Studies on stem rot of Groundnut (*Arachis hypogaea L.)* and its management

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A

Thesis submitted to the Orissa University of Agriculture and Technology in Partial fulfillment of the Requirements for the Degree of Master of Science in Agriculture

(Plant Pathology)

By

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CERTIFICATE- I

This is to certify that the thesis entitled **"Studies on stem rot of Groundnut (*Arachis hypogaea L.*)and its management."** submitted by **Monalisa Pati, Adm. No. 08 PPT/14** to the Orissa University of Agriculture and Technology, Bhubaneswar in partial fulfillment of the requirements for the award of the degree of **Master of Science in Agriculture**(**Plant Pathology)**is a faithful record of bonafide research work carried out by her under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

It is further certified that the assistance and help received by her from various sources during the course of investigation have been duly acknowledged.

DR.ARABINDA DHAL
CHAIRMAN,
ADVISORY COMMITTEE

CERTIFICATE – II

This is to certify that the thesis entitled **"Studies on stem rot of Groundnut (*Arachis hypogaea L.*)and its management"** submitted by **Monalisa Pati, Adm. No. 08PPT/14** to the Orissa University of Agriculture and Technology, Bhubaneswar in partial fulfillment of the requirements for degree of **Master of Science** in **Agriculture (Plant Pathology),** has been approved by the Student's Advisory Committee and the External Examiner.

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Date-

Bhubaneswar (MONALISA PATI)

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ABBREVIATIONS USED

%	:	per cent
@	:	at the rate of
µg	:	Micro gram
BSH	:	Bright Sunshine Hours
cm	:	centimeter
CRD	:	Completely Randomized Design
CD	:	Critical Difference
CV	:	coefficient of variation
DAS	:	Days after sowing
e.g.	:	For example
et al.	:	and others
etc.	:	and so on
EC	:	Emulsifiable concentrate
Fig.	:	Figure
g	:	Gram
g/ha	:	gram per hectare
g/kg	:	gram per kg seed
ha	:	hectare
IDM	:	Integrated Disease Management
ICBR	:	Incremental Cost Benefit Ratio
i.e.,	:	that is
kg	:	Kilogram
l	:	litre
LLS	:	Late leaf spot
m	:	Meter

m^2	:	Meter square
mha	:	million hectare
mt	:	million tonnes
ml	:	milliliter
mm	:	millimeter
NA	:	Nutrient agar
No	:	Number
^0C	:	Degree Centigrade
PDA	:	Potato Dextrose Agar
PDI	:	Per cent Disease Incidence
ppm	:	parts per million
psi	:	pounds per square inch
RBD	:	Randomized Block Design
RD	:	rainy days
RF	:	rain fall
RH	:	relative humidity
SEm	:	Standard error of mean
SMW	:	Standard Meteorological Week
Spp	:	Species
SR	:	Stem rot
ST	:	Seed treatment
viz.	:	namely
WP	:	Wettable powder
ELS	:	Early leaf spot

ABSTRACT

Seed and soil borne diseases have been recognized as major constraint limiting groundnut production. Among the soil borne diseases the stem rot disease incited by *Sclerotium rolfsii* Sacc. assume major threat to farmers of Odisha resulting seedling mortality and ultimately causing reduction in pod yield. Hence the present investigation has been undertaken in the title "Studies on stem rot of Groundnut (*Arachis hypogaea L.*)and its management" aimed at monitoring and survey of groundnut diseases in east and south east coastal tracts of Odisha, influence of weather parameters on stem rot disease incidence, *in vitro* bioassay with biocontrol agents, fungicides and management of the disease through application of soil amendments. Besides, field experiment conducted on integrated management and varietal response to stem rot incidence. The late leaf spot was the most prevailing disease which attained a maximum score of 7, a week before harvest. The stem rot incidence initiated 40 DAS and recorded a maximum 7.4% infection up to pod formation and maturity stage of the crop. The weather parameters as a whole contributed about 48.7% to disease development in Kharif, 2017; where minimum temperature solely contribute to 80% to disease development. During Rabi-summer 2017-18, weather parameters as a whole contributed about 62.2% to stem rot development. The maximum and minimum temperature and number of rainy days contributed 38%, 24% and 22% to disease development respectively. Out of eleven number of fungicides experimented through poison food technique; Carbendazim+Mancozeb at 0.2%,Azoxystrobin+Tebuconazole at 0.1%,Azoxystrobin+Mancozeb at 0.1% and triazole group of fungicides viz.Tebuconazole,Propiconazole and Hexaconazole recorded 100% inhibition of the test pathogen. Antagonist *Trichoderma harzianum* was the most efficacious with 74.4% mycelial growth inhibition,87.1% sclerotial inhibition followed by *T.viride* with 70.6% mycelial growth inhibition and 85.2% sclerotial inhibition. Out of several organic amendments tried through a pot culture experiment, the FYM enriched *T.viride*(0.1%) recorded the least mortality of plants due to Sclerotium pathogen(6.7%).The integrated management approach in field revealed that deep summer ploughing with MB plough+basal application of *T.viride*@4kg/ha enriched with 50 kg FYM+seed treatment with Tebuconazole@1.5g/kg of seed followed by PGPR @6g/kg seed followed by soil application of *Trichoderma* @4 kg/ha enriched in 250 kg FYM per ha at 35 and 70 DAS was found to be the best treatment which increased the germination by 24.9%,reduces stem rot by 65.9% and increased the yield by 38.7% when compared to control, with ICBR of 8.03. Out of the prevailing varieties cultivated in Odisha, eight varieties were taken to find their response to stem rot incidence.It was evident that, the variety Smruti was the best with 71.6% reduction in disease and 32.4% increase in yield when compared over the susceptible local check TMV-2.However TAG-24, TG 38B and K6 may be considered as the next best varieties reducing the disease by 63.6%,68.9%,55.4% and increasing the yield by 34.2%,28.8% and 29.9% respectively.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an important oilseed crop of tropical and subtropical regions of the world. It is native of South America and belongs to annual legume group. It is regarded as "King of oilseed crops" on account of its diversified uses. Groundnut kernel is a rich source of energy because of its high oil content (44 - 50%) and protein content (25 – 33%). It can supply about 5.6 and 5.8 calories per gram of kernel in the raw and roasted forms respectively. It is also very good source of minerals (calcium, magnesium and iron) and vitamins (B1, B2 and Niacin). Groundnut cake is used as cattle and poultry feed. It is also good organic manure because of its high nitrogen content (7.0 – 8.0%) and other nutrients. Groundnut being a legume crop, it fixes a large amount of nitrogen and improves the fertility status of the soil.

The most important groundnut growing countries are China, India, Nigeria, Sudan and USA. India is the largest groundnut growing country and second largest producer after China with a national average of 1486kg/ha considering both Kharif and Rabi season. It is an important oilseed and ancillary food crop in India with production of 6.77 mt from 4.56 mha. Groundnut has good export potential of about 0.78 million tones and earn 4675.37 crores in 2014-15. (Agricultural statistics at a glance, DES, DAC&FW, 2016).

Major groundnut growing states in India are Gujarat, Andhra Pradesh, Karnataka, Rajasthan, Tamil Nadu and Maharashtra. About 80% of groundnut crop is cultivated in rain- fed areas where productivity fluctuates between 500 and 1500 kg/ha.

In Odisha, the crop is cultivated in scattered manner covering 267.68 thousand ha and annual production of 478.33 thousand metric tons with productivity of 17.87q/ha. This groundnut crop is predominantly grown in Jajpur, Bargarh, Ganjam, Malkangiri, Kalahandi, Baleswar and Puri. During Rabi-summer the average productivity is 19.36q/ha compared to 14.62q/ha in Kharif season crop. (Odisha Agriculture Statistics 2013-14,Directorate of Agriculture and Food production,Odisha).

Groundnut is prone to several biotic and abiotic stresses. Among several fungal biotic stresses, diseases like leaf spot (*Cercospora* spp.), rust (*Puccinia arachidis*),

Aspergillus crown rot, *Sclerotinia* blight,*Verticillium* wilt, *Sclerotium* stem rot (southern blight) are the major constraints for groundnut cultivation. These are the most destructive diseases and cause more than 50 per cent yield loss.(Melouk *et al*., 1995)

Stem rot disease caused by *Sclerotium rolfsii* Sacc. is one of the significant factors contributing to yield loss. It is one of the most economically important diseases of groundnut which accounts for 10 to 25 per cent loss in yield annually (Storgeon, 1986). It was first observed by Peter Henry Rolfs in the year 1892 on tomato plants with 70% losses. The hyphae grew upward on the surface of the infected plant covered with a cottony, white mass of mycelium, scattered inside and outside of infected stem nearby the soil surface, The fungus produced numerous small round, white sclerotia of uniform size when immature and dark brown at mature stage (Kwon and Park, 2002).

In India, stem rot incidence is most severe in Maharashtra, Gujarat, Madhya Pradesh, Karnataka, Andhra Pradesh, Odisha and Tamil Nadu, This disease causes severe damage and can reach over 80% in heavily infected fields (Mehan and McDonald 1990).

The fungus *Sclerotium rolfsii* survived in the soil for many years by producing sclerotial bodies and causing the disease either in the form of stem rot or foot rot or root rot in addition to leaf blight on several of its hosts. So it is difficult to manage this pathogen through single chemical management option.

The green revolution has led to intensified agriculture which is practiced at great cost to the environment, resulting in continuous damage of natural ecosystems, ground water and food-stuff pollution and other environmental degradation. Awareness about the health hazards and environmental problems due to the continuous use of pesticides resulted in the development of Integrated Disease Management (IDM). Keeping this in view, studies have been initiated to include crop protection and growth promotion using the native micro-organisms, as a component of IDM.

Biological control represents both the oldest and youngest technology for the control of plant diseases and pest. Yet modern biological control achieved with introduced microorganisms is still in its infancy. The bioagent,*Trichoderma harzianum* is already recommended for control of stem rot as seed treatment and soil application in which whole culture is used for formulation and application. However, the information

on efficiency of culture filtrate alone and its fractionations on *Sclerotium rolfsii* are not available. Management of stem rot is highly dependent on chemical fungicides, as adequate levels of host plant resistance with desirable agronomic traits are scarce in cultivated germplasm. However, the need for repeated application of fungicides coupled with uncertain rainfall remains an obstacle for the wide adoption of chemical management by the poor farmers.Thus, there exists a greater demand for economic and sustainable integrated technology for stem rot management.

Biopesticides are cheaper, ecofriendly and do not pose risk of the pathogen developing resistance. Plant growth promotion and yield increase are the twin additional benefits from PGPR. A convenient means of applying crop protection techniques involves treating the seeds with fungicides. Seed treatments can be particularly useful, since they can provide protection to young plants during a vulnerable stage in their development (Walters *et al*., 2013).Fungicidal seed treatment is a cheap insurance for peanut seed producers and growers. Correct fungicide use can contribute to better performance of the propagation material, increasing the yield. (Zhang *et al.*, 2001)

Keeping in view the significance of stem rot of groundnut which adversely affects the most important oilseed crop of Odisha, a systemic investigation was carried out in Department of Plant Pathology, College of Agriculture, Bhubaneswar and AICRP on Groundnut, OUAT, Bhubaneswar. Emphasis was given to investigate the distribution of the disease, epidemiology and management in integrated approach for a pocket friendly and easy to understand of poor farmers.

Considering the occurrence, incidence and severity of soil borne diseases, the following programme of work have been taken as part of the investigation under the present study.

1. Survey and monitoring of diseases of groundnut in east and southeastern tracts of Odisha.
2. Study the influence of weather parameters on stem rot disease incidence.
3. In-vitro bioassay with biocontrol agents, fungicides and management of the stem rot through soil amendments.
4. Integrated approach for management of stem rot disease of groundnut in field condition.

REVIEW OF LITERATURE

Sclerotium rolfsii is the causal agent of stem rot of groundnut. This disease takes a heavy toll of the crop in both Kharif and Rabi /Summer season. The incidence of disease leads to low plant population in the field. Accordingly significant yield reduction occurs. The disease is wide spread and pathogen is most destructive to crop under favorable conditions. The related reviews on pathogen and the disease pertaining to the present investigation are dealt in this chapter.

2.1 Prevalence of Pathogen and symptom

2.1.1 The pathogen

Sclerotium rolfsii was first reported by Rolfs (1892) on tomato plants with 70% losses and later the pathogen was named as *Sclerotium rolfsii* by Saccardo (1911).

Mc Clintock (1917) and Butler and Bisby (1931) reported occurrence of stem rot of groundnut for the first time in Virginia and India, respectively. Higgins (1927) worked in detail on physiology and parasitism of *S.rolfsii*. This was the first detailed and comprehensive study in USA.

This pathogen *Sclerotium rolfsii* forms brown sclerotia which are very well organized compact structures, built of three layers, the rind, composed of empty melanized cells; the cortex cells, filled with vesicles and the medulla (Chet, 1975).

The pathogen *Sclerotium rolfsii* Sacc., is a soil borne pathogen that commonly occurs in the tropics, sub-tropics and other warm temperate regions of the world causing root rot, stem rot, wilt and foot rot on more than 500 plant species including almost all the agricultural and horticultural crops (Aycock, 196;Farr *et al.,* 1989). Sclerotia initially white in color, later it becomes light brown to dark brown at maturity and they are sub spherical, the surface finely wrinkled, sometimes flattened (Subramanian, 1964 and Mehan, 1995).

Sclerotia may be spherical or irregular in shape and at maturity resemble the mustard seed (Taubenhaus, 1919; Barnett and Hunter, 1972). Sclerotial size was

reported to be varied from 0.1 mm to 3.0 mm (Om Prakash and Singh, 1976; Ansari and Agnihotri, 2000 and Anahosur, 2001).

2.1.2 Symptoms:

Mehan *et al*. (1995) reported that the symptoms of stem and pod rot of groundnut included yellowing and wilting of a branch or the whole plant with dried out chlorotic leaves. They also reported sheaths of white mycelium of *Sclerotium rolfsii* seen around affected plants at or near the soil surface, imparting a white washed appearance to the base of the affected plants. Light to dark brown lesions were observed on the stem and in severe conditions the lesions coalesced girdling the lower stem. The mycelia growth developed spherical sclerotia on the surface of affected plant parts or on the adjoining soil surface. The sclerotia are initially white which turn dark brown on maturity. Pegs were observed to develop light to dark brown lesions that led to shredding and pod detachment. Pods were found to be infected showing light brown lesions and few were covered with white mycelia mat. The typical "Blue damage" on the seeds owing to characteristic bluish grey discoloration of the testa was also noticed.

2.1.3 Survey for the incidence of stem rot

Stem rot causes pod yield losses of 10-25%, but under severe diseased conditions yield losses may range to up 80% (Rodriguez Kabana *et al*., 1975).A random survey conducted by Siddaramaiah *et al*.(1979) during Kharif seasons of 1975-76 and 1976-77 in Dharwad district of Karnataka showed that the mean incidence of groundnut stem rot was 7.8 per cent on various cultivars of 70 to 90 days old crop.

The pod yield losses commonly ranged from 10 to 25 per cent, but could reach over 80 per cent in heavily infected fields. (Porter *et al.* 1982).Patil and Rane (1982) reported yield loss up to 10 to 50% due to this disease.

In India, stem rot occurs in all groundnut growing states. It is most severe in Maharashtra, Gujarat, Madhya Pradesh, Karnataka, Andhra Pradesh, Orissa and Tamil Nadu. It is estimated that over 5, 00,000 ha of groundnut fields are infected with the *S. rolfsii* and yield losses of over 25 per cent have been reported (Mayee and Datar, 1988).

The stem rot (*S. rolfsii*) occurs regularly in all areas wherever groundnut is grown. The disease is wide spread and caused serious losses in Bolivia, China, Egypt,

India, Taiwan, Thailand and USA. In USA damage due to stem rot accounted for 5 to 10 per cent loss in yield, despite the use of disease management practices (Bowen *et al.*, 1992).

In another survey conducted by Gouda (1999) during Kharif 1998 and Rabi 1998-99 in Dharwad,Belgaun and Haveri district of Karnataka , it was observed that the stem rot incidence was 12.57 and 8.68 per cent under rain-fed and irrigated conditions respectively. Adiver (2003) reported the yield loss of 15-70% in groundnut is due to leaf spot, rust and stem rot singly or in combination.

Kadam *et al*.(2011) conducted a field survey in the Marathwada region of Maharastra, India ,during 2003 to determine the prevalence of stem rot disease on various groundnut cultivars. Results indicated that the disease incidence was highest in Renapur Tahsil (17.8%) followed by Udgir (16.7%),Ausa (14.7%) and Lathur (14.3%) where lowest disease incidence was recorded in Nilanga (8.9%). Among the cultivars, JL-24 showed the maximum per cent disease incidence compared to others.

2.1.4 Pathogenic behavior

The fungus *S.rolfsii* has wide host range of 500 species in about 100 families including groundnut, green bean, lima been, onion, pepper, potato, sweet potato, tomato and water melon (Aycock, 1966).

Microscopic examination of the fungal culture revealed the aerial hyaline, thin walled, septate hyphae with profusely branched mycelium showing clamp connections. When fungus attained maturity small mycelial knots were formed which later turned to mustard seed like sclerotia which were deep brown or brownish black, shiny, hard and spherical to irregular in shape (Mirza and Aslam, 1993), Mohan *et al*., 2000.Geographical variability among *S.rolfsii* populations was demonstrated by earlier workers (Harlton *et al*., 1995; Okabe *et al*., 1998).

