10	Mancozeb in cow dung supernatant	15.33
11	Absolute control	15.67
		NS

Table 10. Number of days for the appearance of the leaf blight disease in solarized plots.

Mean of three replications\*

Table 11. Effect of soil solarization along with biocontrol agents, chemical activator, indigenous formulations and new generation fungicides on the foliar blight disease incidence in amaranthus.

Treatment No.	Treatments	Mean disease incidence*	Percent reduction over control
1	Pseudomonas fluorescens (2 %)	34.72	7.80
2	Trichoderma harzianum (2%)	33.61	10.76
3	Fish amino acid (5 %)	34.48	8.45
4	ASM soil application (75 ppm)	30.41	19.23
5	ASM foliar spray (100 ppm)	30.42	19.21
6	Turmeric powder-baking soda combination (10:1)	34.58	8.16
7	Azoxystrobin (0.15 %)	33.59	10.81
8	Copper hydroxide (0.15 %)	36.65	2.69
9	Tebuconazole (0.1 %)	35.42	5.95
10	Mancozeb in cow dung supernatant (0.4 %)	35.42	5.95
11	Absolute control	37.66	
		NS	

Mean of three replications\*

#### **4.5.1.3.** *Disease severity (Disease index)*

Statistical analysis of the data on disease severity revealed (Table 12) (Plate 25) that soil solarization with application of biocontrol agents, chemical activator, indigenous organic formulations and new generation fungicides were effective in suppressing the pathogen when compared to the absolute control. Chemicals recorded the lowest disease index in comparison to rest of the treatments. Among the chemicals used, foliar spray of tebuconazole (0.1 %) recorded lowest disease index 44.76 with a percentage of disease suppression 42.64 which was followed by foliar spray of mancozeb in cowdung supernatant (0.4 %) with a disease index of 48.32 and the percentage disease suppression was 38.08. This was on par with superior treatment foliar spray of tebuconazole (0.1 %). Foliar spray of azoxystrobin (0.15 %) and foliar spray of copper hydroxide (0.15 %) were statistically on par and also gave maximum disease index of 58.84 and 61.50 with percentage disease suppression of 24.59 and 21.19 respectively among the chemicals.

Accordingly soil solarization with the application of biocontrol agents, chemical activator and indigenous formulations gave good control of the pathogen and was lower to the chemical treatment but was superior than the absolute control. Among biocontrol agents, foliar spray of *P. fluorescens* talc formulated product @ 2 % recorded the disease index 50.77 was significantly different from other treatments with the percentage of suppression of 34.93 and was on par with the following treatment *T. harzianum* talc formulated product @ 2 % with a disease index of 56.55 and percentage disease suppression was 27.53.

Soil solarization along with chemical activator and indigenous organic formulations were significantly different from other treatments but are on par with each other. The soil application of ASM (75 ppm) gave the disease index of 68.10 and percentage disease suppression of 12.73 followed by foliar application of ASM (100 ppm), foliar spray of fish amino acid (5 %) and turmeric baking soda

Treatmonte	*Percentage Disease Index			*Percentage disease suppression				
Treatments	30 DAT	40 DAT	50 DAT	60 DAT	30 DAT	40 DAT	50 DAT	60 DAT
Pseudomonas fluorescens (2 %)	22.36 (28.19) <sup>cd</sup>	33.27 (35.20) <sup>e</sup>	45.22 (42.25) <sup>de</sup>	50.77 (45.44) <sup>d</sup> e	22.78	44.64	30.72	34.93
Trichoderma harzianum (2%)	22.03 (27.99) <sup>cd</sup>	33.45 (35.33) <sup>e</sup>	52.00 (46.16) <sup>cd</sup>	56.55 (48.76) <sup>c</sup> d	23.91	44.35	20.32	27.53
Fish amino acid	25.46	39.67	49.51	71.80	12.05	33.99	24.14	7.99
(5 %)	$(30.27)^{abc}$	(39.04) <sup>d</sup>	$(44.72)^{cd}$	$(57.97)^{b}$				
ASM soil application	24.84	43.58	54.86	68.10	14.19	27.49	15.95	12.73
(75 ppm)	$(29.87)^{bcd}$	$(41.30)^{cd}$	$(47.79)^{bc}$	$(55.62)^{b}$				
ASM foliar spray	24.79	47.37	54.60	68.10	14.36	21.18	16.34	12.72
(100 ppm)	$(29.85)^{bcd}$	$(43.49)^{bc}$	(47.66) <sup>bc</sup>	$(55.61)^{b}$				
Turmeric powder-baking	24.22	57.20	63.91	72.10	16.35	4.83	2.08	7.60
soda combination (10:1)	$(29.46)^{bcd}$	$(49.15)^{a}$	$(53.10)^{a}$	$(58.14)^{b}$				
Azoxystrobin	22.22	49.36	54.97	58.84	23.27	17.87	15.77	24.59
(0.15 %)	$(28.12)^{cd}$	$(44.63)^{b}$	$(47.85)^{bc}$	$(50.12)^{c}$				
Copper hydroxide	27.44	45.36	61.36	61.50	5.23	24.51	6.00	21.19
(0.15 %)	(31.54) <sup>ab</sup>	$(42.33)^{bcd}$	$(51.59)^{ab}$	$(51.66)^{c}$				
Tebuconazole	21.50	27.89	37.85	44.76	25.75	53.58	42.01	42.64
(0.1 %)	$(27.60)^{d}$	(31.87) <sup>f</sup>	$(37.96)^{\rm e}$	$(41.98)^{\rm e}$				
Mancozeb in cow dung	21.38	33.00	40.02	48.32	26.13	45.09	38.69	38.08
supernatant (0.4 %)	(27.54) <sup>d</sup>	$(35.05)^{\rm ef}$	$(39.23)^{\rm e}$	$(44.03)^{\rm e}$				
Absolute control	28.94	60.10	65.27	78.03				
Absolute control	(32.54) <sup>a</sup>	$(50.84)^{a}$	(53.91) <sup>a</sup>	$(62.05)^{a}$				
CD (0.05)	2.368	3.321	4.553	3.645				

Table 12. Effect of soil solarization biocontrol agents, chemical activator, indigenous formulations and new generation fungicides on the leaf blight disease severity in amaranthus.

\*mean of three replications, values in the parenthesis are arc sin transformed. Treatments with same alphabets in the superscript do not differ significantly.

combination (10:1) with disease index of 68.10, 68.10, 71.80 and 72.10 with the percentage disease suppression of 12.73, 12.72, 7.99 and 7.66 respectively.

# 4.5.1.4. Days to flowering

Effect of soil solarization along with the different treatments on the number of days taken for flowering was found to be non significant (Table 13). Foliar spray of *P. fluorescens* talc formulated product @ 2 % and fish amino acid 5 % taken maximum number of days for flowering (35) and foliar spray of azoxystrobin (0.15 %) and tebuconazole (0.10 %) taken minimum number of days (30) for the flowering.

### 4.5.1.5. *Height of the plant* (*cm*) – 30 DAT

Effect of soil solarization along with other treatments on height of the plant was statistically found to be non significant (Table 14). The treatment mancozeb in cowdung supernatant (0.40 %) recorded maximun plant height of 45.93 and minimum plant height of 37.00 and 37.60 was recorded by absolute control and *Pseudomonas fluorescens* talc formulated product @ 2 %.

#### 4.5.1.6. Number of leaves- 30 DAT

Statistical analysis of the data on effect of soil solarization along with other treatments on number of leaves was shown to be non significant (Table 14). Foliar spray of ASM and foliar spray of azoxystrobin gave the maximum number of leaves 37.67 and 36.93 respectively. Minimum number of leaves was observed in absolute control and *P. fluorescens* talc formulated product @ 2 % by 24.56 and 24.73 respectively.

# **4.5.1.7.** Height of the plant (cm) – at harvest

Maximum plant height (127.07) was recorded in case of plants treated with mancozeb in cow dung supernatant (0.40 %) which was on par with tebuconazole

(0.1 %), azoxystrobin (0.15 %), turmeric powder-baking soda combination (10:1), and copper hydroxide (0.15 %) with the plant height 124.46, 123.73, 120.36 and 120.13 respectively. The untreated control plants gave the minimum plant height (109.13) (Table 15).

#### 4.5.1.8. Number of leaves – at harvest

With regard to number of leaves, the plants treated with foliar spray of azoxystrobin (0.15 %) registered maximum number of leaves (78.00) which was on par with the treatment foliar spray of mancozeb in cow dung supernatant (0.4 %) (75.93), tebuconazole (0.1 %) (73.67), fish amino acid (5 %) (69.07) and *P*. *fluorescens* (2 %) (68.93) and the soil solarization along with absolute control given the minimum number of leaves (61.40) (Table 15).

### 4.5.1.9. *Root length* (*cm*)

Effect of soil solarization along with other treatments on root length was found to be statistically non significant (Table 15). The plants treated with foliar spray of azoxystrobin (0.15 %) registered the maximum root length (13.10), where as absolute control registered the minimum root length (11.10).

#### 4.5.1.10. *Root weight (kg/ha)*

There was no significant difference among the treatments with respect to root weight (Table 15). The plants treated with foliar spray of mancozeb in cow dung supernatant (0.4 %) shown maximum root weight of 6633.33, whereas absolute control shown minimum root weight of 5733.33.

#### 4.5.1.11. Fresh weight (kg/ha)

Maximum fresh weight (38.038) was noticed among the plants treated with foliar spray of azoxystrobin (0.15 %) which was on par with the treatment tebuconazole (0.1 %) (26208.33), *Pseudomonas fluorescens* (2 %) (24491.67), mancozeb in cow dung supernatant (0.4 %) (24450.00), *Trichoderma harzianum* (2 %) (24041.67) and copper hydroxide (0.15 %) (23583.33). The minimum fresh weight (19541.67) was observed among the absolute control (Table 16).

#### 4.5.1.12. Dry weight (kg/ha)

The highest dry weight of shoot (4233.33) was registered with the plants treated with foliar spray of azoxystrobin (0.15 %), followed by the treatment foliar spray of mancozeb in cow dung supernatant (0.4 %) (3916.66) and foliar spray of tebuconazole (0.1 %) (3879.16) and were on par with each other. The dry weight was minimum (2966.66) in absolute control (Table 16).

# 4.5.1.13. Incidence of other diseases and pests

Statistical analysis of the data on incidence of leaf eating caterpillar and leaf webber was found to be non significant (Table 24) (Plate 29). The maximum incidence of leaf eating caterpillar was observed with the treatment turmeric powder and baking soda combination (10:1) (5.33) or soil application of ASM (75 ppm) (5.33) and the minimum incidence was observed with foliar spray of copper hydroxide (0.15 %) (0.67 %). The maximum incidence of leaf webber was recorded with the treatment turmeric powder and baking soda combination (10:1) (1.66) and minimum was observed in treatment soil solarization along with mancozeb in cow dung supernatant (0.4 %) (0 %), copper hydroxide (0.15 %) (0 %) and *Trichoderma harzianum* (2 %) (0 %).

