Studies on root and stem rot of cowpea (*Vigna unguiculata*) with reference to management

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This is to certify that the thesis **"STUDIES ON ROOT AND STEM ROT OF COWPEA WITH REFERENCE TO MANAGEMENT"** submitted to partial fulfilment of the requirements for the award of the degree of MASTER OF SCIENCE IN AGRICULTURE (PLANT PATHOLOGY) of Orissa University of Agriculture and technology, Bhubaneswar, is a authentic record of bonafide and genuine research work carried by **TRIBIKRAM SAHOO,** Adm.No. 12PPT/14 under my guidance and supervision. No part of this thesis has been submitted for the award of any degree or diploma or published in other form.

It is further certified that the evidence and help obtained by him from various sources during the course of investigation have been duly acknowledged.

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ABSTRACT

The cowpea is also called black eyed peas with the botanical name *Vigna unguiculata (L.) Walp.* The plant has *a* synonym of *Vigna sinensis.* Importance of outbreak of different diseases on this crop are of the major constraints for its low productivity that has been felt seriously. Root and stem rot of cowpea caused by *Rhizoctonia solani* is of wide spread occourance in the country as well as in the state of Odisha. An in depth survey was undertaken in Keonjhar, Cuttack, Khurda and Bhubaneswar area where incidence found 18.4 to 40.8%. The symptomatological results revealed that the pathogen initially infecting the root portion. The lesions rapidly expanded both upward and downward direction. The root and stem became girdled, discoloured and dried subsequently as the disease severity increased. Finally the infection caused withering and death of the plants. The pathogenicity of the fungus *Rhizoctonia solani* was proved on potted cowpea plants, isolated to pure culture and found identical to earlier one. Initially the colour of mycelium was white and later turned to brown followed by formation of microsclerotial bodies. The sclerotia were typically 2140µm in diameter, spherical, rough and light brown to dark brown in colour. The growth of the fungus was found best in potato dextrose agar medium (90.00mm) followed by Sabouraud's dextrose agar medium(87.90mm). The meteorological parameters did not contributed significantly towards disease development. However the soil factors found as sandyloam textured soil and acidic to neutral P^H found responsible for disease development. Regarding the host range study the isolate of cowpea infected rice (*Oryza sativa*), maize (*Zea mays*), tomato (*Solanum lycopersicum*), chilly (*Capsicum annum*), brinjal (*Solanum melongena*), fieldpea (*Pisum sativum)*,cucumber (*Cucumis sataivus*), bengalgram (*Cicer arietinum*), arhar (*Cajanus cajan*), cotton (*Gossypium hirsutum*), and groundnut (*Arachis hypogeae*) in addition to its own host. Garlic and turmeric were proved to be the most effective botanicals at 10% concentration, while garlic and calotropis gave complete growth inhibition at 15% and 20% concentration. Among the bioagents tested, the maximum percentage growth inhibition was found in *Bacillus subtilis* (72.04%) followed by *Trichoderma hamatum* (49.78%).In *in vitro* evaluation of fungicides against *Rhizoctonia solani,* maximum growth inhibition was recorded by Carboxin + Thiram, Hexaconazole, Difenoconazole, Tebuconazole and Tebuconazole + Trifloxystrobin. But in *in vivo* study, least mortality was recorded from Cymoxanil +Mancozeb (1.83%) followed by Difenoconazole (2.0%) , However against maximum mortality was recorded in Carbendazim followed by Propineb registering 82.00% and 65.97% respectively. In pot culture experiment the minimum mortality was recorded in Spent mushroom substrate + Cowdung + Vermicompost treatment (10.0%) followed by Vermicompost (15.0%). The maximum mortality was recorded in control(91.66%) followed by Poultry manure treatment 66.67%. With regard to the plant height Spent mushroom substrate + Cowdung + Vermicompost recorded maximum 89.33cm followed by Vermicompost and Spent mushroom substrate + cowdung registering 81.67cm and 79.00cm respectively.

CHAPTER-1

INTRODUCTION

INTRODUCTION

Vegetable not only adorn the dining table but also enrich the health of human beings by toning of their energy and vigour. Regular intake of vegetables help in providing essential health building and protecting substances such as proteins, vitamins and minerals.

Legumes enrich human nutrition with their rich source of proteins, minerals and vitamins (Dashpande, 1992). Especially for the lower income group, the cowpea serves as a better alternative source for proteins, minerals and ash etc. compared to other legumes.

The cowpea is also called black eyed peas with the botanical name *Vigna unguiculata (L.) Walp.* All cultivated cowpeas are grouped under *Vigna unguiculata* (Padulosi and Ng, 1997). The plant has *a* synonym of *Vigna sinensis.* This plant is a dicotyledon with the following systematic position:

Kingdom	: Plantae
Division	: Magnoliophyta
Class	: Magnoliopsida
Order	: Fabales
Family	: Fabaceae
Sub family	: Faboideae
Genus	: *Vigna*
Species	: *unguiculata*

The botany of the plant describes this as an annual herb with a strong taproot and many lateral roots. Growth may be trailing, climbing or bushy. Leaves are alternate and trifoliate, usually dark green. The stems are smooth or slightly hairy sometimes tinged with purple colour. Flowers are arranged in racemose type of inflorescence. Flowers are conspicuous, self pollinating, borne on short pedicels and corollas variously coloured. Flowers open in the early day and close during mid day. The fruits are pods usually green at young but turns yellow at maturity. The number of seeds per pod varies from 8-20. Seeds vary considerably in size, shape and colour.

The cowpea seeds have an approximate content

Moisture	: 9.40%
Protein	: 24.70%
Carbohydrate	: 56-68%
Fat	: 1.40-2.70%
Ash	: 4.20%

Na, K, P, Fe, Cu, Zn, Mn and Mg are the minerals along with Na:K of 0.08% and Ca:P of 0.59% (Iqbal *et al.*,2006)

Dry cowpea grain cooks quicker than other grain legumes. This is an important consideration in countries with shortage of cooking fuel. Cowpea is consumed in many forms: the young leaves, green pods and green seeds are used as vegetables, dry seeds are used in various food preparations and the haulms are used to feed the livestock as nutritious supplement to cereal fodder. The plant is a good source of fodder where either the green parts are used for grazing or more commonly cut and mixed with dry cereals for stall feeding. The use of cowpea in combination with cereals and other crops in an intensive scheme for lactating cows which helps in maintaining the milk yield >5 lit./cow/day (Relwani,1970). Trials of cowpea fodder varieties in India gave dry matter yields of >4 tons per ha, with crude protein content of 26%. The carbohydrate fraction of cowpea seed is relatively rich in oligosaccharides, which are well known for their flatulence effects, but not as high as common bean. The nutritive composition of cowpea seeds is similar to other grain legumes especially to common bean. Like most other legumes , cowpea grain is deficient in sulphur containing amino acid. Cowpea is one of the best natural sources of Folic acid.

V. unguiculata is said to be originated from West Africa (Ng and Marechal, 1985). The important cowpea growing countries are Nigeria, Mali, Ghana, Sudan, Zambia, USA, Nepal, Cuba and South Africa. The estimated world wide area under cowpea is over 14 m. ha with over 4.5 m. tons annual production and per hectare production comes to 387 Kg. The available data on area, production and average yield of cowpea comes to a total of 11.3 m. ha and 3.6 m. tons respectively. Nigeria is the largest producer and consumer of cowpea with about 5 million hectares area and about 2.4 m. tons production annually. India is the largest producer of cowpea in Asia and other countries. In India, the cowpea is grown in an area of about 3.9 m. ha with an annual production of 2.2 m. tons having a productivity of 567 Kg per ha (Halemani, 2009).

In our state, the Directorate of Horticulture, Odisha, Bhubaneswar, do not have separate statistics for cowpea production and about areas under cultivation. In Odisha Cuttack, Keonjhar, Nabarangpur, Nayagarh, Puri, Boudh and Kalahandi are the leading districts, where significant cowpea production occurs.

Cowpea diseases induced by different pathogens belonging to various pathogenic groups constitute one of the most constraints to profitable cowpea production in all agro-ecological zones where the crop is cultivated. The important diseases which cause considerable loss in the yield are root and stem rot (*Rhizoctonia solani*), rust (*Uromyces phaseoli*), powdery mildew (*Erysiphe polygony*), anthracnose (*Colletotrichum lindemuthianum*) and cowpea golden mosaic virus. As this present study was concentrated on a fungal disease root and stem rot ,the details are given on root and stem rot only. The losses in green fodder and seed yield were estimated to be about 28.8 and 39.7 per cent, respectively (Ram and Gupta, 1988).

Rhizoctonia solani (teleomorph: *Thanatephorus spp.*) is a plant pathogenic fungus with a wide host range and worldwide distribution. *R. solani* frequently exists as thread-like growth on plants or in culture and is considered a soil-borne pathogen. *R. solani* attacks its hosts in their juvenile stages of development. This saprophytic pathogen would survive in the soil and attack the hosts. The pathogen is known to cause serious plant losses by attacking primarily the roots and lower stems of plants. Although it has a wide range of hosts, its main targets are herbaceous plants. *R. solani* would be considered a basidiomycetous fungus if the teleomorph stage were more abundant.

Root and stem rot of cowpea caused by *R. solani* is an important soil borne disease in worldwide. Therefore it attacks seeds of plants present below the soil surface. It can also infect pods, leaves and stems of the host plants. The most common symptom of *Rhizoctonia* is the failure of infected seeds to germinate. *R. solani* may invade the seed before it has germinated to cause pre-emergence mortality or death to very young seedlings soon after they emerge from the soil. Seeds that do germinate before being killed by the fungus have reddish-brown lesions and cankers on stems and roots. There are various environmental conditions that put the plants at higher risk of infection. The pathogen prefers warm and wet climatic condition for successful infection and growth. *Rhizoctonia solani* can survive in the soil for many years in the form of sclerotia. Sclerotia of *Rhizoctonia* have thick outer layers to allow for survival and they function as the overwintering structure for the pathogen. In some rare cases (in the teleomorphic stage) the pathogen may also take on the form of mycelium that resides in the soil as well. The fungus is attracted to the host by chemical stimuli released by it by decomposing plant residues. The process of penetration of a host can be accomplished in a number of ways. Entry can occur either by direct penetration or through the natural openings in the plant. Hypha come in contact with the plant and attaches itself to the plant after which it produces an appressorium that penetrates the plant cell and allows the pathogen to establish in the plant cell. The pathogen releases enzymes that breaks down the cell wall and then continues to colonize and grow inside dead tissue. This break down of the cell walls and colonization of the pathogen within the host. New sclerotia are formed and the disease cycle completed.

Based on this back ground, quite a number of experiments were designed intensively adopting several methodologies in order to propose the management measures of the root and stem rot disease in cowpea cultivated in the country in general and the state of Odisha in particular for agro climatic relevance. The results as conceived might of course, in one hand help to eradicate the disease while ensuring a bumper productivity in the other hand.

The objectives of the study were:

1. Collection of diseased samples from different cowpea growing areas of Odisha.
2. Isolation of pathogen from infected samples and pure culturing.
3. Proving pathogenicity.
4. Morphological study and growth of the pathogen in different culture media.
5. Study of host-range of *R. solani*
6. Effect of weather parameters on disease incidence.
7. Integrated disease management by using Plant product, Bio control agents, Chemicals and Organic amendments against *Rhizoctonia solani* in cowpea.

CHAPTER-2

REVIEW OF LITERATURES

REVIEW OF LITERATURES

Root and stem rot of cowpea caused by *Rhizoctonia solani* and teleomorph *Thanetophorus cucumeris* causing huge loss in cowpea production. Root and stem rot of cowpea is severe not only in India but also in other countries as well. This disease is widely prevalent in Odisha causing great loss in cowpea production. But studies on it have not been conducted in detail in Odisha. However, reports are available on some aspects of the diseases from elsewhere in India and other countries. In this investigation, an attempt has been made to review the details of the somewhat recent literature available on the following aspects in order to bring forward the significant progress made on the diseases, reflecting upon the objectives and findings of the present study.

2.1 Introduction

2.2 Occourance and distribution of diseases

2.3 Economic importance

2.4 Symptomatology

2.5 Morphological characters of *Rhizoctonia solani*

2.6 Pathogenicity

2.7 Cultural characterstics of the fungus

2.8 Environmental factors affecting infection and disease development

2.9 Host range study

2.10 Disease management

2.10.1 Efficacy of botanicals

2.10.2 Biological control

2.10.3 Chemical control in *In vitro* and *In vivo*

2.10.4 Organic amendments

2.1. INTRODUCTION

Species of *Rhizoctonia* are ubiquitous and variable soil inhibiting fungi. Many different *Rhizoctonia* isolates cause worldwide economically important diseases on most of the world's important crop plants. Such as cereals, cotton, sugar beet, potato, vegetables (field beans and peas etc.,), field crops, turf grasses, ornamentals, fruit trees and forest trees. On the other hand many isolates of *Rhizoctonia* are saprophytic in nature. This chapter Taxonomy, Pathogenicity, Ecology, Diseases occurrence and Management aspects have been covered.

Dorrance *et.al.*(2001) reported that *Rhizoctonia solani* infection and disease development can occur over a wide range of soil moisture levels. *Rhizoctonia solani* damage occurs at any time during the growing season; however, it is more severe on young seedlings. They also reported that, *Rhizoctonia solani* is a complex and collective fungal species, which consists of strains that differ in host range, pathogenicity, cultural characteristics and the way they respond to the environment.

Rhizoctonia solani is a soil borne pathogen with worldwide distribution and it is capable of attacking a tremendous range of host plants, causing seed decay, stem cankeraerial leaf blights to rot and seedling damping-off in crops including carrot, soybean, potatoes and cowpea.(Thornton *et. al.*,2004).

Iqbal *et.al.*(2006) studied on composition, mineral constituents and amino acid profile of four important legumes (chickpea, lentil, cowpea and green pea) were studied in order to evaluate their nutritional performance. Significant ($P < 0.05$) variations existed among the legumes with respect to their proximate composition, mineral constituent and amino acid profile. Lentil was found to be a good source of protein, while cowpea was good in ash among the grain legumes tested. All four types of legumes were also better suppliers of mineral matter, particularly potassium, phosphorus, calcium, copper, iron, and zinc. However, the concentration of various mineral constituents was not in good nutritional balance. It was concluded that the four legumes tested were rich in lysine, leucine and arginine and can fulfill the essential amino acid requirement of human diet except for S-containing amino acids and tryptophan. In order to make good, the deficiency of certain essential amino acids in legume protein, they must be supplemented with other vegetables, meat and dairy products

2.2 OCCOURANCE AND DISTRIBUTION OF DISEASES

Kaiser *et.al.* (1970) were found *Rhizoctonia solani* to initiate collar rot and stem canker of mung bean (*Phaseolus aureus*).

Yehia *et.al.* (1985) reported that *Fusarium solani, Fusarium oxysporum ,Rhzioctonia solani* and *Pythium debaryanun* were destructive on lentil plants causing damping- off and root rot diseases in Egypt..

Muyolo *et.al.* (1993) noticed that anastomosis grouping (AG) of 290 *R. solani* isolates from diseased *Phaseolus vulgaris* and cowpea roots and/or hypocotyls and foliage in Ohio (USA) and Zaire were determined. Virulence of 60 isolates, representing 12 isolates each from 1 to 5 origin/host/anastomosis-group categories, was studied on *P.vulgaris* cv. Great Northern and Cowpean cv.Williams with variation in virulence, assessed by inoculating roots, hypocotyls and leaves.

Pedroza-San doval (1994) reported that, *R. solani* was the main severe pathogen causing root rot disease in beans and thus affecting crop density, especially in bean variety of Pinto Americano.