In India, Sharma *et al*. (2002) studied variability among 26 isolates of *S.rolfsii* collected from various hosts/soil samples and localities. Variations in sclerotial colour, shape and size and their ability to infect plants have been reported by different scientists on various hosts and media (Sharma *et al.,* 2002; Palaiah and Adiver, 2006).

2.2 Isolation and purification :

Kokub *et al*. (2007) isolated 8 fungal strains of Sclerotium rolfsii and observed the growing behavior on Potato Dextrose Agar (PDA) plates at 28^0c ranged from 0.86-1.35 mm hour-1. Strains D4, D7 and D8 were found to be comparatively fast growing and produced greater number of sclerotia than others. All strains produced round shaped sclerotia with average diameter of 0.5-2.0 mm.

Surulirajan *et al*. (2007) isolated *Sclerotium rolfsii* [*Athelia rolfsii*] the causal organism of root rot disease of lentil and studied the different morphological characters of the pathogen on two percent potato dextrose agar medium as it was found to be the best medium for both vegetative and reproductive growth.

2.3Pathogenicity tests

Siddaramaiah *et al.* (1978) proved the pathogenicity by sowing 50 seeds which were artificially inoculated with 20 days old Corticium culture and the same quantity of the seeds were sown in sterilized soil as control. Out of 50 seeds, 40 germinated and remaining 10 seeds were not germinated. The fungus started infection after the third day of seed germination and all the 40 seedlings were infected within a week causing post emergence death.Based on the characteristics symptoms, fungus was identified as *Corticium rolfsii*,Curzi(1931).

Haware and Nene (1978) obtained 100 per cent germination in 42 inoculated chickpea seeds. Fifty per cent of the seeds in (21 seeds) the inoculated treatment were failed to germinate. Germinated seedlings were killed within 7 days after emergence. The pathogen was successfully reisolated from the diseased plant.

Such similar results on the pathogenicity of *S. rolfsii* have also been reported on *Edgeworthia papyrifera* from Taiwan (Chang, 1994), maize and apple from Pakistan (Ahmed *et al*., 1984), *Phaius flavus* and *Paphiopedilum venustum* from India (Bag, 2004) chilly from Malaysia (Jomduang, 1995) and apple from USA (Conway and Tomasino, 1985).

Shokes and Gorbet (1998) observed stem and pod rot on groundnut by *S. rolfsii* with potential death and estimated field yield losses of 10% or more in the southern-eastern USA. Similarly, Blum & Rodriguez (2004) also recorded reduction in seed germination and plant growth in soybean by *S. rolfsii.*

Padole *et al*. (2009) investigated variations in 51 isolates of *Sclerotium rolfsii* showed considerable variation with regards to cultural and morphological characters on PDA medium. These isolates may be broadly grouped into three pathotypes. The pathogenicity test showed the isolates to vary in number of days taken to initiate plant mortality and reach upto 100% mortality.

Awasthi *et al.* (2010) investigated the pathogenicity of different isolates of *S. rolfsii Sacc*. on groundnut. Observations revealed that all the isolates were found to be pathogenic towards groundnut but extent of their pathogenicity in respect of their disease severity differs in some isolates. Result revealed a marked variation in the virulence of the different isolates. Isolate 10 showed highest disease severity of 54.4% which was superior as compared to all other isolates. Whereas, Isolate 2 and Isolate 8 showed the least disease severity of 40.8 and 40.9% respectively.

2.4 Epidemiology:

Temperature and moisture are very important factors in the spread and development of this pathogen. Hyphal growth occurs over a temperature range of 8-40ºC / 46-104º F, but optimal growth and sclerotia production occurs between 27-35ºC / 81-95ºF. In addition to temperature effects, hyphal growth and sclerotia germination require a water-saturated soil. High humidity also favours fungal development. At 27ºC / 81ºF on Potato Dextrose Agar, the hyphal growth rate of *S. rolfsii* has been observed to be 0.8-0.9 mm per hour. Sclerotia form after 5-7 days. Host penetration and infection will proceed optimally at 27-30ºC / 81-86ºF, provided that moisture and high humidity are present (Tu and Kimbrough, 1978)

The prevalence of *S. rolfsii* in warm regions of the world is a reflection of the high temperature optimal for its growth and sclerotial production. The temperature range for hyphal extension and dry weight production is 8-40°C (Zoberi,1980) maximum growth and sclerotial formation occur at 27-30°C (Mihail and Alcorn,1984). Hanumanthegowda (1999) observed 92.50 per cent pre-emergence collar rot of groundnut at two per cent inoculum level and above two per cent lead to 100 per cent seedling mortality.

Sclerotium rolfsii develops at intermediate soil moisture level (70%) of field capacity and at temperature range between 25^0C to 30^0C(Pattanapipitpaisal and Kamlandham,2012).

2.5 Management studies

2.5.1 Biological control:

Biswas and Sen (2000) tested 11 isolates of *Trichoderma harzianum* and out of these, the isolate of wheat field, groundnut field, and potato field were effective against stem rot of groundnut caused by *S. rolfsii* and they overgrew the pathogen by 92, 85 and 79% respectively in dual culture technique.

Chandrasehar *et al*. (2005) reported that the antagonists *T. harzianum* and *T.viride* overgrew and completely suppressed the growth of *S.rolfsii*. The least zone of inhibition (5 mm) was produced by *A. flavus* while *P. fluorescens* produced the maximum inhibition zone of 11 mm. Pot culture experiments indicated that seed treatment and soil drenching with antagonists increased the percent survival of treated seedlings. Seed treatment was more effective than soil application. The maximum percent survival of 89.5 was recorded by treating the seeds with *T. viride* followed by *P. fluorescens* with 86.4% survival. Soil application with *T. viride* (77.9%) and *P.fluorescens* (69.5%) enhanced survival of seedlings in both treatments. Pre emergence mortality was nil in soil and seed treatments with *Trichoderma* sp. and *P. fluorescens.*

Khosla and Kumar (2005) reported that 82.58% disease control by combination of *T. viride* (0.5%) and thiram (0.4%) in comparison to *Trichoderma* individually (72.9%) against root rot disease of strawberry caused by *S. rolfsii*.

Karthikeyan *et al*. (2006) reported that *Trichoderma viride, T. harzianum* and *Pseudomonas fluorescens* were inhibitory bioagents against the growth of *Sclerotium rolfsii* (Sacc.) in stem-rot of groundnut. *T. viride* inhibited the mycelial growth of the pathogen by 69.40%, while *P. fluorescens* inhibited 64.40%. Radawan *et al*. (2006) reported that *Trichoderma harzianum* and *Trichoderma hamatum* were most effective against *Sclerotium rolfsii* and inhibited the mycelial growth by 79%.

Varadharajan *et al.*(2006) evaluated the three isolates of *Trichoderma viride*, one isolate each of *T. harzianum* and *Pseudomonas fluorescens* in dual culture and found inhibitory to the growth of *S. rolfsii* (*Corticium rolfsii*), the causal agent of stem rot of groundnut.

Trichoderma species have also been used in commercial enzyme productions like cellulases, hemicellulases, proteases and β-1, 3-glucanase.The combination of *Rhizobium* and *T. harzianum* were significantly effective against *S. rolfsii* which caused stem rot disease and promote the plant growth and increase seed production of groundnut. Ganesan *et al.* (2007) performed the integrated management of stem rot disease of groundnut using a combined application of *Rhizobium* and *T. harzianum.* The results indicated that the application of these native micro-organisms successfully decreases the stem rot incidence and also increases the growth of the groundnut plants.

Mundhe *et al*.(2009) investigated that the bio-control activity of *Trichoderma harzianum, Trichoderma viride, Pseudomonas fluorescens* and *Bacillus subtilis* against *Sclerotium rolfsii* causing foot rot of Finger millet. All the bio-agents tested were found effective in reducing the growth of the test pathogen. Maximum inhibition of *Sclerotium rolfsii* was achieved due to *T. harzianum,* (P) followed by *T. harzianum*(JCR), *Pseudomonas fluorescens* and *Bacillus subtilis* with 73.77, 73.00, 72.66, 71.55 and 63.33 percent inhibition over control respectively.

Pastor *et al*. (2010) observed that *Pseudomonas sp*. were more potent antagonistic activity against *Sclerotium rolfsii* in the rhizospheric soil of groundnut. Several species of Pseudomonas produce antifungal antibiotics such as 2, 4 di acetylphloro glucinol, oligomycin, phenazine, pyolyteorin, pyrrolnitrin and pyocyanin which inhibit fungal activity. (Muthukumar *et al.*, 2010)

Samsuzzaman *et al*. (2012) reported that *Trichoderma harzianum* reduced the mortality in tomato plants inoculated with *S. rolfsii* in soil and increased height and production of tomato, so biofungicide inhibit growth of *S. rolfsii* causes collar rot disease of tomato and no risk of environmental pollution than chemical control. Rekha (2012) reported that *Trichoderma sp.* inhibited the mycelial growth and formation of sclerotial bodies of *S. rolfsii.*

Khalid (2013) isolated four bioagents viz, *Bacillus subtilis, Pseudomonas fluorescens,* yeast and *Trichoderma viride* which inhibited damping-off disease of bean caused by *S. rolfsii*. *Bacillus subtilis, T. viride,* and *P. fluorescens* control 88.7%, 83.7% and 86.3% respectively as bioagents against *S. rolfsii* and plants survived by 90.3%, 86.1% and 87.6% respectively.

Darvin *et al*. (2013) selected three species of Trichoderma (*T. viride, T. harzianum and T. longibrachiatum*) for inhibition of radial growth of *S. rolfsii*. *T. viride* and *T. harzianum* have highest radial growth inhibition and *T. longibrachiatum* has lowest radial growth inhibition of *S. rolfsii* using dual culture technique under in vitro condition. *Pseudomonas aeruginosa* is significantly active and inhibits the growth of *Sclerotium rolfsii* by 73.7% under *in vitro* condition. (Babu GP and Paramageetham, 2013).

Adhilakshmi *et al*. (2014) isolated 30 actinomycetes from the rhizospheric soil of groundnut from different area of Tamil Nadu, India and tested for their antagonistic activity against *Sclerotium rolfsii* Sacc. causing stem rot of groundnut following dual culture technique under *in vitro*. Among the various isolates tested *in vitro,* five isolates ie. CBE, ANR, MDU, SA and PDK were found significantly effective to control the mycelial growth of *S. rolfsii*.Swathi *et al*. (2015) reported that *T. harzianum* and *T. virens* were more active against *S. rolfsii* with 100% inhibition under *in vitro* condition.

Pseudomonas species act as a biocontrol agent and produce antifungal metabolites such as 2,4-diacetylphloroglucinol(DAPG), pyoluteorin, pyrrolnitrin and phenazines which inhibit the growth of *S.rolfsii*.(Jothi *et al*.,2015)

Basumatary *et al*. (2015) have selected six fungal species viz. *Penicillum* sp. ,*Curvularia* sp. ,*Aspergillus niger*,*Trichoderma harzianum*,*Trichoderma viride* and *Fusarium* spp. as bio- agents against *Sclerotium rolfsii*. The maximum percentage of growth inhibition of *Sclerotium rolfsii* was observed with *Trichoderma harzianum* (77.39%) and *Trichoderma viride* (76.54%), while *Penicillum* sp. (29.05%), *Aspergillus niger* (30.48%),*Curvularia* sp. (13.57%) and *Fusarium* sp. inhibited the growth by 3.02% under *in vitro* condition. Trichoderma species produced β-xylosidase, α- glycosidase, β-glucosidase, cellobiohydrolase, trypsin, chymotrypsin and chymoelastase-like proteases and Nacetyl β-glucosaminidase which are responsible for the biocontrol activity of *Sclerotium rolfsii*.

Senapati *et al*.,(2017) reported that seeds treated with tebuconazole @ 1.5g/kg recorded the highest percentage of germination (80.15%) and obtained yield of 1880 kg/ha whereas the untreated control plot recorded 67.74% germination and a minimum yield of 1547kg/ha.

2.5.2Chemical control:

Chowdhury *et al.* (1998) carried out *in vitro* evaluation of triazoles (i.e., hexaconazole, triademefon,propiconazole and bitertanol) and some non-systemic fungicides against *S. rolfsii* causing sclerotium wilt in bell pepper. Out of which non-systemic fungicides like captan, thiram and mancozeb tested were found completely inhibitory to the pathogen at 1000 ppm concentration and proved that hexaconazole was significantly superior.

Charde *et al.* (2002) found that seed treatment with propiconazole and hexaconazole were superior in checking stem rot of groundnut caused by *S. rolfsii* and increasing the shoot and root length.Tajane *et al.*(2002) found that seed treatment of soybean with hexaconazole and propiconazole inhibited *S.rolfsii*. These fungicides were found to be absorbed by roots and translocated to shoot and leaf.

Toorray *et al*. (2007) evaluated that seven fungicides (each at 1000, 1500 and 2000 ppm) against *Sclerotium rolfsii* under *in vitro* condition. Complete inhibition of growth of *S. rolfsii* was recorded by captan, thiram, mancozeb, hinosan (edifenphos) and antracol (propineb)whereas, kavach showed partial inhibition at low concentration. Bavistin (carbendazim) did not show much inhibition at all concentrations. In *in-vivo* captan, kavach and thiram showed reduction in pre emergence mortality.

Yaqub and Shahzad (2006) reported that six fungicides viz. benomyl, sancozeb, thiovit, dithane M-45, carbendazim, and topsin-M were effective against *Sclerotium rolfsii*. No fungicide inhibited the growth of *Sclerotium rolfsii* at low concentration while high concentration of dithane M-45 and sancozeb significantly reduced the growth of *Sclerotium rolfsii.*

Adiver (2007) who reported that triazoles suchas tebuconazole, cyperconazole, difenconazole and diniconazole provide excellent control of foliar fungal diseases and some soil borne diseases including stem rot.

Perez *et al*. (2009) evaluated the *in-vitro* reaction of *S. rolfsii* to five fungicides used for its control .The results showed that Tebuconazole and TCMTB inhibited mycelial growth and sclerotia production.

The fungicides hexaconazole, propiconazole, difenoconazole; combi product, avatar (hexaconazole 4% + zineb 68%), nativo (tebuconazole 50%+ trifloxystrobin

25%) and vitavax power (thiram 37.5%+carboxin 37.5%) and bioagents *Trichoderma harzianum* inhibits *Sclerotium rolfsii* under field condition.(Manu *et al*.,2012)

The bio-efficacy of formulations of tebuconazole 2% DS was evaluated for the management of stem rot of groundnut and its effect on dry pod yield. Application of tebuconazole 2%DS @ 1g/kg seed to groundnut kernels prior to sowing was found to be highly effective in the management of stem rot of groundnut with least disease incidence (7.31%) with higher pod yield (2664 kg/ha) and benefit cost ratio (5.42). The fungicide was also very effective in farm and large scale demonstration trials in controlling the stem rot and also resulted higher percentincrease in yield (9.95%) over recommended fungicide carbendazim (3g/kg seed).(Gururaj,2012)

Khan and Javaid (2015)reported that four fungicides tegula (tebuconazole), thiophanate methyl, ridomil gold (metalaxyl+mancozeb) and mancozeb significantly inhibits the radial growth of *S. rolfsii* under *in vitro* condition. Besides it, two fungicides thiophanate methyl and mancozeb substantially control the growth of *S. rolfsii* under *in vivo* condition responsible for causing collar rot disease in Chickpea.

2.5.3 Management through organic amendments:

FYM application to the soil +tuber treatment with *T. harzianum* prior to planting helped in reducing the sclerotial wilt of potato in field as well as in storage has been reported by Anahosur (2001).

Siddanagoudar Radder (2005) evaluated that soil application and culture filtrate formulation with FYM was found to be effective to enhance the seed germination against *Sclerotium rolfsii.*

Vinoddange(2006) evaluated that, FYM was found to be most effective against the root rot of chilli caused by *S. rolfsii* and recorded the least disease (19.34%) incidence . Johnson, and Reddy (2008) reported that integration of *Pseudomonas fluorescence* and in combination with tryptophan and Farm Yard Manure (FYM), has reduced the stem rot incidence and the highest yields were recorded against *S. rolfsii.*

Dhingani *et al*. (2013) evaluated the organic extracts against *M. phaseolina* by poisoned food technique *in vitro.* Significantly least growth of mycelium and maximum mycelium inhibition was recorded in extracts of neem cake (59.40 %) followed by farm yard manure (42.56 %) castor cake and mustard cake.

Meena *et al*.(2014) evaluated the extracts of two oil cakes at different concentrations for their inhibitory effect on *M. phaseolina*. The neem cake extract at a concentration of 20% allowed minimum growth (35.60mm) of the pathogen followed by neem cake extract 15% (45.39mm), neem cake extract 10% (58.40mm) and mustard cake extract 20% (68.58mm). On contrary, mustard cake extract at 15% concentration of (71.36mm) and 10% (75.80mm) also reduced the growth but not at par as neem cake extract at similar concentration. The maximum mycelial growth inhibition (52.40%) was recorded with neem cake extract at the concentration of 20% followed by 42.61% and 29.60% with concentration of 15% and 10%, respectively. Likewise, the maximum mycelial growth inhibition (19.42%) was recorded with mustard cake extract at the concentration of 20% followed by 16.64% and 12.20% at the concentration of 15% and 10% respectively. In general mycelial growth inhibition was dose dependent and it was higher in case of neem cake extract than mustard cake extract.

Mahato and Mondal (2014) reported that the plaster of Paris, *Azotobacter chroococcum*, vermicompost, *Pseudomonas*, FYM and *Trichoderma viride* were highly effective against *S.rolfsii*.

