Integrated Management of Pythium Stem Rot of Vegetable Cowpea (*Vigna unguiculata* sub sp. *sesquipedalis* (L.) Verdcourt)

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by

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(2012-11-125)

THESIS
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DECLARATION

I hereby declare that this thesis entitled '**Integrated Management of Pythium Stem Rot of Vegetable Cowpea (*Vigna unguiculata* sub sp. *sesquipedalis* (L.) Verdcourt)**' is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associate ship, fellowship or other similar title of any university or society.

Vellayani
23-8-2014

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CERTIFICATE

Certified that this thesis entitled "**Integrated Management of Pythium Stem Rot of Vegetable Cowpea (*Vigna unguiculata* sub sp. *sesquipedalis* (L.) Verdcourt)**" is a record of research work done independently by Mr. Mohamed Anees. M. (2012-11-125) under my guidance and supervision that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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Mohamed Anees. M

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LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Per cent
µm	Micro meter
µl	Micro litre
@	At the rate of
°C	Degree Celsius
CD	Critical difference
cm	Centimeter
DAT	Days after transplanting
DAI	Days after inoculation
et al.	And other co workers
Fig.	Figure
g	Gram
ha	Hectares
h.	Hours
g-1	Per gram
i.e.	that is
kg.	Kilogram
l.	Litre
m	Meter
mm	Milli meter
mg	Milli gram
ml	Milli litre
sec	Seconds
SE	Standard error
sp. or spp.	Species (Singular and plural)
viz.	Namely
dia.	Diameter
max.	Maximum

d	Days
DI	Disease Incidence
RH	Relative Humidity
ppm	Parts per million
min	Minutes

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Introduction

1. INTRODUCTION

Vegetable cowpea, also known as yard long bean (*Vigna unguiculata* sub. sp. *sesquipedalis* (L.) Verdcourt) is an important leguminous crop grown in different parts of the world. This crop has come to occupy a prime position among the vegetable crops raised in Kerala in coverage and popular preference. The tender green pods used as vegetable are rich in protein, minerals, vitamins and dietary fibre. However, the crop is susceptible to a wide range of pests and pathogens that attack the crop at all stages of growth. These include insects, bacteria, fungi and viruses.

Pythium stem rot caused by *Pythium aphanidermatum* (Edson) Fitzp. is an important soil borne disease of cowpea (Onuorah, 1973). Pythium stem rot is known to occur and infect cowpea at all stages of the plant growth and moreover, it is lethal in nature and is a major constraint to increase production during warm humid conditions.

The *Pythium* species (Kingdom: Straminopila; Phylum: Oomycota; Class: Oomycetes; Subclass: Peronosporomycetidae; Order: Pythiales and Family: Pythiaceae) (Hawksworth, 2001) are worldwide in distribution and associated with a wide variety of habitats ranging from terrestrial or aquatic environments, in cultivated or fallow soils, in plants or animals, in saline or fresh water (Van der Plaats-Niterink, 1981; Dick, 1990). Among the *Pythium* species, *P. aphanidermatum* is cosmopolitan in distribution and one of the most common plant parasitic pathogen of a number of different crop plants in warmer parts of the world. *P. aphanidermatum* is known to cause infection on a wide range of plant species, belonging to different families viz. Amaranthaceae, Cucurbitaceae, Leguminosae, Solanaceae, Zingiberaceae etc (Waterhouse and Waterston, 1964).

In Kerala, Pythium stem rot of vegetable cowpea is a new disease and is found to be very severe in lowland cowpea cultivation. Since it is a new disease in Kerala, not much work has been done so far regarding the etiology of the disease, its epidemiological aspects and management strategies. Thus the present work, "Integrated management of Pythium stem rot of vegetable cowpea (*Vigna*

unguiculata sub sp. *sesquipedalis* (L.) Verdcourt)", was undertaken to investigate the stem rot disease of cowpea caused by *Pythium* sp. and to evolve an integrated management package for the control of the disease.