Litterick *et. al.* (1995) were found that *Rhizoctonia*-like mycelia was isolated from the foliage, stem-base and roots of *Calluna vulgaris* and *Erica* spp. collected from nurseries in Scotland. Isolated fungi were identified as either bi-nucleate *Rhizoctonia* spp. or *R. solani.* All isolates tested for pathogenicity causing foliar browning and webs of mycelial growth were observed on dead and dying foliage.

Karaca *et.al.*(2002) stated that 50% of the isolated pathogens obtained from the roots and rhizosphere soil of bean plants grown in Samsun(Turkey) were identified as *R. solani.*

Majchrzak and Kurowski (2002) reported that *R. solani,* the causative of damping-off, was found to be the most frequently isolated fungus from the infected roots and stem base of bean plants in Poland.

Adandonon *et.al.* (2004) reported that the disease incidence was higher in the South (0.074) and Centre zones (0.063) than in the other zones (<0.02) in the country. Among factors influencing the disease incidence, cultural practices such as sole crop and no-till systems appeared to be most important. Isolated fungi included *Sclerotium rolfsii, Fusarium spp., Pythium ultimum, Rhizoctonia solani, Phoma sp., Rhizopus sp.* and *Trichoderma harzianum.* None of the *Fusarium, T. harzianum* or *Rhizopus sp.* isolates were pathogenic in the greenhouse. *Pythium ultimum, R. solani* and *Phoma sp.* were infrequently isolated and few isolates caused the disease symptoms in the greenhouse. It is the first report of *Phoma sp.* causing damping-off and stem rot of cowpea in Benin. *Sclerotium rolfsii* was by far the most common species isolated from all the agro-ecological zones and all isolates were pathogenic on cowpea in the greenhouse. *Sclerotium rolfsii* was considered to be the main causal agent of cowpea damping-off and stem rot in the Republic of Benin due to its wide distribution, high incidence and predominance on plants with damping-off and stem rot symptoms.

2.3. ECONOMIC IMPORTANCE

Sumner *et.al.* (1985) studied on crop rotation on severity of crown and root rot in infested field with anastomosis group (AG)-2 or (AG)-4 of *Rhizoctonia solani. Rhizoctonia* like bi-nucleate fungi or un-infested and planted to 3-yr cropping system of corn –peanut –corn, peanut-corn-corn, cucumber-cowpea –peanut-corn. Crown and root rot of corn was more severe each year in soil infested with AG-2 than with other treatments, but there were no difference among cropping system. Corn yield for 3-yr average 22% less in soil infested with AG-2 than in un-infested soil. Root and hypocotyle disease severity in peanut was slight each year in all soil treatments and there were in no difference in yield *R. solani* AG-2 type 2 was isolated from 1.5-11% of the seed from unblemished attached of loose peanut pods during the last 2-yr of the study. *Rhizoctonia* like CAG-2 and CAG-4 were isolated from both unblemished and loose peanut pods in the third year. Only AG-4 caused fruit rot and reduced plant stand in cucumber. There were no difference in root disease severity or yield among soil treatments in turnip and cowpea. *R. solani* AG-2 was recovered from soil after the first year of crops but not thereafter.

Estevez de Jensen *et.al.* (2002) isolated *R. solani* from the infected roots of bean plants in Minnesota, USA. The root rot disease in dry bean was increasingly important in North Central region of the United States. In Minnesota it caused primarily by *Fusarium solani* f.sp. *phaseoli* in a complex with *R. solani* and *F. oxysporum*. The yield losses in the severely infested areas in Minnesota reached up to 50 %.

Dubey *et.al.*(2012) surveyed the morphological isolates showing variable atmospheric temperature and relative humidity and low to medium levels of soil organic carbon and high level of available phosphorus influenced the development of the disease incidence from 2-48%. Seventy three cultivars of mungbean, twenty eight cultivars of urdbean and eight cultivars of cowpea were evaluated against virulent isolate of *R. solani* (RASC 30) to design a set of differential cultivars for virulence analysis. Two cultivars of urdbean, namely, NDU3-4 and IPU2-14, one cultivar of mungbean, namely, HUM 1 and three cultivars of cowpea, namely, V240, V585 and DCP7 showed resistant reactions. Four cultivars of urdbean, namely, TU94-2, KU323, KUG216 and B3-8-8, one cultivar of mungbean, namely, PDM54 and two cultivars of cowpea, namely, V578 and DCP13 were moderately resistant against the *R. solani.*

2.4. SYMPTOMATOLOGY

Gupta (1985) reported a severe incidence of root and collar rot due to *Rhizoctonia solani* in different mustard varieties. It appeared when the plants were approximately two months old. About 20-40% plants were found infected. It was characterized by formation of reddish brown lesions on the collar region of the plants. The lesions increased in size downwards damaging the roots. With the advancement of this disease the plants started drying and in a few days, the leaves dried up. The flowers and immature pods also have been reported to dry up causing a serious loss to the crop.

Jones *et.al.* (1997) observed that Cowpea pod that come in contact with the soil in warm wet areas develop brown rot often with alternating light and dark colored concentric bands. Brown coarse mycelium of the fungus appeared on the surface of infected plant parts under moist conditions.

Olsen and Young (1998) reported that *R. solani* caused damping-off with reddish-brown lesions, which appear on the stem at soil level and girdle the stem when conditions are favorable. The stem may sometimes be water soaked and soft causing the plant to collapse.

Dorrance *et.al.* (2001) stated that *Rhizoctonia solani* damage occurs at any time during the growing season and the pathogen attacks mostly young seedlings and also causes seed rot, root rot and lesions on hypocotyls. When older plants are infected they become chlorotic resembling plants with nitrogen deficiency.

Adandonon *et.al.* (2004) reported whitish or brownish lesions at the base of stem followed by wilting. But the wilted plant remain upright. The lesions on the infected plant found covered with white mycelial growth in which brown sclerotia were often embedded. This severity was reported at flowering stage where as no diseased plants were observed at pod formation stage.

2.5. MORPHOLOGICAL CHARACTERS

Parmeter and Whiteny (1969) were reviewed the findings of Matsumoto (1921), Thomas (1925), Schultz (1936), Richter and Schneider (1953), Palo (1926), Monteith and Dahi (1928) and indicated that most isolates fall within 5-14µm in range with an average of about 6-10 um in hyphal diameter. The compositions of the medium and incubation temperatures were reported to affect the hyphal diameters. Refering to the work of Bracker and Butler (1963) they further stated that the septal pore apparatus is a prominent feature in *Rhizoctonia solani.* Also they considered that the multinucleate condition of young actively growing hypha of *R. solani* is a stable characteristic that can be used to distinguish *R. solani* from similar fungi with regularly bi-nucleate cells.

Thakur *et.al.* (1992) conducted the studies on 19 isolates of *R.solani* from 13 plant hosts, divided them in to three groups based on sclerotial characters, Macrosclerotial group (with sclerotia more than 1mm in diameter), microsclerotial group (sclerotia less 1 mm in diameter). *Rhizoctonia solani* is known as a basidiomycete fungus that does not produce asexual spores (conidia) and occasionally produces basidiospores. The mycelium of *R. solani* is colourless at first but becomes brown as the hyphae grow.

Laemmlen (2004) reported that *Rhizoctonia solani* is found in most agricultural soils and survives on plant residues and as micro sclerotia. It is in the soil or seeds, it moves quickly through the seedling skilling those in its path. The symptoms start with seeds turning dark brownthen decaying until the brown, threadlike mycelium may be seen with a lens on the surface of the lesion or canker(1 mm in diameter) and isolates that did not form sclerotia.

Lal *et.al.* (2009) studied the morphological variability of 25 isolates of *R. solani* in characteristically having hyphal branching at right angle, constriction at the point of branching of the mycelium and presence of a septum near the branching junction which is of immense taxonomical importance. It was an obvious observation for the mycelial branching at right angles as a known feature of *R. solani.* Hyphal width from 4.75-7.43 µm. Maximum hyphal width (7.43µm) was observed in isolate RS-22 (New Delhi) while minimum(4.75µm) was observed in isolate RS-20 (Sikkim). Based on pigmentation of sclerotia, the isolates were assigned to 4 groups- Dark brown, (9 isolates), dark yellowish brown (6 isolates), olive brown (6 isolates), and light brown (3 isolates). No sclerotium was formed in isolate RS-22 (New Delhi). The texture of sclerotia, was classified in two groups smooth and rough. In the smooth category, 12 isolates and remaining 12 isolates in rough category were grouped. The diameter of sclerotia is 1.30-2.03 mm. Sclerotia were formed in aerial mycelium in 9 isolates and on the surface of the mycelium in all isolates except RS-22. In 9 isolates sclerotia were formed on both aerial and on surface of the mycelium. Formation of sclerotia was observed in the Petri dish and classified into three groups. Sclerotia were formed in the central region in eight isolates and in remaining isolates they were found in periphery.

Manashi et al (2015) were found that formation of sclerotia was observed in the Petri dish and classified into three groups: peripheral, scattered and ring at periphery. Sclerotia was formed both in surface mycelium in four isolates (RS-1, RS-4, RS-5 and RS-6). The texture of sclerotia was classified into two groups smooth and rough. Based on the pigmentation of the sclerotia, the isolates were assigned to 2 groups -willow green and greyed yellow. The first sclerotial initiation was observed 9 days after inoculation by RS-1. Initiation of sclerotial production started in 11 days after inoculation for RS-4 ,15 days for RS-5 and 18 days for RS-6. The diameter of sclerotia ranged from 1.4-2.5 mm.

2.6. PATHOGENICITY

Amira (2008) studied to identify fungi associated with ten varieties of legumes seeds in an eastern region of the kingdom of Saudi Arabia: Lupine (*Lupinus albus* L.), dry and kidney beans (*Phaseolus vulgaris*), cowpea and mungbeans (*Vigna radiate* L.), faba and field beans (*Vicia faba* L.), brown and green lentil (*Lens culnaris*), chickpea (*Cicer judaicum*). The highest percentage of fungal infection was associated with mung bean (23.29% infection) followed by faba beans (15.75%), field beans (13.87%), dry beans (11.89%), brown lentil (11.13%), lupine (6.69%), kidney beans (6.16%), green lentil (5.14%), cowpea(3.25%) and chick pea (2.74%). Several fungi associated with these were isolated at the following frequencies. *Rhizoctonia solani* (21.18%), *Pythium aphanidermatum* (17.8%), *Alternaria alternate* (12.18%), *Asergillus flavus* (9.43%) and *Phytophthora spp.* (4.12%). The potency of the first three most frequent fungi to infect all studied seeds was ranked is decreasing order, as *R.solani*(35.33%), *P.aphanidermatum* (28.67%), *A. alternata* (27.67%).The weight of infected plant was significantly lower than that of healthy plants.

Khedri *et.al.* (2014) studied pathogenicity of cotton plant by taking eight isolates of *R.solani* obtained from cotton, sugar beet and potato on commonly used cotton varieties in Iran. Sixteen seeds from each variety were sown in pots containing 4 kg of pasteurized field soil pre-inoculated with each *R. solani* isolate. Pots were placed in the greenhouse with 12 h photo period and were watered as needed. Pathogenicity of different *R. solani* isolates on the seedlings of various cotton varieties was evaluated by counting the number of survived seedlings in each treatment 60 days after sowing. The pathogenicity of *R. solani* varied on different cultivars. The isolates R.s-Sb-3 and R.s-Po-1 showed the highest pathogenicity on Bakhteghan variety while, R.s-Sb-1 and R.s-Sb-2 showed the lowest pathagenicity on this variety. For Mehr variety, R.s-Po-1 and R.s-Sb-3 were the most pathogenic, and isolates R.s-Sb-2 and R.s-Co-2 were the least pathogenic isolates. In the Sahel variety isolates R.s-Po-2 and R.s-Sb-3 revealed the highest pathogenicity while, R.s-Sb-1 and R.s- Co-1 caused the least pathogenicity. In the Varamin variety, isolates R.s-Co-2 and R.s-Po-2 were the most pathogenic. The variety, R.s-Sb-3 and R.s-Sb-1 showed the lowest pathogenicity. In general, isolates R.s-Sb-3 and R.s-Po-1 showed the highest pathogenicity on most varieties.

2.7. CULTURAL CHARACTERSTICS

Ranganathan *et.al.* (1973) have reported blackgram and cowpea to be seriously affected by collar rot disease, caused by *R.solani*. He also reported that the pathogen isolated from the infected plants on oat meal agar, when inoculated in to 9 days old plant, showed collar rot after 6-7 days of inoculation, the percentage of infection ranged from 17 to 90.

Dash (1987) reported that amongst liquid media tried potato-dextrose, Sabouraud's, oat-meal, Richard's media supported maximum mycelial growth. As far as linear mycelial growth was concerned, Sabouraud's media supported maximum linear growth.

2.8. ENVIRONMENTAL FACTORS AFFECTING INFECTION AND DISEASE DEVELOPEMENT

Hemmiaud and Yokogi (1927) has reported that the optimum temperature to be 30^0 C , maximum 40-42^0 C with little or no growth occouring at 10^0 C.

Beker (1947) and Neergard (1958) revealed that he pathogen invades the seed while still in the pod decomposing the pod, or may infect from infested soil during planting. The problem of seed decay starts immediately after planting before germination. Damping-off might result from insufficient inoculum for fast infection to take place or from unfavourable conditions that do not favour the pathogen or the host .

Post-emergence damping-off occurs when the stem starts decaying at about soil level causing it to fall because of lack of supporting tissues. The pathogen sometimes attacks the roots of young plants causing root rot.

Parmeter (1970) and Harikrishnan and Yang (2004) reported that the mildly virulent strain causes most disease at 24°C, while the moderately virulent strain causes disease at 32°C and the highly virulent strain causes disease at 24, 27, and 32°C.

Homma *et.al.* (1983) were found that the optimum temperature for growth was 28-35^{0}C for *R.solani* isolated from Japanese raddish fields.

Sinha and Ghufran (1988) have reported the optimum temperature for growth *in vitro* was 25-40^{0}C.

Harikrishnan and Yang (2004) studied the optimum temperature range for sclerotia production in *R. solani* is between 18 and 25 °C.

Goswami *et.al.*(2011) conducted an experiment to find out variation in isolates of *R.solani* based on radial mycelial growth and sclerotial production. Five isolates of *R.solani* representing five clusters group were selected and were grown at different levels of temperature and pH on potato dextrose agar (PDA).It was observed that optimum temperature and pH for growth and scierotial production varied among the isolates. The rates of growth and sclerotial formation were not uniform at the same levels of the two growth factors. The maximum mycelial growth of all isolates was found at 30°C. At 35°C, only GAZ-9 and GAZ-18 showed initiation of growth, but the rate was very slow. The optimum temperature for sclerotial production of the isolates GAZ-9, JES-16, GAZ-18 SYL-26 at 30°C and for the isolate DIN-8 at 25°C. The optimum pH for maximum radial growth was 6 for DIN-8 and 7 for other four isolates. The maximum number of sclerotia was produced by DIN-8, GAZ-9,and SYL-30 at pH 8, 4, and 7, respectively. The optimum pH for sclerotia formation in JES-16 and GAZ-18 was pH 6.0.