2.5.4 Integrated Disease Management:

Garren (1961)reviewed the cultural practices for the control of *S. rolfsii*. Newhall (1955) described several cases in which ploughing the soil during the hot season in order to expose it to the sun's rays, resulted in decrease in disease incidence. This effect is apparently due, among others, to physical killing of the pathogen propagules occurring at elevated temperatures and can be regarded as dry-soil solarization.

Deep ploughing is practised to reduce the contact between plant roots and pathogen structures, to enhance pathogen killing by burying it, or to expose the inoculum to natural heating and desiccation and control of *Sclerotium rolfsii* in peanuts (Garren and Duke, 1958). Similarly, deep ploughing, compared to shallow disking, reduced incidence of southern blight in tomato (caused by *S. rolfsii*) and increased yields (Worley et al., 1966).

Asghari and Mayee (1991), reported that, application of *T. harzianum* inoculum and soil drenching with 0.2 per cent carbendazim reduced the stem rot of groundnut caused by 44-60 per cent and increased the pod yields by 17-47 %.

The control practices of stem rot disease include cultural methods such as plant rotation, deep soil processing and weed control as well as soil solarization, using antagonistic microorganisms or fungicides treatments after sowing on the plant rows (Damicone& Jackson, 1994).

Kulkarni (1994) and Prabhu *et al.* (1997) showed that, seed and soil treatment with *T. viride* and *T.harzianum* were the most effective in reducing the mortality percentage of groundnut incited by *S. rolfsii.*

Biswas *et al.*(2000) observed that application of *T. harzianum* inoculum to soil and seed dressing at the time of sowing in the pots exhibited percent disease reduction through seed dressing was 33 to 50% and through direct soil application was 72 to 83%.

Sclerotial wilt of potato caused by *Sclerotium rolfsii* was effectively reduced when *T. harzianum* was applied @ 4 g/kg to the soil and FYM Anahosur (2001).

Vanitha and Suresh (2002) observed that application of the inoculum *T. harzianum* in the seed treatment and soil application of Adathoda leaf powder and FYM exhibited , the lowest collar rot of brinjal (9.44%) caused by *Sclerotium rolfsii.*

Vanith and Suresh (2002) conducted a study to investigate efficacy of biological control agents and organic amendments in controlling while a combination of *Trichoderma viride*+ FYM + dry Adathada leaf powder was found effective against collar rot of brinjal caused by *Sclerotium rolfsii.*

Bhardwaj and Raj (2004) reported that soil solarization with transparent polyethylene mulch (25 μm) for 40 days (June to July) was effective for the control of collar and root rot of strawberry caused by *S. rolfsii*.

Bacteria isolated from the rhizosphere and belonging to a wide variety of genera have the potential to suppress diseases caused by a diversity of soil-borne plant pathogens. Some symbiotic N_2 fixing Rhizobium strains not only fix atmospheric N_2 in the nodules but also show an antagonistic effect against soil-borne pathogens (Deshwal *et al*., 2003; Bardin *et al.,* 2004).

Culture filtrates of Bradyrhizobial strains significantly inhibited the growth of *Macrophomina phaseolina* as reported by Chakraborty and Purkayastha (1984) and

Deshwal *et al.* (2003). Inhibition of the pathogen by Rhizobium may be due to the production of siderophore and Rhizobiotoxin (Chakraborty and Purkayastha, 1984).

Chakraborty *et al*.(2003) reported that combined application of *Bradyrhizobium japonicum* and *Trichoderma harzianum* significantly reduced root rot disease in soyabean. They also showed that activity of PAL and peroxidase enzymes and Phytoalexin (Glyceollin) activity increased in treated plants.

Integrated management of stem rot disease of groundnut using a combined application of Rhizobium and *Trichoderma harzianum*(ITCC – 4572) was performed, and it was observed that the application of these native micro-organisms successfully decreases the stem rot incidence and also increases the growth of groundnut plants (Ganesan, 2006).

Kulkarni (2007) evaluated fungicides and bioagents alone or in combination as seed dressers along with soil amendments as components for integrated management of potato wilt caused by *S. rolfsii* under glasshouse conditions and recorded least disease incidence(13.33%) in treatment consisting carboxin + *T.harzianum*+ farm yard manure.

Banyal *et al*. (2008) selected ten fungicides viz., carbendazim 50 WP, carbendazim + mancozeb 75 WP, captan 50 WP, chlorothalonil 80 WP, thiabendazole 80 WP, mancozeb 75 WDG, carboxin 75 WP, propineb 70 WP, mancozeb 75 WP and tebuconazole 5 DS; five bioagents *Trichoderma harzianum*(local strain), *Trichoderma viride*(local strain), *Gliocladium virens*(local strain), *Paecilomyces lilacinus*(Bhubanes war strain) and *T. viride*(Ecoderma). These inhibited *Sclerotium rolfsii* causing collar rot of tomato, but the combination of tebuconazole and *T. viride*(local strain) controls 100% effective against *Sclerotium rolfsii.*

Johnson and Reddy (2008) reported that integration of *Pseudomonas fluorescence* and in combination with tryptophan and Farm Yard Manure (FYM), has reduced the stem rot incidence and the highest yields were recorded against *S. rolfsii.*

Vinod Babu (2008) observed seed treatment with fungicide (mancozeb) + soil application of potential native antagonist + soil application with potential bacterial antagonist (Pf-1) recorded least percentage disease incidence of 6.67%, maximum plant height of 30.66 cm, maximum root length of 29.13 cm of groundnut.Madhavi and

Bhattiprolu (2011) reported that tebuconazole and combination of carbendazim and mancozeb were very efficient against *Sclerotium rolfsii* causing root-rot in chilli.

Soil solarization is most effective for soil pathogen *S. rolfsii* during the hot summer months where soil temperature level increased that kill many important soil-borne fungal and bacterial plant pathogens. Soil solarization is a hydrothermal procedure which used transparent film to capture solar radiation in the soil. In hot arid region of India solarization is effective against soil pathogen particularly during April – June months.(Lodha,2011)

The fungicides viz., hexaconazole, tebuconazole, propiconazole, difenoconazole, vitavax, carbendazim along with captan and mancozeb and various combinations were applied as seed treatment at recommended doses. The results indicated that tebuconazole 2DS 1.5g /kg of seed,mancozeb 75%WP@ 3g/kg of seed,carbendazim 12%+mancozeb 63%wp@ 3g/kg of seed, were very effective in the management of soil borne diseases when used separately, with apparent yield advantage over untreated plots. (Jadon, 2015)

2.6 Varietal reaction of different varieties against stem rot of groundnut

Gopal *et al.* (2006) reported that twenty groundnut genotypes were evaluated in Jagtial, Andhra Pradesh, India, for resistance to pod rot disease (caused by soil borne organisms, such as *Rhizoctonia solani, Fusarium solani, Sclerotium rolfsii* [*Corticium rolfsii*]*, Fusarium oxysporum and Macrophomina phaseolina*). Based on percent disease index (PDI), the cultivars were classified as immune (disease-free), resistant (0.10-10.0 PDI), moderately resistant (11.1-30.0 PDI), susceptible (30.1-50.0 PDI), and highly susceptible (>50.0 PDI). PDI, percent incidence (PI), and percentage of pods infected (PPI) ranged from 38.8 to 65.9, 39.9 to 94.4, and 9.3 to 90.7%, respectively. The lowest PDI values were recorded for INS 9013 and R 8808 (38.8% for each), followed by R 8972 (38.9%) and ICGV 86885 (39.2%). PI was lowest in R 8972 (39.9), followed by ICGS 11 (53.6%), ICGV 86885 (55.9%) and R 8806 (61.2%). R 8972, R 8808, ICGV 86885, R 8806 and ICGS 11 registered the lowest PPI (9.3, 10.4, 11.8, 13.1 and 15.1, respectively).

Kale *et al.* (2007) observed that TG 38 showed resistance to stem rot (*Sclerotium rolfsii* [*Corticium rolfsii*]) and dry root rot (*Macrophomina phaseolina*).

Varietial screening of groundnut against stem and pod rot was conducted during Kharif 2006 and 2007 in field conditions. Fourteen groundnut cultivars viz., J-11, GG-2, GG-4, GG-5, GG-6, GG-7, JL-24, TAG-24, TG-26, GG-20, GG-13, GG-11, BAU-13 and ICGV-86564 were screened for their resistance against *S. rolfsii*. Spreading type groundnut GG-11 and GG-13 were moderately resistant, while eight varieties viz., J-11, GG-4, GG-6, JL-24, TG-26, TAG-24, BAU-13 and ICGV-86564 were susceptible. Four varieties viz., GG-2, GG-5, GG-7 and GG-20 were highly susceptible to *S. rolfsii*.(Rakholiya and Jadeja ,2010)

Evaluation of inter specific derivatives of groundnut was carried out under field and laboratory condition for stem rot caused by *Sclerotium rolfsii* during 2005-2008.During initial screening 42 lines were found to be promising with no disease incidence. Advance screening of these promising lines was carried out. Interspecific lines,NRCGCS-47,NRCGCS-99,NRCGCS-131 and NRCGCS-319 were found promising against stem rot during early stage of crop growth. Out of which line NRCGCS-319 was found to be most suitable one with comparatively low pooled disease.(Bera *et al*.,2014)

Forty groundnut advanced breeding lines along with susceptible checks JL-24, J-11 and TMV-2 were used for collar rot (*Aspergillus niger*) and stem rot (*Sclerotium rolfsii*) disease screening. Based on the per cent number of plants affected by the collar rot pathogen, the advanced breeding lines were categorized into four groups. The lines present in group I (Resistant) having < 15% incidence, group II (Moderately resistant) having 15.1 to 30%, group III (Susceptible) having 30.1 to 45 % and group IV (Highly susceptible) having > 45% incidence. Similarly among 40 breeding lines only three lines (ICGV86699, ICGV91114 and ICGV 89280) have shown stem rot disease reaction below 3 (up to 25 % plants were symptomatic) and considered to be moderately resistant to stem rot pathogen. The advanced breeding line ICGV99058 has recorded a disease reaction of 5 scale (> 50 % of the plants symptomatic) equal to the susceptible checks which is considered to be highly susceptible to stem rot pathogen.(Divya Rani *et al*.,2018)

MATERIALS AND METHODS

The present investigation on stem rot of groundnut and its management were carried out in the laboratory of Department of plant pathology, Bhubaneswar and at AICRP on Groundnut, Orissa university of Agriculture and Technology, Bhubaneswar during the period 2016-17 to 2017-18.The details of materials used and the methodology followed during the course of the present investigation are described in the subsequent pages.

3.1 Survey and monitoring of groundnut diseases

The survey was conducted to monitor both soil borne as well as foliar diseases of groundnut during the crop season of Rabi summer 2016-17 as per the guideline specified for Random Field Survey provided by Directorate of Groundnut Research (Manual for groundnut pest surveillance; "NICRA" jointly published by NCIPM, New Delhi; CRIDA Hyderabad and DGR Gujarat, 2011)

Ten groundnut fields @ 2 fields per village of two different farmers growing the same variety of groundnut; in five villages with similar agro ecological situations in the districts of Puri, Khordha, Cuttack and Jagatsingpur were selected randomly. Care was taken to select the farmer growing common groundnut variety DEVI (ICGV-91114) in each selected field.

Five spots were randomly selected so that four were from four corner and one from the centre of the field. Five feet distance all along side of boundary of the field in all directions was left as buffer space during observations. At each spot ten plants were selected adjacent to each other for observations. Relating to all and whole plant observations during different stages of the crop viz. Vegetative, first flowering, pegging+ pod setting and development/maturity. Date of sowing, date of first appearance of disease, percentage incidence for soil borne pathogens and scores (1-9) assigned to foliar disease were recorded.

Table.1-Disease monitoring and survey of four districts during Rabi summer 2016-17

s.no.	Name of Farmer	Village	Block	District	Date of sowing
1	Sukanta Bhoi	Mauzpur	Adaspur	Cuttack	5.11.2016
2	Pravat ku Pradhan	Mauzpur	Adaspur	Cuttack	5.11.2016
3	Nrusingh Panda	Alipingala	Nimapada	Puri	9.11.2016
4	Surendra Muduli	Alipingala	Nimapada	Puri	9.11.2016
5	Akrur Pal	Choudaman	Pipili	Puri	12.11.2016
6	Ramesh Mekap	Choudaman	Pipili	Puri	12.11.2016
7	Nirmal Bastia	Bhakarsahi	Balipatna	Khurda	7.11.2016
8	Dipak Rout	Bhakarsahi	Balipatna	Khurda	7.11.2016
9	Bankabihari Das	Derakana	Tirtol	Jagatsinghpur	13.11.2016
10	RabindraKhuntia	Derakana	Tirtol	Jagatsinghpur	13.11.2016

3.2 Weather parameters in relation to stem rot disease incidence

In order to study the relationship of weather parameters on the natural occurrence of stem rot disease and its severity in groundnut, observations were taken at AICRP Groundnut Research Station at OUAT, Bhubaneswar during Kharif 2017,and Rabi-summer 2017-18.

The weekly incidence and severity of *Sclerotium rolfsii* were recorded as sampling method for counting number of stem rot infected plants out of 10 plants in a spot and recorded. Similarly 20 places were taken for observations and averaged (NICRA Manual for groundnut pest surveillance, 2011).

Standard meteorological parameters such as maximum and minimum temperatures (^{o}c), Relative Humidity(morning), Relative Humidity (afternoon),Rainfall (mm), Number of rainy days and Bright Sunshine Hours were obtained from Meteorological observatory, OUAT, Bhubaneswar.

A correlation-regression analysis of disease incidence with the parameters of Standard Meteorological Week (SMW) was studied to ascertain the contribution of weather parameters for perpetuation of the disease under natural conditions.

3.3 General laboratory procedure

3.3.1 Glassware cleaning

Borosil glass wares such as culture tube, petridishes, conical flasks, beakers etc. were used for laboratory studies. These were kept submerged overnight in cleaning solution prepared by dissolving 60g potassium dichromate (K_2 Cr_2O_7) and 60 ml of concentrated sulphuric acid (H_2SO_4) in one litre of water. Then they were washed with detergent solution followed by rinsing several times in tap water and finally in distilled water. Then they were kept in inverted position to remove excess water and dried.

3.1.2 Sterilization

All the glasswares were sterilized in hot air oven at 160^0C for two hours after wrapping with newspaper or carbon paper. All the liquid and solid media were sterilized in autoclave at 15psi or 1.1kg/cm^2 pressure for 15minutes. Inoculation needle, forceps, cork borer were flame sterilized under spirit lamp. Inoculation chamber was sterilized by putting formalin inside the chamber.

3.1.3 Preparation of stains and surface disinfectants

3.1.3.1 Preparation of cotton blue

For preparation of cotton blue (1%), 1g of cotton blue crystals were dissolved in 100ml of lacto phenol and mixed thoroughly in suitable container for use.

3.1.3.2 Preparation of Lacto phenol

Chemicals	Quantity(ml)
Lactic acid	100
Phenol	100
Glycerine	100
Distilled water	100

Lacto phenol solution was made by dissolving phenol in water without heat to prevent oxidation. Then glycerine was added to it followed by lactic acid.

3.1.3.3 Preparation of 0.1% mercuric chloride

Stock solution:

Mercuric chloride	20g
Conc. Hydrochloric acid	100ml

Working solution:

Stock solution	5ml
Distilled water	995ml

For preparation of stock solution 20g of mercuric chloride dissolved in 100ml conc. hydrochloric acid. At the time of use 5ml of stock solution was mixed with 995ml of distilled water and used for surface sterilization of plant samples.

3.1.3.4 Preparation of 4% formalin solution

100ml of commercial formaldehyde was added with 900ml distilled water and mixed thoroughly.

3.1.4 Preparation of culture media

Potato dextrose agar

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

200g peeled sliced potatoes were boiled in 400ml distilled water. This was filtered with double layered muslin cloth. 20g agar agar was melted in 400ml distilled water separately. Both the solutions were mixed thoroughly and 20g of dextrose was added to the mixture. The final volume was made up to 1000 ml with distilled water and autoclaved at 15psi pressure for 15minutes.

After sterilization 250 mg of streptomycin sulphate was added to one litre sterilized media to avoid bacterial contamination. Twenty ml of the medium was poured into sterilized petridish and solidified under laminar airflow.

3.2 Collection, Isolation and Purification of the pathogen

3.2.1 Collection of disease sample and microscopic observation

A severe incidence of groundnut stem rot was observed and diseased samples were collected from AICRP on Groundnut, Bhubaneswar. Affected plant parts were collected and examined under the microscope which revealed the presence of characteristic mycelia of the fungus as well as sclerotial bodies were also detected.

3.2.2 Isolation of the pathogen

The infected stem samples were cut into small pieces with both healthy and diseased portion. These bits were surface sterilized using 0.1% mercuric chloride for 30 seconds and then washed three times with sterilized distilled water. These bits were then transferred to Potato Dextrose Agar(PDA) plates (four bits at the four corners of plate).These plates were then incubated at 27 $\pm 1^0$C for seven days. The observations were taken periodically. Colony of fungal mycelium was developed on plates. From the colony, hyphal tip of the fungus was transferred into PDA slant with the help of sterilized inoculating needle. Hyphal tip method was followed three times to get the pure culture of the fungus .The slant was incubated at room temperature for further growth of the fungus and stored for future study and use.

3.2.3Purification of the pathogen

The culture thus obtained was purified with hyphal tip methods as described below.

Hyphal tip method

The fungus was grown in a sterile petridish containing potato dextrose agar medium. An 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 the 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.