Sl.No	Treatments	Number of days to flowering after transplanting*
1	Pseudomonas fluorescens (2 %)	35.00
2	Trichoderma harzianum (2 %)	31.67
3	Fish aminoacid (5 %)	35.00
4	ASM soil application (75 ppm)	29.33
5	ASM foliar spray (100 ppm)	28.67
6	Turmeric powder-baking soda combination (10:1)	33.33
7	Azoxystrobin (0.15 %)	30.00
8	Copper hydroxide (0.15 %)	31.67
9	Tebuconazole (0.1 %)	31.67
10	Mancozeb in cow dung supernatant (0.4 %)	30.00
11	Absolute control	31.67
		NS

Table 13. Number of days taken for the flowering of amaranthus in solarized plots

Mean of three replications\*

Table 14. Effect of soil solarization, biocontrol agents, chemical activator, indigenous formulations and new generation fungicides on the plant height and number of leaves of amaranthus 30 days after transplanting (DAT).

Treatments	Plant height* (cm)	% increase over control	No. of leaves / plant*	% increase over control
Pseudomonas fluorescens (2 %)	37.60	1.62	24.73	0.68
Trichoderma harzianum (2%)	37.93	2.52	27.93	13.71
Fish amino acid (5 %)	39.53	6.85	36.00	46.54
ASM soil application (75 ppm)	37.93	2.52	34.93	42.20
ASM foliar spray (100 ppm)	42.60	15.14	37.67	53.33
Turmeric powder-baking soda combination (10:1)	42.00	13.51	29.00	18.05
Azoxystrobin (0.15 %)	40.66	9.91	36.93	50.34
Copper hydroxide (0.15 %)	42.13	13.87	31.60	28.63
Tebuconazole (0.1 %)	42.06	13.69	30.20	22.93
Mancozeb in cow dung supernatant (0.4 %)	45.93	24.14	30.93	25.92
Absolute control	37.00		24.56	
CD (0.05)	NS		NS	

Mean of three replications\*

Table 15. Effect of soil solarization, biocontrol agents, chemical activator, indigenous formulations and new generation fungicides on the plant height, number of leaves, root weight and root length of amaranthus at harvest (60 DAT).

Treatments	Plant height (cm)*	% Variation over control	No. of leaves / plant*	% Variation over control	Root weight (Kg/ha)*	% Variatio n over control	Root length (cm)*	% Variation over control
Pseudomonas fluorescens (2 %)	110.43 <sup>d</sup>	1.19	68.93 <sup>abcd</sup>	12.27	5916.67	3.20	23.23	4.65
Trichoderma harzianum (2%)	110.03 <sup>d</sup>	0.82	66.87 <sup>bcd</sup>	8.90	5816.67	1.45	22.53	1.50
Fish amino acid (5 %)	116.41 <sup>bcd</sup>	6.67	69.07 <sup>abcd</sup>	12.49	5883.33	2.62	25.56	15.16
ASM soil application (75 ppm)	116.36 <sup>bcd</sup>	6.63	63.13 <sup>d</sup>	2.82	6283.33	9.59	25.43	14.56
ASM foliar spray (100 ppm)	114.23 <sup>cd</sup>	4.68	66.33 <sup>bcd</sup>	8.03	5933.33	3.49	23.83	7.35
Turmeric powder-baking soda combination (10:1)	120.36 <sup>abc</sup>	10.30	65.93 <sup>cd</sup>	7.38	5816.67	1.45	24.10	8.56
Azoxystrobin (0.15 %)	123.73 <sup>ab</sup>	13.38	$78.00^{a}$	27.04	5933.33	3.49	26.20	18.11
Copper hydroxide (0.15 %)	120.13 <sup>abc</sup>	10.08	63.67 <sup>d</sup>	3.69	5966.66	4.07	25.40	14.41
Tebuconazole (0.1 %)	124.46 <sup>ab</sup>	14.05	73.67 <sup>abc</sup>	19.98	6083.33	6.10	24.80	11.71
Mancozeb in cow dung supernatant (0.4 %)	127.07 <sup>a</sup>	16.44	75.93 <sup>ab</sup>	23.67	6633.33	15.70	22.83	2.85
Absolute control	109.13 <sup>d</sup>		61.40 <sup>d</sup>		5733.33		22.20	
CD (0.05)	9.116		9.884		NS		NS	

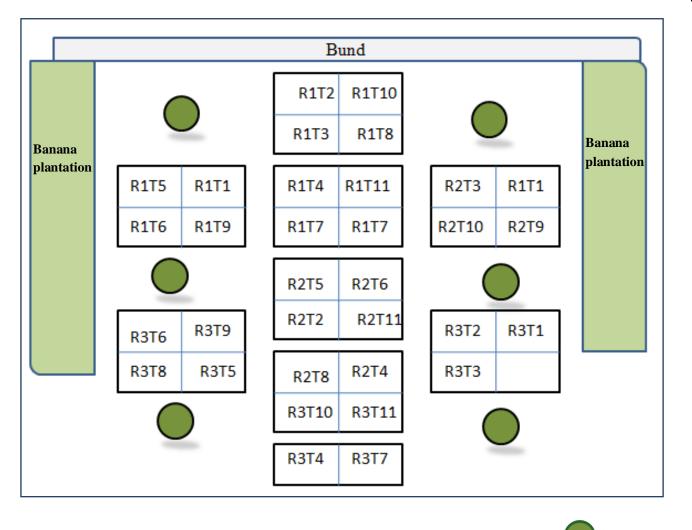
Mean of three replications\*, treatments with the same alphabets in the superscript, do not differ significantly.

Table 16. Effect of soil solarization, biocontrol agents, chemical activator, indigenous formulations and new generation fungicides on the yield of amaranthus.

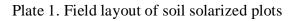
Treatments	Fresh weight Kg / ha *	% Variation over control	Dry weight Kg / ha*	% Variation over control
Pseudomonas fluorescens (2 %)	24491.67 abc	25.33	3233.33 <sup>d</sup>	8.99
Trichoderma harzianum (2 %)	24041.67 abc	23.03	3241.66 <sup>cd</sup>	9.27
Fish amino acid (5 %)	22916.67 bcd	17.27	3212.50 <sup>d</sup>	8.29
ASM soil application (75 ppm)	22416.67 <sup>cd</sup>	14.71	3225.00 <sup>d</sup>	8.71
ASM foliar spray (100 ppm)	23250.00 <sup>bc</sup>	18.98	3175.00 <sup>d</sup>	7.02
Turmeric powder-baking soda combination (10:1)	23183.33 <sup>bc</sup>	18.64	3475.00 bcd	17.13
Azoxystrobin (0.15 %)	26975.00 <sup>a</sup>	38.04	4233.33 <sup>a</sup>	42.70
Copper hydroxide (0.15 %)	23583.33 abc	20.68	3533.33 <sup>bcd</sup>	19.10
Tebuconazole (0.1 %)	26208.33 <sup>ab</sup>	34.12	3879.16 <sup>abc</sup>	30.76
Mancozeb in cow dung supernatant (0.4 %)	24450.00 <sup>abc</sup>	25.12	3916.66 <sup>ab</sup>	32.02
Absolute control	19541.67 <sup>d</sup>		2966.66 <sup>d</sup>	
CD (0.05)	3483.542		644.345	

Mean of three replications\*, treatments with the same alphabets in the superscript, do not differ significantly.





- Coconut plants



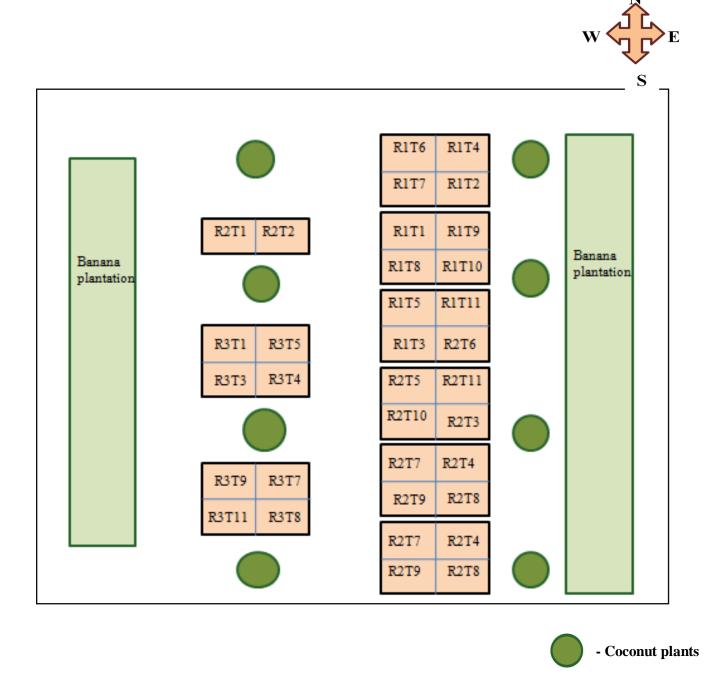


Plate 2. Field layout of non solarized plots

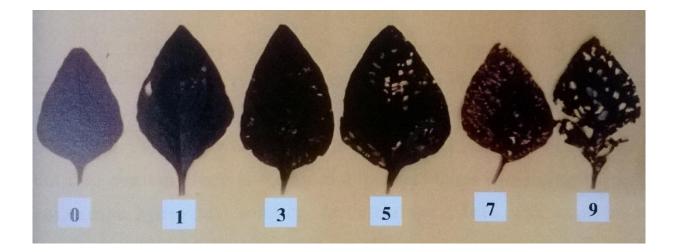


Plate 3. The scale of 0-9 for grading disease severity



Vellayani – RS1

Kalliyoor - RS2



Venganoor - RS3



Kakkamoola - RS4

Plate 4. Field samples of amaranthus showing leaf blight symptoms.

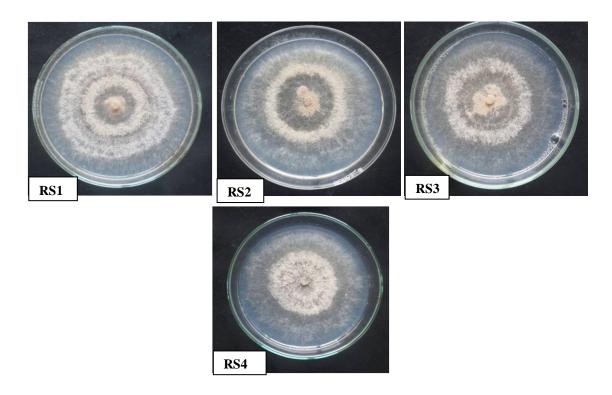


Plate 5. Growth of different isolates of Rhizoctonia solani on PDA.

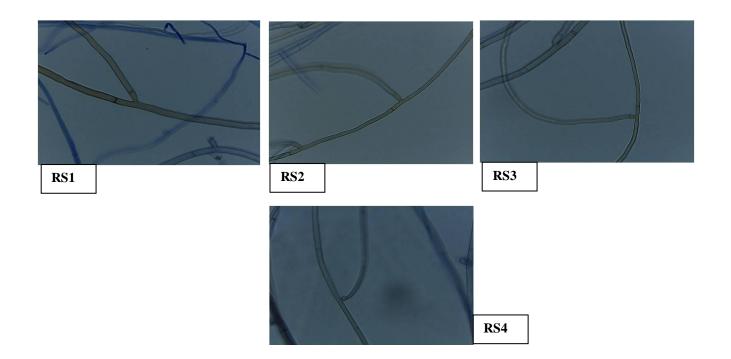


Plate 6. Hyphae of *Rhizoctonia solani* showing right angle branching in 5-day-old culture on PDA.