2.9. HOST RANGE

Panwar *et.al.*(2012) have studied the host range and pathogenic variation of 7 isolates of *Rhizoctonia solani* collected from Rajasthan, Haryana and New delhi. All 7 isolates tested for their pathogenicity using susceptible varieties like C-235 of chickpea, RC-101 of cowpea, RMO-225 of mothbean and RMG-344 of mungbean, had varying disease incidence levels in pot culture experiment using sterilized and unsterilized soil. The isolate Hisar-2775 on chickpea, Delhi-4097 on cowpea and Hisar-2775 on mothbean were highly virulent in both types of soil, while Delhi-4097 was highly virulent on mungbean in sterilized soil but not in unsterilized soil. Contrary to this the isolate Hisar-2775 was highly virulent in unsterilized soil. The incidence of disease appeared first on mungbean followed by chickpea, mothbean and cowpea in both soil types.

2.10. DISEASE MANAGEMENT

2.10.1. EFFICACY OF BOTANICALS

. The possibilities of controlling plant disease by the integration of several methods have been the subject of extensive research. An integrated control which denotes the rational use of available control measures will have to be considered especially with crops which are infected simultaneously by various types of pathogen. It does offer the possibilities of making up for the deficiencies of any single method. Integration of chemicals, plant extracts and biotic agents along with resistance for managing plant disease has been considered as a novel approach as it requires low amount of chemical by reducing the cost of control as well as pollution hazardous, with minimum interference of biological equilibrium (Papavizas,1973).

Sharma *et.al.*(2003) studied that seed treatment with Datura and Neem extracts showed good seed germination, seedling vigour and least mortality due to *F. oxysporum* f.sp. *pisi, R. solani, Macrophomina phaseolina* and and *Alternaria alternata.*

Yadav *et.al.*(2005) found that 20 per cent extract of *Datura stramonium* gave 44.8 per cent growth inhibition of *R. solani.*

Shashidhara *et.al.*(2008)tested the effectiveness of *Clerodendron inerme, Duranta plumeri, Euphatorium* spp., *Allium sativum, Vitex negundo, Lantana camara* and *Azadirachta indica* against *Phytophthora capsici* in black pepper. Among these, Duranta and Garlic (10%) provided 35.5 and 26.6 per cent mycelial inhibition of *P. capsici*, respectively.

Sehajpal *et.al.*(2009) recorded that total number of 44 plant extracts tested, *Allium sativum* gave strong inhibition i.e. 5.75 mm at 10 ppm concentration followed by *A. cepa* and *Emblica officinalis* i.e. 3.25 mm each against *R. solani* isolated from rice.

Shahnazdawar *et.al.*(2010) studied the effect of *Datura alba* and *Cynodon dactylon* against *Macrophomina phaseolina* and *R. solani* in okra and cow pea. Both pathogens were completely suppressed by *D. alba*extract.

Dawar *et.al.*(2010) found that *Macrophomina phaseolina* (Tassi) Goid and *Rhizoctonia solani* Kuhn were completely suppressed when *D. alba* extract was used as soil drenching and seeds of okra and cowpea were coated with *P.variotii* while *Fusarium* spp., was suppressed when *C. dactylon* extract was used in combination with *P. variotii* as seed treatment. Growth parameters were maximum when soil was drenched with *D. alba* and *C. dactylon* extracts and seeds were treated with *P. variotii.*

2.10.2.BIOLOGICAL CONTROL

Elad *et.al.* (1983) reported that bio-control agents *Trichoderma harzianum* and *Trichoderma hamatum* were effective against soil borne plant pathogens like *Sclerotium rolfsii* and *Rhizoctonia solani.* TEM studies parasitisation of the bio-agent showed that hyphae of the parasite contact their hosts, either producing appressorium like bodies or coiling around the host hyphae. Then enzymatically dissolve the host cell wall.

Chang and Choi (1990) observed that mycoparasitism between the antagonist *Trichoderma viride* and the pathogen was observed after mycelial contact in dual culture, Coiling and penetration of the antagonist in the hyphae of the pathogen resulted in breaking, lysis and abnormal vacuolation.

Schmiedeknecht (1993) reported that, when he treated stemcanker and black scurf caused by *R. solani* with microbial antagonists of the genera *Bacillus*, *Gliocladium* and *Streptomyces*, the disease was reduced to 50% up to 78%. It seems that especially bacterial antagonists of the genus Bacillus are preferably suitable for biological control of *Rhizoctonia* disease on potato.

Nagarajkumar *et.al.* (2004) were reported that fourteen strains of *Pseudomonas fluorescens* isolated from rhizosphere soil of rice were tested for their antagonistic effect towards *Rhizoctonia solani*, the rice sheath blight fungus. Among them, PfMDU2 was the most effective in inhibiting mycelial growth of *R. solani in vitro.*

Sharma *et.al.* (2009) studied with twenty seven endophytic fungi as bio agent against *Rhizoctonia solani* among the fast growing isolates, *Trichoderma atroviride-II* produced a high amount of volatile and moderate amount of non volatile metabolites. *T. atroviride-II* was the most effective isolate in inhibiting the growth of *Rhizoctonia solani* while *T. atroviride-I* was the most effective against *Sclerotium rolfsii.* The slow growing isolates, *T. lignorum* that produced a high amount of non-volatile and a moderate amount of volatile metabolites was the least effective against the pathogens. Therefore, both the level of growth and the production of metabolites were important factors involved in the determination of antagonistic efficacy of *T. atroviride* against *R.solani* and *S. rolfsii.*

Bhat *et.al.* (2009) reported that sheath blight disease of rice in wet season of kharif with bio-control agents, like, *Trichoderma harzianum, Trichoderma viride, Gliocladium virens, Gliocladium roseum* and *Trichoderma sp.* In dual culture studies maximum inhibition of *R.solani* was recorded by *Trichoderma harzianum*(65.87%) followed by *T. viride* , *G. virens* and *Trichothecium sp.*, while *G. roseum* exhibited least effect against radial growth of pathogen.

Akhtar *et.al.*(2010) conducted experiment on four bio-agents such as *T. harzianum, T. viride, T. virens* and *Pseudomonas fluorescens* were evaluated against *R. solani* causing banded leaf and sheath blight of maize 86.9 per cent mycelial inhibition was shown by *T. harzianum* followed by *T. viride*(77.9%) in dual culture.

Panwar *et.al.*(2012) tested two fungi *Trichoderma harzianum* and *T.viride*, and one bacterium *Bacillus subtilis* for their antagonistic activity against seven isolates of *Rhizoctonia solani* causing wet root rot of chickpea on Czapek'sdox medium. Inhibition of the mycelium growth of *R.solani* isolates by *T. harzianum*, *T. viride* and *B. subtilis* varied from 56.9 to 70.1, 44.6 to 69.4 and 43.5 to 55.6%, respectively. Maximum growth inhibition was caused by *T. harzianum*. In a greenhouse experiment, these antagonists reduced root rot incidence in a susceptible chickpea variety C-235 up to 60, 65 and 50%, respectively as compared to control where 100% mortality was observed. *T. viride* was most effective in reducing the disease followed by *T. harzianum* and *B. subtilis.*

2.10.3. CHEMICAL CONTROL

Sahu (1986) has reported that out of ten fungicides tested, Bavstin, Brassicol, Vitavax , Ziram and Ceresan wet performed best followed by Dithane Z-78 and Dithane M-45 in controlling *Rhizoctonia solani* blight of rapeseed and mustard.

Mefanoxam with fludioxonilor a combination of mefanoxam, fludioxonil and azoxystrobin were effective in reducing *R. solani* root rot in soybean (Bucher & Pedersen, 2004).

Sunder *et.al.*(2009) were conducted an experiment applying carbendazim and tolclofos-methyl in three types of soils. Disease control with fungicides reduced in heavy textured soils particularly at lower concentrations of both the fungicides (0.5 or 1.0. g a.i/Kg seed). Carbendazim proved better than tolclofos –methyl in sandy soil but in heavy structured soils, tolclofos-methyl was found superior. More amounts of fungicides were required in heavy structured soils and only two higher dosages of both fungicides provided 50% or more disease control. Seedling mortality of mungbean was found to increase with the increase in amount of inoculum of *R.solani* in the soils.

2.10.4. ORGANIC AMENDMENTS

El-Mohamedy *et.al.*(2006) reported in the greenhouse trails that soil amendment with *Trichoderma harzianum* formulated on sugar cane bagasse at the rate of 10 % (w/w) of soil shown a highly effect in reducing root rot incidence caused by *F. solani, R. solani* and *M. Phaseolina* by 73.9 %, 73.9 % and 78.6 % at pre-emergence stage, respectively. The same treatment reduced post-emergence damping-off by 76.4 %, 71.8 % and 72.2 % respectively. Bio-priming seed treatment reduced root rot diseases by 60.8, 60.8 %, 75.0 % at pre-emergence stage, respectively and by 58.8 %, 75.0% and 58.8 % at post-emergence damping-off respectively.Coating cowpea seeds with *T.harzianum* or dressing with Rizolex-T caused a moderate effect in reducing root rot disease incidence. Under field conditions, amended soil with *T. harzianum*+ bagasse at the rate 10 % (w/w) of soil reduced root rot disease by 71.2 % and 68.2 % at pre-emergence stage. After 40 and 60 days from sowing the same treatment reduced root rot disease by 79.1, 76.6 % and 71.4, 66.7 % during 2004 and 2005 seasons. Moreover, fresh pod yield of cowpea was increased by 60.8% and 53.7 % during the same seasons, respectively. Bio-primed seed treatment reduced root rot incidence by 64.0 % and 56.3 % at pre-emergence stage and by 68.0, 60.1 % and 57.1, 64.0 % at post-emergence stage after 40 and 60 days of sowing during 2004 and 2005 seasons, respectively. Therefore, fresh pod yield was increased by 44.0 and 36.1 % compared with 19.5 and 11.2 % in the case of Rizolex-T treatment during the same seasons, respectively. It could be noted that practical use of soil amendment with agricultural wastes formulated with bio control agents and/or bio-priming seed treatments to control soil borne plant pathogens as a substitute of chemical fungicides is possible without any risk to human, animal and the environment.

Ashlesha *et.al.* (2009) found that PDA amended with dried powder of cow dung pots resulted in 0.5-1.3 mm (9.00mm check) growth of mycelium at its different concentrations. Pea seeds dipped in cow dung slurry and milk up to 120 minutes and in cow urine up to 30 minutes in 1:1 and 1:2 (v/v) dilutions with water showed 100 percent germination. Pea seeds dipped in cow dung slurry and milk (1:1) and cow urine (1:2, 1:1) concentrations resulted in complete control of root rot of pea in pot culture. Pea seeds treated with cow pat ash at all the concentrations and of cow pat powder @ 0.5 and 1.0 per cent supported 100 percent seed germination and control of pea root rot. Okra seed treatment with cow pat powder @2.5 and 5.0 g/Kg seed and seed dip in cow dung slurry (1:1) for 1 and 2 hour supported very good seed germination and also provided complete control of damping off of seedlings.

Fresh cow dung, urine, milk and cow dung based preparations namely cow dung slurry, dried powder and ash were evaluated against *R. solani* causing damping-off of okra and root rot of pea pathogens. Complete inhibition in mycelial growth was obtained by amending potato dextrose agar with cow dung and cow dung ash @ 5 g/100 ml medium followed by cow dung powder (0.5 mm radial mycelial growth) (Ashlesha *et. al.*,2009). Out of twelve organic inputs tested, eight inputs *viz.* biosol, matkakhad, agnihotra ash + cow urine, panchgavya, vermicompost, cow pat pit compost, NADEP compost and biodynamic compost showed 60.2-100 per cent inhibition in mycelial growth of *S. sclerotiorum* without autoclaving (Shalika 2009).

Sinha *et.al.*(2010)studied the antifungal properties of vermicompost and vermiwash against soil borne pathogens (*Pythium ultimum*, *R. solani* and *Fusarium* spp.) and recorded 51-72 per cent inhibition in mycelial growth of pathogens.

CHAPTER-3

MATERIALS AND METHODS

MATERIALS AND METHODS

Rhizoctonia solani causes root and stem rot of cowpea. It is a wide spread disease in Odisha causing huge economic loss.Considering the importance of disease, research work has been undertaken to study the pathogen and its management through application of bio agents and chemicals.

The present investigation was carried out both in the laboratory and field during 2014-16.The field experiment was conducted at the AICRP on vegetable crops,Orissa University of Agriculture and Technology,Bhubaneswar and Research field of Department of Plant Pathology,OUAT,Bhubaneswar while the laboratory experiments were carried out in the Department of Plant Pathology, College of Agriculture, Bhubaneswar, Odisha.

The details of materials used and methodology followed in conducting experiments are described below.

3.1.GENERAL LABORATORY PROCEDURES

3.1.1 Cleaning and Sterilization of glass wares:

Borosil glasswares such as culture tubes,Petri dishes,conicalflasks,beakers etc., were used throughout the period of investigation.Standard procedures for cleaning and sterilization of glass wares (Riker and Riker,1936) were adopted. All the glass wares were cleaned in dilute solution of potassium dichromate and sulphuric acid (60g potassium dichromate per liter distilled water, 60 ml of concentrated sulphuric acid added slowly to it). The glass wares were kept in the solution for 4-6 hours followed by through washing with tap water and subsequent rinsing with distilled water before use. Petri dishes, pipettes etc. wrapped with paper were sterilized in hot air oven at 160^0C for 2 hours.The micro-slides and cover slips were exposed to flame just before use.

3.1.2 Preparation of sterilized materials:-

1.Sterilized water :- Tap water was sterilized in autoclave at 15 psi for 20 minutes. The mouth of the conical flasks and test tubes were wrapped with paper prior to sterilization and stored until use.

1. Sterilized blotter paper:- Blotter paper was sterilized in hot air oven.
2. Sterilized Petri dishes:- Properly washed Petri dishes were wrapped with news papers and sterilized in hot air oven at 160^0C for 2 hours.
3. Sterilized moist chamber :- Three layers of circularly sterilized blotter paper were aseptically placed in Petri dishes and sterile cotton swab was placed in it. It was moistened with sterile water.
4. Sterilization of plant materials:- The surface sterilization for the elimination of surface-borne microbes of the test materials was accomplished by dipping the materials in 0.1% $HgCl_2$ solution for 2 minutes and rinsed with 3 changes of sterile water to remove traces of the $HgCl_2$ solution.
5. Sterilization by flame:- The spear-head needle, prickling needle, inoculation needle, glass rod and nichrome wire loop etc. were sterilized by dipping them in 70% Ethanol followed by flaming over spirit lamp.

3.1.3 Preparation of stains and surface disinfectants:-

1 Preparation of Lacto phenol:-

Lacto phenol was prepared and used primarily to mount and for observing them with their natural pigmentation.

Chemicals:-

Lactic acid	20 ml
Phenol	20 ml
Glycerin	40 ml
Distilled water	20 ml

For the Preparation of this mounting fluid,phenol was dissolved in water without heat to prevent oxidation.Then glycerin was added to it followed by lactic acid.

2 Preparation of cotton blue:-

For staining the fungal structures,cotton blue crystals were dissolved in lacto phenol at the rate of 1 g per 100 ml (1%).Mounting material with this preparation followed by slight warming for about 30 seconds and washing with lacto phenol imparted a blue color to the fungal protoplasm.

3. Preparation of MercuricChloride:-

Tissue for isolation of micro organisms was disinfected with 0.1% mercuric chloride to eradicate the unwanted surface-borne organisms.

Chemicals:-

(a) Stock solution

Mercuric chloride	20g
Conc. Hydrochloric acid	100 ml

(b) Disinfecting solution

Stock solution	5 ml
Distilled water	995 ml

Mercuric chloride was dissolved in conc. HCl and was maintained as the stock solution.At the time of use, 5 ml of stock solution was added to 995 ml of distilled water.

4. Preparation of formalin 4% solution:

One hundred ml of commercial formalin (40%) was added to 900 ml of distilled water for obtaining 4% formalin.