3.3 Morphological studies

The mycelial tip was taken from pure culture plate and put over a clean glass slide. A drop of cotton blue was put over it and covered with cover slip. The slide was observed under compound microscope. The typical structure of the fungus was observed and compared with the references. The thread like hyaline mycelium with presence of knots and gradual transformation of knots to sclerotia was observed both under low and high power magnification and microphotographs were taken. Sclerotial bodies of *Sclerotium rolfsii* characters are noted by visual observations. *Sclerotium rolfsii* is the anamorphic stage of the pathogen .the teleomorph stage i.e. the sexual stage is rarely observed. The teleomorph, *Athelia rolfsii* (Curzi) C.C. Tu & Kimbr. is a basidiomycete classified in the following order(Liamngee *et al*., 2015)

Kingdom- Fungi
Phylum-Basidiomycota
Subphylum-Agaricomycotina
Class-Agaricomycetes
Order-Atheliales
Family-Atheliaceae
Genus-Athelia or Sclerotium
Species-rolfsii

3.4 Identification and maintenance of pure culture

Pure culture of the desired fungus was sub-cultured on PDA slants and grown at 27 ± 1^0C for 10 days and such slant was preserved in refrigerator and sub -cultured once in 30 days. The fungus was identified according to available literature.

3.5 Pathogenicity test

a)Soil inoculation method

The fungus was multiplied on 100g wheat grains in sterilized bottle for 10 days at 28±1°c.The inoculum was then mixed thoroughly with upper 8 cm of double sterilized sand soil mixture and incubated for 3 days. The inoculum mixed soil was kept moist. Five surface sterilized groundnut seeds were sown in each pot. After seven days seeds were examined and again fungus was reisolated from disease sample.

b)Seedling inoculation method

Twelve days old seedlings were inoculated at collar region with mixture of mycelium and sclerotial bodies of the fungi multiplied in PDA medium for 5 days by pin prick method and also by surface inoculation method. Inoculated collar region was covered with wet soil to keep inoculum moist. After observation of symptoms ,the fungus was reisolated from diseased samples.

3.6 Management studies

3.6.1 *In vitro* evaluation of bio control agents

The efficacy of bio control agents were tested against causal organism by dual culture technique. Biocontrol agents like *Trichoderma viride,Trichoderma harzianum, Trichoderma hamatum* and *Pseudomonas fluorescens* were tested against the fungus. The fungal antagonist were grown in Potato Dextrose Agar media and bacterial antagonist in Nutrient Agar media to get fresh active culture for the experiment.

Dual culture technique

About 20 ml of Potato Dextrose Agar media for fungus and Nutrient Agar media for bacteria was poured into petridishes and allowed to cool down. The fungal mycelia disc(5mm) was transferred to one end of the plate and fungal antagonist culture disc placed opposite to it leaving 5-6mm distance from the periphery of the plates. In case of bacterial antagonist, the bacterium was streaked at one side of the plate and fungal culture disc at other side of the plate. Each treatment was replicated four times. The inoculated plates were incubated at room temperature. After five days the observation were taken. The data were analysed statistically. The efficacy of bio-control agents were expressed as percentage inhibition of mycelia growth over control. The percent inhibition over control was calculated according to formula given by Vincent (1947) as follows.

$$I = \frac{C-T}{C} \times 100$$

I= Percent inhibition

C= Radial growth in control

T= Radial growth in treatment

Completely Randomized Design (CRD) was followed in all the *in vitro* experiments and statistical analysis were calculated as per procedure in Gomez and Gomez (1984).

Efficiency of antagonistic fungal species were identified by scoring on modified Bell's scale (Bell *et al*., 1982) R1=100% overgrowth; R2=75% overgrowth; R3=55% overgrowth; R4= blocked at point of contact and R5=pathogen overgrow the antagonist.

3.6.2 *In vitro* evaluation of fungicides

The fungicides were tested initially under *in vitro* conditions by using poisoned food technique (Nene and Thapliyal, 1973) at desired concentration. Required amount of fungicides were added to sterilized Potato Dextrose Agar medium. Twenty ml of poisoned medium was poured into sterilized petridishes. Mycelial disc of five mm from actively growing zone of ten days old culture were inoculated into each plate and placed at the centre of petriplate. Control petriplate was maintained without adding any fungicide. Each treatment was replicated thrice. The plates were incubated at 27± 1^0C temperature and radial growth of fungal mycelium was measured from both direction and radial growth was calculated. The data were analysed statistically and efficacy of fungicides were expressed as percentage of inhibition of mycelia growth over control. The percent inhibition over control was calculated according to formula given by Vincent (1947) as follows.

$$I = \frac{C-T}{C} \times 100$$

I= Percent inhibition

C= Radial growth in control

T= Radial growth in treatment

Table.2: List of fungicides used in management of the test pathogen

Sl. No.	Chemical name	Trade name	Dose(%)	Concentration (g or ml/litre)
1	Carbendazim 50%WP	BAVISTIN	0.15%	1.5g/litre
2	Mancozeb 75% WP	DHANUKA M-45	0.25%	2.5g/litre
3	Carbendazim12%+ Mancozeb63%WP	SIXER	0.2%	2g/litre
4	Tebuconazole 250EW	FOLICUR	0.1%	1ml/litre
5	Azoxystrobin 23%EC	AMISTAR	0.1%	1ml/litre
6	Azoxystrobin 11%+Tebuconazole 18.3%	CUSTODIA	0.1%	1ml/litre
7	Azoxystrobin 8.3%+Mancozeb 66.7%	AVANCER GLOW	0.1%	1g/litre
8	Propiconazole 250EC	TILT	0.05%	0.5ml/litre
9	Hexaconazole 5% EC	CONTAF	0.05%	0.5ml/litre
10	Chlorothalonil75%WP	DACONIL	0.2%	2g/litre
11	Cymoxanil 8%+Mancozeb 64%	CURZATE	0.15%	1.5g/litre

3.6.3 *In vivo* efficacy of Organic products

Different organic products were taken to evaluate their efficacy in field condition for controlling the stem rot. The organic products selected for experiment as per their local availability viz. Mustard cake, Groundnut cake, Neem cake, Vermicompost, FYM, FYM enriched with *Trichoderma viride*. Each treatment was replicated thrice. soil was sterilized at 15p.s.i at temperature of 121°c for 20 minutes. The fungus was multiplied on 100g of wheat grains taken in a flask for 10 days at 28±1°c. The inoculum was then mixed thoroughly with upper 8 cm of previously sterilized soil and incubated for 3 days. The inoculum mixed soil was kept moist. The poly pots were closed with rubber bands & kept under shade. The organic products were mixed thoroughly with previously incubated soil @10 gms per kg of soil. After mixing the organic products, the poly pots were closed with rubber bands and kept for 7 days. Five surface sterilized groundnut seeds were sown in each pot and

watering was carried on thrice a week to promote germination and growth of the plants. Regular observations were recorded regarding mortality percentage of seeds/germinated seedlings.

The order of the treatments were T_1-Groundnut cake, T_2-Mustard cake, T_3-Neemcake. T_4-FYM,T_5-FYM enrichrd *Trichoderma viride*, T_6-Vermicompost,T7-Control.

3.6.4 Intregrated management of stem rot pathogen under field conditions

In order to develop a cost effective disease management module against stem rot pathogen as well as other soil borne and foliar diseases, field experiments were conducted for Kharif 2017 in the experimental site of All India Coordinate Research Project on groundnut, OUAT,Bhubaneswar,Odisha.

The trial was laid out in Randomized Block Design with 3 replications and 7 treatments in a plot size of (4×3)m^2 for each treatment.

Treatment details:

T_1:Deep summer ploughing with mould board plough+ seed treatment with Tebuconazole 2DS 1.5g/kg seeds followed by PGPR@ 6g per kg of seeds+soil application of *Trichoderma viride* @4kg/ha enriched in 250 kg FYM/ha at 35 and 70 DAS.

T_2:Deep summer ploughing with mould board plough+seed treatment with *T.viride* @10kg/seeds followed by PGPR @6g per kg of seeds+soil application of *T.viride* @4kg/ha enriched in 250kg FYM/ha at 35 and 70 DAS.

T_3:Deep summer ploughing with mould board plough+seed treatment with Tebuconazole 2DS 1.5g/kg seeds+soil application of *T.viride*@4kg/ha enriched in 250kg FYM/ha at 35 and 70 DAS.

T_4:Deep summer ploughing with mould board plough+soil application of *T.viride* @4kg/ha enriched in 250kg FYM/ha+seed treatment with Tebuconazole 2DS@1.5g/kg of seeds followed by seed treatment with PGPR@6g per kg seeds+soil application of *T.viride*@4kg/ha enriched in 250 kg FYM/ha at 35 and 70 DAS.

T_5: Deep summer ploughing.

T_6:Farmers practice(Seed treatment with Carbendazim 0.2%+Drenching base of the plant with Carbendazim 12%+ Mancozeb63%@0.2%.

T_7:Control(without any treatment)

The crop was planted in a row to row spacing of 30cm and plant to plant spacing of 10 cm in Kharif . All the agronomic practices as generally recommended were followed with recommended dose of fertilizer($N:P_2O_5:K_2O$::20:40:40 kg/ha).The disease severity for stem rot and collar rot were calculated on percentage count basis.Observations were taken on germinability, stem rot(%),collar rot(%), foliar disease like tikka,rust and Alternaria blight and pod yield were taken. ICBR was also calculated as per the following formula

Incremental cost benefit ratio=additional income obtained/additional expenditure incurred

For foliar diseases (1-9) scale was adopted as per the guide line of NICRA Groundnut pest surveillance (2011).

Table.3 : Disease severity rating for foliar diseases

Rating	Description of severity
1	No disease
2	1-5% leaf area of lower leaves affected
3	6-10% leaf area of lower and middle leaves affected
4	11-20% leaf area of lower and middle leaves affected
5	21-30% leaf area of all lower and middle leaves affected
6	31-40% leaf area of all lower and middle leaves affected
7	41-60% leaf area of lower and middle leaves affected
8	61-80% damage to lower and middle leaves
9	81-100% leaf area affected,almost all leaves withered and bare stem seen

The percent disease incidence PDI was worked out by using the standard formula by wheeler (1969)

$$PDI = \frac{\text{Sum of individual ratings} \times 100}{\text{Number of leaves examined} \times \text{maximum scale}}$$

The data recorded were assigned with corresponding angular transformed values and analysed as per standard statistical rules.

3.7 Varietal response

Varietal resistance against stem rot incidence, germinability and pod yield in groundnut

One field trial was conducted during Rabi-summer 2017-18 to test the reaction of some prevailing varieties grown by the farmers of Odisha against stem rot disease caused by *Sclerotium rolfsii*. Seeds are collected from AICRP, Groundnut and from Jageswari Krushak club, Pipili of distric Puri were used for conducting the experiment at research field of AICRP groundnut, Beramunda, Bhubaneswar as per the details given below

Number of varieties: 8
Number of replication: 3
Design of experiment: RBD
Plot size: 5m×3m
Spacing: 25cm×10cm
Fertilizer dose : 20:40:40 kg N_2:P_2O_5:K_2O
Date of sowing: 05/12/2017

Agronomical practices as generally recommended were followed. The disease severity for stem rot were calculated on percent count basis. Observations on germinability were taken 15 DAS. Observations on stem rot incidence was taken at 20 DAS and then after in every 15 days interval. The pod yield was recorded after harvesting, threshing and sundrying of the pods. The data recorded on percentage were assigned with corresponding angular transformed values and analysed as per standard statistical rules. The duration, growth characters and resistance/tolerance characters expressed by those varieties are presented in table-4(Manual on crop production technology, 2015, DEE,OUAT,Bhubaneswar)

Table 4: Growth characters of groundnut varieties under evaluation.

SL.NO.	Variety	Duration in (days)	Year of release	Type of growth	Oil content %	Special characters
1	SMRUTI(OG-52-1	110	1995	Erect	51	Red colour kernels, suitable for rain fed , irrigated and residual moisture situation. Tolerant to collar rot and stem rot
2	DEVI(ICGV-91114)	115	2007	Erect	49	Tolerant to mid season and end off season drought
3	TAG-24	110	1992	Semi-erect	49	Tolerant to PBND
4	TG-38 B	125	2006	Bunchy	49	Tolerant to leaf spot and rust
5	K6(K-1240)	105	2005	Bunchy	51	Tolerant to ELS and LLS
6	K9	110	2009	Bunchy	50	Tolerant to early and end off season drought
7	G3(PBS-12160)	110	2010	Bunchy	45	Tolerant to leaf minor and thrips
8	TMV 2 (Local check)	115	1940	Bunchy	51	Moderately resistant to tikka

RESULTS

4.1 Monitoring and Survey of major groundnut diseases

The survey was conducted in five villages comprising of two from each village in five blocks of Cuttack, Puri, Khordha and Jagatsingpur district . The soil condition of these area was almost sandy to sandy loam. It was also found that in coastal tracts of Odisha ,30% of farmers were growing old varieties such as AK 12-24, and TMV-2.Rest of the farmers were growing new varieties such as SMRUTI,DEVI,G-2,,K-6,TAG-24 and TG-38B.The farmers had sown their crops in between 5.11.16 to 13.11.16.During the season of survey i.e. Rabi-summer 2016-17, it was found that farmer of Adaspur block of Cuttack had sown the crop in first week of November where as farmers of Nimapada&Pipili block of Puri,Balipatna block of Khordha and Tirtol block of Jagatsingpur had confined their sowing during second week of November 2016.

During the survey, incidence/intensity of ELS, LLS, Rust, Alternaria leaf blight, Collar rot, Stem rot ,Dry root rot and PBND were taken. The incidence of ELS appeared 27 DAS which scored a maximum of 4 towards 55 DAS.The LLS was most prevailing foliar disease which appeared towards fourth week of December and attained a maximum score of 7 in between 90 to 100 DAS i.e. a week before harvest. The incidence of both Rust and Alternaria blight appeared in traces towards 50 DAS and recorded a maximum score of 3 and 4 respectively. Incidence of pea nut bud necrosis disease (PBND) was maximum up to 5.5 %.(Table.5)

Monitoring the infection of soil borne pathogens, it was found that the collar rot incidence was mostly within 17-40 DAS, recording a maximum of 6% in farmers' field. The incidence of stem rot was noticed around 40 DAS towards third week of December and recorded a maximum 7.4% of infection which is a cumulative effect of stem rot disease up to pod formation and maturity period of crop i.e. 90-100 days of crop. The dry root rot incidence was initiated around 45 DAS and continued to cause root rot and wilt of groundnut crop up to harvest recording 3.5% death of the plants.

Considering the major soil borne diseases of groundnut as a whole it was found that the stem rot incidence was the highest(44%) followed by collar rot (36%) and dry root rot (20%).Among the major foliar diseases infecting groundnut crop in Rabi-summer season it was revealed that the incidence of LLS was the highest (39%) followed by ELS(25%), Alternaria blight(19%) and Rust(17%).

Table.5: Disease occurrence in farmers field during Rabi-summer 2016-17 at different growth stages of groundnut.

Sl.no.	Disease	Date of first appearance	Days after sowing	Vegetative stage(15-30 DAS)	Flowering/Pegging stage (45-60 DAS)	Pod formation/ Maturity(a week before harvest 90 DAS)
1	Collar rot (%)	26.11.16	17DAS	6.0	-	-
2	Stem rot(%)	19.12.16	40DAS	-	5.5	7.4
3	Dry root rot(%)	24.12.16	45DAS	-	2.4	3.5
4	Early leaf spot (Grade)	6.12.16	27DAS	1-2	3-4	-
5	Late leaf spot (Grade)	25.12.16	46 DAS	-	3-4	4-7
6	Rust(Grade)	29.12.16	50DAS	-	1-2	2-3
7	Alternaria leaf spot(Grade)	29.12.16	50 DAS	-	2	3-4
8	PBND(%)	10.1.17	62 DAS	-	1.8	5.5

4.2 Isolation and identification of the pathogen

The diseased samples of groundnut rotted plants were collected from farmers' field during survey as well as AICRP on Groundnut field of Bhubaneswar.