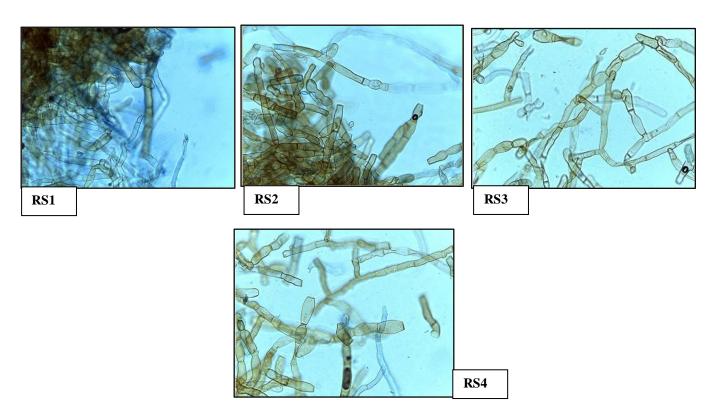
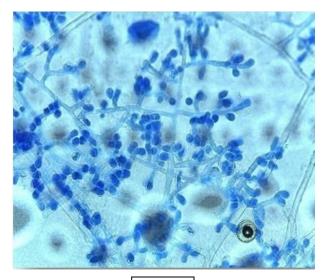


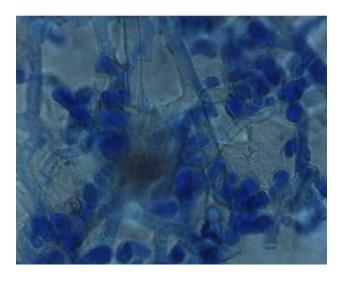
Plate 7. Monilioid cells observed in 9 days old culture forming sclerotia.



Hymenium layer (under surface)



10 X



40 X

Plate 8. Teleomorph of R. solani – Thanatephorus cucumeris



Plate 9. Pathogenicity test of RS1 isolate of *R. solani* on amaranthus seedlings.



Plate 10. Pathogenicity test of RS2 isolate of R. solani on amaranthus seedlings

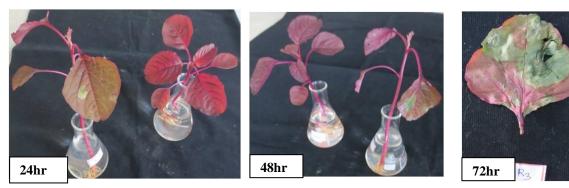


Plate 11. Pathogenicity test of RS3 isolate of R. solani on amaranthus seedlings

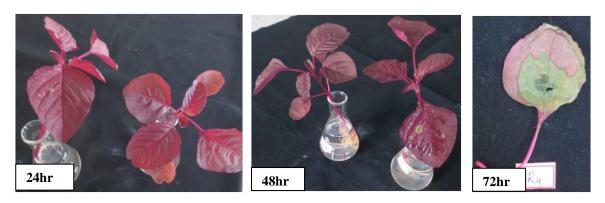


Plate 12. Pathogenicity test of RS4 isolate of R. solani on amaranthus seedlings

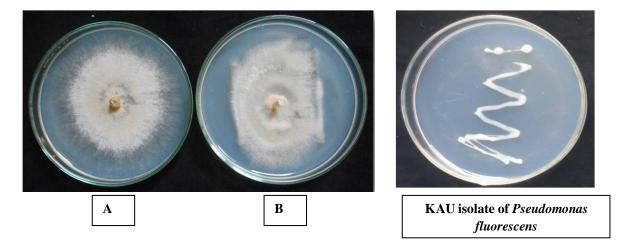


Plate 13. Biological control of R. Solani by Pseudomonas fluorescens.A. ControlB. Dual culture

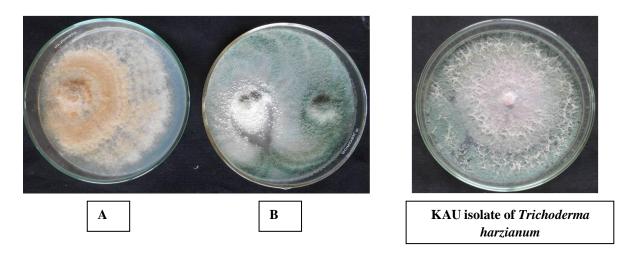


Plate 14. Biological control of Rhizoctonia solani by Trichoderma harzianum

A. Control B. Dual culture

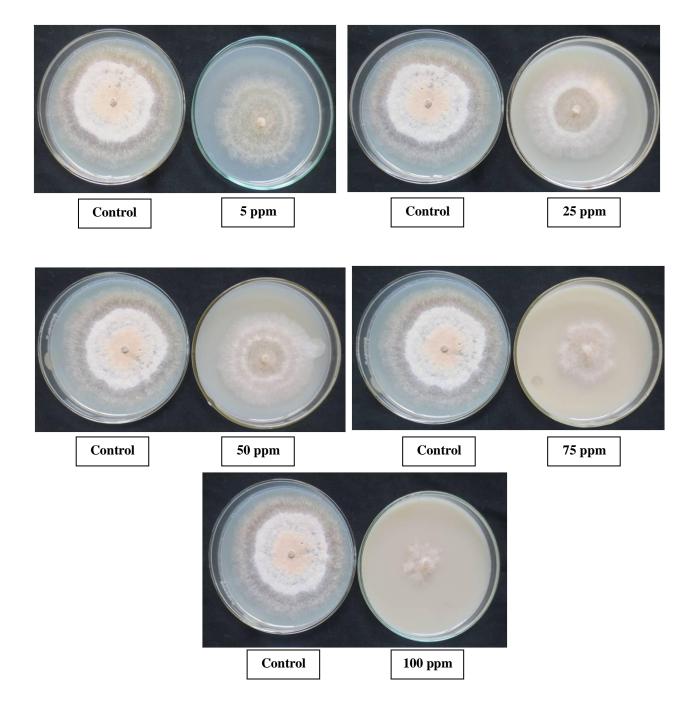


Plate 15. Effect of Acibenzolar -S -methyl on the growth of R. solani

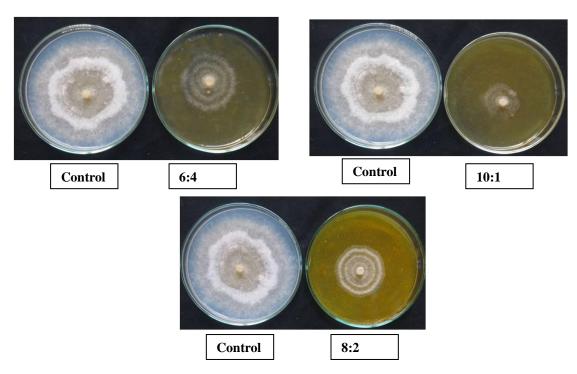


Plate 16. Effect of turmeric powder-baking soda combination on the growth of *R. solani* 

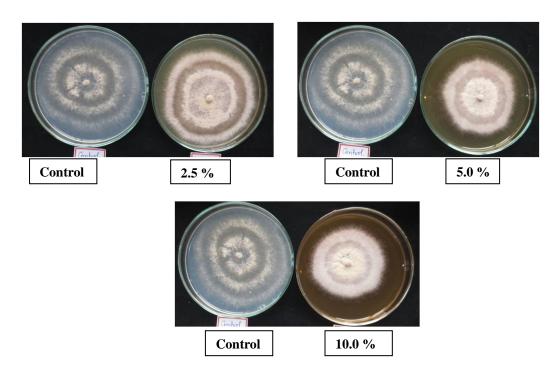


Plate 17. Effect of fish amino acid on the growth of R. solani

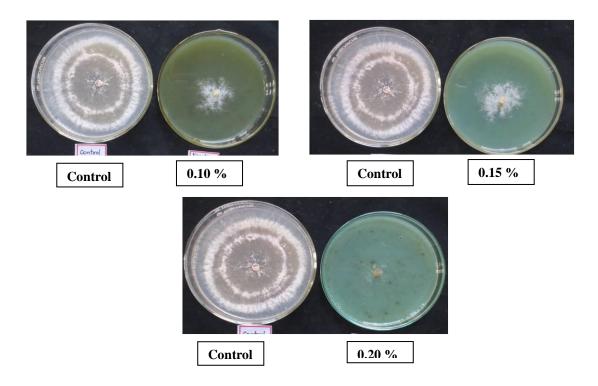


Plate 18. Effect of Copper hydroxide on the growth of R. solani

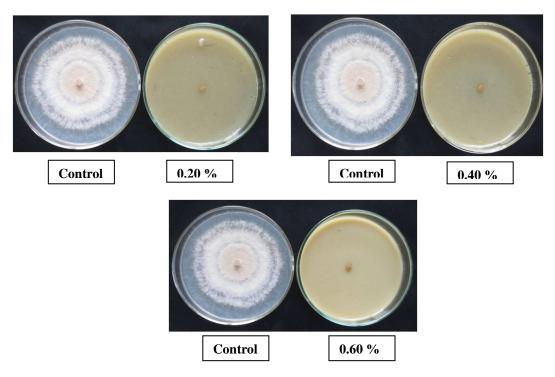


Plate 19. Effect of mancozeb in cow dung supernatant on the growth of R. solani

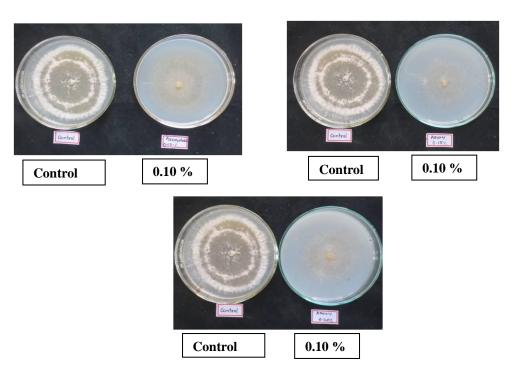


Plate 20. Effect of azoxystrobin on the growth of R. solani

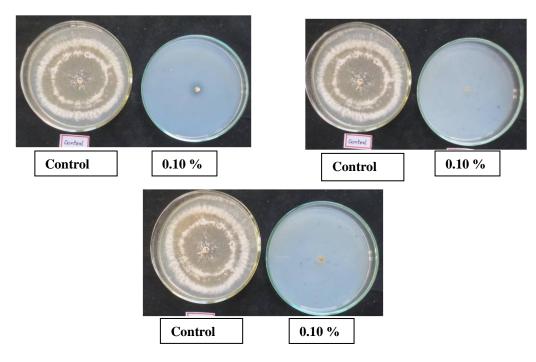


Plate 21. Effect of tebuconazole on the growth of R. solani

## 4.5.2. In vivo suppression of Rhizoctonia leaf blight of amaranthus

The effect of biocontrol agents, chemical activator, indigenous organic formulations and new generation fungicides on the suppression of Rhizoctonia leaf blight of amaranthus was studied under field condition with 11 treatments (Plate 26). All the treatments were given to the plants after the natural infection by the pathogen.