The following items of work were undertaken:

- Isolation and identification of the pathogen
- Study on the symptomatology of the disease
- Studies on the cultural and physiological characteristics of the pathogen
- Studies on the epidemiology of the disease
- Isolation and *in vitro* testing of fungal antagonists
- *In vitro* evaluation of bioagents, organic preparations and chemical fungicides
- *In vitro* seedling assay of different agents
- Field evaluation of selected agents from seedling assay for disease management

Review of literature

2. REVIEW OF LITERATURE

Cowpea (*Vigna unguiculata* (L.) Walp.) is one of the most important food legumes which serve as vital source of protein in the diet of the people of developing countries. It is widely grown in the third world for its cheap source of dietary protein (Ibrahim *et al*., 2010). In Kerala, the crop is cultivated in an area of 7317 ha (Anon., 2014) .

Pythium stem rot of cowpea caused by *Pythium aphanidermatum* showed a field incidence ranging from 0.5 -10% and occasionally up to 30% in Nigeria (Williams and Ayanaba, 1975).

2.1. ISOLATION OF THE PATHOGEN

Ali-Shtayeh (1986) isolated *Pythium* spp. from infected plant tissue by plating on the VP3 medium plates or on plates of 2% water agar containing 5 mg/l pimaricin. Excised plant material was first washed under running tap water for about 2 h. surface-sterilised in 0.5% sodium hypochlorite for 3 min, rinsed in sterile distilled water, blotted dry on sterile filter paper, and then plated on the agar plates. The plates were then incubated at 22-25^{0}C for 24-48 h. and examined for the presence of fungal colonies.

Adandonon *et al*. (2004) isolated *Pythium* sp. by rinsing the diseased plants in tap water, were surface disinfected for 1 min in 0.53% sodium hypochlorite, rinsed with sterile water and plated on to potato dextrose agar (PDA) and incubated in the dark at 27±^{0}C.

El-Mohamedy and El-Mougy (2009) isolated *Pythium ultimum* by rinsing the root and basal parts of plant samples in ethanol (5% v:v) for 30 sec, washed with tap water to remove the adhering soil particles, followed by sterile tap water, dried between two sheets of sterilized filter paper, and then cut to small pieces (2 sq cm each) and placed into petri dishes containing sterilized media. The media used were Martin's medium, VP3 selective medium and Pythium selective agar medium. All plates were incubated at 20±2°C for 4–6 days until fungal colonies appeared.

Suleiman (2010) isolated *P. aphanidermatum* by cutting small pieces of root from the advancing margin of lesions and immersed in 0.1 % mercuric chloride for 30 sec, washed three times in sterile distilled water, and blotted dry before being placed on PDA. Mycelium growing out of root tissue was sub-cultured and incubated for one week under the same condition before examination and identification.

2.2. IDENTIFICATION OF PATHOGEN

According to Onuorah (1973) *P. aphanidermatum* (Edson) Fitzpatrick has been associated with seed decay and severe stem rot of cowpea in Nigeria.

Van der Plaats-Niterink (1981) observed that the morphological features considered for the identification of *Pythium* spp. were

(1) Oogonia – ornamentation, size, color, shape, wall thickness.

(2) Sporangia – size, shape, presence/absence.

(3) Antheridia – shape, monoclinous /diclinous, number, size.

(4) Chlamydospores – presence/absence, wall thickness.

(5) Hyphae – diameter, ornamentation.

(6) Mycelia – colour, growth rate/pattern, texture.

Van der Plaats-Niterink (1981) has described *P. aphanidermatum* (Edson) Fitzp. described as given below.

Colonies on corn meal agar grew with cottony aerial mycelium, while those on potato-carrot agar appeared with some loose aerial mycelium without a special pattern. Main hyphae up to 10 µm wide. Sporangia consisting of terminal complexes of swollen hyphal branches of varying length and up to 20 µm wide. Zoospores formed at 25-30°C. Encysted zoospores 12 µm dia. Oogonia terminal, globose, smooth, (20-) 22-24(-25) µm (av. 23 µm) dia. Antheridia mostly intercalary, sometimes terminal, broadly sac-shaped, 10-14 µm long and 10-14 µm wide, 1or2 per oogonium, monoclinous or diclinous; oospores aplerotic, (18-) 20-22 µm (av. 20.2 µm) dia., wall 1-2 µm thick.