3.1.4 Preparation of culture media

Potato Dextrose Agar (PDA) medium was prepared by following standard procedure postulated by Ricker and Ricker (1936).

Ingredients:-

Peeled and thinly sliced potato	200 g
Dextrose	20 g
Agar agar	20 g
Water	1000 ml

3.1.5 Preparation of Potato Dextrose Agar medium (PDA)

Potato Dextrose Agar, the routine laboratory medium for growing test fungus, was prepared as follows.Two hundred gram of peeled and sliced potato was boiled in 500 ml of distilled water till potatoes became soft. Then the extract was filtered through cheese cloth and was collected in a graduated cylinder.Twenty gram of agar agar powder was boiled in 500 ml of distilled water till the agar agar was dissolved completely. Both the solutions were subsequently mixed.Twenty gram of dextrose was added and the volume was restored to 1000 ml by adding fresh distilled water .

Before sterilization,5-6 ml were taken in culture tubes for preparation of agar slants.Media to be poured into Petri dishes were taken in Erlenmeyer conical flasks.The culture tubes and conical flasks were plugged with non absorbent cotton and autoclaved at 15 psi for 15-20 minutes. Streptomycin sulphate was added to the medium at the rate of 250 mg per 1000 ml before autoclaving for suppression of bacterial contamination.Slants were prepared by putting hot tubes in slating position for solidification and were stored in a wire basket until further use.

3.2 PHYTOPATHOLOGICAL ANALYSIS:-

3.2.1 Survey of disease incidence

A survey was conducted during Kharif 2015 at AICRP on vegetable crops, OUAT Bhubaneswar, Farmers' field in different places of Odisha, viz. Khamangsasan (Balianta Block, Khurdha district), Chittalpur (Cuttack district) and Anandapur (Block- Anandapur, Keonjhar district) to know the incidence of *Rhioctonia* root and stem rot by visual observation on cowpea crop. The incidence of other diseases like Anthracnose and Cercospora leaf spot were also recorded by visual observation. During survey, the characteristic symptoms of diseases were recorded. Samples belonging to the surveyed locations having rot symptom were collected in separate polythene bags and brought to laboratory for isolation of pathogens

3.2.2 Microscopic examination:

The affected root and stem portions were examined under microscope by preparing squash mount in lacto phenol and by cutting hand sections. Cut pieces of affected plant tissue were cleaned with sterile water and kept in moist chamber for 2-3 days, after which squash mount of the tissue as well as frutification on stem were observed under microscope. The mycelia and sclerotia bodies were observed under microscope and microphotographs were taken.

3.2.3 Isolation of pathogen:

The pathogen was isolated from the stem sample collected from the field in the following procedures.First the samples were washed with distilled water to remove the foreign materials.A small disease portion (2-3) of stem along with healthy portion were cut into pieces and surface sterilized with mercuric chloride (0.1%) solution for 2 minutes followed by thorough washing with sterile water for 3 times to remove residues of mercuric chloride from cut pieces.Then the diseased cut pieces were aseptically transferred to the middle of Petri dishes containing host extracts/potato dextrose agar medium. The Petri dishes were incubated at room temperature (25^0C) for 7-10 days.Pure culture of fungus was appeared after 3-4 days of inoculation.

3.2.4 Purification and identificationof fungi:

Each isolate of fungus maintained on PDA slants or Petri dishes. Mycelia from the periphery of young colonies were carefully examined and transferred to PDA slants.This process was repeated for 2-3 times till the concerned fungus was found to be free from other fungi and bacteria. Pure culture of fungus was obtained through 'hyphal tip' method. The characteristics of the fungal colony on PDA and the details of their morphology were recorded for each isolate. Each isolate was taxonomically identified with the help of available cultures and literature.

3.2.5.Hyphal tip culture:

The fungus was grown in a sterile Petri dish containing potato dextrose agar medium. As isolated, hyphal tip was located under the microscope and marked with the help of sharp glass marking pencil.The tip was carefully lifted up and transferred by sterilized inoculating needle to a potato dextrose agar slant at room temperature. After 2-3 days, the growth of fungus was observed in the culture tube and thus a pure culture of the fungus was obtained.After getting the fungus in pure culture, it was maintained in potato dextrose agar medium and sub cultured in 2 weeks intervals.

3.2.6 Inoculation and incubation:

Aseptic conditions were maintained in the inoculation chamber at the time of inoculation.The inoculation consisted of a small piece of medium with pure culture taken out of the culture slants with the help of inoculating needle.The inoculated cultures were incubated at $25\pm1^{0}C$ unless otherwise specified.

3.2.7 Maintenance of culture:

The pure culture of the fungus was maintained on potato Dextrose Agar (PDA) slants throughout the period of investigation. The fungus was sub cultured at an interval of two months and stored at $25\pm1^{0}C$. Fifteen days old pure mycelial cultures of the test fungus were used in various studies.

3.2.8 Identification of pathogen:

The identification of fungal pathogen was done based on the mycelial growth and sclerotia bodies formation.The culture was further purified by following hyphal tip techniques.

3.2.9 Pathogenicity Test:-

In order to determine the parasitic nature of fungus (*Rhizoctonia sp.*), pathogenicity tests were carried out.Cowpea plants were raised in polythene bags containing sterilized soil and watering was done once in a day. Fifteen days old plants were inoculated with the test pathogen adopting following procedures.

Seedlings were surface sterilized and were pin pricked.For inoculation, fungal mycelium was transferred from the actively grown edges of the colony and inoculated on the incision given on the pin pricked area.Such inoculated plants were covered with polythene bags and kept in dark for 48 hours at temperature of 25^0C and 100% RH. After the end of 48 hours,the pots were kept in green house under natural humidity.Regular observation were made for the appearance and development of symptoms.

After development of typical disease symptoms in inoculated plants, the fungus was re-isolated. Its morphological characters were studied again and compared with that of the isolate obtained originally from the naturally infected plants.

3.3 STUDY OF MORPHOLOGICAL CHARACTERISTICS

A bit of isolated fungal colony of seven days old pure culture was properly stained and mounted on slide for examination under microscope for its different morphological and taxonomic characteristics.The measurements of mycelia and sclerotia were recorded.

3.3.1 Study of cultural characteristics

The fungus isolated from the diseased parts of cowpea root and stem were subjected to incubation at a temperature of 28 ± 1^0C for growth. The fungus was grown in Petri dishes by transferring 5 mm of mycelial disc of seven days old pure culture.The characteristics of the fungal colony including colony shape and growth habits were observed by naked eye and by compound microscope.

3.4 PHYSIOLOGICAL STUDIES OF *R. solani*

3.4.1 Growth in different media.

In order to find out growth of test fungus, five different culture media viz.,potato dextrose agar, Sabouraud's agar, oat meal agar, malt extract agar and potato dextrose rose bengel agar were selected. The media were prepared as described by Riker and

Riker (1936) with slight modifications where ever necessary. The composition and general preparation of different media have been given below.

1. **Potato Dextrose agar medium:**

Peeled and sliced potato	200g
Dextrose	20g
Agar agar	20g
Distilled water	1000ml

2.Oat meal agar:

Oat flake	40g
Agar agar	20g
Distilled water	1000ml

3. **Malt extract agar:**

Malt extract	20g
Agar agar	20g
Distilled water	1000ml

4. Potato Dextrose Rose bengel agar:

Rose bengel	0.0084g
Dextrose	20g
Agar agar	15g
Distilled water	1000ml

5. Sabouraud's agar:

Peptone	10g
Dextrose	40g
Agar agar	15g
Distilled water	1000ml

The p^H of different media was adjusted to 6.0 before autoclaving. The Petri dishes were inoculated with equal sized mycelial discs made by 5mm cork borer from margin of young growing colonies and incubated at 25 ± 1^0C. Three replications were maintained.

The experiment was designed in Complete Randomized Design. Mycelial growth was recorded after seven days of incubation(mm).

3.5. EPIDEMIOLOGY

3.5.1 Meteorological parameters in relation to disease incidence

In order to study the relationship of meteorological parameters on the natural occurance of *Rhizoctonia solani* in cowpea, observations were taken from 10/08/2015 to 11/10/2015 consisting of 2 month meteorological months (2015 Kharif) under field condition at experimental plot of AICRP on vegetable crops, OUAT, Bhubaneswar. The weather parameters like maximum and minimum temperature (^{0}C), maximum and minimum RH (%), rainfall (mm) and bright sunshine hours (hr) were co-related with *Rhizoctonia* disease incidence. The mortality was recorded basing on the intensity of disease appearance on cowpea through visual estimation. Per cent disease incidence (PDI) was calculated by using the formula as mentioned below

$$PDI= \frac{\text{No of plants wilted}}{\text{Total no. of observations}} X\ 100$$

3.6 STUDY OF HOST RANGE

Different plant spp. i.e. Rice (*Oryzae sativa*), Maize (*Oryzae sativa*), Tomato (*Solanum lycopersicum*), Okra (*Abelmuschus esculentus*), Brinjal (*Solanum melongena*), Sunflower (*Helianthus annus*), Coriander (*Coriandrum sativum*), Chilli (*Capsicum annuum*), Field pea (*Pisum sativum)*, Raddish (*Raphanus sativus*), Cucumber (*Cucumis sataivus*), Bengal gram(*Cicer arietinum*), Arhar (*Cajanus cajan*), Cotton (*Gossypium hirsutum*), and Groundnut (*Archis hypogeae*) with suspected fungal infection (root and stem rot) were collected and examined under microscope. The fungal pathogen *R. solani* was brought into pure culture on PDA medium and pathogen was established. Above plant spp. were grown in poly pots and the pathogenic fungal isolates from each plant including cowpea were cross inoculated with each other. The reaction was recorded after seven days of inoculation.

Different host plant seeds used in this experiment are enlisted below.

Table-1:List of different seeds used for host range study

Sl No	Common name	Scientific name	Family
1	Rice	*Oryza sativa*	Poaceae
2	Maize	*Zea mays*	Poaceae
3	Tomato	*Solanum lycopersicum*	Solanaceae
4	Okra	*Abelmoschus esculentus*	Malvaceae
5	Brinjal	*Solanum melongena*	Solanaceae
6	Sunflower	*Helianthus annus*	Asteraceae
7	Coriander	*Coriandrum sativum*	Apiaceae
8	Chilli	*Capsicum annuum*	Solanaceae
9	Field pea	*Pisum sativum*	Leguminaceae
10	Raddish	*Raphanus sativus*	Brassicaceae
11	Cucumber	*Cucumis sativus*	Cucurbitaceae
12	Bengal gram	*Cicer arietinum*	Leguminaceae
13	Arhar	*Cajanus cajan*	Fabaceae
14	Cotton	*Gossypium hirsutum*	Malvaceae
15	Groundnut	*Arachis hypogeae*	Fabaceae

3.7 MANAGEMENT STRATEGIES

3.7.1 *In vitro* evaluation of plant extracts in inhibiting the growth of the fungus by poison food technique-

Fresh plant materials were collected and washed first in tap water and then in distilled water. These leaves were allowed to dry under air. Hundred grams of fresh sample was chopped and then crushed in a surface sterilized pestle and mortar by adding 100ml sterile water (1:1 w/v). The extract was centrifuged at 10000 rpm at 26^0C for 20 minutes. Finally filtrate thus obtained was used as stock solution.

To study the antifungal mechanism of plant extracts, the poisoned food technique was used (Nene and Thapliyal, 1973) in *in vitro*. Ten ml, fifteen ml and twenty mlof the stock solution was mixed with 90, 85 and 80 ml of sterilized molten PDA medium respectively so as to get 10, 15 and 20 percent concentration. The medium was thoroughly shaken for uniform mixing of extract. Twenty ml of medium was poured into sterile Petri dishes. Mycelium of 5 mm size discs from periphery of actively growing culture were cut out by sterile cork borer disc, was placed on the center of each agar dish. Controls were also maintained by growing the only pathogen on PDA dishes. Each treatment was replicated thrice and dishes were incubated at 28 ± 1^0C till control dishes reached the maximum radial growth of 90 mm.

Different plant extracts used in these experiments are enlisted below.

Table 2:List of plant extracts used in *In vitro* evaluation against *R.solani*

Sl. No	Common name	Botanical name	Plant part used
1	Neem	*Azadirachta indica* A.	Leaf
2	Garlic	*Allium sataivum* L.	Clove
3	Turmeric	*Curcuma longa*	Rhizome
4	Karanja	*Pongamia glabra*	Leaf
5	Begunia	*Begunia nirgundi*	Leaf
6	Datura	*Datura stramonium*	Leaf
7	Milkweed	*Calotropis spp.*	Leaf

The per cent inhibition over control was calculated according to formula given by Vincent (1947) as follows.

$$I = \frac{(C-T)}{C} x100$$

I = Per cent inhibition of mycelium

C = Growth of mycelium in control

T = Growth of mycelium in treatment

3.7.2 *In vitro* evaluation of bioagents by Dual culture technique

The antagonistic microorganisms like *Trichoderma viride, Trichoderma harzianum, Trichoderma hamatum* and *Bacillus subtilis* were maintained in medium potato dextrose agar and evaluated for their antagonistic effect under *in vitro* conditions against *Rhizoctonia solani* by dual culture technique.

Dual culture technique

In dual culture technique, twenty ml of sterilized and cooled potato dextrose agar was poured into sterile Petri dishes and allowed to solidfy. Fungal antagonistics inoculating the pathogen at one side of Petridish and the antagonist inoculated at exactly opposite side of the same plate by leaving 3-4 cm gap. For this, actively growing cultures were used. Each treatment was replicated 2 times. After required period of incubation i.e. after control plate reached growth of 90 mm diameter, the radial growth of pathogen was measured. Per cent inhibition over control was worked out according to formulae given by Vincent (1947).

$$I = \frac{(C-T)}{C} x100$$

I = Per cent growth inhibition of mycelium

C = Growth of mycelium in control

T = Growth of mycelium in treatment

3.7.3. Evaluation of fungicides

The fungicides were tested initially under *in vitro* condition by poison food technique (Nene and Thapliyal,1973) at desired concentration in *in vivo* condition by pot culture method.

3.7.3.1 *In vitro* evaluation of fungicides by poisoned food technique

All the fungicides were tested at specified concentrations by adopting poisoned food technique (Nene and Thapliyal,1973). The required concentrations of chemicals were prepared and incorporated into sterilized, cooled potato dextrose agar medium.

Twenty ml of medium was poured into 90 mm sterilized Petri dishes and all plates inoculated with actively growing 5 mm mycelial disc of test fungus. Three replications were maintained for each treatment. These plates were incubated at 25 ± 1^0C for seven days and then colony diameter was recorded. Per cent inhibition of mycelial growth over control was calculated by using the formulae given below by Vincent (1947).

3.7.3.2 *In vivo* evaluation of fungicides

Soil was collected from the research plot of Department of Plant Pathology. The soil was sterilized in autoclave at 121^{0}C at 15 psi for 2 hours. Sterilized soils were put into polythene bag mixing with inoculum of actively growing culture of *R. solani*. Then it was incubated for 10days. After that seeds of cowpea at the rate of ten seeds per pot were sown. After thirteen days, thinning was done. Then the required concentrations of chemicals were prepared and incorporated into pot. Three replications were maintained for each treatment.