## **4.5.2.1.** Number of days for the appearance of the leaf blight disease

None of the treatments differed significantly from each other with respect to number of days for the appearance of the leaf blight disease (Table 17) (Plate 27). The maximum days was taken by the *P. fluorescens* (2 %) plots with 14 days and minimum days were taken by the plots allotted for the treatment application of ASM (75 ppm) and *T. harzianum* (2 %).

# 4.5.2.2. Disease incidence

There was no significant difference among the treatments with respect to disease incidence (Table 18). Plants treated with foliar application of ASM registered minimum disease incidence (37.06 %) where as turmeric powder and baking soda combination (10:1) registered the maximum disease incidence (45.57 %).

#### 4.5.2.3. Disease severity

The data on disease index revealed that all the treatments were significantly superior to the absolute control. The lowest disease index (42.93) with the percentage inhibition of 44.18 was recorded by the plants treated with mancozeb in cow dung supernatant (0.4 %) which was on par with the treatment tebuconazole (0.10 %) with the disease index of 44.03 and the percentage inhibition of 42.75. The

Trt.No	Treatments	Number of days for first appearance of leaf blight disease*
1	Pseudomonas fluorescens	14.00
2	Trichoderma harzianum	13.00
3	Fish aminoacid	13.33
4	ASM soil application	13.00
5	ASM foliar spray	13.67
6	Turmeric powder-baking soda combination	13.33
7	Azoxystrobin	13.67
8	Copper hydroxide	13.33
9	Tebuconazole	13.67
10	Mancozeb in cow dung supernatant	13.67
11	Absolute control	13.33
		NS

Table 17. Number of days for the appearance of the leaf blight disease in non solarized plots.

Mean of three replications\*

Table	18.	Effect	of	biocontrol	agents,	chemical	activator,	indigenous	formulations	and	new
	gene	eration f	ùng	icides on th	e foliar l	blight disea	ase inciden	ce in amaran	thus.		

Treatment No.	Treatments	Mean disease incidence*	Percent reduction over control
1	Pseudomonas fluorescens (2 %)	43.78	4.73
2	Trichoderma harzianum (2%)	41.92	8.78
3	Fish amino acid (5 %)	43.23	5.93
4	ASM soil application (75 ppm)	38.84	15.48
5	ASM foliar spray (100 ppm)	37.06	19.35
6	Turmeric powder-baking soda combination (10:1)	45.57	0.83
7	Azoxystrobin (0.15 %)	44.80	2.51
8	Copper hydroxide (0.15 %)	43.44	5.47
9	Tebuconazole (0.1 %)	39.22	14.66
10	Mancozeb in cow dung supernatant (0.4 %)	40.16	12.61
11	Absolute control	45.95	
		NS	

Mean of three replications\*

Treatments	*]	Percentage D	visease Index		*Percentage disease suppression			
Treatments	30 DAT	40 DAT	50 DAT	60 DAT	30 DAT	40 DAT	50 DAT	60 DAT
Pseudomonas fluorescens	31.76	41.60	51.66	57.20	33.73	34.74	27.09	25.62
(2 %)	$(34.30)^{cd}$	(40.17)e	$(45.95)^{d}$	$(49.14)^{\rm e}$				
Trichoderma harzianum	33.04	45.87	55.06	59.80	31.07	28.04	22.29	22.24
(2%)	$(34.91)^{cd}$	$(42.63)^{d}$	$(47.91)^{cd}$	$(50.66)^{\rm e}$				
Fish amino acid (5	37.66	62.84	63.59	73.06	21.42	1.41	10.25	5.00
%)	$(37.81)^{bcd}$	(52.45)a	$(52.89)^{b}$	$(58.74)^{b}$				
ASM soil application (75	32.38	49.51	55.80	65.88	32.43	22.33	21.25	14.35
ppm)	$(34.62)^{cd}$	$(44.72)^{cd}$	$(48.33)^{cd}$	$(54.28)^{d}$				
ASM foliar spray (100	34.46	54.18	57.96	68.68	28.11	15.00	18.20	10.70
ppm)	$(35.83)^{bcd}$	$(47.40)^{b}$	$(49.58)^{c}$	$(55.97)^{cd}$				
Turmeric powder-baking	40.62 (39.59) <sup>abc</sup>	63.06	66.54	72.03	15.25	1.07	6.08	6.34
soda combination (10:1)	(39.39)	$(52.57)^{a}$	(54.66) <sup>ab</sup>	(58.07) <sup>bc</sup>				
Azoxystrobin (0.15	39.71	50.92	55.95	61.12	17.15	20.11	21.03	20.53
%)	$(39.01)^{abc}$	$(45.53)^{bc}$	$(48.43)^{cd}$	$(51.44)^{\rm e}$				
Copper hydroxide	42.50	62.09	64.48	66.30	11.34	2.59	9.00	13.79
(0.15 %)	$(40.68)^{ab}$	$(52.00)^{a}$	$(53.43)^{b}$	$(54.51)^{d}$				
Tebuconazole	29.39	36.17	39.28	44.03	38.68	43.25	44.56	42.75
(0.1 %)	$(32.78)^{d}$	(36.959) <sup>f</sup>	$(38.80)^{\rm e}$	$(41.56)^{\rm f}$				
Mancozeb in cow dung	31.56	40.70	42.98	42.93	34.16	36.15	39.34	44.18
supernatant (0.4 %)	$(34.14)^{cd}$	$(39.95)^{\rm f}$	$(40.96)^{\rm e}$	$(40.93)^{\rm f}$				
Absolute control	47.93	63.74	70.85	76.91				
	$(43.77)^{a}$	$(52.98)^{a}$	$(57.33)^{a}$	$(61.31)^{a}$				
CD (0.05)	5.490	2.306	2.928	2.482				

Table 19. Effect of biocontrol agents, chemical activator, indigenous formulations and new generation fungicides on the leaf blight disease severity in amaranthus.

\*mean of three replications, values in the parenthesis are arc sin transformed, Treatments with same alphabets in the superscript do not differ significantly. absolute control recorded the maximum disease index of 76.91 and differed significantly from all other treatments (Table 19) (Plate 28).

# 4.5.2.4. Days to flowering

There was no significant difference among the treatments with respect to number of days taken for floweing (Table 20). Maximum days of 31.67 were taken for the flowering by the plants treated with Fish amino acid, azoxystrobin, copper hydroxide, mancozeb in cow dung supernatant and absolute control. Soil application of ASM recorded the minimum number of days taken for flowering (27.67).

# 4.5.2.5. Height of the plant (cm)- 30 DAT

Effect of different treatments on height of the plant was statistically found to be non significant (Table 21). The maximum height (40.46) was recorded by the plants treated with foliar application of ASM (100 ppm) and minimum plant height (36.87) was recorded by the absolute control.

# 4.5.2.6. Number of leaves – 30DAT

With regard to the number of leaves, all the treatments were found to be non significant (Table 21). The maximum number of leaves per plant (25.43) was registered by the plants treated with azoxystrobin, whereas minimum number of leaves per plant (20.47) was registered by absolute control.

# 4.5.2.7. Height of the plant (cm) – at harvest

Maximum plant height (116.77) was recorded in case of plants treated with mancozeb in cow dung supernatant (0.40 %) which was significantly superior than other treatments. The absolute control plants recorded the minimum plant height (99.25) (Table 22).

#### 4.5.2.8. Number of leaves - at harvest

The plants treated with azoxystrobin (0.15 %) recorded the highest number of leaves (67.67) followed by Tebuconazole (0.1 %) (64.13), ASM foliar spray (100 ppm) (63.73) and mancozeb in cowdung supernatant (0.4 %) (61.80) which are on par with the superior treatment azoxystrobin (0.15 %) and differed significantly from all other treatments. However plants with absolute control recorded the minimum number of leaves (57.73) (Table 22).

# **4.5.2.9.** *Root length* (*cm*)

No significant difference was observed among the treatments with regard to root length (Table 22). The longest roots were recorded by the plants treated with tebuconazole (0.10 %) and the minimum root length was observed by the plants with absolute control (10.86).

# 4.5.2.10. *Root weight (kg/ha)*

The plants treated with tebuconazole (0.10 %) recorded the highest root weight (4750.00), where as lowest root weight was recorded by the absolute control (3054.00) and all the treatments were significantly not different from each other (Table 22).

# 4.5.2.11. Fresh weight (kg/ha)

The maximum fresh weight (23375.00) was observed among the plants treated with tebuconazole (0.10 %) which was on par with the treatments mancozeb in cowdung supernatant (0.4 %) (22816.67), azoxystrobin (0.15 %) (22625.00), *Pseudomonas fluorescens* (2 %) (21791.67), *Trichoderma harzianum* (2 %) (21416.67), fish amino acid (5 %) (20800.00) and turmeric powder-baking soda

combination (10:1) (20500.00). The minimum fresh weight was observed in plants with absolute control (17500.00) (Table 23).

## 4.5.2.12. Dry weight (kg)

The highest dry weight of shoot (3501.67) was observed with the plants treated with azoxystrobin (0.15 %) followed by treatment with either mancozeb in cowdung supernatant (0.4 %) (3491.67) or tebuconazole (0.10 %) (3362.50) and were on par with each other. The minimum dry weight was observed among the plants with absolute control (2537.50) (Table 23).

# 4.5.2.13. Incidence of other diseases and pests

The data on statistical analysis of incidence of leaf eating caterpillar and leaf webber was found to be non significant (Table 24). The maximum incidence of leaf eating caterpillar was observed with the treatment turmeric and baking soda combination (10:1) (3.66) and the minimum or no incidence was observed with the treatment tebuconazole (0.10 %) (0.00). The maximum incidence of leaf webber was recorded with the treatment absolute control (2.0) where as minimum or no incidence was recorded by the treatment azoxystrobin (0.15 %), mancozeb in cowdung supernatant (0.4 %), tebuconazole (0.10 %) and copper hydroxide (0.15 %) (Plate 29).

Table 20. Number of days taken for the flowering of amaranthus in non solarized plots

Sl.No	Treatments	Number of days to flowering after transplanting
1	Pseudomonas fluorescens (2 %)	31.00
2	Trichoderma harzianum (2 %)	30.00
3	Fish aminoacid (5 %)	31.67
4	ASM soil application (75 ppm)	27.67
5	ASM foliar spray (100 ppm)	28.33
6	Turmeric powder-baking soda combination (10:1)	30.00
7	Azoxystrobin (0.15 %)	31.67
8	Copper hydroxide (0.15 %)	31.67
9	Tebuconazole (0.1 %)	31.00
10	Mancozeb in cow dung supernatant (0.4 %)	31.67
11	Absolute control	31.67
		NS

Mean of three replications\*

Table 21. Effect of biocontrol agents, chemical activator, indigenous formulations and new generation fungicides on the plant height and number of leaves of amaranthus 30 days after transplanting (DAT).