The pathogen was isolated from stem region of affected plants using tissue segment method on Potato Dextrose Agar (PDA) medium. The culture was purified by single hyphal tip method and were maintained on PDA at 27±2°C. The isolated fungi were identified on the basis of following morphological characteristics. The young growing mycelia mass, *in-vivo* and *in-vitro*, is snow white with a silky – luster, later turn dull white with radial spreading giving fan like appearance Microscopic examination of the fungal culture revealed the pathogen is septate and hyaline with conspicuous branching at acute angles. The well developed mycelium had cord-like strands. The hyphae have clamps in the form of forks and hooks or H-like connections .When fungus was at maturity, small mycelial knots were formed which later turned to mustard seed like structures known as sclerotia, which are at first white,

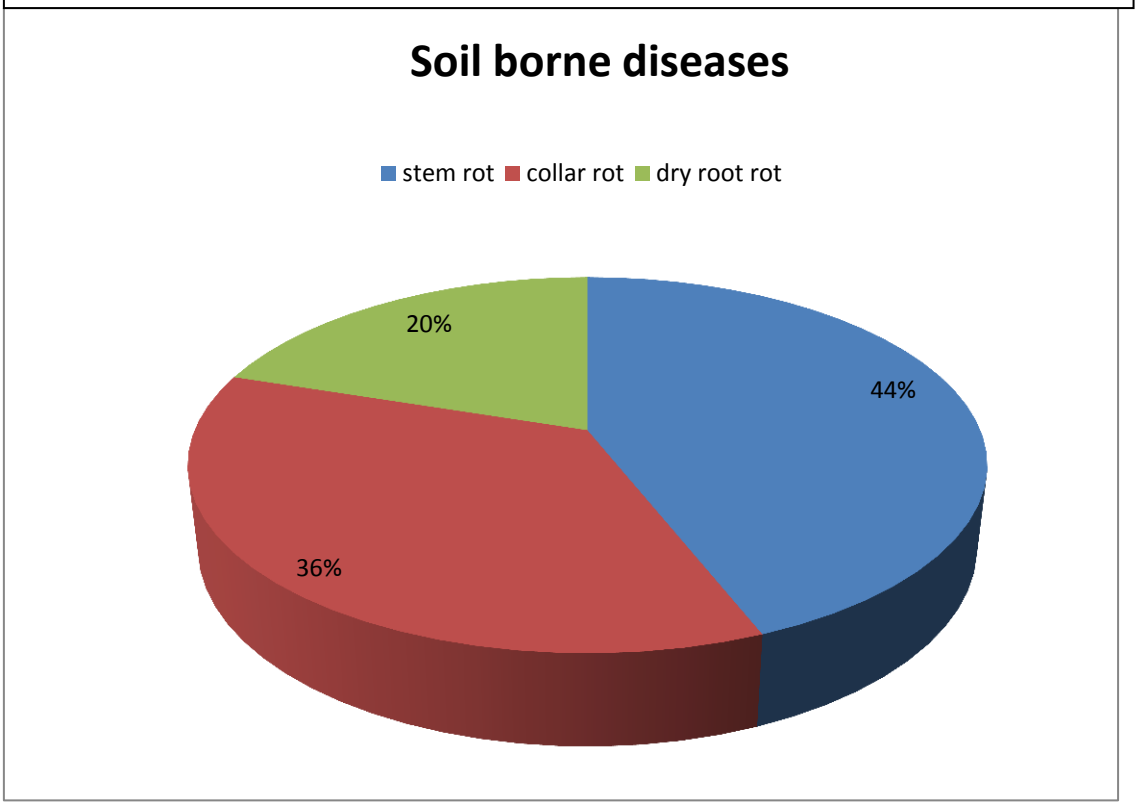
Fig.1:Stem rot infected plant in the survey field

Fig.2:Appearance of sclerotia bodies in infected plant

Fig.3: Reduced plant stand in field showing severity of disease

Fig.4: Field survey during Rabi – summer 2016-17 at Nimapada, Puri

Fig.5 Major diseases of Groundnut in coastal districts of Odisha during survey



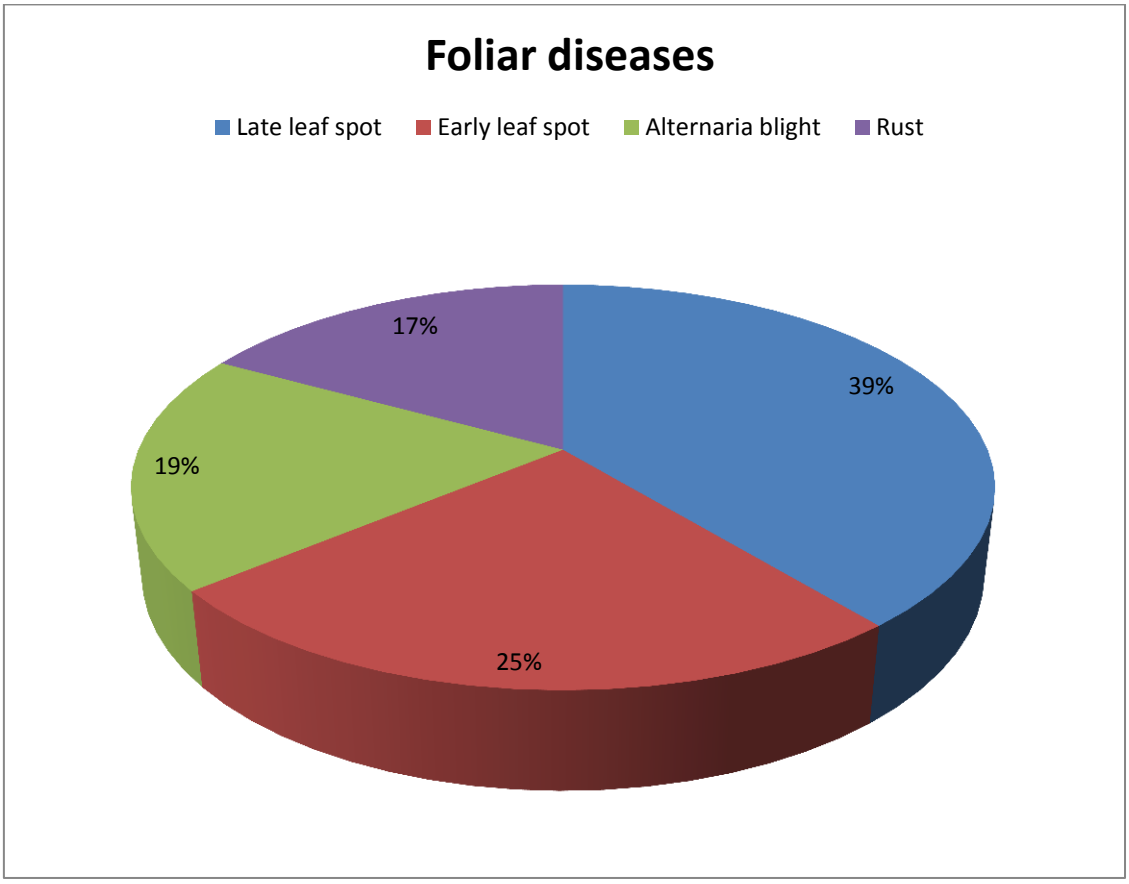


Fig.6:Pure culture of *Sclerotium rolfsii* at 7 days(left)& formation of Sclerotial bodies at 25 days(right)

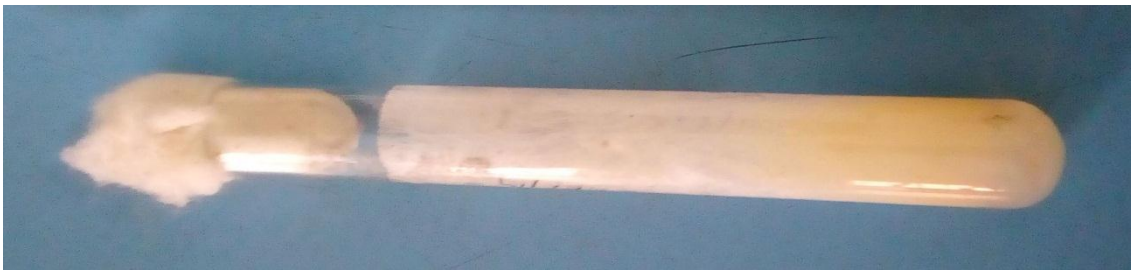


Fig.7: Pure culture of *S. rolfsii* in agar slant

Fig.8: Microphotograph of *S.rolfsii*

becoming light brown to dark brown at maturity . They are sub-spherical, the surface being finely wrinkled or pitted, sometimes flattened, and were easily detached from the mycelium.

4.3 Symptomatology

Following symptoms of stem rot disease were observed in groundnut under natural incidence in field condition. (survey areas)

Stem rot appeared in groundnut after one month of seed sowing. The first symptom of stem rot was noticed as the yellowing and subsequent wilting of the whole plant. Leaves became chlorotic and turned to brown as they rapidly dry out within 5 to 10 days. Infected plants were scattered randomly over the field and it was an important indication of stem rot infection. White mycelium of *Sclerotium rolfsii* was seen around the affected plants at or near the soil surface, imparting a 'white washed' appearance to the base of the affected plants. The infected area of the collar portion was shredded and mycelium quickly produced abundant spherical sclerotia on the collar portion of the affected plant. The sclerotia were initially white and turned light brown to dark brown in colour towards maturity .The Stem rot fungus also attacked the roots, pegs and pods of groundnut. Pods on damaged pegs were shed before harvest. Diseased pods turned dark brown and some of the pods disintegrated in the soil. Severely infected pods were completely covered with a white fungal growth and decayed.

4.4 Pathogenicity test

The seeds in the inoculated pots have shown both pre-emergence and post-emergence mortality of seedlings. Some plants have shown wilting symptoms 45 days after germination. After germination in seedling stage, the initial symptoms were appeared on lower leaves which become dull and gradually increased towards the upper leaves and slowly turned yellowish. After 4-5 days white mycelial growth was observed around the collar region of seedlings and thus it moved upward which resulted in aggregation of mycelia in stem portion which was followed by white sclerotia formation and gradually it turned to light brown and moved to dark brown and black appearance. The collar region later turned brown, shrunken and gradually dried. After 10 days stem was broken down from the collar region which resulted in seedling death .The pathogen was reisolated from the infected plants and the characters of the pathogen tallied with the original one isolated from the field there by fulfilling the Koch's postulates,for proving pathogenicity.

Fig.9 Mass multiplication of *Sclerotium rolfsii* on wheat grain

Fig.10 Pathogenicity test of *Sclerotium rolfsii*

Sclerotium infected plant

Control

4.5 Weather parameters with occurrence of stem rot during crop growth period

Table 6. I ncidence of stem rot disease in groundnut during kharif 2017

Week	SMW	T_{max} (°c)	T_{min} (°c)	RF (mm)	RH_1 (7 hrs)	RH_2 (14hrs)	No. of rainy days	BSH (hrs)	Stem rot(%)
9-15 JULY	28	32	25.4	145.4	94	76	7	1.6	0
16-22 JULY	29	31.7	26.1	164.3	93	85	7	2.8	0
23-29 JULY	30	31.8	26.3	34.3	92	78	4	2.7	0
30 JUL-05AUG	31	33.8	26.1	56.6	91	71	5	3.7	0
06-12 AUG	32	33.1	25.6	85.1	90	76	5	6.1	1.33
13-19 AUG	33	32.4	25.8	76.9	93	84	6	3.5	1.87
20-26 AUG	34	33.6	26	63.2	87	71	5	7.3	2.8
27Aug-02 SEP	35	32.1	25.3	161.4	96	78	6	3.3	4.53
03-09 SEP	36	33.6	26	24.9	92	74	2	5.7	5.63
10-16 SEP	37	34.4	25.7	77.9	90	65	3	6.6	6.2
17-23 SEP	38	32.6	25.8	44.9	92	71	3	3.1	7.67
24-30 SEP	39	33.7	25.5	33.7	92	69	4	3.4	8.2
01-07 OCT	40	30.7	25.1	77.7	94	80	5	2.3	6.33
08-14 OCT	41	33.3	25.4	24.6	94	64	1	6.1	5.67

Table. 7:Incidence of stem rot disease in groundnut during Rabi- 2017-18

Week	SMW	T_{max} (°c)	T_{min} (°c)	RF (mm)	RH_1 (7 hrs)	RH_2 (14hrs)	No. of rainy days	BSH (hrs)	Stem rot (%)
05-11 NOV	45	31.4	19.5	0	86	52	0	9.3	0
12-18 NOV	46	27.3	20.7	55.2	87	66	4	3.5	0
19-25 NOV	47	29.6	18.7	0	91	56	0	6.5	1.87
26 NOV-2 DEC	48	29.4	13.9	0	92	40	0	8.4	3.53
03-09 DEC	49	27.1	14	36.3	88	49	1	5.6	4.33
10-16 DEC	50	29.5	18.3	0	94	59	0	6.3	5.53
17-23 DEC	51	28	13.6	0	92	43	0	7.8	5.83
24-31DEC	52	28	12.5	0	93	43	0	7.8	6.17
01-07 JAN	1	26.5	12.5	0	91	38	0	6.6	6.4
08-14 JAN	2	28	11.2	0	91	34	0	7	6.63
15-21 JAN	3	27.6	11	0	95	35	0	7.8	6.83
22-28JAN	4	29.1	13.4	0	93	35	0	7.2	7.11
29JAN-04 FEB	5	31.1	12	0	91	24	0	8.9	7.4
05-11 FEB	6	33.5	17.1	0	93	31	0	7.7	0
12-18FEB	7	31.6	15	0	90	33	0	7.8	0
19-25FEB	8	35.2	16.5	0	94	29	0	8.8	0
26-04 MAR	9	37.4	19.6	0	93	27	0	8.5	0

Table.8 Correlation studies of weather parameters with incidence of stem rot disease in kharif 2017

	Tmax	Tmin	RF	RH1	RH2	RD	BSH	Stem rot
Tmax	1							
Tmin	0.25415	1						
RF	-0.45695	-0.23825	1					
RH1	-0.59895	-0.51548	0.38200	1				
RH2	-0.75870	0.11179	0.55490	0.34990	1			
RD	-0.50744	-0.03046	0.80859	0.15558	0.72999	1		
BSH	0.73903	0.14791	-0.38329	-0.65906	-0.57005	-0.52916	1	
Stem rot	0.24196	-0.47477	-0.41751	0.08801	-0.50880	-0.61873	0.20840	1

The prediction equation (y) for stem rot incidence

Stem rot(y) =251.15+1.99(Tmax)-8.32(Tmin) +0.04(RF)-1.21(RH1) +0.4(RH2)-3.01(RD)-1.62(BSH)

R^2=0.7631

R^2 (Adjusted) =0.4866

Table.9: Regression values of weather parameters to incidence of stem rot in Kharif 2017

Regressors	Coefficients	Coeff.sq	% Contribution
Intercept	252.14711		
Tmax	1.99254	3.970216	4.58424
Tmin	-8.32401	69.28914	80.00524
RF	0.03771	0.001422	1.64E-05
RH1	-1.21397	1.473723	1.70164
RH2	0.39663	0.157315	0.18164
RD	-3.01583	9.095231	10.50188
BSH	-1.61824	2.618701	3.023703
Total		86.60575	

The correlation study between percent disease incidence of stem rot disease in groundnut and weather parameters viz.Maximum and Minimum temperatures (°c),Relative humidity % in morning and afternoon,Rainfall(mm),Number of rainy days and Bright Sunshine Hours revealed that the disease incidence is positively correlated with Tmax(0.24196),morning Relative humidity(0.08801), Bright Sunshine Hours(0.20840) and negatively correlated with Tmin(-0.47477),afternoon relative humidity(-0.50880),rainfall(-0.41751) and no of rainy days(-0.61873).

From the regression analysis it was concluded that the weather parameters as a whole contribute about 48.7%to disease development. whereas minimum temperature solely contribute to 80% disease development..Number of rainy days contribute only 10% disease incidence and all other factors show insignificant contribution for incidence of disease in groundnut.

Table.10: Correlation studies of weather parameters with incidence of stem rot disease in Rabi 2017-18

	Tmax	Tmin	RF	RH1	RH2	RD	BSH	Stem rot
Tmax	1							
Tmin	0.46778	1						
RF	-0.33914	0.32024	1					
RH1	0.23633	-0.33741	-0.59003	1				
RH2	-0.49251	0.50515	0.56631	-0.48189	1			
RD	-0.28852	0.40747	0.93869	-0.53407	0.57732	1		
BSH	-0.02164	-0.22006	-0.79180	0.32340	-0.67768	-0.77580	1	
Stem rot	-0.62844	-0.79846	-0.22841	0.33745	-0.13863	-0.28510	-0.02164	1

The prediction equation(y) for stem rot incidence

Stem rot (y) = -3.3-0.52(Tmax)-0.42(Tmin)-0.00074(RF)+0.31(RH1)+0.008(RH2)-0.4(RD)+0.02(BSH)

R^2=0.7873

R^2 (Adjusted) =0.6219

Table.11: Regression values of weather parameters to incidence of stem rot in Rabi 2017-18

Regressors	Coefficients	Coeff.sq	% Contribution
Intercept	-3.28196		
Tmax	-0.52111	0.271556	38.53504
Tmin	-0.41608	0.173123	24.56692
RF	-0.00073926	5.47E-07	7.76E-05
RH1	0.31218	0.097456	13.82952
RH2	0.00819	6.71E-05	0.009518
RD	-0.40258	0.162071	22.9986
BSH	0.02062	0.000425	0.060336
Total		0.704698	

The correlation study between stem rot percent disease incidence and weather parameters in Rabi-summer revealed that the disease incidence is positively correlated only with morning Relative humidity(0.33745) and negatively correlated with all other weather factors viz. Tmax(-0.62844),Tmin(-0.79846)),afternoon Relative humidity(-0.13863),rainfall(-0.22841)) and number of rainy days(-0.28510).

From the regression analysis it was concluded that the weather parameters as a whole contributed about 62.2% to stem rot disease development in groundnut during winter season of 2017-18,among which maximum and minimum temperature contribute 38%and 24% respectively, number of rainy days contributed 22% to stem rot disease incidence.

4.6 Management of stem rot disease

4.6.1 Antagonistic effects of Bioagents against *Sclerotium rolfsii* under *in vitro* condition

Three *Trichoderma spp.* viz., *Trichoderma viride, Trichoderma harzianum,Trichoderma hamatum* and one bacterial antagonist, *Pseudomonas fluorescens* were tested to find out their antagonistc potential and type of colony interaction against test pathogen *Sclerotium rolfsii*. The data are presented in (Table.12) revealed that all the isolates of *Trichoderma* in dual culture significantly inhibited mycelial growth of *Sclerotium rolfsii* and inhibition ranged from 63.00 to 74.00 per

cent over control. It is observed that, antagonist *Trichoderma harzianum* is most efficacious with 74.4% mycelial growth inhibition, 87.1% sclerotial inhibition followed by *Trichoderma viride* with 70.6% mycelial growth inhibition and 85.2% sclerotial inhibition. The bacterial antagonist also have 63.5%mycelial inhibition and 71.2% sclerotial inhibition. *Trichodema harzianum* is more efficacious as compared to *Trichodema viride* but *Pseudomonas fluorescens* is statistically at par with *Trichoderma hamatum.*

Trichodema harzianum showed better ranking from modified Bells score which depicted ability of antagonist to overgrew and caused lysis of mycelium of test pathogen that is R1(100% growth),where as*Trichoderma viride, Trichoderma hamatum and Pseudomonas fluorescens* all showed R2(75% growth) ranking.