Table 3: List of different fungicides tested for efficacy in *R.solani*

Fungicide tested	Commonname/Trade name	Chemical name	Concentration (%)
1	Bavistin	Carbendazim 50%WP	0.2
2	Vitavax power	Carboxin 37.5% + Thiram 37.5%	0.2
3	Sheathmar 3L	Validmycin 3%L	0.1
4	Onestar	Azoxystrobin 23% SC	0.1
5	Sixer	Mancozeb 63%WP + Carbendazim 12% WP	0.2
6	Curzate	Cymoxanil 8% WP+Mancozeb 64% WP	0.15
7	Roko	Thiophanate Methyl 70% WP	0.15
8	Antracol	Propineb 70% WP	0.2
9	Contaf	Hexaconazole 5% EC	0.05
10	Score	Difenconazole 25% EC	0.05
11	Folicur	Tebuconazole 25%EC	0.05
12	Tilt	Propiconazole 25% EC	0.05
13	Nativo	Tebuconazole50%WP+ Trifloxystrobin25%WP	0.05

3.7.4. *In vivo* efficacy of organic products against *R. solani*

Different organic products were taken to evaluate their efficacy in field condition for controlling the *Rhizoctonia solani* infecting cowpea. Organic products were selected for experimental purpose as per their local availability viz. Mustard oil cake, Karanj oil cake, Neem oil cake, Mahua oil cake, Poultry manure, Goat manure, VAM, Vermicompost, Spent Musroom Substrate and fresh cow dung. Each treatment was replicated thrice. Soil was sterilized at 15 psi at temperature of 121^{0}C for 20 minutes.The soil was inoculated with the culture of *Rhizoctonia solani.* The poly pots were closed with rubber bands and kept under shade. The organic products were amended thoroughly with previously incubated soil at the rate of 10gms per kg of soil. After mixing the organic products, the poly pots were closed with rubber bands and kept for seven days. Ten cowpea seeds were sown in each pot and watering was carried out thrice a week to promote germination and growth of the plants. The regular observation was recorded with respect to mortality percentage and plant height.

Table 4: List of organic products used against *Rhizoctonia soalni*

Sl. No	Name	Quantity (gm)/Kg of soil
1	Mustard oil cake	10
2	Karanja oil cake	10
3	Poultry manure	10
4	Goat manure	10
5	Vescicular arbuschular mycorrihiza(VAM)	10
6	Vermi compost	10
7	Spent mushroom substrate (SMS)	10
8	SMS + Cow dung	10
9	SMS + Vermi compost	10
10	SMS + Vermicompost +Cow dung	10
11	Neem oil cake	10
12	Mahua oil cake	10

3.8 STATISTICAL ANALYSIS

Statistical analysis was carried out by following the standard procedures (Panse and Sukhatme ,1967). Data in percentage were transformed to angular values before analysis.

CHAPTER-4

RESULTS

RESULTS

4.Experimental results

The experimental results of the studies on root and stem rot of cowpea caused by *Rhizoctonia solani* conducted both *in vitro* and *in vivo* are presented below.

4.1. Disease incidence survey

A survey was conducted during Rabi 2015 in different Cowpea growing areas,particularly in the farmer's field of Khamangasasan (Khurdha) Chittalpur (Cuttack) Anandapur (Keonjhar) and AICRP on vegetable crops,OUAT, Bhubaneswar. The survey results revealed the incidence of *Rhizoctonia* root and stem rot ranging from 18.4 to 40.5%. In Keonjhar the least percentage of incidence was recorded (18.4%), whereas the maximum incidence was noticed at AICRP on vegetable crops, OUAT, Bhubaneswar(40.5%). Besides *Rhizoctonia* root and stem rot other diseases like Anthracnose and Cercospora leaf spot were also noticed. The data relating to survey is presented in table 5.(Figure 1)

Table-5: Survey for the incidence of *Rhizoctonia solani* of cowpea in different places

S.No	Place	District	Disease Survey		
			Percentage of disease incidence		
			Rhizoctonia solani	Anthracnose	Cercospora leaf spot
1	AICRP on vegetable crops, OUAT	Khurda	40.5	24.6	10.8
2	Anandapur	Keonjhar	18.4	21.2	24.6
3	Chittalpur	Cuttack	26.3	6.8	14.0
4	Khamangasasana	Khurda	20.0	4.0	12.6

'igure: 1 Survey of root and stem rot of cowpea

A) Initiation of disease in cowpea (30 day after sowing) , (B) and (C) Later tages of root and stem rot symptom at AICRP on vegetable crops,OUAT, 3hubaneswar

4.2. SYMPTOMATOLOGY

It was observed that the pathogen girdled the stem and root portions. Initially infected root and stem showed water soaked lesions at the infected portion. However, the lesions rapidly expanded both up and down. The root and stem became girdled, discoloured and dried as the disease advanced. Finally, the infection caused withering and death of the plants. On the infected portion while mycelial growth was noticed in which brown sclerotial bodies were found embeded.

4.3 ISOLATION AND IDENTIFICATION OF THE FUNGUS

Infected root and stem rot samples were collected and thin transverse section of the affected parts were prepared and examined under microscope to observe the morphological characters of the fungus. From the fresh affected root and stem rot samples, the fungal pathogen *Rhizoctonia solani* could be observed consistently on microscopic study.

The pure culture of the fungus was also maintained in the Petri dishes and also in slants after isolation from the disease samples and cultural characteristics were studied.

4.3.1 Study of cultural characteristics

The fungus in pure culture produced colonies of scattered mycelium imparting whitish colour after seven days of inoculation.(Figure-2)

4.3.2 Study of morphological and taxonomic characteristics

Table 6: Mesurement (μm) of sclerotia and mycelium of *Rhizoctonia solani* from host and culture

Sl. No.	Characterstics	From Host	From Culture
1	Formation of sclerotia	Scattered	Scattered
2	Location of sclerotia	Aerial and surface	Aerial and surface
3	Texture of sclerotia	Rough	Rough
4	Colour of sclerotia	Light to dark brown	Light to dark brown
5	Average diameter of sclerotia (μm)	1798-2056μm and average 1942μm	1961-2265μm and average 2140μm
6	Sclerotia per plate after ten days of inoculatrion)	142	155
7	Hyphal width	5.75μm	5.81μm
8	Colony colour	Whitish brown	Whitish brown
9	Growth pattern	Abundant	Abundant
10	Colony diameter	90mm	90mm

Microscopic examination revealed that hyphae are multinucleate, hyaline, septate and branched at right angles. Hyphae are 5.81μm wide in culture and 5.75μm in host. On agar media, *R. solani* produced light to dark brown scattered mycelium. Sclerotia were produced abundantly in aerial as well as surface part of culture. Sclerotia were produced in Petri dishes with average 155 number per plate in culture and 142 in host after ten days of inoculation. Sclerotia diameter measured 1798-2056μm with an average of 1942μm from host and 1961-2265μm with an average 2140μm from culture. They were observed spherical, rough and light brown to dark brown in colour. From the above morphological characters, the pathogen was identified as *Rhizoctonia solani.* The taxonomic position of *Rhizoctonia solani* is

Domain: Eukarya

Kingdom: Fungi

Phylum: Basidiomycota

Class: Agaricomycetes

Order: Cantharellales

Family: Ceretobasidiaceae

Genus: *Rhizoctonia*

Species: *solani*

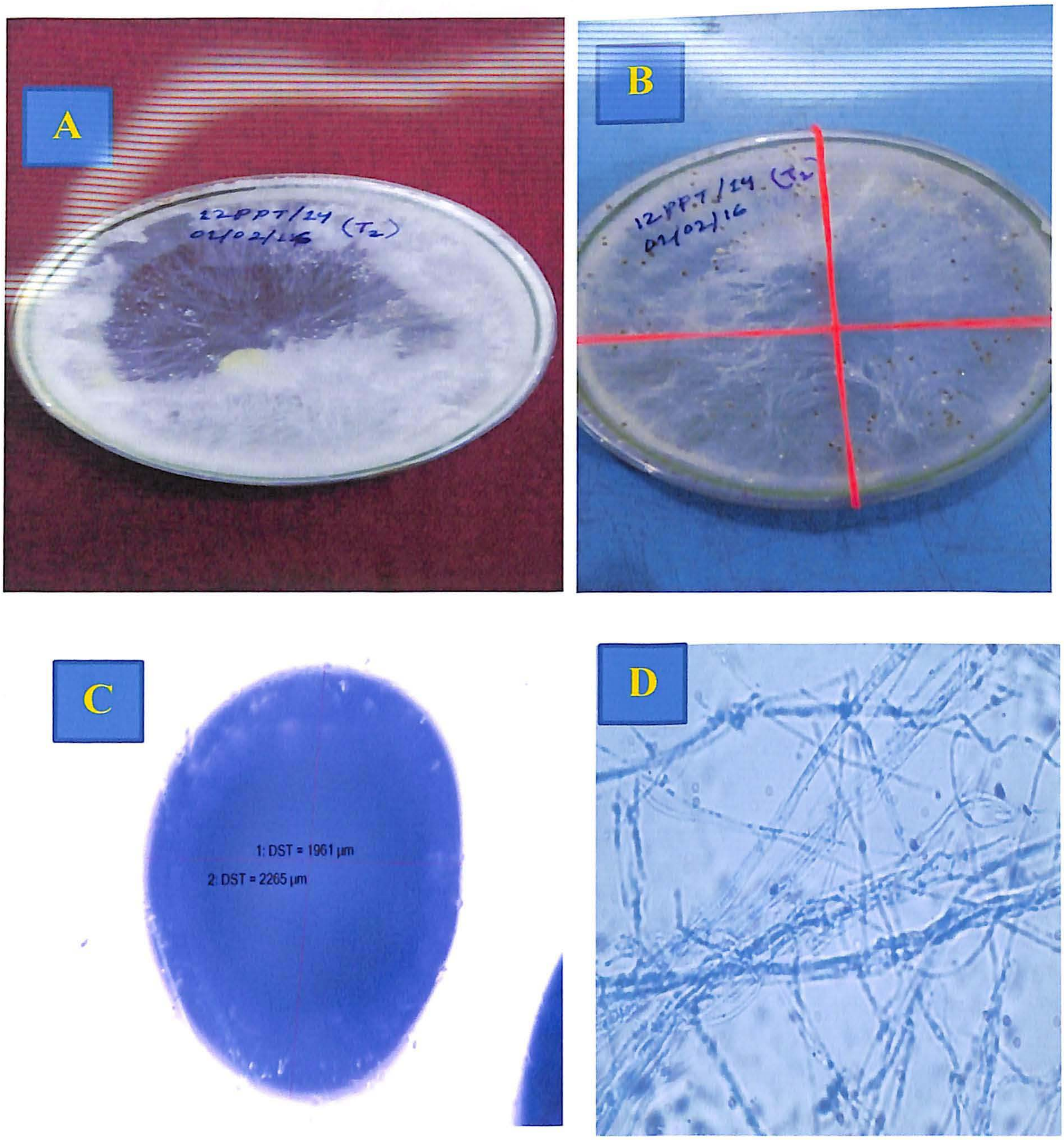


Figure 2: (A) Pure culture of *Rhizoctonia solani* after 7 days, (B) Sclerotial bodies 12 days after inoculation (C) Sclerotia body diameter and (D) Mycelial structure under microscope

Rhizoctonia soalni having teleomorphic stage is = *Thanatephorus cucumeris*

Taxonomic position

Domain: Eukarya

Kingdom: Fungi

Phylum: Basidiomycota

Class: Agaricomycetes

Order: Cantharellales

Family: Ceretobasidiaceae

Genus: *Thanatephorus*

Species: *cucumeris*

4.4 PATHOGENICITY TEST

To test the pathogenicity of the fungus, the cowpea plants were grown in polythene bags following Koch's postulates as the procedure described in 'Materials and Methods'. Symptoms were observed after seven days of inoculation where the control plants did not show any symptoms (Figure-3).

The symptoms produced by the fungus as well as its cultural, morphological and taxonomic characteristics were studied in detail and compared with the symptoms occurred in nature and fungus isolated originally from the diseased samples. In both the cases it was found similar and identified as *Rhizoctonia solani.*

Figure 3: Pathogenicity test

Figure 3: Pathogenicity test

4.5 GROWTH OF *Rhizoctonia solani* IN DIFFERENT SOLID MEDIA

In order to find out a suitable medium for *Rhizoctonia solani,* five different solid media were tried. Observations on radial growth of the mycelium were recorded after seven days of inoculation.The data obtained are presented in Table 7.

The growth of the pathogen *Rhizoctonia solani* was significantly different in culture media. In the media under study, the growth increased with an increase in incubation period. Potato dextrose agar gave maximum growth of the fungus (90.00mm). The most effective supporting medium for growth of the fungus was the Sabouraud's dextrose agar which showed 87.90 mm diameter mycelial growth of the pathogen after an incubation period of seven days followed by Potato dextrose rose bengal agar (82.40 mm). The minimum growth obtained in Malt extract agar (70.98mm). The result showed that, the rate of growth in solid media was significantly different from each other. (Figure-4)

Table-7:Growth of *Rhizoctonia solani* in different solid media

S.No.	Name of solid medium	Radial growth of colony (mm)
1	Oat meal agar	76.60*
2	Malt extract agar	70.98
3	Potato dextrose rose bengal agar	82.40
4	Sabouraud's dextrose agar	87.90
5	Potato dextrose agar	90.00
	SE(m)±	1.73
	CD(0.05)	5.14

***Mean of five replication**

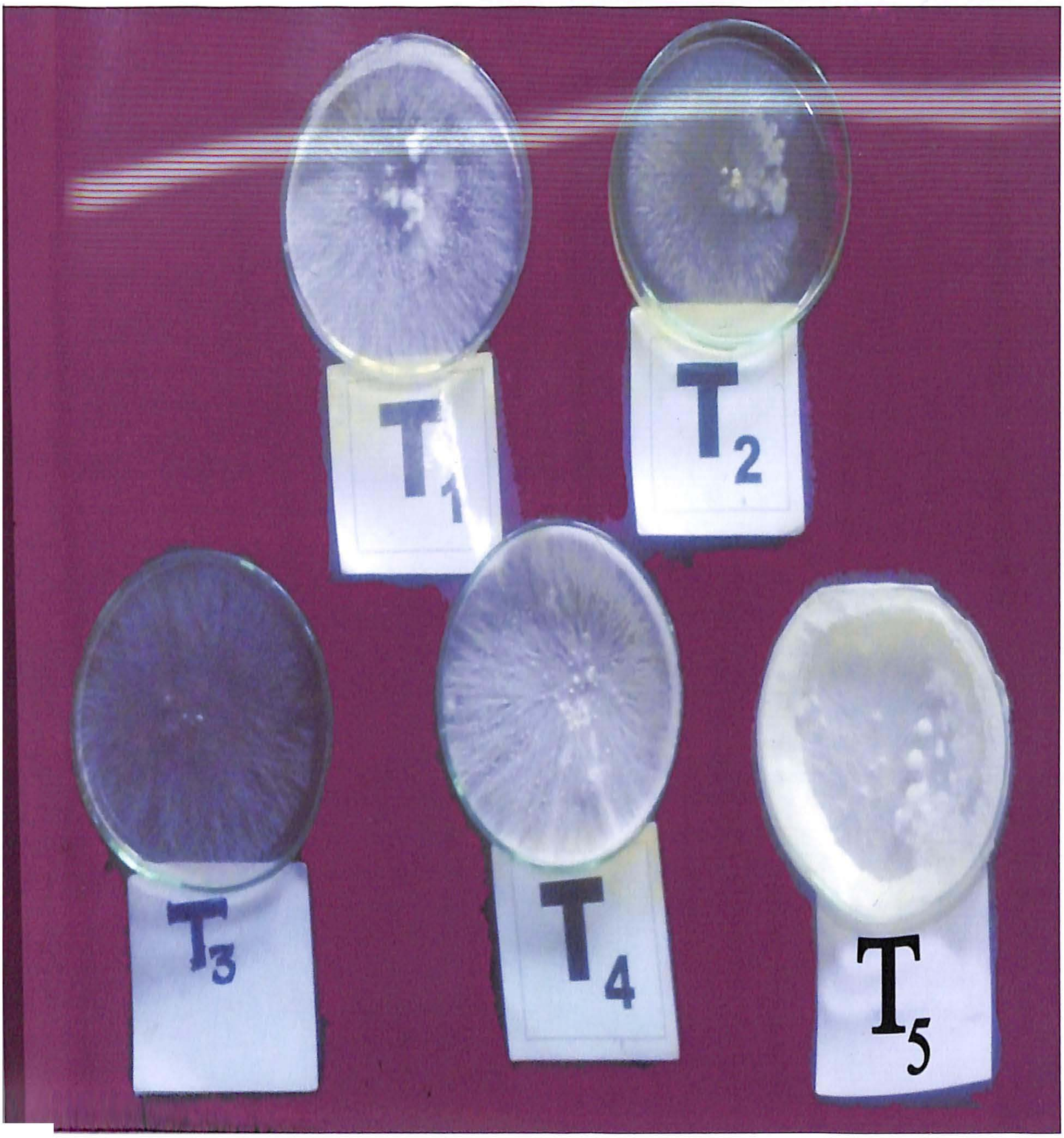


Figure-4: Growth of test fungus in different media

T_1: Oat meal agar,T_2: Malt extract agar,

T_3: Potato dextrose rose Bengal agar, T_4: Sabouraud's dextrose agar, T_5: Potato dextrose agar.