Treatments	Plant height* (cm)	% increase over control	No. of leaves / plant*	% increase over control
Pseudomonas fluorescens (2 %)	37.80	2.53	23.93	16.94
Trichoderma harzianum (2%)	39.13	6.15	22.00	7.50
Fish amino acid (5 %)	37.00	0.36	21.26	3.91
ASM soil application (75 ppm)	37.60	1.99	21.93	7.17
ASM foliar spray (100 ppm)	40.46	9.77	24.93	21.83
Turmeric powder-baking soda combination (10:1)	37.60	1.99	22.67	10.75
Azoxystrobin (0.15 %)	38.96	5.70	25.43	24.27
Copper hydroxide (0.15 %)	39.83	8.05	23.10	12.87
Tebuconazole (0.1 %)	40.27	9.22	23.87	16.61
Mancozeb in cow dung supernatant (0.4 %)	37.93	2.89	23.13	13.03
Absolute control	36.87		20.47	
CD (0.05)	NS		NS	

Mean of three replications\*

Table 22. Effect of biocontrol agents, chemical activator, indigenous formulations and new generation fungicides on the plant height, number of leaves, root weight and root length of amaranthus at harvest (60 DAT).

Treatments	Plant height (cm)*	% Variation over control	No. of leaves / plant*	% Variation over control	Root weight (Kg/ha)*	% Variatio n over control	Root length (cm)*	% Variation over control
Pseudomonas fluorescens (2 %)	104.70 <sup>bc</sup>	5.50	59.17 <sup>cd</sup>	2.48	3466.67	13.51	22.06	1.59
Trichoderma harzianum (2%)	113.75 <sup>ab</sup>	14.61	59.27 <sup>cd</sup>	2.66	3433.33	12.42	22.98	5.80
Fish amino acid (5 %)	104.33 <sup>bc</sup>	5.12	62.27 <sup>bcd</sup>	7.85	3116.67	2.05	24.47	12.64
ASM soil application (75 ppm)	113.67 <sup>ab</sup>	14.52	61.60 <sup>bcd</sup>	6.70	3533.33	15.70	25.20	16.02
ASM foliar spray (100 ppm)	107.03 <sup>abc</sup>	7.85	63.73 <sup>abc</sup>	10.39	3716.67	21.70	22.46	3.43
Turmeric powder-baking soda combination (10:1)	105.07 <sup>bc</sup>	5.86	59.13	2.42	3625.00	18.70	21.96	1.13
Azoxystrobin (0.15 %)	117.60 <sup>a</sup>	18.49	67.67 <sup>a</sup>	17.21	4616.67	51.17	24.00	10.50
Copper hydroxide (0.15 %)	109.93 <sup>abc</sup>	10.77	57.93 <sup>d</sup>	0.34	3333.33	9.15	23.91	10.09
Tebuconazole (0.1 %)	114.4 <sup>ab</sup>	15.30	64.13 <sup>ab</sup>	11.09	4750.00	55.53	25.27	16.32
Mancozeb in cow dung supernatant (0.4 %)	116.77 <sup>a</sup>	17.65	61.80 <sup>abc</sup>	7.04	4500.00	47.35	23.37	7.58
Absolute control	99.25 <sup>°</sup>		57.73 <sup>d</sup>		3054.00		21.72	
CD (0.05)	11.099		4.785		NS		NS	

Mean of three replications\*, treatments with the same alphabets in the superscript, do not differ significantly.

Treatments	Fresh weight Kg / ha *	% Variation over control	Dry weight Kg / ha*	% Variation over control
Pseudomonas fluorescens (2 %)	21791.67 abc	24.52	2851.67 <sup>cd</sup>	12.38
Trichoderma harzianum (2 %)	21416.67 <sup>abc</sup>	22.38	2906.67 <sup>cd</sup>	14.55
Fish amino acid (5 %)	20800.00 <sup>abc</sup>	18.85	2894.17 <sup>cd</sup>	14.06
ASM soil application (75 ppm)	19891.67 bcd	13.66	2725.00 <sup>d</sup>	7.39
ASM foliar spray (100 ppm)	19958.33 bcd	14.04	2716.67 <sup>d</sup>	7.06
Turmeric powder-baking soda combination (10:1)	20500.00 abc	17.14	2961.67 bcd	16.72
Azoxystrobin (0.15 %)	22625.00 <sup>ab</sup>	29.28	3501.67 <sup>a</sup>	37.99
Copper hydroxide (0.15 %)	18941.67 <sup>cd</sup>	8.23	2860.83 <sup>cd</sup>	12.74
Tebuconazole (0.1 %)	23375.00 <sup>a</sup>	33.57	3362.50 <sup>abc</sup>	32.51
Mancozeb in cow dung supernatant (0.4 %)	22816.67 <sup>ab</sup>	30.38	3491.67 <sup>ab</sup>	37.60
Absolute control	17500.00 <sup>d</sup>		2537.50 <sup>d</sup>	
CD (0.05)	2950.834		534.514	

Table 23. Effect of biocontrol agents, chemical activator, indigenous formulations and new generation fungicides on the yield of amaranthus.

Mean of three replications\*, treatments with the same alphabets in the superscript, do not differ significantly.

	Solarize	ed plot*	Nonsola	rized plot*
Treatments	Leaf eating caterpillar	Leaf webber	Leaf eating caterpillar	Leaf webber
Pseudomonas fluorescens (2 %)	3.00	0.33	1.33	0.33
Trichoderma harzianum (2%)	3.33	0.00	3.33	1.33
Fish amino acid (5 %)	4.00	1.33	2.00	1.00
ASM soil application (75 ppm)	1.33	0.33	1.67	1.00
ASM foliar spray (100 ppm)	5.33	1.33	3.00	1.33
Turmeric powder-baking soda combination (10:1)	5.33	1.67	3.67	1.00
Azoxystrobin (0.15 %)	1.67	0.33	2.67	0.00
Copper hydroxide (0.15 %)	0.67	0.00	0.67	0.00
Tebuconazole (0.1 %)	2.00	0.33	0.00	0.00
Mancozeb in cow dung supernatant (0.4 %)	1.00	0.00	2.00	0.00
Absolute control	4.67	1.33	3.33	2.00
CD (0.05)	NS	NS	NS	NS

Table 24. Incidence of other pest and diseases.

\*Mean of three replications





Plate 22. Land preparation, soil solarization and transplanting



Plate 23. First appearance of the leaf blight disease in solarized plots



Plate 24. Stunting effect of ASM on amaranthus



Plate 25. Field view of solarized plot



Plate 26. Land preparation and transplanting



Plate 27. Number of days taken for first appearance of leaf blight disease



Plate 28. Field view of non solarized plot



Plate 29. Incidence of leaf webber and leaf eating caterpillar

# 4.6. EFFECT OF SOIL SOLARIZATION ON THE FOLIAR BLIGHT DISEASE SEVERITY AND YIELD IN AMARANTHUS.

#### **4.6.1.** Effect of soil solarization on the foliar blight of amaranthus.

Foliar blight disease severity in amaranthus for soil solarized and non solarized field plots is depicted in Table 25. Foliar spray of tebuconazole (0.1 %) recorded the minimum per cent disease index 44.76 and the maximum disease index was recorded by the absolute control (62.05 %) in solarized plots. In case of non solarized plots the minimum per cent disease index of 42.93 was recorded by foliar spray of mancozeb in cow dung supernatant (0.40 %) and maximum per cent disease index of 76.91 was recorded by the absolute control. In the interaction effects, foliar spray of tebuconazole (0.10 %) recorded the minimum per cent disease index of 41.77 and the maximum per cent disease index 61.69 was recorded by the absolute control.

#### **4.6.2.** Effect of soil solarization on the yield of amaranthus.

In the interaction effect, maximum yield of 24800.00 kg/ha was recorded by foliar spray of azoxystrobin (0.15%) and the minimum yield of 18812.50 kg/ha was recorded by the absolute control (Table 26).

Trt. No.	Treatments	(A1)	(A2)	(A2-A1)
T1	Pseudomonas fluorescens (2 %)	50.77 (45.44)	57.20 (49.14)	6.43
T2	Trichoderma harzianum (2%)	56.55 (48.76)	59.80 (50.66)	3.25
T3	Fish amino acid (5 %)	71.80 (57.97)	73.06 (58.74)	1.26
T4	ASM soil application (75 ppm)	68.10 (55.62)	65.88 (54.28)	-2.22
T5	ASM foliar spray (100 ppm)	68.10 (55.61)	68.68 (55.97)	0.58
Т6	Turmeric powder- baking soda combination (10:1)	72.10 (58.14)	72.03 (58.07)	-0.07
Τ7	Azoxystrobin (0.15 %)	58.84 (50.12)	61.12 (51.44)	2.28
Т8	Copper hydroxide (0.15 %)	61.50 (51.66)	66.30 (54.51)	4.8
Т9	Tebuconazole (0.1 %)	44.76 (41.98)	44.03 (41.56)	-0.73
T10	Mancozeb in cow dung supernatant (0.4 %)	48.32 (44.03)	42.93 (40.93)	-5.39
T11	Absolute control	78.03 (62.05)	76.91 (61.31)	-1.12
	Mean	51.94	52.43	
	CD (0.5 %)	T - 2.115 A - 0.905 T x A -2.993		

Table 25. Effect of soil solarization on the foliar blight disease severity in amaranthus

\*mean of three replications, values in the parenthesis are arc sin transformed.

A1- Solarized plots

A2- Non solarized plots

Trt. No.	Treatments	(A1)*	(A2)*	A1-A2
T1	Pseudomonas fluorescens (2 %)	24491.67	21791.67	2700.00
T2	Trichoderma harzianum ( 2 % )	24041.67	21416.67	2625.00
Т3	Fish amino acid (5 %)	22916.67	19966.67	2950.00
T4	ASM soil application (75 ppm)	22416.67	18766.67	3650.00
T5	ASM foliar spray (100 ppm)	23250.00	19125.00	4125.00
T6	Turmeric powder- baking soda combination (10:1)	23183.33	21333.33	1850.00
Τ7	Azoxystrobin (0.15 %)	26975.00	22625.00	4350.00
Т8	Copper hydroxide (0.15 %)	23583.33	18941.67	4641.66
Т9	Tebuconazole (0.1 %)	25291.67	23375.00	1916.67
T10	Mancozeb in cow dung supernatant (0.4 %)	24450.00	22816.67	1633.33
T11	Absolute control	20125.00	17500.00	2625.00
	Mean			
CD (0.5 %)			T- 3020.433 A- 1287.918 T x A- 4271.545	

Table 26. Effect of soil solarization on the yield (kg/ha) of amaranthus.

\*Mean of three replications

A1- Solarized plots

A2- Non solarized plots

# **5. DISCUSSION**

Amaranthus is one the most cultivated, consumed and cheapest leafy vegetable in Kerala. It has been rediscovered as a promising food crop mainly because of its resistance to pest and diseases and high nutritional value. Rhizoctonia leaf blight of amaranthus causes reduced marketability which leads to considerable economic loss of the produce. The recommended measure was foliar spray of mancozeb in cow dung supernatant (0.4 %) at fortnight intervals mainly to lessen the severity of disease (Nayar *et al.*, 1996). In order to minimize fungicide residues on produce and also to explore alternative and ecofriendly methods of disease management, studies have been conducted.