Table.12: Efficacy of Bioagents on inhibiting the growth of *Sclerotium rolfsii*

	Treatment	Radial growth(mm)	%inhibition	%inhibition of sclerotial bodies	Score (Bell scale)
T1	*Trichoderma viride*	25	70.6* (57.17)	85.2* (67.37)	R2
T2	*Trichoderma harzianum*	21.75	74.4 (59.60)	87.1 (68.95)	R1
T3	*Trichoderma hamatum*	30.25	64.4 (53.37)	84.1 (66.50)	R2
T4	*Pseudomonas fluorescens*	31.00	63.5 (52.83)	71.2 (57.54)	R2
T5	Control	85.00	0 (0.00)	0 (0.00)	
	CD %(0.05)	1.443	0.928	2.727	
	SE(m)±	0.474	0.305	0.896	

***Figures in the parenthesis are arc sine transformed value**

4.6.2 Bioassay of fungicides against *Sclerotium rolfsii*

Antifungal activity of various chemicals were assessed *in-vitro* by poison food technique. Results revealed that all the systemic fungicides were capable of inhibiting growth of test fungus at recommended dosage as compared to check

Mixed fungicides such as Carbendazim+Mancozeb at 0.2%, Azoxystrobin+Tebuconazole at 0.1%,Azoxystrobin+Mancozeb at 0.1% and triazole group of fungicides viz., Tebuconazole,Propiconazole,Hexaconazole recorded inhibition of *Sclerotium rolfsii* growth by 100% at 0.1% concentration followed by Cymoxanil+Mancozeb at 0.2%(82.6%), Azoxystrobin at 0.1%(75.6%) and Mancozeb at 0.25% concentration(53.4%).Least antifungal activity was expressed by Carbendazim at 0.15% concentration(22.6%) followed by Chlorothalonil at 0.2%(31.1%).(Table.13)

Table.13: Evaluation of fungicides against *Sclerotium rolfsii in vitro*

	Ttreatment	Dosage	Radial growth(mm)	%inhibition
T1	Carbendazim 50%WP	0.15%	63.5	22.6*(28.39)
T2	Mancozeb 75%WP	0.25%	38.2	53.4(46.95)
T3	Carbendazim12%+ Mancozeb63%WP	0.2%	0	100(90.00)
T4	Tebuconazole 250EC	0.1%	0	100(90.00)
T5	Azoxystrobin 23%EC	0.1%	20	75.6(60.40)
T6	Azoxystrobin11%+Tebuconazole18.3%	0.1%	0	100(90.00)
T7	Azoxystrobin8.3%+Mancozeb66.7%	0.1%	0	100(90.00)
T8	Propiconazole 250EC	0.05%	0	100(90.00)
T9	Hexaconazole 5% EC	0.05%	0	100(90.00)
T10	Chlorothalonil 75%WP	0.2%	56.5	31.1(33.90)
T11	Cymoxanil8%+Mancozeb64%	0.15%	14.7	82.6(65.35)
T12	Control		82	-
	CD(0.05)		0.269	3.656
	SE(m)±		0.092	1.245

***Figures in the parenthesis are arc sine transformed values**

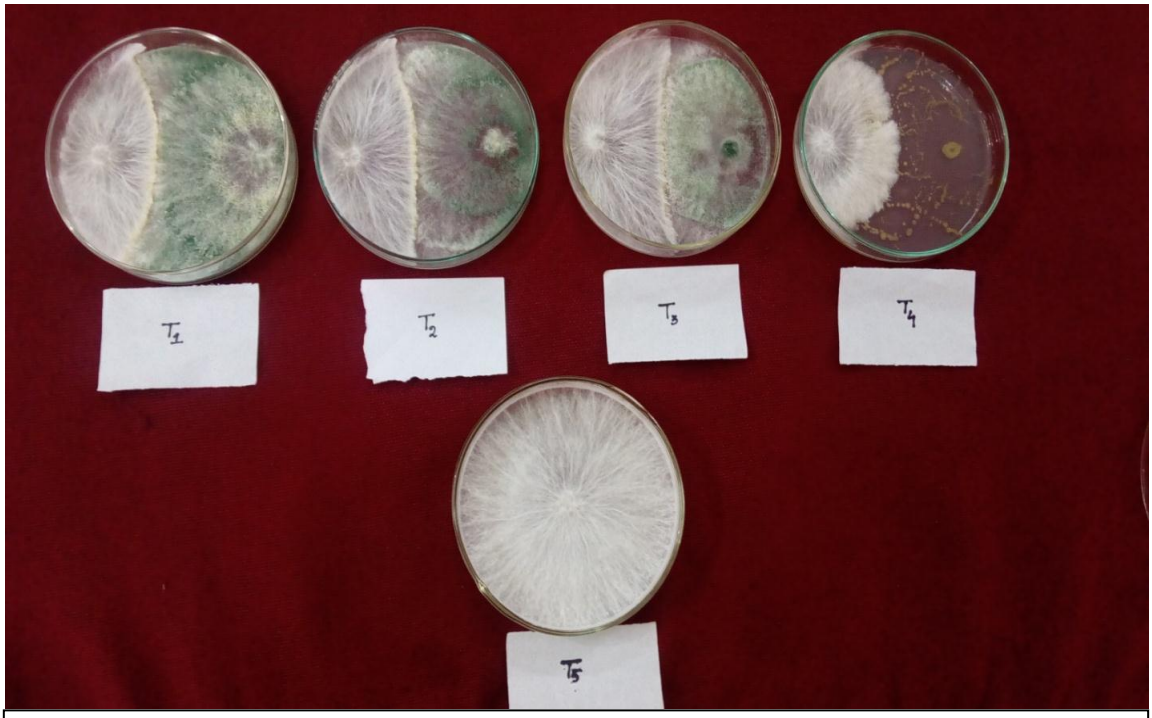


Fig.11 *In vitro* efficacy of bioagents against *S.rolfsii*.T1-*T. viride*;T2-*T.harzianum*;T3-*T.hamatum*;T4-*P. fluorescens*;T5-Control

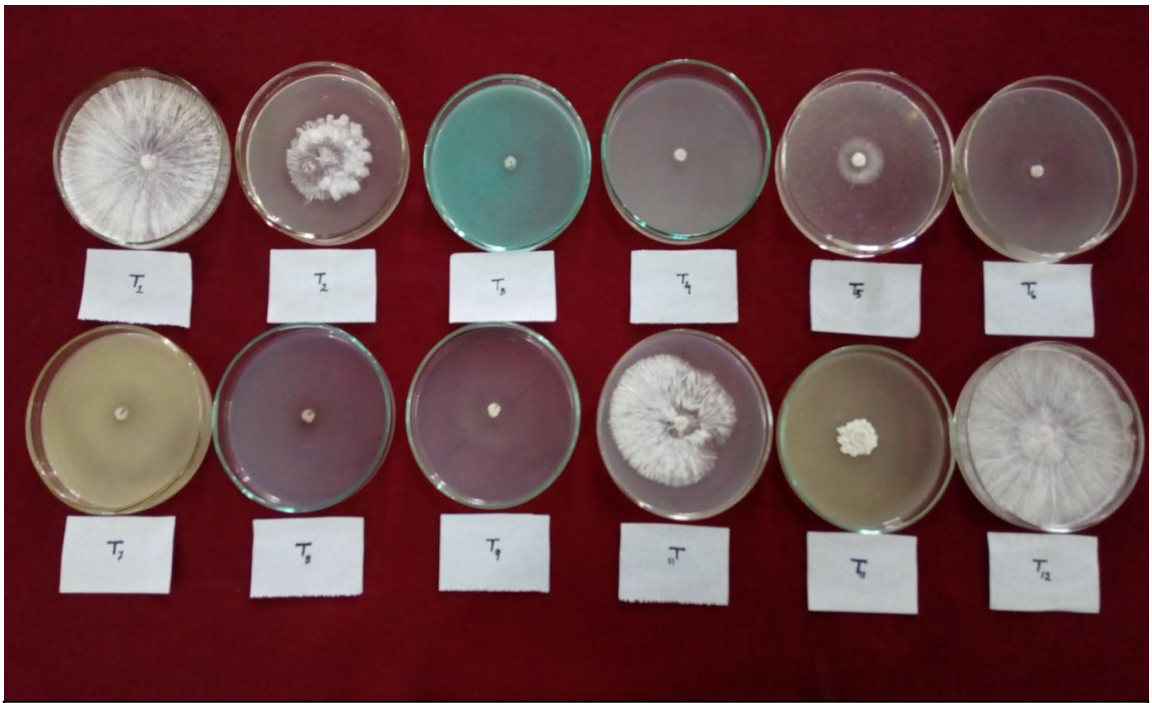


Fig.12 *In vitro* efficacy of fungicides against *S.rolfsii* T1-Carbendazim;T2-Mancozeb;T3- Carbendazim+Mancozeb;T4-Tebuconazole;T5-Azoxystrobin;T6-Azoxystrobin+Tebuconazole;T7-Azoxystrobin+Mancozeb;T8-Propiconazole;T9-Hexaconazole;T10- Chlorothalonil;T11- Cymoxanil+Mancozeb;T12-Control

4.6.3 Evaluation of organic products against the test pathogen, *Sclerotium rolfsii*

The present experiment was conducted to study the effect of different organic amendments on stem rot disease of groundnut. Different organic products viz. Groundnut cake,Mustard cake,Neem cake, FYM,FYM enriched *T.viride*,Vermicompost were incorporated in sterile soil as described in "Materials and Method". The efficacy of the organic products against *Sclerotium rolfsii* was evaluated. The mortality per cent was recorded at 15 DAS. Among all the treatments FYM enriched *T.viride* recorded the least mortality (6.7%) followed by neem cake treated pot(26.7%).Vermicompost amended pot shows 40% mortality where as mustard cake and FYM both expressed 53.3% mortality due to stem rot. Groundnut cake treated pots showed highest mortality of 66.7% among all other treatments and also at par with the control untreated pot (73.3%).

Table.14: Effect of organic amendments in the management of stem rot of groundnut in pot culture experiment

	Treatment	Concentration	Mean mortality(%) of plants due to stem rot
T1	Groundnut cake	1.00	66.7 (54.76)*
T2	Mustard cake	1.00	53.3 (46.89)
T3	Neem cake	1.00	26.7 (31.11)
T4	FYM	1.00	53.3 (46.89)
T5	FYM enriched T.viride	1.00	6.70 (15.00)
T6	Vermicompost	1.00	40.00 (39.23)
T7	Control	-	73.30 (58.89)
	CD (0.05)		16.78
	SE(m)±		5.481

***Figures in parenthesis are angular transformed value**

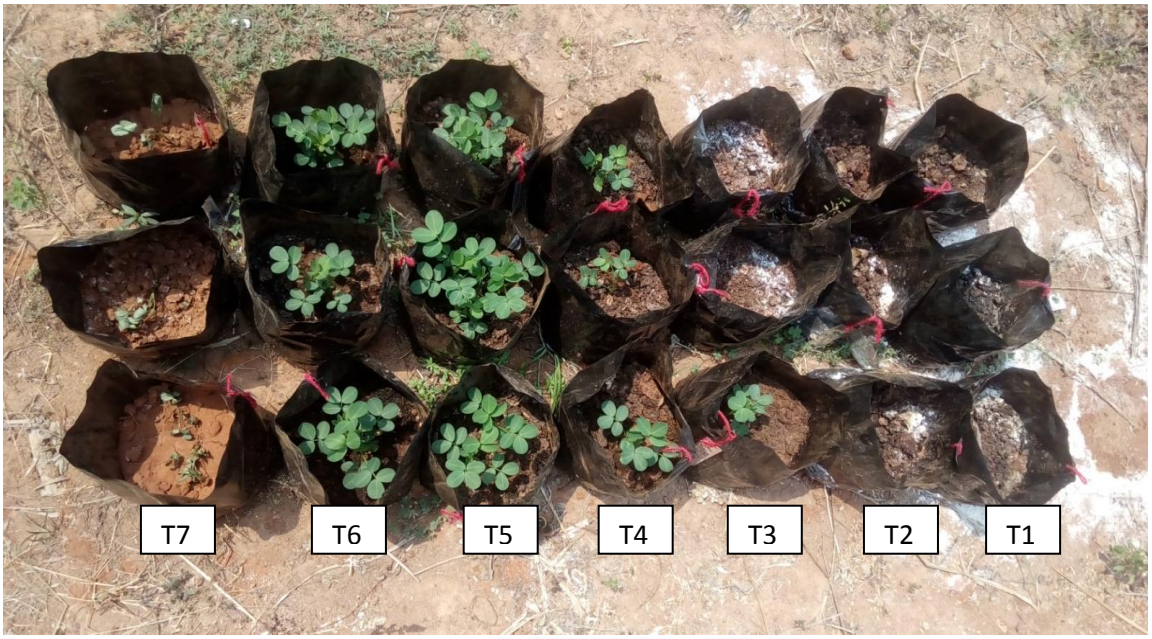


Fig.13 Pot culture experiment of organic amendments

T1; Groundnut cake;T2- Mustard cake;T3- Neem cake;T4- FYM;T5- FYM enriched *T.viride*;T6- Vermicompost;T7- Control

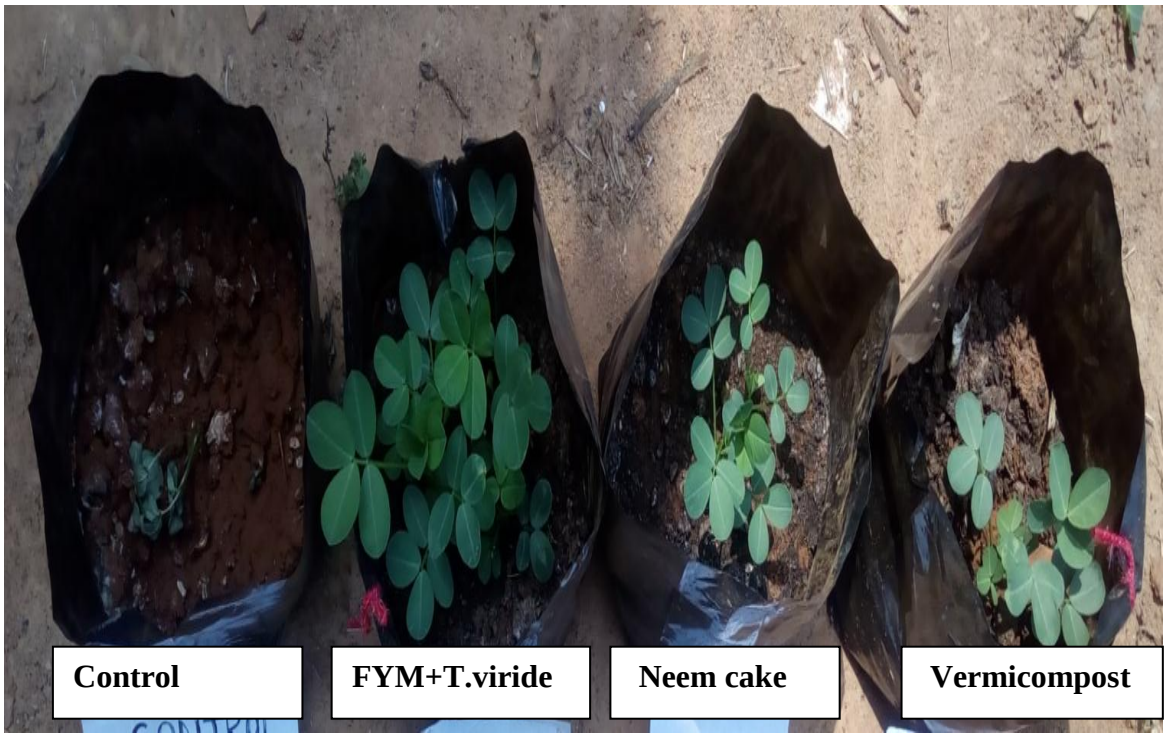


Fig.14 Comparison of different organic treatments over control

4.6.4 Integrated management approach for stem rot disease during Kharif 2017

The results of integrated approach on stem rot disease management in groundnut during Kharif 2017 revealed that Deep summer ploughing with mould board plough+ basal application of *Trichoderma@* 4 kg/ha enriched with 50 kg FYM+seed treatment with tebuconazole @1.5g/kg of seed and by PGPR@6g/kg seed followed by soil application of *Trichoderma@* 4kg/ha enriched in 250 kg FYM per ha at 35 and 70 DAS was found to be the best. It increased the germination by 24.9%, reduced the stem rot incidence by 65.9% and increased the yield by 38.7%.