4.6 ENVIRONMENTAL FACTORS AFFECTING GROWTH AND DISEASE DEVELOPMENT BY *Rhizoctonia solani*

4.6.1 Meteorological parameters in relation to disease incidence

Observations on the incidence of *Rhizoctonia solani* of cowpea was recorded at weekly intervals as per procedures described under 'Materials and Methods'. The data of meteorological parameters and disease intensity are presented in Table-8

Table -8 Meteorological parameters

It may be seen from the table 8 that, the disease appeared more or less throughout the season in cowpea crop. The least infection was found during august when the temperature and RH were in the range 24-34^{0}C and 73-94 per cent respectively.

Table 8: Climatic parameters and disease incidence recorded from 13.08.2015 to 14.10.2015

Meterological week	Max. Temp. (^0C)	Min.Temp.(^0C)	Max. RH(%)	Min. RH(%)	Rainfall (mm)	BSH(hr)	PDI
13/08/2015-19/08/2015	32.1	24.9	94	84	36.8	3.8	1.6
20/08/2015-26/08/2015	33.3	24.8	91	73	124	4.3	6.8
27/08/2015-02/09/2015	32	25.3	94	81	54.4	4.1	11.4
03/09/2015-09/09/2015	33.4	24.2	91	69	58.5	3.4	26.8
10/09/2015-16/09/2015	32.8	24.8	92	83	59.7	4.3	32
17/09/2015-23/09/2015	32.3	25.5	94	78	23.9	4.4	38.6
24/09/2015-30/09/2015	33.7	25.5	89	67	9.4	5.7	42.2
01/10/2015-07/10/2015	33.9	24.7	92	75	35.8	6.4	44.6
08/10/2015-14/10/2015	32.7	22.9	92	64	36.4	5.8	51

Table 9: Co-relation matrix of climatic parameters on disease incidence (Kharif,2015)

	X_1	X_2	X_3	X_4	X_5	X_6	Y
X_1	1.000						
X_2	-0.090	1.000					
X_3	-0.836	0.107	1.000				
X_4	-0.576	0.536	0.699	1.000			
X_5	0.062	-0.116	-0.066	0.096	1.000		
X_6	0.516	-0.211	-0.353	-0.463	-0.401	1.000	
Y	0.436	-0.330	-0.353	-0.573	-0.570	0.730	1.000

The Prediction Equation,

$$Y= -151.266+6.372x_1-4.308x_2+1.093x_3-0.512x_4-0.248x_5+5.583x_6$$

Co-efficient of determination $R^2 = -1.80$

Where, X_1=Maximum Temperature

X_2 =Minimum Temperature

X_3 = Maximum RH

X_4 =Minimum RH

X_5 = Rainfall

X_6 = Bright sunshine Hours

Y = Per cent disease incidence

It is revealed from the correlation study that, all the weather factors (Maximum Temperature, Minimum Temperature, Maximum RH, Minimum RH, Rainfall and Bright sunshine hours) did not yield any result towards disease development. The maximum disease infection was found in the month of October at maximum temperature 32.7^{0}C and minimum temperature 22.9^{0}C with maximum RH 92% and minimum RH 64% respectively. Therefore the soil factor was responsible for disease development. Sandy loam textured soil and acidic to neutral P^H contributed root and stem rot disease as high as 51.00% in second week of October 2015.

4.7HOST RANGE

The fungal pathogen isolated into pure culture from different hosts as described earlier in ''Materials and Methods'' were cross inoculated and the reaction of each host species after seven days of inoculation have been presented in Table10(Figure 5a and 5b).

It might be seen (Table 10) that, *R. solani* isolate from cowpea could infect rice (*Oryza sativa*), maize (*Zea mays*), tomato (*Solanum lycopersicum*), chilly (*Capsicum annuum*), brinjal (*Solanum melongena*), fieldpea (*Pisum sativum)*, cucumber *(Cucumis sataivus),* bengalgram (*Cicer arietinum*), arhar (*Cajanus cajan*), cotton (*Gossypium hirsutum*), and groundnut (*Arachis hypogeae*) in addition to its own host. Similarly the isolate of rice and maize could infect cowpea in addition to their own host. The isolate of tomato (Solanum *lycopersicum*), brinjal (*Solanum melongena*) and chilly (*Capsicum annuum*) could infect each other along with cowpea(*Vigna unguiculata*). The isolate of okra (*Abelmuschus esculentus*) and fieldpea (*Pisum sativum)* could infect each other. Sunflower (*Helianthus annus*) and groundnut (*Archis hypogeae*) isolates, cucumber (*Cucumis sataivus*), Bengal gram (*Cicer arietinum*) and arhar (*Cajanus cajan*) could infect each other in addition to cotton. However, raddish (*Raphanus sativus*) and coriander(*Coriandrum sativum*) confined to their own hosts only.

It was evident from foregoing observation that the isolates of rice, maize, tomato, brinjal, chilly, fieldpea, cucumber, Bengal gram, arhar, cotton and groundnut were identical in their pathogenicity as they caused similar infection. The symptoms subsequent upon infection by these isolates were similar comprising of formation of reddish-brown lesions just at the soil line i.e. collar region of plant. The lesion increased in size both upward to the stem and downward to the roots with the advancement of the disease, the plants started dying in a few days.

Table 10: Reaction of different host species to *Rhizoctonia solani* occour after 7 days of inoculation

	Cowpea	Rice	Maize	Tomato	Okra	Brinjal	Sunflower	Coriander	Chilly	Fieldpea	Raddish	Cucumber	Bengel gram	Arhar	Cotton	Ground nut
Cowpea	+	+	+	-	-	-	-	-	-	+	-	+	-	+	+	+
Rice	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Maize	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Tomato	+	-	-	+	-	+	-	-	+	-	-	-	-	-	-	-
Okra	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-
Brinjal	+	-	-	+	-	+	-	-	+	-	-	-	-	-	-	-
Sunflower	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-
Coriander	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Chilly	+	-	-	+	-	+	-	-	+	-	-	-	-	-	-	-
Fieldpea	+	-	-	+	+	-	-	-	-	+	-	-	+	+	-	-
Raddish	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-
Cucumber	+	-	-	+	-	+	-	-	-	-	-	+	-	-	-	-
Bengel gram	+	-	-	-	-	-	-	-	-	+	-	-	+	+	+	+
Arhar	+	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
Cotton	+	-	-	-	+	-	-	-	-	-	-	-	+	+	+	+
Ground nut	+	-	-	-	-	-	+	-	-	-	-	-	-	-	+	+

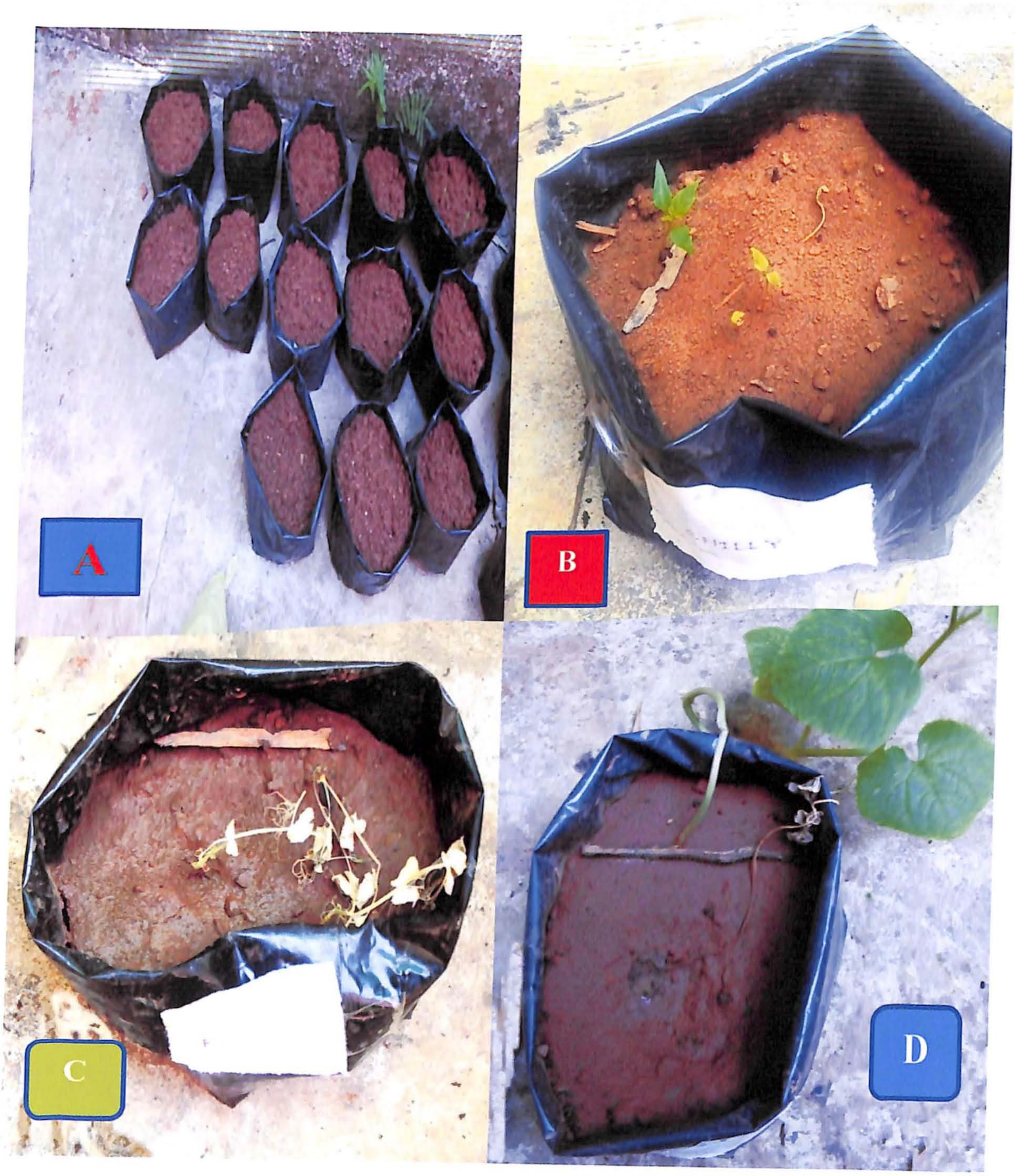


Figure 5(a) : Cross inoculation for host range study

(A) Sowing of seed in pot

(B), (C) and (D) Damping off of chilly, field pea and cucumber plant

gure 5(b): *Cross* inoculation for host range study

otting of seed occour in (E) arhar, (F) ground nut, (G) bengal gram and (H) maize in *R. lani* inoculated pots

4.8 DISEASE MANAGEMENT

4.7.1 Efficacy of plant extracts in inhibiting the growth of the fungus

Seven plant extracts, which are easily available in the locality were evaluated for their fungitoxicity activity against the growth of *Rhizoctonia solani* at 10,15 and 20% concentration as per the procedure described under ''Materials and Methods''. The radial growth of the fungal colony on plant extract mixed medium were. measured after seven days of incubation and the data obtained were presented in Table 11(Figure 6).

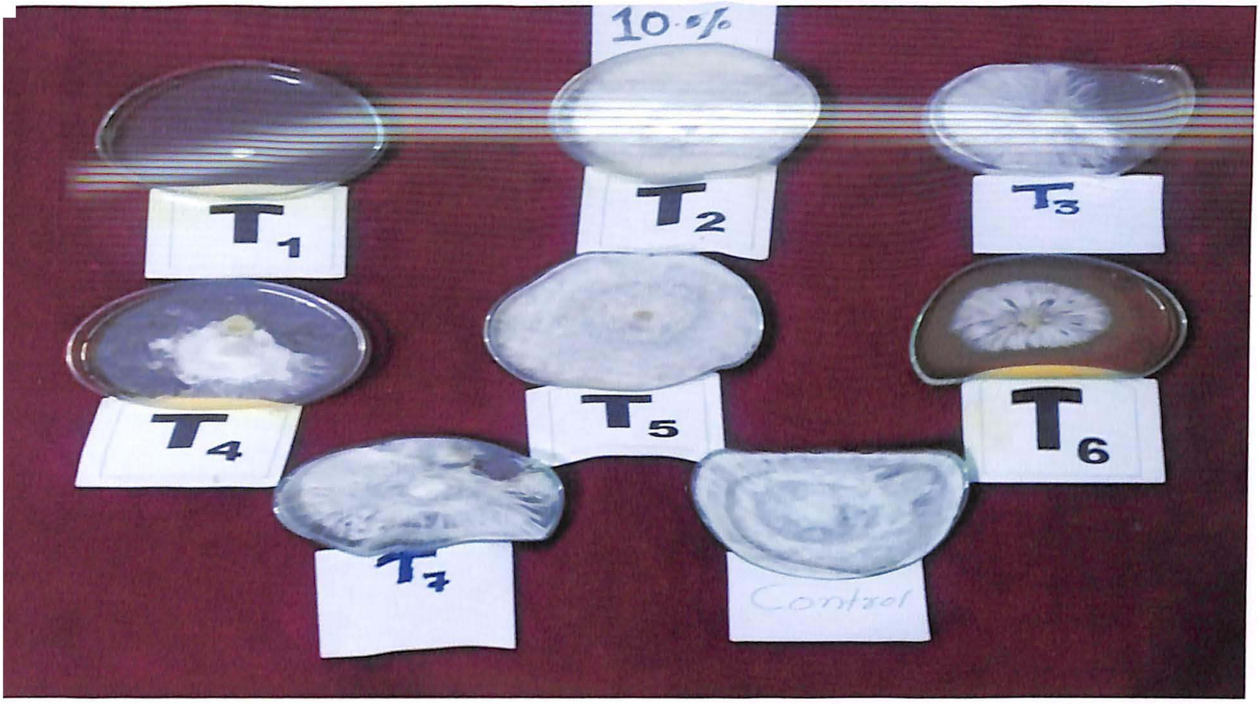
All the plant extracts under study inhibited mycelial growth of *Rhizoctonia solani* at 10, 15 and 20 per cent concentrations and were significantly superior over control. The results indicated that, the maximum inhibition was recorded in Garlic (100%) followed by Turmeric (34.08) at 10% concentration. At 15% concentration, a total inhibition was noticed in Garlic and Calotropis(100%) followed by Turmeric (41.11%). At 20% concentration, a total inhibition was noticed in Garlic and Calotropis (100%) followed by Turmeric (68.33%). The least reduction of growth was observed in Begunia (0.38%) at 10% concentration and in Karanja 5.56% and 9.82% at 15% and 20% concentration respectively. In general all the leaf extracts tested, significantly inhibited the mycelia growth of *Rhizoctonia solani* above 1% at 10% concentration, 5% at 15% concentration and 9% at 20% concentration except Begunia and Karanja.