The present study 'Integrated management of Rhizoctonia leaf blight of amaranthus' involved the use of soil solarization, biocontrol agents, chemical activator, indigenous formulations and new generation fungicides. Previous works on the management of Rhizoctonia leaf blight of amaranthus carried out in the Department of Plant pathology, College of Agriculture, Vellayani, include (Smitha, 2000) use of *Trichoderma longibrachiatum* and *Pseudomonas* spp. Pot culture experiment conducted by Priyadarsini (2003) to manage the disease using *T. harzianum, P. fluorescens,* and *Piriformospora indica* and indigenous materials like rice husk ash and turmeric baking soda combination. Nair (2005) used plant growth promoting Rhizobacteria and a chemical activator for managing the foliar blight of amaranthus.

Field samples of the infected leaves showing Rhizoctonia leaf blight in amaranthus were collected from four different locations. Initially mycelial growth of pathogen in pure culture was creamy colour and finally turns to hyaline brown in all the isolates. Mature hyphae showed right angle branching with cross septa which were found close to the branching point. Different isolates vary with their time of sclerotial formation on PDA as RS1 and RS2 isolates taken only 6 days after inoculation for sclerotial formation. With regards to growth rate of pathogen on PDA, significantly higher growth rate of 2.88, 4.48 and 8.03 cm were observed in case of RS1 isolate at 24, 48 and 72 HAI respectively compared with other isolates. Present report on the variation in the growth rate of the fungus at 28 °C in different media is in accordance with Ullstrup (1936), Ogoshi (1987) and Chen *et al.* (1990). The species of *R. solani* were distinguished from one another primarily by cultural, hyphal morphology, size of sclerotia, monilioid cells and on the basis of pathogenicity. However, strains within the species may vary in host range, virulence, survival at various depths of soil and cultural characteristics (Lal, 1985). The cultural and morphological characteristics confirmed to the description of *R. solani* given by Parmeter *et al.* (1969), Lucas (1975), Blazier and Conway (2004), Agrios (2005). Curtis (1939), Warcup and Talbot (1962), Anderson (1982), Ogoshi (1987) Currah *et al.* (1987) and Xiag *et al.* (2008) reported existence of genetically diverse group of *R. solani* while studying biology and pathology of the pathogen.

Koch's postulates were proved for the pathogenicity of different isolates of *R. solani*. All the four isolates have taken three days for the first symptom development. Progression of lesion size of RS1 isolate was maximum compared to all other isolates. Among the four isolates, RS1 therefore selected as the most virulent isolate for use in further studies. Similar type of experiments were carried out by Devaki (1991) and Akhtar *et al.* (2009) on *R. solani* infecting maize causing banded leaf and sheath blight and Henz *et al.* (2007) on the same pathogen causing rot of okra pods. Previously, similar variations of pathogenicity across distinct geographical areas were reported (Parissa *et al.*, 2007). Akhtar *et al.* (2009) has also reported that *R. solani* isolates collected from different locations of Jharkhand showed variation in their morphological characteristics. This work gives clear evidence for existence of variations in a much smaller geographical region stretching

100 km itself. This knowledge calls for the need for evolving a better and comprehensive control measure as the pathogen shows more diversity.

In the present investigation, *T. harzianum* and *P. fluorescens* were tested for their effect on the growth of *R. solani* by dual culture studies under *in vitro* conditions. *T. harzianum* found to overgrow the pathogen, initially there was a clear zone of inhibition between the paired cultures. *T. harzianum* showed per cent inhibition of 49.56 against the pathogen. *In vitro* studies of Monga (1993) and Naeimi *et al.* (2010) Showed that *T. harzianum* was excellent bicontrol agent against *R. solani*. Gandhi and Kumar (2006) reported that *T. harzianum* significantly inhibited the growth *of R. solani* up to 60 % under *in vitro* conditions. Elzerjawi (2015) conducted antagonistic test and showed that *T. harzianum* isolate was significantly reduced the mycelial diameter of *R. solani*, which reach to 16.10 mm on PDA medium in compare with *R. solani* Kuhn by *T. harzianum* was 82.21.

Antagonism of *P. fluorescens* was tested against *R. solani. P. fluorescens* showed *in vitro* antagonism of 28.3 %. Dantre *et al.* (2003) reported that fifteen strains of *P. fluorescens* showed inhibition of the mycelial growth of *R. solani* under *in vitro* condition. Effectiveness of *P. fluorescens* is related with inherent quality to produce antibiotics, hydrogen cyanide and siderophores, which are involved in suppression of plant root pathogens (Kloepper *et al.*, 1980; O'Sullivan and O'Gara, 1992) and their ability to compete with indigenous microflora, may explain their ability to colonize the rhizosphere (Weller. 1988; Mazzola and Cook, 1991). The consortium of *P. fluorescens* and *T. viride* was found superior against pre-emergence damping off, powdery mildew, fruit rot, wilt/root rot of chilli crop in addition to higher yield (Naik *et al.*, 2013).

Effect of different concentrations of ASM (Actigard) ranging from 5 ppm to 100 ppm was tested against growth of the pathogen under *in vitro* condition. It was observed that ASM significantly inhibited the growth of *R. solani*. In the present study maximum per cent mycelial inhibition of 75.67 was observed for 100 ppm concentration of ASM and minimum inhibition of 27.70 % was observed with the 5 ppm concentration of ASM. Maximum mycelial growth inhibition of *R. solani* at different concentrations of ASM was recorded with 37.5 ppm concentration of ASM (Nair and Anith, 2009). The direct inhibitory effect of ASM on mycelial growth of *R. solani* has been previously reported by Meyer *et al.* (2006).

Indigenous organic formulations such as fish amino acid and turmeric powder baking soda combination significantly inhibited the growth of the pathogen under *in vitro* condition. Turmeric powder and baking soda combination inhibited the maximum growth of the pathogen by 64.40 % and fish amino acid recorded less suppression of the pathogen by 29.00 %. According to Dhanya (2000), three levels of turmeric powder – baking soda 0.05, 0.10 and 0.15 per cent were tested against *Xanthomonas axonopodis pv dieffenbachiae* causing bacterial blight of anthurium and inhibition to a lesser extent was observed.

In the *in vitro* studies with new generation fungicides, mancozeb in cow dung supernatant (0.4 %) and tebuconazole (0.1 %) recorded the 100 % mycelial inhibition of the pathogen. Tebuconazole has shown excellent efficacy against Rhizoctonia limb rot (Brenneman *et al.*, 1991; Besler *et al.*, 1996) and Rhizoctonia pod rot (Grichar and Jaks, 1995). Rauf *et al*, (2007) reported that mancozeb (Dithane M 45) was effective at 500 ppm against *R. solani* causing black scurf disease in potato. Sheela *et al.*, 2015 reported that mancozeb (@ 0.4 % has given 76.75 % control against *R. solani* causing sheath blight in rice.

The present study was carried out to investigate the effect of soil solarization, biocontrol agents, chemical activator, indigenous organic formulations and new generation fungicides on disease severity of Rhizoctonia leaf blight, growth and yield of amaranthus under field condition.

The number of days taken for first appearance of Rhizoctonia leaf blight disease in soil solarized plot ranged from 15 to 17 days after transplanting, whereas for the non solarized plots it was ranged from 13 to 14 days after transplanting. The principle mode of action of soil solarization was direct thermal inactivation of soil borne pathogens and pests (Schrader, 2000). Stapleton and Devay (1982, 1984) reported that soil solarization has reduced the fungal population (Meron *et al.*, 1989; Gamliel and Katan, 1991) increased the beneficial Pseudomonads in the soil.

Soil solarization + soil application of ASM (75 ppm) and soil solarization+ foliar application of ASM (100 ppm) recorded the lowest disease incidence of 30.41 % and 30.42 % respectively. Among non solarized plots the highest per cent reduction of Rhizoctonia leaf blight incidence of amaranthus was recorded in plants treated with foliar spray of ASM and soil application of ASM by 19.35 and 15.48 respectively compared over the absolute control. ASM has been reported to be effective against plant diseases caused by fungal, bacterial, and viral pathogens (Métrauxs, 2001). Greenhouse experiments carried out by Pradhanang et al. (2005) in which ASM was applied as foliar spray and/or soil drench (3µg/ml) before and as foliar spray (30µg/ml) after transplanting. Results of the studies indicated that ASM significantly enhanced resistance in cultivars with moderate resistance to bacterial wilt such as Neptune and BHN 466. The leaves of pepper, Capsicum anuum L. were inoculated with Phytophthora capsici three day after treatment with ASM and resistance to Phytophthora blight disease was evaluated. Results showed that P. capsici was significantly inhibited by ASM treatment by up to 45 % in field condition (Baysal et al., 2005). According to Nair et al. (2007) and Nair and Anith, (2009) Combined application of PGPR and ASM recorded excellent control of Rhizoctonia

leaf blight disease with respect to disease incidence and disease severity of 42 and 21 % respectively.

Soil solarization + foliar spray of tebuconazole (0.1 %) was recorded the maximum percentage disease suppression of 42.64 over soil solarization + absolute control at regular intervals which was followed by the treatment soil solarization + mancozeb in cow dung supernatant (0.4 %) with percentage disease suppression of 38.08 (Fig 1). Chandran (1989) and Sainamol (1992) suggested that the soil fungal population was reduced by soil solarization. Soil solarization improved disease control due to a generation and accumulation of toxic volatiles (Rubin *et al.*, 2007). The results are in accordance with Osman and Sahab (1983), Katan (1987), Shukla and Dwivedi (2011). Grichar and Jaks (1995) reported that tebuconazole reduced Rhizoctonia pod rot from 17 to 70 % over the untreated check. Sheela *et al.* (2015) reported that among the new six fungicides tested against sheath blight disease tebuconazole (0.1%) gave superior control of disease with 20-30 per cent increase in yield. Raju *et al.* (2008) found that tebuconazole reduced disease severity of *R. solani* causing sheath blight disease. Hegde (2015) and Raji *et al.* (2016) also supported the above results with increase in yield levels.

The per cent disease index was recorded at 10 days interval from 30 DAT to 60 DAT. Mancozeb in cow dung supernatant (0.4 %) recorded the maximum per cent disease suppression of 44.08 over the absolute control, followed by teboconazole (0.1 %) with per cent suppression of 42.75 (Fig 2). Priyadarsini (2003) reported that mancozeb in cow dung supernatant (0.4 %) was effective in reducing the disease intensity of foliar blight of amaranthus. Results of the Thangasamy and Rangaswamy (1989) revealed that mancozeb showed better control for sheath blight disease. Gupta *et al.* (2000) reported that mancozeb has effectively controlled root rot disease in French bean. Rauf *et al.* (2007) reported that mancozeb was highly effective in the

control of potato black scurf disease. Sheela *et al.* (2015) reported that mancozeb @3g/l was effective in controlling Rhizoctonia leaf blight disease in amaranthus.