The germination percentage was found the highest (90.6%) in T4 followed by T1, T3,T2,T6 and T7.The germination percentage was the lowest (72.5%)in untreated control which is significantly less than all other treatments. The stem rot incidence was the lowest in T4 i.e. 1.63% at 40-45 DAS, 2.60% at 75DAS and 2.80% at 90DAS and significantly less than all other treatments. The effect of integrated management on other soil borne diseases revealed that the T4 recorded the least disease incidence of collar rot, dry root rot and pod rot which recorded the incidence of 1.80%, 2.76%,and 1.50% respectively. The untreated control plot recorded the incidence of 7.9% collar rot,6.73% dry root rot and 5.4% pod rot which expressed significantly higher disease than other treatments. The treatments though confined to seed treatment and soil application of bioagent at various stages of growth had an effect on late leaf spot, the most prevailed foliar disease of the region. The T4 recorded a minimum score of 3 in (1-9) point scale against the highest score of 8 in untreated control for late leaf spot disease.

The pod yield of 1301.7 kg/ha was recorded in control plot and the highest being 1806.7 kg/ha in T4.The T4 recorded the highest pod yield and haulm yield of 1806.7 and 4106.7 kg/ha respectively with ICBR of 8.03 and hence found to be superior over other treatments. Only deep summer ploughing expressed less reaction towards incidence of stem rot and other soil borne diseases. Deep summer ploughing with MB plough(T5) also recorded pod yield of 1491.7 kg/ha and was found significantly higher than both farmer practice as well as untreated control.

Fig.15 Integrated approach for management of stem rot disease in field

Fig.16 Comparison of most effective treatment with untreated plot

T4:MB PL+Soil *T.viride*+ST Tebuconazole+PGPR+Soil *T.viride* 35 and 70 DAS

T7:Untreated control

Table.15:Integrated approach on stem rot disease management in groundnut during Kharif 2017

	Treatment	Germination (%)	Stem rot incidence(%)			Pod yield (kg/ha)	Hulm yield (kg/ha)	ICBR
			40-45 DAS	70-75 DAS	90 DAS			
T_1	MB PL.+ST Tebuconazole +PGPR+Soil T.viride 35 &70 DAS	89.10 (70.71)*	1.90 (7.85)*	2.80 (9.60)*	3.03 (10.02)*	1755.0	3706.7	7.63
T_2	MB PL+Seed T.viride +PGPR+Soil T.viride 35 & 70 DAS	86.76 (68.71)	2.93 (9.83)	3.20 (10.23)	3.96 (11.48)	1576.7	3465	5.58
T_3	MB PL+ST Tebuconazole +Soil T.viride 35 & 70 DAS	88.86 (70.58)	3.00 (9.70)	3.90 (11.40)	4.23 (11.86)	1635.0	3291.7	6.61
T_4	MB PL+Soil T.viride+ST Tebuconazol + PGPR+Soil T.viride 35 & 70 DAS	90.60 (72.20)	1.63 (7.33)	2.00 (8.04)	2.80 (9.62)	1806.7	4106.7	8.03
T_5	Deep summer ploughing with MB plough	87.83 (69.61)	2.83 (9.70)	4.10 (11.60)	4.53 (12.29)	1491.7	3230	6.61
T_6	Farmers practices(ST Carbendazim followed by sparaying of Saaf	84.70 (67.00)	3.80 (11.20)	5.00 (12.92)	6.16 (14.37)	1355.0	2826.7	5.52
T_7	Untreated control	72.50 (58.35)	4.20 (11.8)	5.63 (13.70)	8.23 (16.67)	1301.7	2723.3	_
	CD(0.05)	2.874	0.852	0.989	0.944	33.71	630.101	
	SE(m)±	0.923	0.273	0.318	0.303	11.43	202.352	

***Figures in the parenthesis are arc sine transformed value**

Table.16: Effect of integrated stem rot management on other major diseases of groundnut during Kharif 2017

	Treatment	Collar rot (%)	Dry root rot(%)	Pod rot(%)	Late leaf spot Score(1-9) at harvest	Yield
T_1	MB PL.+ST Tebuconazole +PGPR+Soil T.viride 35 &70 DAS	2.60 (9.20)*	3.23 (10.35)*	1.90 (7.85)*	5	1755.0
T_2	MB PL+Seed T.viride +PGPR+Soil T.viride 35 & 70 DAS	3.90 (11.43)	3.73 (11.13)	2.73 (9.50)	6	1576.7
T3	MB PL+ST Tebuconazole +Soil T.viride 35 & 70 DAS	3.00 (9.96)	3.13 (10.19)	3.06 (10.07)	6	1635.0
T_4	MB PL+Soil T.viride+ST Tebuconazol + PGPR+Soil T.viride 35 & 70 DAS	1.80 (7.70)	2.76 (9.60)	1.50 (6.93)	3	1806.7
T_5	Deep summer ploughing with MB plough	4.80 (12.74)	4.70 (12.50)	3.8 (11.20)	6	1491.7
T_6	Farmers practices(ST Carbendazim followed by sparaying of saaf	6.20 (14.45)	5.70 (13.80)	4.5 (12.24)	7	1355.0
T7	Untreated control	7.90 (16.31)	6.73 (15.03)	5.4 (13.50)	8	1301.7
	CD(0.05)	0.91	0.56	0.72	1.03	33.71
	SE(m)±	0.31	0.19	0.24	0.33	11.43

***Figures in the parenthesis are arc sine transformed values**

Fig 17.Effect of treatments on the stem rot incidence and yield

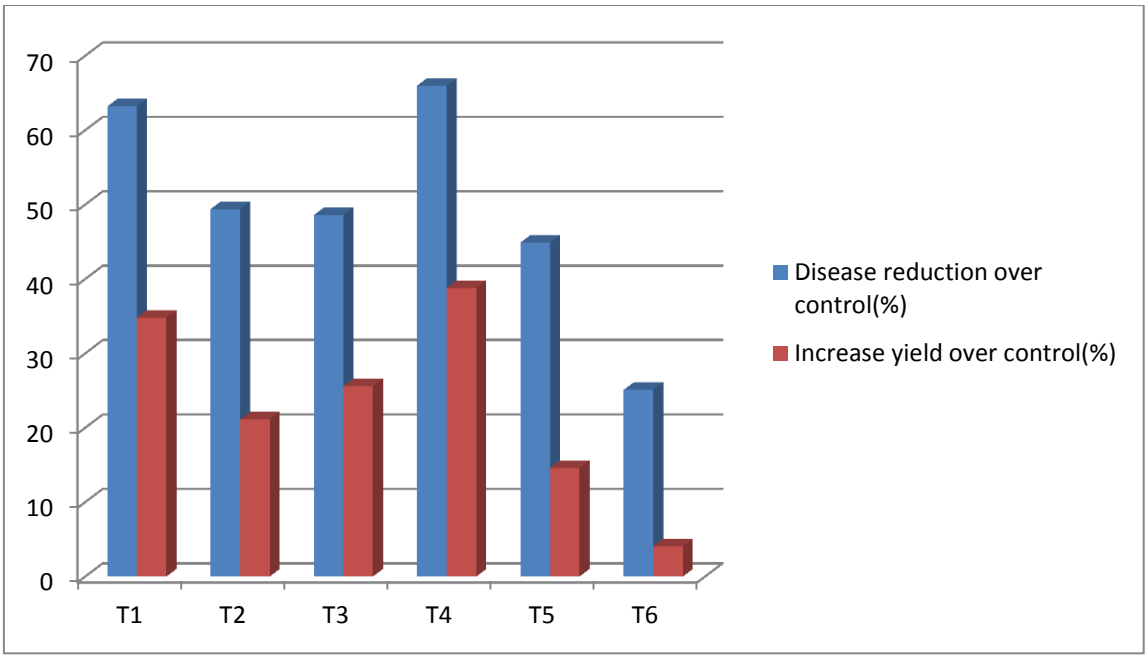
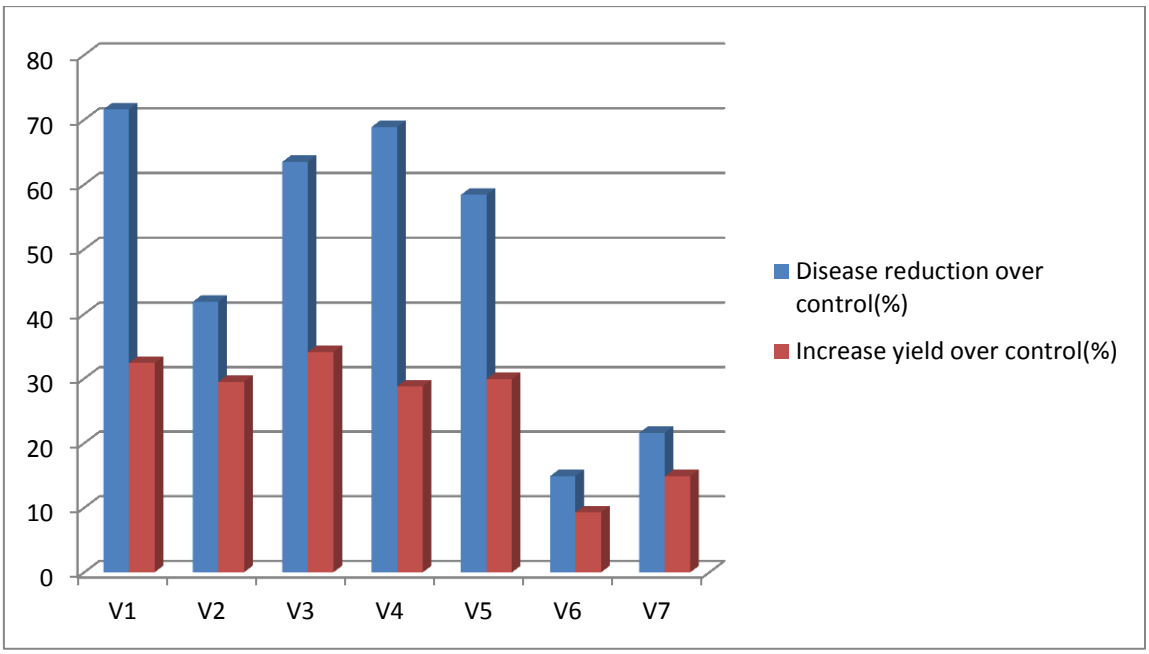


Fig 18 .Response of varieties on the stem rot incidence and yield



4.7 Varietal reaction

Varietal evaluation against stem rot incidence, germinability and pod yield in groundnut.

The reaction of eight cultivars of groundnut against stem rot disease was studied under field condition as per the procedure described in materials and method and are presented in table.17

From the data presented in table-17,it is evident that no cultivar was found to be immune to stem rot disease out of the eight cultivars tested. The least incidence of 2.1% was recorded from variety Smruti which is at par with TG 38 B having incidence of 2.3%.Varieties TAG-24, K6 and DEVI recorded less than 5% of disease incidence. The maximum disease incidence was recorded with variety TMV-2 which is significantly higher than all other varieties followed by K9 and G3 recording incidence of 7.4%,6.3% and 5.8% respectively. The variety Smruti expressed a disease reduction of 71.6% and TG-38 B with a disease reduction of 68.9% when compared with TMV-2, the susceptible check.

Out of the eight varieties tested ,TAG-24 recorded the highest germination percentage of 88% followed by K6(86.3%),DEVI(84.3%) and Smruti(83.7%).From germinability point of view Smruti and Devi were statistically at par .TMV-2 recorded lowest germination of 73.3%, which is statistically different from all other varieties under test.

Considering the pod yield, the maximum pod yield of 1885kg/ha was recorded from the variety TAG-24 followed by Smruti(1860kg/ha),K6(1825kg/ha) and DEVI(1820kg/ha) which are statistically at par. The susceptible check TMV-2 recorded the lowest yield of 1405kg/ha followed by K9(1535kg/ha) and G3(1615kg/ha).Considering both stem rot incidence and yield, the variety Smruti was found the best with 71.6% reduction in disease and 32.4% increased in yield when compared over the check.TAG-24 and TG-38 B may be considered as the next best varieties reducing the disease by 63.5%,68.9% and increasing the yield by 34.2% and 28.8% respectively.

Table.17: Evaluation of groundnut varieties against germinability, stem rot incidence and pod yield.

SL.NO.	VARIETY	Germination %	Increased in germination %	Stem rot incidence %	Disease reduction over check%	Yield(kg/ha)	Increased yield over check%
1	SMRUTI(OG-52-1	83.7(66.19)*	14.2	2.1(8.33)*	71.6	1860	32.4
2	DEVI(ICGV-91114)	84.3(66.66)	15.0	4.3(11.97)	41.9	1820	29.5
3	TAG-24	88.0(69.73)	20.1	2.7(9.46)	63.5	1885	34.1
4	TG-38 B	82.8(65.50)	12.9	2.3(8.72)	68.9	1810	28.8
5	K6(K-1240)	86.3(68.28)	17.7	3.3(16.47)	55.4	1825	29.9
6	K9	74.9(59.93	2.2	6.3(14.54)	14.9	1535	9.3
7	G3(PBS-12160)	78.3(62.24)	6.8	5.8(13.94)	21.6	1615	14.9
8	TMV 2 (Local check)	73.3	-	7.4(15.79)	-	1405	-
	CD(0.05)	1.030		0.521		71.346	
	SE(m)±	0.336		0.170		23.296	

***Figures in the parenthesis are arc sine transformed values**

DISCUSSION

Disease caused by soil borne pathogens especially cause a threat to groundnut production due to the similarity of symptoms, which create problems in diagnosis. Groundnut is susceptible to losses incited by soil borne pathogens due to the close association of the pods with the soil. Soil borne diseases are especially complicated to manage due to the deficiency of dispersing fungicides through the peanut canopy to the soil profile. Among the soil borne diseases collar rot, stem rot, afla rot and dry root rot are having major importance. These can cause severe seedling mortality resulting in patchy crop stand in sandy loam soils and reduction of pod yields from 25-40%.(Ghewande *et al*., 2002) In coastal districts of Odisha as high as 20.3% incidence of soil borne pathogens were reported out of which the stem rot incidence was high as 8.3%.(Vineela,2017)

5.1 Monitoring and survey of major groundnut diseases

During the season of survey i.e. Rabi-summer 2016-17, it was found that among the foliar diseases late leaf spot was the most severe which attend a maximum score of 7 in between 90-100 DAS i.e. a week before harvest

Monitoring the infection of soil borne pathogens, it was found that the incidence of stem rot of groundnut caused by *Sclerotium rolfsii* appeared around 40 DAS towards third week of December recording a maximum of 7.4% of infection which is a cumulative effect of stem rot disease upto pod formation and maturity stage of the crop i.e. towards 90-100 days of the crop growth stage. Similar type of occurrence, distribution and incidence of stem rot pathogen was also earlier reported by Rodriguez Kabana *et al*.,1975 ; Siddaramaiah *et al*.,1979;Patil and Rane,1982; Mayee and Datar,1988.Gouda in 1999 reported 8.68% stem rot incidence in Dharwad,Belgaun and Haveri district of Karnataka during Rabi 1998-99,which corroborates the occurrence and incidence of stem rot pathogen in Odisha condition as surveyed in the present investigation.

Adivar,2003 and Kadam *et al*.,2011 also conducted field survey and reported similar type of prevelance of stem rot disease singly or in combination with foliar diseases on various groundnut cultivars.

5.2 Isolation and identification of the pathogen, symptomatology and pathogenicity test

The diseased samples of groundnut rotted plants were collected from farmers field The pathogen was isolated from stem region of affected plants on potato dextrose agar (PDA) medium The isolated fungi were identified on the basis of morphological characteristics like snow white with a silky – luster mycelia mass, later turn dull white with radial spreading giving fan like appearance. Microscopic examination of the fungal culture revealed the pathogen is septate and hyaline with conspicuous branching at acute angles. The hyphae have clamps in the form of forks and hooks or H-like connections. The results of Mirza and Aslam,1993 and Mohan *et al*.,2000 are also in agreement with the present findings .When fungus was at maturity, small mycelia knots were formed which later turned to mustard seed like structures known as sclerotia.Sclerotia are at first white, becoming light brown to dark brown at maturity .Similar results also obtained by Chet,1975;Subramanian,1964; Ansari and Agnihotri, 2000 and Anahosur, 2001.Symptoms obtained from present study like stem rot appeared in groundnut after one month of seed sowing , yellowing and subsequent wilting of the whole plant, leaves became chlorotic and turned to brown as they rapidly dry out within 5 to 10 days matched with findings of Mehan *et al.*,1995.The seeds in the inoculated pots have shown both pre-emergence and post- emergence mortality of seedlings. Some plants have shown wilting symptoms 45 days after germination The pathogen was reisolated from the infected plants and the characters of the pathogen tallied with the original strain isolated from the field there by fulfilling Koch's postulates which support the earlier reports of Curzi(1931);Haware and Nene (1978);Padole *et al*. (2009);Awasthi *et al.*(2010) .

5.3 Influence of weather parameters on occurrence of stem rot

The meteorological parameters such as maximum and minimum temperatures (^{o}c),Relative Humidity(morning),Relative Humidity(afternoon),Rainfall(mm),Number of rainy days and Bright Sunshine Hours during the crop growth period in Kharif 2017 and Rabi-summer 2017-18 were correlated with the incidence of stem rot disease. The maximum temperature, relative humidity (morning), Bright sunshine Hours were positively correlated with development of stem rot incidence during Kharif 2017,the highest incidence of 8.2% towards 39 SMW i.e 24th-30th September.

High temperature and moisture correlation with hyphal growth,sclerotial formation were also previously reported by Tu and Kimbrough, 1978; Mihail and Alcorn,1984; Pattanapipitpaisal and Kamlandham,2012.

However the minimum temperature contributed 80% to the disease development in Odisha condition. Vinnela in 2017 reported that the minimum temperature solely contributed 98.51% to the development of stem rot which has close relevance with the present findings. It was also revealed from its regression analysis that the weather parameter as a whole contributed about 48.7% to disease development.