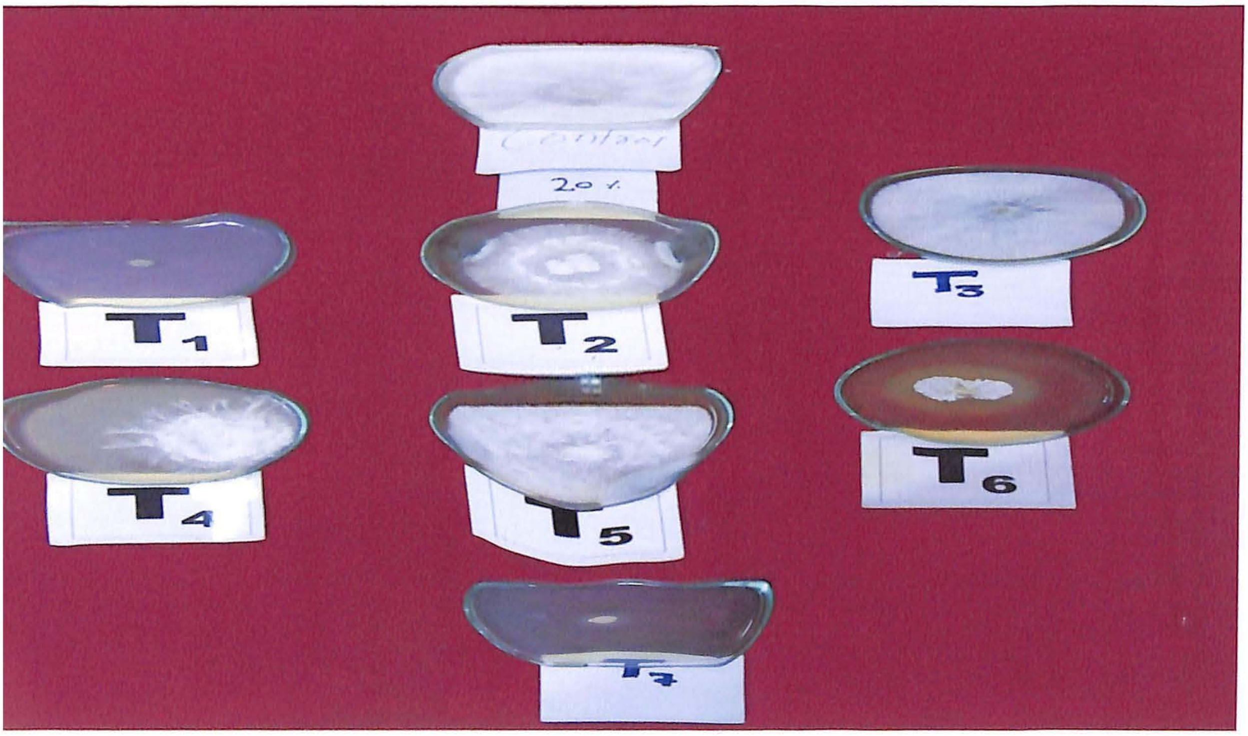
Table 11. Efficacy of plant extracts against *Rhizoctonia solani* in *in vitro*

Sl. No	Common name	Percent inhibition at 10% conc.	Percent inhibition at 15% conc.	Percent inhibition at 20% conc.
1	Garlic(*Allium sataivum* L.)	*100 (90.02)	100 (90.02)**	100 (90.02)
2	Neem *(Azadirachta indica* A.)	1.11 (6.05)	17.41 (24.67)	21.11 (27.36)
3	Begunia (*Vitex nirgundi*)	0.38 (3.52)	6.67 (14.97)	20.87 (27.20)
4	Datura (*Datura stramonium*)	30.93 (33.80)	33.33 (35.27)	41.67 (40.21)
5	Karanja (*Pongamia glabra*)	9.82 (18.27)	5.56 (13.64)	9.82 (18.27)
6	Turmeric(*Curcuma longa*)	34.08 (35.72)	41.11 (39.89)	68.33 (55.77)
7	Milkweed (*Calotropis spp.*)	7.78 (16.20)	100 (90.02)	100 (90.02)
8	Control	0	0	0
	SE(m)±	0.79	0.66	0.55
	CD(0.05)	2.36	1.88	1.65

* Mean of three replications

** Figures in parenthesis are angular transformed value





gure 6:. Efficacy of plant extracts at 10% and 20% concentration against *Rhizoctonia solani* in *in vitro*

: Garlic, T_2 : Neem, T_3 : Begunia, T_4 : Datura, T_5 :Karanja, T_6 : Turmeric, T_7: Milk weed, C-Control

4.7.2.EFFECT OF BIOCONTROL AGENTS ON MYCELIAL GROWTH OF *Rhizoctonia solani.*

An experiment was conducted to explore the capabilities of four bio-agents against the test pathogen. The antagonistic nature of these bioagents against the test fungus was studied adopting dual culture technique and data obtained are presented in Table 12 (Figure 7)

Antagonistic studies revealed that, growth of pathogen was significantly checked over control by the antagonistic nature of all the antagonists tested. The antagonists also restricted the growth of the pathogen and didn't allow it to grow further. The lowest growth of the pathogen was observed in *Bacillus subtilis*(25.16mm) followed by *Trichoderma hamatum* (45.20mm),*Trichoderma harzianum* (48.60mm), *Trichoderma viride*(50.60mm).However, the growth of *Rhizoctonia solani* in *Trichoderma viride* was maximum.

Regarding the degree of growth inhibition, *Bacillus subtilis* inhibited the maximum growth of the pathogen (72.04%) followed by *Trichoderma hamatum, Trichoderma harzianum* but such inhibition of the growth of the pathogen with *Trichoderma viride* was relatively least among all the antagonists. The range of growth inhibition was from 43.78% to 72.04% which undoubtly positively establishes a check in the growth of pathogen without treatment with fungicides.

Table 12:*In vitro* bio assay of bio control agent

Sl.no.	Name of antagonist	Mean diameter of *Rhizoctonia solani* (mm)	*Mean diameter in dual culture (mm)	Per cent growth inhibition (%)
1	*Trichoderma hamatum*	90	45.20	49.78
2	*Trichoderma viride*	90	50.60	43.78
3	*Trichoderma harzianum*	90	48.60	46.00
4	*Bacillus subtilis*	90	25.16	72.04
5	Control	90	90.00	0.00

*Mean of three replications

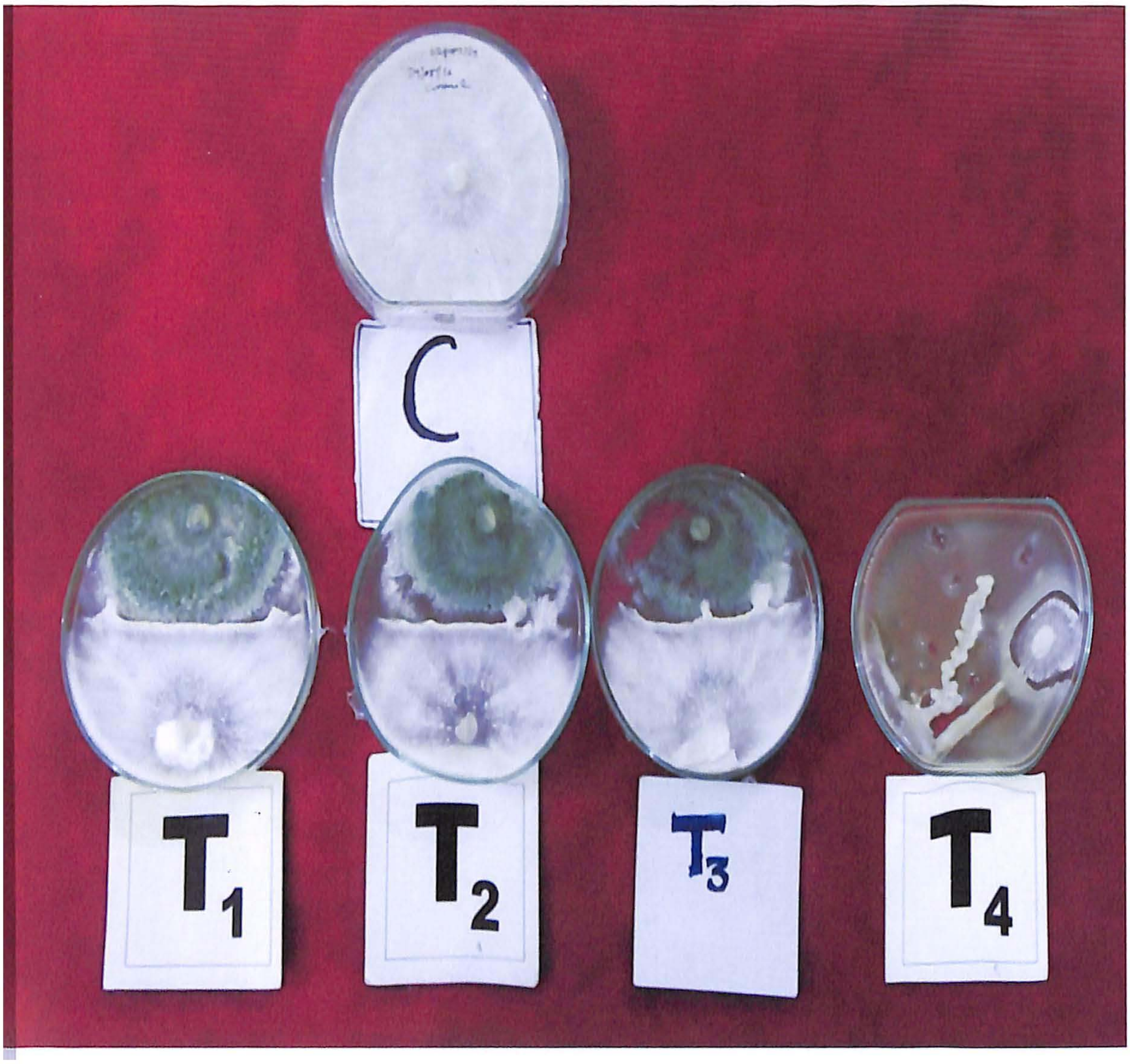


Figure 7: ***In vitro*** **bio-assay of biocontrol agents against** ***R. solani.***

C: Control, T1: ***Trichoderma hamatum*****, T2:** ***Trichoderma viride*****, T3:** ***Trichoderma harzianum*****, T4:** ***Bacillus subtilis***

4.7.3. Efficacy of fungicides on growth of *Rhizoctonia solani* in *in vitro*

In order to evaluate the efficacy of some selected fungicides on growth of *Rhizoctonia solani*, the experiment was conducted as per the procedure mentioned earlier in ''Materials and Methods'' following posion food technique. The colony diameter and per cent growth inhibition have been presented in Table 13 It was observed from the data that, all the fungicides tested in solid medium significantly reduced the fungal colony in comparision to control. (Figure 8).

It was revealed that fungicides namely Carboxin 37.5% + Thiram 37.5% along with Hexaconazole 5% EC, Difenoconazole 25% EC, Tebuconazole 25% EC and Tebuconazole 50% + Trifloxystrobin 25% proved efficacious in inhibiting the mycelial growth with 100 per cent inhibition. The next effective fungicides were Cymoxanil 8% WP+Mancozeb 64% WP and Propiconazole 25% EC which recorded 79.81 and 77.57 per cent inhibition respectively. However Validamycin, Mancozeb 63%WP + Carbendazim 12% WP Azoxystrobin 23% SC and Propineb 70 WP registered 54.63, 52.78 and 5.07 respectively.

Table 13 : Efficacy of fungicides on growth of *Rhizoctonia solani* in *in-vitro*

Fungicide tested	Chemical name	Concentration (%)	Per cent growth inhibition
1	Carbendazim 50%WP	0.2	0.00* (0)
2	Carboxin 37.5% WP + Thiram 37.5% WP	0.2	100 (90.02)
3	Validamycin 3%L	0.1	54.63 (47.67)
4	Azoxystrobin 23% SC	0.1	8.37 (16.82)
5	Mancozeb 63%WP + Carbendazim 12% WP	0.2	52.78 (46.60)
6	Cymoxanil 8% WP+ Mancozeb 64% WP	0.15	79.81 (63.31)**
7	Thiophanate Methyl 70% WP	0.15	1.26 (6.44)
8	Propineb 70% WP	0.2	5.07 (13.02)
9	Hexaconazole 5% EC	0.05	100 (90.02)
10	Difenoconazole 25% EC	0.05	100 (90.02)
11	Tebuconazole 25% EC	0.05	100 (90.02)
12	Propiconazole 25% EC	0.05	77.57 (61.74)
13	Tebuconazole50% WP+ Trifloxystrobin25%WP	0.05	100 (90.02)
	SE (m)±		0.90
	CD(0.05)		2.71

*Mean of three replication

**Figures in parenthesis are angular transformed value

Figure 8: ***In vitro*** **evaluation of different chemicals against** ***R. solani***

T_1-Carbendazim 50%WP, T_2-Carboxin 37.5%WP +Thiram 37.5%WP,T_3-Validamycin 3%L, T_4-Azoxystrobin 23%SC, T_5-Mancozeb 63%WP+Carbendazim12%WP, T_6-Cymoxanil8%WP+Mancozeb64% WP, T_7-ThioPhanate Methyl 70% WP, T_8-Propineb 70% WP, T_9-Hexaconazole 5%EC, T_{10}-Difenoconazole 25%EC, T_{11}-Tebuconazole 25%EC, T_{12}-Propiconazole 25%EC, T_{13}-Tebuconazole 50%WP+Trifloxystrobin 25%WP, C-Control

Table 14: Efficacy of fungicides on *Rhizoctonia solani* in *in-vivo*

Fungicide tested	Chemical name	Concentration (%)	*Mortality(%)
1	Carbendazim 50%WP	0.2	82.00*
2	Carboxin 37.5%WP + Thiram 37.5%WP	0.2	2.60
3	Validamycin 3%L	0.1	8.93
4	Azoxystrobin 23% SC	0.1	48.77
5	Mancozeb 63%WP + Carbendazim 12% WP	0.2	7.73
6	Cymoxanil 8% WP+ Mancozeb 64% WP	0.15	1.83
7	Thiophanate Methyl 70% WP	0.15	60.07
8	Propineb 70% WP	0.2	65.97
9	Hexaconazole 5% EC	0.05	2.67
10	Difenoconazole 25% EC	0.05	2.00
11	Tebuconazole 25% EC	0.05	12.63
12	Propiconazole 25% EC	0.05	12.10
13	Tebuconazole50%WP+ Trifloxystrobin25%WP	0.05	3.47
14	Control		99.07
	SE (m)±		1.51
	CD(0.05)		4.54

*Mean of three replication

4.7.4 Efficacy of fungicides on growth of *R. solani* in *in-vivo*

The fungicides which were studied in laboratory, they were further studied under pot culture experiment as per the procedure described under 'Materials and Methods' and presented in table 14.