Accordingly, soil solarization + biocontrol agents gave good results compared with soil solarization + absolute control. Among biocontrol agents foliar spray of *P*. *fluorescens* recorded percentage disease suppression of 34.93 and also foliar spray of *T. harzianum* gave percentage disease suppression of 27.53 over absolute control of soil solarized plots. Regarding biocontrol agents in non-solarized plots, foliar spray of *P. fluorescens* (2 %) and *T. harzianum* (2 %) gave comparatively good results with the per cent disease suppression of 25.62 and 22.24 over the absolute control. De Freitas and Germida (1991) and Dantre *et al.* (2003) reported that *P. fluorescens* found to suppress *R. solani* under the field condition. The consortium of *P. fluorescens* and *T. viride* was found superior against pre-emergence damping off, powdery mildew, fruit rot, wilt/root rot of chilli crop in addition to higher yield (Naik *et al.*, 2013). The results of Sudhakar *et al.* (2013) and Taylor *et al.* (2013) are also in accordance with the above results.

According to Chet *et al.*, (1982) use of *T. harzianum* in solarized field infested with *R. solani* has been shown to improve disease control while delaying the buildup of pathogen inoculum. *Trichoderma* use different mechanisms for the control of plant pathogens which include mycoparasitism, competition for space and nutrients, secretion of antibiotics and fungal cell wall degrading enzymes (Kubicek *et al.*, 2001; Howell, 2003; Benítez *et al.*, 2004; Harman *et al.*, 2004). Prasad and Kumar (2011) evaluated three isolates of *Trichoderma* spp. against sheath blight disease of rice and reported that *T. harzianum* found to be effective in reducing the disease incidence under field condition.

The number of days taken for flowering in soil solarized plots ranged from 28.67 to 35 days where as the number of days taken for the flowering of amaranthus in non solarized plots was ranged from 27.27 to 31.67 days. Soil solarizations

enhances plant growth by improving soil structure, temperature, moisture and also improve germination and helps in the development of healthy seedlings (Schrader, 2000).

Biometrical characters such as height of the plant, number of leaves were observed at 30 and 60 DAT (Fig 3, 4). With regard to plant height soil solarization + mancozeb cow dung supernatant (0.4 %) recorded maximum per cent increase in plant height of 24.13 and 16.44 over absolute control at 30 and 60 DAT respectively. Soil solarization + azoxystrobin (0.15 %) recorded the highest per cent increase in number of leaves by 50.34 and 27.04 over the absolute control in solarized plots at 30 and 60 DAT. In the present field study of non solarized plots biometric characters were observed and the plant height and number of leaves were recorded at 30 DAT and 60 DAT. The maximum per cent increase in plant height of 9.77 and 9.22 was recorded at 30 DAT by the plants treated with foliar spray of ASM (100 ppm) and tebuconazole (0.1 %). At 60 DAT the plants treated with azoxystrobin (0.15 %) showed maximum per cent increase in plant height of 18.49 cm over the absolute Application of azoxystrobin was effective in promoting plant growth control. parameters in terms of plant height, number of leaves and yield (Devaraju et al., 2013; Kumar *et al.*, 2014)

The numbers of leaves were maximum at both 30 DAT and 60 DAT in case of azoxystrobin (0.15 %) treated plants with the per cent increase of 24.27 and 17.21 over the absolute control respectively. Increased colonization of (183-631 %) of plant roots by plant growth promoting fluorescent pseudomonads from inoculated seed also occurred in solarized soil (Stapleton and Devay, 1984). Increased growth response is observed in plants cultivated in solarized soil. This was mainly evident as increase in plant height, number of leaves, better root formation and yield. Several soil borne pathogens can be controlled by solarization. (KAU, 2011). Root weight and root length of the plants were observed in solarized plots at the time of harvest. Mancozeb in cow dung supernatant (0.4 %) recorded 15.69 increase in per cent root weight over the absolute control. In case of root length maximum per cent increase over control was observed in azoxystrobin (0.15%) by 18.11. The results of Katan (1981) and Nair *et al.* (1990) are in accordance with the above findings. The maximum root weight and root length in non solarized plots was observed in the plants treated with tebuconazole (0.1 %) with 55.53 and 16.32 per cent increase over the absolute control. Barry *et al.* (2003), Biswas (2004) and Sundravadana *et al.* (2007) found that azoxystrobin increased the yield levels by improving the biometrical traits of the plants.

Soil solarization + foliar spray of azoxystrobin @ 0.15 % recorded the highest per cent increased yield in terms of fresh weight by 38.04 and dry weight of 42.70 over the absolute control of solarized plot (Fig 5). Soil solarization is found to improve nutrient status of the soil and theirby improves yield levels (Nair *et al.*, 1990), by destruction of pathogens and nullification of toxins in soil (Katan, 1981) and production of beneficial chemicals like fulvic acid (Davis and Sorensen, 1986). Stapleton and Devay (1984) and KAU (2011) found the similar results.

The yield of the non solarized plots recorded in terms of fresh weight and dry weight during harvest of the crop (Fig 6). Maximum fresh weight was observed in case of plants treated with tebuconazole @ 0.1 % by 33.57 per cent increase over the absolute control whereas, in case dry weight, azoxystrobin (0.15 %) recorded 37.99 per cent increase over the absolute control. Sheela *et al.* (2015) evaluated six new fungicides against rice sheath blight disease and found that tebuconazole (0.1 %) given maximum control with 20-30 per cent improved yield levels. Barry *et al.* (2003) in their nine years of trials, application of azoxystrobin against the Rhizoctonia crown and root rot of sugar beet given excellent control with increased yield levels. Sundravadana *et al.* (2007) investigated the effect of azoxystrobin on *R*.

*solani* causing rice sheath blight under field conditions and the results revealed that yield levels of rice improved more than 60 % compared with that of control.

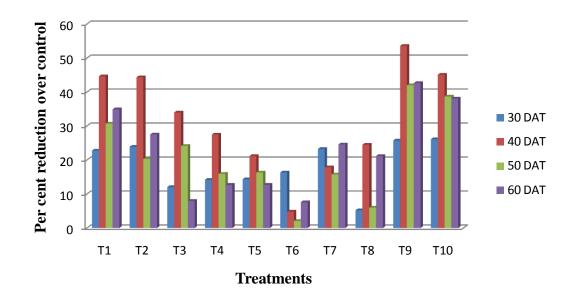


Fig:1 Effect of soil solarization along with biocontrol agents, chemical activator, indigenous formulations and new generation fungicides on the foliar blight disease severity in amaranthus.

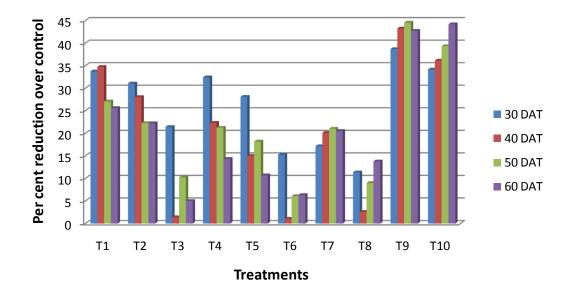


Fig:2 Effect of biocontrol agents, chemical activator, indigenous formulations and new generation fungicides on the foliar blight disease severity in amaranthus.

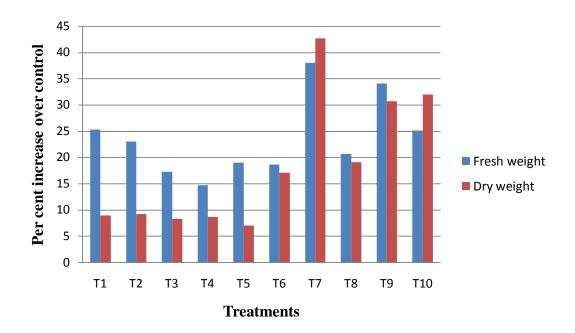


Fig:5 Effect of soil solarization along with biocontrol agents, chemical activator, indigenous formulations and new generation fungicides on the yield of amaranthus.

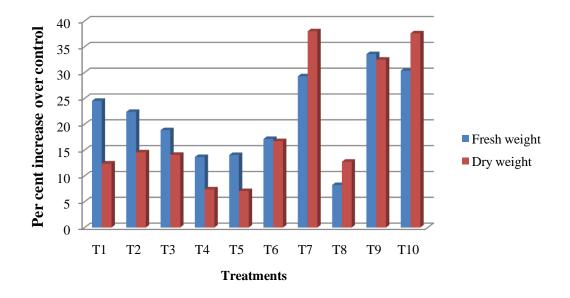
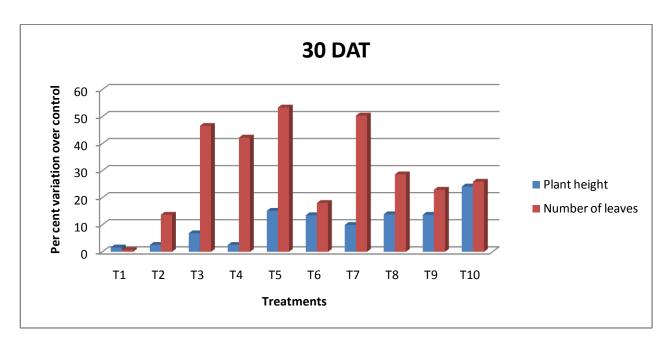


Fig: 6 Effect of biocontrol agents, chemical activator, indigenous formulations and new generation fungicides on the yield of amaranthus.



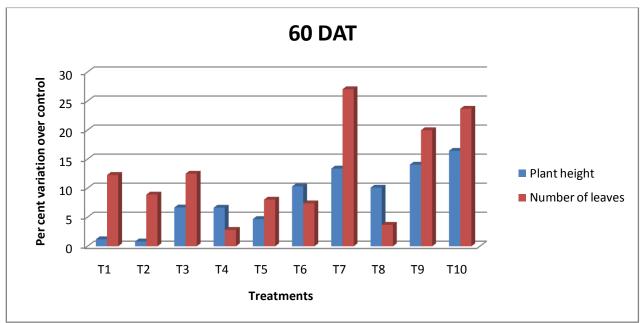
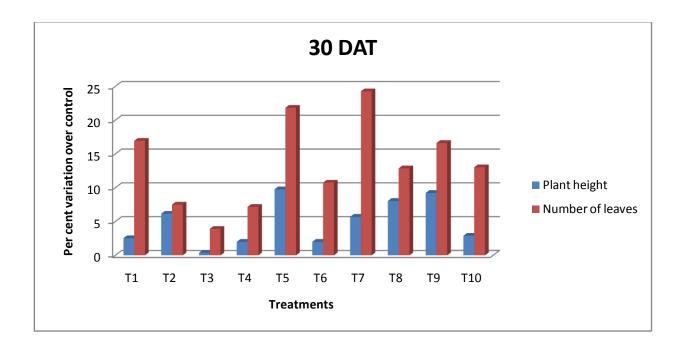


Fig: 3 Effect of soil solarization along with biocontrol agents, chemical activator, indigenous formulations and new generation fungicides on the biometric characteristics of amaranthus at 30DAT and 60DAT.



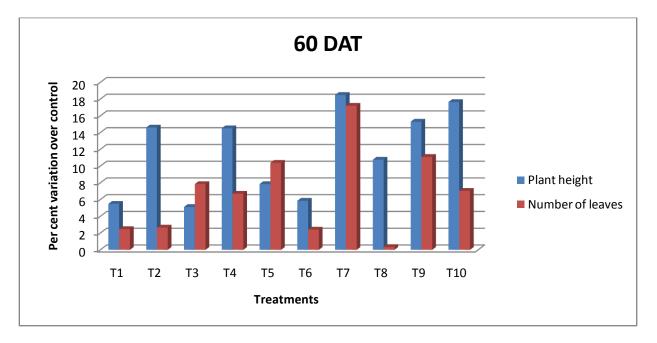


Fig: 4 Effect of biocontrol agents, chemical activator, indigenous formulations and new generation fungicides on the biometric characteristics of amaranthus at 30DAT and 60DAT.