During Rabi-summer 2017-18, the maximum and minimum temperature, morning relative humidity and number of rainy days had significant contribution like 38.5%,24.5%,13.8% and 22.9% respectively to disease development. From regression analysis it was concluded that the weather parameters as a whole contributed 62.2% to stem rot development.

It may be inferred from both seasons that relative humidity, soil moisture had a key role and contribution to fungal growth and development which phenomenon was also evident from the findings of Zoberi,1980.During Kharif the disease development period coincides to a temperature range of 25°c to 35.4°c and during rabi season it ranged from 11.2°c to 24.5°c. Mihail and Alcorn, 1984 also reported that maximum fungal growth and development occoured in between 27-30°c, which has similarity but wider range of occurrence in Odisha condition.

5.4 Management of stem rot disease

5.4.1 Antagonistic effects of Bioagents against *Sclerotium rolfsii* under *in vitro* condition

Present investigation include three species of Trichoderma viz. *T viride,T harzianum,T hamatum* and one bacteria species, *Pseudomonas fluorescens* to find out their antagonistic potential and type of colony interaction against *Sclerotium rolfsii*.The results are similar with the findings of Mundhe *et al*.(2009),where maximum inhibition percentage on growth of *sclerotium rolfsii* was achieved by *Trichoderma harzianum*(73.77%) followed by *Trichoderma viride* (72.66%) and

Pseudomonas fluorescens(71.55%). Darvin *et al.*(2013) reported that *T.harzianum* and *T.viride* shows highest radial growth inhibition and *T longibrachiatum* has lowest radial growth inhibition in dual culture with *sclerotium rolfsii in vitro*.Basumatry *et al.*(2015) reported similar result showing maximum percent of growth inhibition of *Sclerotium rolfsii* by *Trichoderma harzianum*(77.39%) followed by *Trichoderma viride*(76.54%).Khalid(2013) reported *Pseudomonas fluorescens* and *Trichoderma viride* to control 83.7% and 86.3% respectively as bioagents against *S. rolfsii*.Karthikeyan *et al.*(2006) reported *T.viride* inhibited the mycelia growth of the pathogen by 69.40%, while *P.fluorescens* inhibted 64.4% which support the present findings.Present study also revealed that Trichoderma species inhibited sclerotia formation which is similar with the findings of Rekha(2012).Though *Trichoderma hamatum* shows the least mycelial inhibition (64%)among bioagent but have very good potential of in inhibiting sclerotial formation by 84% which is in agreement with the findingsof Radawan *et al.*(2006).

5.4.2 Bioassay of fungicides against *Sclerotium rolfsii*

Antifungal activity of various chemicals were assayed *in-vitro* by poison food technique. Results revealed that all the triazole group of fungicides viz., tebuconazole, propiconazole, hexaconazole recorded inhibition of *Sclerotium rolfsii* growth by 100% at 0.1% concentration which supports the reports made by Charde *et al.*(2002) and Adiver(2007).Tebuconazole at 0.1% was most effective fungicide found in this study and have similarity with the findings of Perez *et al.*(2009) and Khan and Javaid(2015).From the present experiment it revealed that at low concentration , carbendazim and chlorothalonil recorded less inhibition of 22.6%and 31.1% respectively which matched with results of Toorray *et al.*(2007) , who also demonstrated the partial inhibition effect of kavach(chlorothalonil) at low concentration .Yaqub and Shahzad,during 2006 and reported the ineffectiveness of carbendazim *S.rolfsii* which corroborates the present result.

5.4.3 Evaluation of organic product

Different organic products viz. Groundnut cake,Mustard cake,Neem cake, FYM,FYM enriched *T.viride*,Vermicompost were incorporated in sterile soil to study the efficacy of the organic amendments against *Sclerotium rolfsii* . Among all the

treatments FYM enriched *T.viride* showed the least mortality(6.7%) which is similar with the results of Chandrasehar *et al.*(2005),who had found that maximum survival of 89.5% by treating seeds with *T.viride* as well as soil application giving 77.9%survival of seedlings.Similar findings also obtained by Anahosur(2001),who reported that FYM+tuber treatment with *T. harzianum* prior to planting helped in reducing sclerotial wilt of potato in field.Neem cake treated pot gave 26.7% mortality,which was supported by findings of Meena *et al*.(2014),who reported maximum mycelia growth inhibition 29.6% by application of neem cake at concentration of 10% .Similarly, Dhingani *et al*.(2013) reported maximum inhibition of pathogen (59.4%) using neem cake extract. However Groundnut cake showed highest mortality percentage against *Sclerotium rolfsii*, and so found quite ineffective to be used as organic product against stem rot disease.

5.4.4 Integrated management approach for stem rot during kharif 2017

One integrated approach on stem rot disease management in groundnut during kharif 2017 was taken combining the different cultural practice, application of biocontrol agent and seed treatment with chemicals to find out a suitable module for management of stem rot as well as other soil borne diseases in groundnut. The results obtained in the present experiment revealed that deep summer ploughing with MB plough,soil application with *T. viride* @ 4 kg/ha enriched with 50kg FYM as basal application,seed treatment with tebuconazole 2DS @1.5g/kg seed and PGPR @6g/kg followed by soil application of *T.viride*@ 4kg/ha enriched with 250 kg FYM applied at 35 and 70 DAS was the best treatment which recorded 90.6% germination,2.8% incidence of stem rot and the highest pod yield of 1806.7 kg/ha.

This treatment also recorded 1.8%,2.7% and 1.5% of other soil borne diseases like collar rot, dry root rot and pod rot respectively.It also recorded a score of 3 in (1-9)scale against the late leaf spot disease compared to untreated control i.e 8.The reduction of soil borne diseases in general and the pathogen Sclerotium rolfsii in particular by deep summer plough was demonstrated by Garren (1961); Newhall (1955) as well as Damicone& Jackson in 1994 which supports the present findings.

Soil application of *T.viride* and there by increasing the germination% and reducing the stem rot disease was also demonstrated by Kulkarni ,1994 and Prabhu *et al.*,1997. Which is agreement with present result.

Application of *Trichoderma* spp. enriched with FYM was quite effective in reducing incidence of S. rolfsii was well reported by Vanitha and Suresh, 2002; Kulkarni ,2007; Banyal et al. ,2008 Vinod Babu ,2008, which is in close agreement with the present findings.The seed treatment with tebuconazole proved to be much efficacious in reducing the disease and increasing the germination % and hence the yield. Madhavi and Bhattiprolu ,2011;Jadon,2015;Senapati *et al*.,2017 experimented the efficacy of different chemicals as seed treating agent in groundnut and found tebuconazole was the best in reducing the disease and increasing the yield.

Senapati *et al*.,2017 reported seed treatment with tebuconazole @ 1.5g/kg seeds recorded 80.15% seed germination and maximum yield of 1880kg/ha,which corroborates the present findings.

Deep summer ploughing with MB plough with seed treatment and follow up spray with tebuconazole found to be more effective than seed/soil application with *T. viride*(Annual Groundnut Workshop Report,2016) was in agreement with the present findings,where deep summer ploughing with MB plough,seed treatment with tebuconazole and follow up soil application of *T. viride* resulted in minimum disease incidence and higher pod yield than any other treatment.

5.5 Varietal response against stem rot incidence and yield

Keeping in pace with pesticide free environment the IDM measure have gone a long way with different organic practices. However for a suitable economical and sustainable measure; option of selecting resistance/tolerance variety is the prime need of the time.

Oil seed grower of Odisha have been growing some old varieties as well as newer ones with different duration and growth habit in coastal and interior parts.An experiment was felt need to assess their response to stem rot; an emerging and wide spread disease of groundnut. Out of the varieties under test, none of them was found immune. The variety TAG-24 was the highest yielder(1885kg/ha) with only 2.7%

incidence of stem rot. This finding contradicts to the result of Rakholiya and Jadeja ,2010 who screened fourteen cultivars and grouped TAG-24 to be susceptible to stem rot disease.

The least incidence of stem rot (2.1%) was recorded with Smruti with yield of 1860kg/ha.TG-38 B,K6 and DEVI were the next best varieties with a stem rot incidence of 2.3%,3.3% and 4.3% respectively, where as K9,G3 and TMV-2 expressed more than 5% incidence of the disease.

Divya Rani *et al*.,2018 also had taken TMV-2 as susceptible check as it is being taken in the present trial. She also found the moderate resistance in ICGV-91114 which is popularly known as DEVI in odisha and also corroborates to the performance of DEVI in the present findings.

Kale *et al*.,2007 observed TG 38 B;showing resistance to stem rot and eventually the present experiment also revealed TG38 B to be an average yielder(1810 kg/ha) with less incidence (2.3%) of the stem rot pathogen.

Out of eight varieties tested Smruti,Tag-24 and K6 were the best performer with respect to germinability, reducing the disease and increasing the yield, which can be recommended to the farmers of the state.

Future thrust

1. The population of *Sclerotium rolfsii* may be estimated from the native soil and the native Trichoderma and Rhizobacteria may be isolated to study their antagonistic nature and plant growth stimulation.

2.More number of organic amendments with different concentrations are to be tried for management of stem rot and other soil borne pathogens in field condition.

3. To study more and more biocontrol agents and their integration with different cultural practice to formulate suitable IDM measures.

SUMMARY AND CONCLUSION

The present investigation; "Studies on stem rot of Groundnut (*Arachis hypogaea L.*)and its management" were carried out in the Department of Plant Pathology, College of Agriculture and AICRP on Groundnut station OUAT, Bhubaneswar. The investigation mainly consists of four objectives: 1.Survey and monitoring of diseases of groundnut in east and south eastern tracts of Odisha,2.Influence of weather parameters on stem rot disease incidence,3.*In-vitro* bioassay with biocontrol agents, fungicides and management of the disease through organic soil amendments,4.Integrated approach for management of the disease in field condition.

The survey was conducted to monitor both soil borne as well as foliar diseases of groundnut during the Rabi summer 2016-17 in four districts of Odisha viz.,Puri,Khordha,Cuttack, and Jagatsingpur. Five villages comprising two fields per village with similar agro ecological situations and growing common variety DEVI were selected for survey. Observations like date of sowing ,date of first appearance of disease, percentage incidence for soil borne pathogens and scores (1-9) assigned to foliar disease were recorded during different stages of crop growth.The incidence of ELS appeared 27 DAS which scored a maximum of 4 towards 55 DAS. The LLS was most prevailing foliar disease which attained a maximum score of 7 in a week before harvest. The incidence of both Rust and Alternaria blight appeared in traces towards 50 DAS. Among soil borne diseases, the collar rot incidence was mostly within 17-40 DAS, recording a maximum of 6% .The incidence of stem rot was noticed around 40 DAS recorded a maximum 7.4% of infection upto pod formation and maturity period The dry root rot incidence was initiated around 45 DAS .Among soil borne diseases,the stem rot incidence was the highest(44%) followed by collar rot (36%) and dry root rot (20%).Among the major foliar diseases incidence of LLS was the highest (39%) followed by ELS(25%), Alternaria blight(19%) and Rust(17%).

In order to study the relationship of weather parameters on the natural occurrence of stem rot disease and its severity in groundnut, weekly incidence and severity of *Sclerotium rolfsii* were taken at AICRP Groundnut Research Station at

OUAT, Bhubaneswar during kharif 2017,and Rabi-summer 2017-18. The Standard meteorological parameters such as Maximum and Minimum Temperatures (°c),Relative Humidity(morning),Relative Humidity(afternoon),Rainfall(mm),Number of rainy days and Bright Sunshine Hours were obtained from Meteorological observatory, OUAT, Bhubaneswar. From the regression analysis it was concluded that the weather parameters as a whole contributed about 48.7%to disease development in Kharif ,where minimum temperature solely contribute to 80% disease development.It was concluded that the weather parameters as a whole contributed about 62.2% to stem rot disease incidennce in groundnut during winter season of 2017-18. During the season maximum and minimum temperature contributed 38%and 24% respectively and number of rainy days contributed 22% to stem rot disease incidence.

The symptoms of Stem rot were observed under natural field conditions on collar region, leaves, stems and pods as yellowing and subsequent wilting of whole plant.Isolation of the test fungus was made from infected plants of Groundnut, collected from the field showed the presence of *Sclerotium rolfsii* Sacc. and identified on the basis of its morphological characters. Pathogenicity of the fungus was tested by soil inoculation method.

The fungicides were tested initially under *in vitro* conditions by using poisoned food technique at desired concentration. Efficacy of fungicides were expressed as percentage of inhibition of mycelial growth over control. Results revealed that mixed fungicides such as Carbendazim+Mancozeb at 0.2%,Azoxystrobin+Tebuconazole at 0.1%,Azoxystrobin+Mancozeb at 0.1% and triazole group of fungicides viz., tebuconazole,propiconazole,hexaconazole recorded inhibition of *Sclerotium rolfsii* growth by 100% at 0.1% concentration followed by cymoxanil+mancozeb at 0.2%(82.6%),azoxystrobin at 0.1%(75.6%) and mancozeb at 0.25% concentration(53.4%).Least antifungal property was shown by carbendazim at 0.15% concentration(22.6%) followed by chlorothalonil at 0.2%(31.1%).

The efficacy of biocontrol agents like *Trichoderma viride, Trichoderma harzianum,Trichoderma hamatum* and *Pseudomonas fluorescens* were tested against the fungus.All the isolates of *Trichoderma* significantly inhibited mycelial growth of *Sclerotium rolfsii* and inhibition ranged from 63 to 74% per cent over control. Antagonist *Trichoderma harzianum* was the most efficacious with 74.4% mycelial

growth inhibition,87.1% sclerotial inhibition followed by *Trichoderma viride* with 70.6% mycelial growth inhibition and 85.2% sclerotial inhibition.The bacterial antagonist also have 63.5%mycelial inhibition and 71.2% sclerotial inhibition.*Trichodema harzianum* was more efficacious compared to *Trichodema viride* but *Pseudomonas fluorescens* is statistically at par with *Trichoderma hamatum.Trichodema harzianum* showed better ranking from modified Bells scoreR1(100% growth) and other bioagents expressed R2(75% growth) ranking.

Organic products like Mustard cake, Groundnut cake, Neem cake,Vermicompost,,FYM,FYM enriched with *Trichoderma viride* were taken to evaluate their efficacy for controlling the stem rot. FYM enriched *T.viride* showed the least mortality(6.7%) followed by neem cake treated pot(26.7%).Vermicompost, mustard cake,FYM and Groundnut cakes amended pots with more than 40% mortality of plants were not proved efficacious as organic amendments for management of stem rot in groundnut.

In order to develop a cost effective disease management module against stem rot pathogen as well as other soil borne and foliar diseases of groundnut ,a field experiment was conducted during Kharif 2017 in the experimental site of All India Coordinate Research Project on groundnut,OUAT,Bhubaneswar,Odisha. The results of integrated approach on stem rot disease management revealed that Deep summer ploughing with mould board plough+ basal application of *Trichoderma@* 4 kg/ha enriched with 50 kg FYM+seed treatment with tebuconazole @1.5g/kg of seed followed by PGPR@6g/kg seed followed by soil application of *Trichoderma@* 4kg/ha enriched in 250 kg FYM at 35 and 70 DAS was found to be the best. It increased the germination by 24.9%, reduces the stem rot incidence by 65.9% and increased the yield by 38.7% with ICBR of 8.03. The treatments though confined to seed treatment and soil application of bioagent at various stages of growth had an effect on late leaf spot, the most prevailed foliar disease of the region, which recorded a score 3 in T4, compared to score 8 in control.

One field trial was also conducted during Rabi-summer 2017-18 to evaluate the reaction of some prevailing varieties grown by the farmers of Odisha against stem rot disease.The least incidence of 2.1% was recorded from variety Smruti which is at par with TG 38 B having incidence of 2.3%.Varieties TAG-24,K6 and DEVI recorded less

than 5% of disease incidence. The variety Smruti expressed a disease reduction of 71.6% and TG-38 B with a disease reduction of 68.9% when compared with TMV-2,the susceptible check. Out of the eight varieties tested TAG-24 recorded the highest germination percentage of 88% followed by K6(86.3%),DEVI(84.3%) and Smruti(83.7%).Considering the pod yield, the maximum pod yield of 1885kg/ha was recorded from the variety TAG-24 followed by Smruti (1860kg/ha), K6 (1825kg/ha) and DEVI(1820kg/ha) which are statistically at par. The susceptible check TMV-2 recorded the lowest yield of 1405kg/ha followed by K9(1535kg/ha) and G3 (1615kg/ha) .Considering both stem rot incidence and yield, the variety Smruti was found to be the best with 71.6% reduction in disease and 32.4% increased in yield when compared over the check.TAG-24,TG-38 B,K6 may be considered as the next best varieties reducing the disease by 63.5%, 68.9%,55.4% and increasing the yield by 34.2% ,28.8% and 29.9% respectively.

RECOMMENDATION

Integrated approach of using deep summer ploughing with mould board plough+ basal application of *Trichoderma@* 4 kg/ha enriched with 50 kg FYM+seed treatment with tebuconazole @1.5g/kg of seed AND by PGPR@6g/kg seed followed by soil application of *Trichoderma@* 4kg/ha enriched in 250 kg FYM per ha at 35 and 70 DAS should be followed, for management of stem rot as well as other soil borne diseases in groundnut.

The groundnut varieties Smruti,Tag-24,TG 38 B and K6 are best performer in reducing stem rot and increasing yield compared to other ruling varieties which can be grown successfully in both Kharif and Rabi-summer season by the farmers of Odisha.

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