This study reaveled that least mortality was recorded from Cymoxanil 8% WP+mancozeb 64% WP (1.83%) followed by Difenoconazole 25% EC (2.00%) and Carboxin 37.5% + Thiram 37.5% (2.60%). The maximum mortality was recorded in Carbendazim 50%WP(82.00%) followed by Propineb 70% WP (65.97%)and Azoxystrobin 23% SC (60.07%). However the control pots recorded as high as 99.07% mortality.

4.7.4 EFFECT OF ORGANIC AMENDMENT ON GROWTH OF *Rhizoctonia solani*

This study reaveled that, least mortality was recorded from spent mushroom substrate+ cowdung+ vermicompost (10.00%) followed by Vermicompost (15.00%). The mortality was recorded in poultry manure amendment(66.67%) followed by mustard oil cake (58.33%). Fifty per cent mortality was recorded in Spent mushroom substrate +vermicompost, Karanja oil cake and Mahua oil cake amended treatments. However control pots recorded as high as 91.66% mortality.(Figure 9)

So far as plant height was concerned the result was obtained in the same trend. Spent mushroom substrate + cowdung +vermicompost has recorded the maximum plant height (89.33cm) followed by vermicompost (81.67cm). However control pot recorded only (11.00cm).(Figure 16)

Figure 9: Different organic products amended with soil in poly pots

(A) Growth of pathogen in pot after 10 days of inoculation

(B) Mixing of organic products (Mixture of SMS+ Cowdung + Vermicompost) with inoculum pathogen in sterilized soil

(C) After mixing organic products in poly pots

(D) Thirty days after sowing of cowpea seeds

CHAPTER-5

DISCUSSION

DISCUSSION

Cowpea suffers from about many a diseases on global basis. The importance of outbreak of different diseases on this crop are the major constrains of its low productivity. A large number of pathogens have been reported in cowpea inducing root and stem rot, anthracnose, cercospora leaf spot and cowpea mosaic virus diseases. Under favourable climatic condition, diseases occour in alarming proportions causing deterioration in yield and quality of produce. In the present investigation, root and stem rot of cowpea caused by *Rhizoctonia solani* was observed in and around Bhubaneswar, which was studied with various aspects like isolation, identification and proving pathogenicity of pathogen, cultural and morphological characterstics, epidemiology, management practices exploring phytoextracts, bioagents, chemicals and organic products both *in vitro* and *in vivo* condition.

Root and stem rot of cowpea caused by *Rhizoctonia solani* was surveyed during 2014-15. The incidence of *Rhizoctonia solani* was recorded ranging from 18.4 to 40.5 per cent. During the survey, anthracnose and cercospora leaf spot were also recorded. The data revealed that anthracnose incidence ranged from 4.0 to 24.6 per cent while cercospora leaf spot incidence was ranging from 10.8 to 24.6 per cent.This findings were confirmed with the findings of Karaca *et. al.* (2002) from Turkey, Adandonon *et. al.*(2004) from Benin and Majchrzak and Kurowski (2002) from Poland.

In the pathogenicity test the cowpea has shown systemic infection from root to stem portion. Initially symptoms were observed in the root portion, the water soaked lesion at the infected point and it expanded both upward and downward direction. Finally the infection caused withering and death of plant with formation of white mycelial growth and brown colour sclerotia. This finding was also in confirmity with the findings of Jones *et. al.* (1970), Olsen and Young (1998), Dorrance *et. al.*(2001) and Adandonon *et. al.*(2004). The findings were *R.solani* caused root and stem rot, pre and post emergence damping-off, girdling of the stem and also seed rot.

Rhizoctonia solani (teleomorph: *Thanatephorus spp.*) is a plant pathogenic fungus with a wide host range and world wide distribution. The pure culture of *Rhizoctonia solani* produced the whitish mycelial growth initially, later it turned to brown with micro sclerotial bodies, which were produced abundantly in aerial and surface part of the culture. Micro sclerotia were produced in Petri dishes with an average of 155 number per plate after ten days of inoculation. Sclerotia were typically 2140µm in diameter, spherical rough and light brown to dark brown in colour. It supports the results of various researchers viz. Parmeter and Whiteny (1969) and Lal *et. al.* (2009). They found the hyphal branching at right angle constriction at the point of branching of the mycelium and presence of septum near the branching junction. The sclerotial formation was found in scattered as well as peripheral regions which is also in agreement with the present investigation.

During present investigation, the pathogenicity of test fungus was proved on potted cowpea plants following the postulates demonstrated by Robert Koch(1887).In pathogenicity test, *Rhizoctonia solani* caused infection on root as well as stem portion of cowpea plant. It also caused the infection as rotting on seed, seedling, root and stem. This finding was in conformity with the findings of Khedri *et. al.* (2014), who proved pathogenicity of *R. solani* in cotton.

In order to obtain adequate knowledge about the pathogen concerning growth characterstics, it was grown on a variety at solid media. The best one for *Rhizoctonia solani* was potato dextrose agar medium. The most effective supporting medium for the growth of *Rhizoctonia solani* was Sabouraud's dextrose agar medium. This finding coraborated with the earlier findings of Dash (1987). However Ranganathan *et. al.*(1973) reported that oat meal agar is suitable medium for growth of *R.solani* which is contradiction to the present finding.

The incidence of *Rhizoctonia soalni* of cowpea was recorded at weekly interval in the month of August when the temperature and relative humidity were in the range 24-34^{0}C and 73-94 per cent respectively. The maximum disease incidence was found in the month of October when temperature and relative humidity were in the range 22.9 to 32.7^{0}C and 64 to 92 per cent respectively. It supports the findings of Parmeter (1970), Harikrishna and Yang (2004) and Goswami *et.al.* (2011). This finding is also in agreement with the findings of Hemmiaud and Yokogi (1927) who reported the optimum temperature to be 30^{0}C. The reports of Parmeter (1970), Homma *et.al.* (1983), Sihna and Ghufran (1988) and Goswami *et. al.* (2011) regarding temperature requirement by *R. solani* is in agreement with the present observation.

Rhizoctonia solani was more contrast and variable soil inhibiting organism. It has the wider host range such as cereals, cottons, sugarbeet, potato, beans, peas, field crops, horticultural crops, flowers, grasses and forestries. In this studies the isolate of rice, maize, tomato, brinjal, chilly, pea, cucumber, bengal gram, cotton and groundnut found identical in their pathogenicity and caused similar infection. These expressed the symptoms like pre and post emergence damping off formation of reddish-brown lesions just above the soil line i.e. collar region of plant. The lesion increased in size both upward and downward directions on the plant. At severe infection, the plant started dying and collapse within a few days. It has been ascertained by earlier workers viz. Panwar *et. al.* (2012). on studing the host range of *R. solani* as chickpea, moth bean and mung bean in addition to cowpea.

All the phytoextracts under study inhibited mycelial growth of *Rhizoctonia solani* at 10%, 15% and 20% concentration as compared to control. The maximum inhibition was recorded in garlic (100%) at 10% concentration. Garlic and calotropis were recorded the maximum per cent growth inhibition (100%) in both 15% and 20% concentration respectively. Garlic, a potential phytoextract effective against a wide range of diseases including *R. solani* has been reported earlier by Shashidhara *et.al.*(2008) and Sehajpal *et.al.* (2009) which supports our findings. Shahnazdawar *et.al.*(2010) and Dawar *et.al.* (2010). reported potentiality of *Datura alba* against *R.solani* as efficacious one which also corroborating the present investigation.

In the present study the antifungal characterstics of bio-agents viz. *Trichoderma viride, Trichoderma hamatum, Trichoderma harzianum* and *Bacillus subtilis* were tested *in vitro* to study the effectiveness against *R.solani* employing dual culture technique. Maximum growth inhibition was found in *Bacillus subtilis* (72.04%) followed by *Trichoderma hamatum* (49.78%) and *Trichoderma harzianum* (46.00%). The effectiveness of *Trichoderma spp.* in field of plant disease management was confirmed earlier by various workers like Elad *et.al.* (1983), Chang and Choi(1990), Nagarajkumar *et.al.*(2004), Sharma *et.al.* (2009), Bhat *et.al.* (2009), Akhtar *et.al.* 2010) and Panwar *et.al.* (2012). The antagonistic nature of *Bacillus subtilis* also upports the findings of Schmiedeknecht (1993), who reported that *B. subtilis* is a uitable antagonists for biological control of *Rhizoctonia* disease on potato.

In vitro bio assay of fungicides against *Rhizoctonia solani* revealed that, Carboxin 37.5% + Thiram 37.5% @ 0.2%, Hexaconazole 5% @ 0.05%, Difenoconazole 25% @ 0.05%, Tebuconazole 25% @ 0.05% and Tebuconazole 50% + Trifloxystrobin 25% @ 0.05% recorded maximum growth inhibition however Validamycin 3% @ 0.1%, Cymoxanil 8% + Mancozeb 64% @ 0.15%, Mancozeb 63% + Carbendazim 12% @ 0.2%, Propiconazole 25% @ 0.05% concentration also checked the growth of *Rhizoctonia solani* ranging 52.78% to 91.81% inhibition. It was supported by earlier report of Sahu (1986), who reported that Carboxin performed best against *R.solani* in Rapeseed and mustard.

In *in vivo* study with chemicals in pot culture experiment, the minimum mortality was recorded in Cymoxanil 8% + Mancozeb 64% @ 0.15% followed by Difenoconazole 25% @ 0.05% and Carboxin 37.5% + Thiram 37.5% @ 0.2 % concentration registered the mortality as 1.83%, 2.0% and 2.60% respectively. The maximum mortality was recorded in Carbendazim 50% @ 0.2 % followed by Propineb 70% @ 0.2% recording mortality as with 82.0% and 65.97% respectively. The effectiveness of Carbendazim was found to be less against *R.solani* , but Sunder *et.al.* (2009) reported that Carbendazim was proved effective against *R.solani* in mung bean, which contradicts the present findings.

In pot culture experiment, the effectiveness of organic products were explored on the growth of *Rhizoctonia solani* which revealed that, the minimum mortality was recorded from Spent mushroom substrate + Cowdung + Vermicompost followed by Vermicompost with 10% and 15% respectively. The maximum mortality was recorded from Poultry manure 66.67% as compared to rest of the treatments including control. With regard to the plant height the result was also obtained in same trend. Spent mushroom substrate + Cowdung + Vermicompost recorded maximum plant height followed by Vermicompost with 89.33cm and 81.67cm respectively. Such findings are also in agreement with the findings of El-Mohamedy *et.al.* (2006), who amended soil with *Trichoderma hazianum*. Ashlesha *et.al.* (2009) amended soil with dry powder of cowdung and fresh cowdung urine and milk and reported effective against soil borne *Rhizoctonia* and *Fusarium* pathogens.The efficacy of Vermicompost against *R.solani* has been reported earlier by Sinha *et.al.* (2010) which is also in agreement with the present finding.

Therefore the phytoextracts and organic products may be included in integrated disease management strategy against *Rhizoctonia* root and stem rot management in cowpea to the farming community of Odisha.

CHAPTER-6

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

Keeping in view the outline of objectives, studies on root and stem rot of cowpea caused by *Rhizoctonia solani* were conducted during 2014-15. An intensive survey was carried out during Kharif 2014-15 in cowpea fields of AICRP on vegetable crops, farmer's field of keonjhar, cuttack and khurda district and found that the disease incidence ranged from 18.4 to 40.5 per cent. Disease samples were collected and analysed for disease causing agents following standard procedure, microscopically detected and maintained in pure culture for further study.

The symptomatological studies revealed that cowpea has showned systemic infection from root and stem portion. Initially symptoms were observed on the root portion. The water soaked lesion found at the infected point and it expanded both upward and downward direction. Finally the infection caused withering and death of plant with white mycelial growth followed by formation of brown coloured sclerotia on the infected portion.

In pathogenicity test of *Rhizoctonia solani* was proved on potted cowpea plants. The test fungus was re-isolated and confirmed through microscopic observation and found the same.

The pure culture of *Rhizoctonia solani* produced whitish mycelial growth initially, later it turned to brown with formation of micro sclerotial bodies. Micro sclerotia were produced in Petri dishes with an average of 155 number per plate after ten days of inoculation. Sclerotia were typically 2140μm in diameter, spherical, rough and light brown to dark brown in colour.

Among five different media, Potato dextrose agar(90.00mm) was found to support the maximum radial growth followed by Sabouraud's dextrose agar medium (87.90mm), Potato dextrose rose Bengal agar (82.40mm), Oat meal agar(76.60mm) and Malt extract agar (70.98mm).

In study of meteorological parameters in relation to disease development the maximum incidence was recorded in October at maximum temperature of 32.7^{0}C, minimum temperature 22.9^{0}C accompanied by night RH 92% and day RH 64%. However, weather parameters under study did not yield any significant effect on disease development. However, the soil factors like sandy loam textured soil and acidic to neutral P^H contributed towards this soil borne disease.

Among host range studies, *Rhizoctonia solani* isolated from cowpea could infect rice (*Oryza sativa*), maize (*Zea mays*), tomato (*Solanum lycopersicum*), chilly (*Capsicum annum*), brinjal (*Solanum melongena*), fieldpea (*Pisum sativum)*,cucumber (*Cucumis sataivus*), Bengal gram (*Cicer arietinum*), arhar (*Cajanus cajan*), cotton (*Gossypium hirsutum*), and groundnut (*Arachis hypogeae*) in addition to its own host.

Out of seven phytoextracts tested against *Rhizoctonia solani*, the maximum inhibition was recorded in garlic(100%) followed by turmeric(34.08%) at 10 % concentration,where as maximum inhibition 100% was recorded in both 15% and 20% concentration in garlic and calotropis respectively.

The comparative fungitoxic potentially of four biological agents were tested against *Rhizoctonia solani. Bacillus subtilis* was recorded maximum growth inhibition of 72.04% as compared to *Trichoderma hamatum* (49.78%) and *Trichoderma harzianum* (46.00%).

In vitro studies of 13 fungicides against *Rhizoctonia solani* revealed that Carboxin 37.5% + Thiram 37.5%, Hexaconazole 5% , Difenoconazole 25% , Tebuconazole 25% and Tebuconazole 50% + Trifloxystrobin 25% recorded maximum growth inhibition(100%), however Validamycin 3%, Cymoxanil 8% + Mancozeb 64%, Mancozeb 63% + Carbendazim 12% and Propiconazole 25% recorded least growth inhibition.

In vivo studies of 13 fungicides in pot culture experiment against *Rhizoctonia soalni* revealed that Cymoxanil 8% + Mancozeb 64%, Difenoconazole 25% followed by Carboxin 37.5% + Thiram 37.5% registered 1.83%, 2.0% and 2.60% mortality respectively. The maximum mortality was recorded from Carbendazim 50% followed by Propineb 70% (82% and 65.97%) respectively.

Among the organic products tested against *Rhizoctonia solani*, the minimum nortality was recorded from Spent mushroom substrate + Cowdung + Vermicompost ollowed by Vermicompost with 10% and 15% mortality respectively. The maximum nortality was recorded from Poultry manure (66.67%) as compared to rest of the reatments and control. With regarding to the plant height Spent mushroom substrate +

Cowdung + Vermicompost recorded maximum plant height (89.33cm) followed by Vermicompost (81.67cm) and Spent mushroom substrate + cowdung (79.00cm).

Use of phyto extracts as a means of non-chemical crop disease management is found imperative in view of its eco-friendly nature. However use of chemicals in integrated disease management schedule has been the last resort, so far as crop disease management is concerned. But exploration of antagonists and organic products found to be efficacious and economic and non-hazardous to environment. The farming community of Odisha may be educated to use indigenous products like botanicals and organic products in crop disease management programme.

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