#### 6. SUMMARY

The present study entitled "Integrated management of Rhizoctonia leaf blight of Amaranthus (*Amaranthus tricolor* L.)" was carried out in the department of Plant Pathology, College of Agriculture, Vellayani and Coconut Research Station, Balaramapuram during the year 2014-2016. The study has been conducted with the objective to investigate the effect of soil solarization, biocontrol agents, chemical activator, indigenous formulations and new generation fungicides on growth, yield and severity of foliar blight of amaranthus.

The research work includes laboratory studies and field studies. The salient results of laboratory work are summarized below.

Field samples of the infected leaves showing Rhizoctonia leaf blight in amaranthus were collected from Vellayani, Kalliyoor, Venganoor and Kakkamoola locations. Studies on isolation, purification and identification of the pathogen revealed that, initially mycelial growth of pathogen in pure culture was creamy colour and finally turns to hyaline brown in all the isolates. With regard to growth rate, among the four isolates of the pathogen, the Vellayani isolate shown significantly superior growth rate. Different isolates vary with their time of sclerotial formation on PDA as RS1 and RS2 isolates taken only 6 days after inoculation for sclerotial formation. Mature hyphae showed right angle branching with cross septa which were found close to the branching point.

Koch's postulates were proved for the pathogenicity of different isolates of *Rhizoctonia solani*. All the four isolates have taken three days for the first development of symptoms but symptoms progression of isolate RS1 was very fast compare to all other isolates. The inoculated leaves of RS1 defoliated 72 hours after first symptom development where as RS2, RS3 and RS4 isolates inoculated leaves

defoliated 96 hours after first symptom development. Re-isolation of the pathogen from the artificially inoculated amaranthus leaves yielded *R. solani* identical to the original culture. Among the four isolates, RS1 therefore selected as the most virulent isolate for use in further *in vitro* studies.

Under in vitro evaluation of biocontrol agents against R. solani, T. harzianum showed per cent inhibition by 49.56 under *in vitro* condition. Regarding the nature of reaction, T. harzianum found to overgrow the pathogen when subjected to dual culture. The KAU isolate of P. fluorescens was used in the study to test its antagonism against R. solani. P. fluorescens caused 28.3% inhibition of the pathogen under in vitro condition. Effect of different concentrations of chemical activator i.e ASM (Actigard) ranging from 5 ppm to 100 ppm was tested against growth of the pathogen under in vitro condition. It was observed that ASM significantly inhibited the growth of R. solani with maximum per cent mycelial inhibition of 75.67 was observed for 100 ppm concentration of ASM and minimum inhibition of 27.70 % was observed with the 5 ppm concentration of ASM. Indigenous organic formulations recorded significant inhibition the growth of the pathogen under in vitro condition. Turmeric powder and baking soda combination inhibited the maximum growth of the pathogen by 64.40 % and fish amino acid recorded less suppression of the pathogen by 29.00 %. In the *in vitro* studies with new generation fungicides, mancozeb in cow dung supernatant (0.4 %) and tebuconazole (0.1 %) recorded the 100 % mycelial inhibition of the pathogen.

The salient results of the field study are summarized below

The present field study was carried out to investigate the effect of soil solarization, biocontrol agents, chemical activator, indigenous organic formulations and new generation fungicides on disease severity of Rhizoctonia leaf blight, growth and yield of amaranthus under field condition.

The number of days taken for first appearance of Rhizoctonia leaf blight disease in soil solarized plot ranged from 15 to 17 days after transplanting, whereas for the non solarized plots it was ranged from 13 to 14 days after transplanting. Soil solarization along with soil application of ASM (75 ppm) and foliar application of ASM (100 ppm) recorded the lowest disease incidence of 30.41 % and 30.42 % respectively, which was superior when compared with foliar application of ASM (100 ppm) and soil application of ASM (75 ppm) with the disease incidence of 37.06 % and 38.84 %.

The per cent disease index was recorded at 10 days interval from 30 DAT to 60 DAT. Soil solarization + foliar spray of tebuconazole (0.1 %) recorded the minimum disease index of 37.85 % which was superior compared to foliar spray of tebuconazole (0.1 %) with the disease index of 39.28 %. Among the biocontrol agent's soil solarization + foliar spray of *P. fluorescens* (2 %) gave minimum disease index of 45.22 % which was greater compared to foliar spray of *P. fluorescens* (2 %) with the disease index of 51.66 %. In case of indigenous organic formulations, soil solarization + foliar spray of fish amino acid (5 %) given the maximum control of the disease with the disease index of 49.51 % which was superior to foliar spray of fish amino acid (5 %) with disease index of 63.59 %.

The number of days taken for flowering in soil solarized plots ranged from 28.67 to 35 days where as the number of days taken for the flowering of amaranthus in non solarized plots was ranged from 27.27 to 31.67 days.

Biometrical characters such as height of the plant, number of leaves were observed at the time of harvest revealed that soil solarization + mancozeb in cow dung supernatant (0.4 %) recorded maximum plant height of 127.07 cm which was higher compared to foliar spray of azoxystrobin (0.15 %) with plant height of 117.60 cm. Maximum of 78.00 number of leaves were recorded by soil solarization + foliar

spray of azoxystrobin (0.15 %) which was greater compared to foliar spray of azoxystrobin (0.15 %) with 67.67 number of leaves.

The yield of the crop recorded in terms of fresh weight and dry weight at the time of harvest of the crop. Soil solarization + foliar spray of azoxystrobin (0.15 %) gave the highest yield in terms of fresh weight by 26975.00 kg/ha and dry weight of 4233.33 kg/ha which was superior when compared with foliar spray of tebuconazole (0.1 %) with the fresh weight of 23375.00 kg/ha and dry weight of 3362.50 kg/ha.

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## **APPENDIX I**

# Composition of different media

# A) Potato dextrose agar

Potato	:	200.00 g
Dextrose	:	20.00 g
Agar	:	20.00g
Distilled water	:	1 litre

# **B) Kings B medium**

Peptone	:	20.00 g
Dipotasium hydrogen phosphate	:	1.50 g
Magnesium sulphate	:	1.50 g
Glycerol	:	10 ml
Distilled water	:	1 litre

## **APPENDIX II**

# A) Weather parameter taken during the period of field study

Month	Max. temperature	Min. temperature	Total rain (mm)	No. of rainy days
January	36.70	21.30	21.80	1
February	34.08	26.50	2.40	0
March	35.50	23.26	39.8	2
April	36.20	22.90	5.8	2
May	34.40	22.74	496.8	16

# INTEGRATED MANAGEMENT OF RHIZOCTONIA LEAF BLIGHT OF AMARANTHUS (Amaranthus tricolor L.)

GIREESH

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Abstract of the thesis

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#### ABSTRACT

The study entitled "Integrated management of Rhizoctonia leaf blight of Amaranthus (*Amaranthus tricolor* L.)" was conducted at the College of Agriculture, Vellayani and Coconut Research Station, Balaramapuram during 2014-2016 with the objective to investigate the effect of soil solarization, biocontrol agents, chemical activator, indigenous formulations and new generation fungicides on growth, yield and severity of foliar blight of amaranthus.

Samples of the infected leaves showing Rhizoctonia leaf blight in amaranthus were collected from Vellayani, Kalliyoor, Venganoor and Kakkamoola locations. Among the four isolates of the pathogen, the Vellayani isolate gave significantly superior growth rate with minimum of six days for sclerotial formation. Koch's postulates were proved for the pathogenicity of different isolates of *Rhizoctonia solani*. All the four isolates have taken three days for the first symptom development but the progression of lesion size of Vellayani isolate was maximum compared to all other isolates, hence the Vellayani isolate was selected as the most virulent isolate for use in further *in vitro* studies.

Evaluation of biocontrol agents for *in vitro* suppression of *R. solani* showed that *Trichoderma harzianum* completely overgrown the pathogen with maximum inhibition of 49.56 % compared to *Pseudomonas fluorescens* (28.30 %). Under *in vitro* evaluation of chemical activator, different concentrations of Acibenzolar-S-Methyl (ASM) against pathogen, 100 ppm concentration recorded the maximum mycelial inhibition of 75.67 % and 5 ppm concentration recorded the minimum mycelial inhibition of 27.70 %. Among indigenous organic formulations, turmeric powder and baking soda combination inhibited the maximum growth of the pathogen by 64.40 %. In the *in vitro* studies with new generation fungicides,

mancozeb in cow dung supernatant (0.4 %) and tebuconazole (0.1 %) recorded the 100 % mycelial inhibition of the pathogen.

Field studies on disease suppression and plant growth promotion was carried out as two experiments, one in soil solarized plots and the other in non solarized plots. Soil solarization along with soil application of ASM (75 ppm) and foliar application of ASM (100 ppm) recorded the lowest disease incidence of 30.41 % and 30.42 % respectively, which was superior when compared with foliar application of ASM (100 ppm) and soil application of ASM (75 ppm) with the disease incidence of 37.06 % and 38.84 %.

Soil solarization + foliar spray of tebuconazole (0.1 %) recorded the minimum disease index of 37.85 % which was superior compared to foliar spray of tebuconazole (0.1 %) with the disease index of 39.28 %. Among the biocontrol agents soil solarization + foliar spray of *Pseudomonas fluorescens* (2 %) gave minimum disease index of 45.22 % which was greater compared to foliar spray of *P. fluorescens* (2 %) with the disease index of 51.66 %. In case of indigenous organic formulations, soil solarization + foliar spray of fish amino acid (5 %) given the maximum control of the disease with the disease index of 49.51 % which was superior to foliar spray of fish amino acid (5 %) with disease index of 63.59 %.

The number of days taken for flowering in soil solarized plots ranged from 28.67 to 35 days where as the number of days taken for the flowering of amaranthus in non solarized plots was ranged from 27.27 to 31.67 days.

At the time of harvest, soil solarization + mancozeb in cow dung supernatant (0.4 %) recorded maximum plant height of 127.07 cm which was higher compared to foliar spray of azoxystrobin (0.15 %) with plant height of 117.60 cm. Maximum of 78.00 number of leaves were recorded by soil solarization + foliar spray of azoxystrobin (0.15 %) which was greater compared to foliar spray of azoxystrobin (0.15 %) which was greater compared to foliar spray of azoxystrobin (0.15 %) with 67.67 number of leaves.

Soil solarization + foliar spray of azoxystrobin (0.15 %) gave the highest yield in terms of fresh weight by 26975.00 kg/ha and dry weight of 4233.33 kg/ha which was superior when compared with foliar spray of tebuconazole (0.1 %) with the fresh weight of 23375.00 kg/ha and dry weight of 3362.50 kg/ha.

It is concluded that soil solarization for 31 days with the foliar application of tebuconazole (0.1%) can effectively control the Rhizoctonia leaf blight disease severity with plant growth and yield promotion under field conditions.