Ali-Shtayeh (1986) observed that *P. aphanidermatum* colonies isolated from soil on CMA formed a diffuse growth pattern with cottony aerial mycelium. Main hyphae up to 11 µm wide. Sporangia mostly terminal consisting of complexes of

swollen torulated hyphal bodies. Zoospores formed at room temperature. Oogonia are terminal, globose, smooth 18-29 (av. 22.7 ± 0.1-95% C.I), µm dia. Antheridia mostly intercalary, occasionally monoclinous or diclinous, 1(-2)/oogonium. Oospores: aplerotic 15-25 (av. 19.2 ± 0.6-95% C.I) µm dia. Wall 1-2 (av. 1.2 ± 0.3-95% C.I.) µm thick. Ooplast 5-12 (av. 9.3) µm dia. Temperature-growth relationships: Minimum 10^0C, optimum 35-40^0C, maximum 40^0C Daily growth rate on CMA at 25^0C 28 mm.

Mostowfizadeh-Ghalamfarsa and Banihashemi (2005) reported that *P. aphanidermatum* (Edson) Fitzp. isolates from soil sample produced colonies with cottony aerial mycelium on CMA (Corn Meal Agar) and no aerial mycelium on PCA (Potato Carrot Agar) and HAS (Hemp Seed Agar), without a special pattern on all of the three media; main hyphae 6-9 µm wide; sporangia consisting of a terminal complex of swollen hyphal branches of varying length; oogonia terminal, globose, smooth, 24-29 µm dia.; antheridia mostly intercalary sometimes terminal, broadly sac-shaped 9-12 µm wide; oospores aplerotic, 18-25 µm dia., wall 2-3 µm thick (Fig. 1, a-c). Daily growth rate on PCA was 30 mm at 25°C.

Suleiman (2010) reported that the fungus causing root rot of cowpea has coenocytic hyphae with whitish vegetative mycelium that is slender, cylindrical, profusely branching, hyaline and rapidly growing mycelium. The mycelium gives rise to terminal, or intercalary sporangia visible at 400X magnification. The sporangia, which are usually produced in vesicles during sexual reproduction, are globose to oval or irregular in shape and germinate directly by producing one to several germ tubes.

Al-Sheikh and Abdelzaher (2012) reported that *P. aphanidermatum* colonies isolated from rhizosphere soil of cereal crops on cornmeal agar have a cottony aerial mycelium while on potato-carrot agar they have some loose aerial mycelium without a special pattern. Main hyphae are up to 10 µm wide. Zoosporangia consist of terminal complexes of swollen hyphal branches of varying length and up to 22 µm wide. Zoospores are formed at 15-30°C. Encysted zoospores have a diameter of 12 µm. Oogonia are terminal, globose, smooth and of (20-) 22-25(-26) µm (av. 24 µm) dia. Antheridia are mostly

intercalary, sometimes terminal, broadly sac-shaped, 11-15 µm long and 9-15 µm wide, 1or 2 per oogonium and monoclinous or diclinous. Oospores are aplerotic, (19-)20-23 µm (av. 21 µm) in dia. and their walls are 1-2 µm thick. Daily growth rate on Bacto-CMA was 19 mm.

2.2.1. Morphological Studies of *Pythium* spp.

For production of sporangia and zoospores, dishes were half-filled with sterile distilled water (DW) or with an autoclaved mixture of one part filtered pond water and two parts distilled water (Emerson, 1958). Ali-Shtayeh (1986) studied morphological characters by boiling two grass blades for 15 minutes were then placed on or near the hyphal tips. Colonized grass blades were then transferred to sterile dishes half-filled with water (DW) incubated for 24-48 h. at 10-22^{0}C, rinsed daily with water from the same source, and examined periodically for 2-7 days for zoosporangia. If the zoospores were not produced at these temperatures, cultures were put in a refrigerator at 2-5^{0}C for 1-2 h. before they were examined for zoospore production. Changing the water and chilling the cultures enhanced the production of sporangia and the discharge of zoospores.

Ali-Shtayeh (1986) reported that sexual structures could be induced by flooding, blocks of agar containing hyphal tips with distilled water. Two boiled corn kernels were placed adjacant to the hyphal tips and cultures were then incubated at 22^{0}C for 7-14 days before they were examined. When oogonia were not produced in water culture, isolates were grown on CMA supplemented with wheat germ oil (500 mg/ l.) to provide the sterols needed for sexual reproduction. Schmitthenner's medium and CMA supplemented with wheat germ oil were found to be satisfactory for oogonial production and microscopic examination was easy because of the clear substrate.

Mostowfizadeh-Ghalamfarsa and Banihashemi (2005) studied morphological characters of asexual organs of *Pythium aphanidermatum* were on PCA and V8A (V8 vegetable juice 200 ml, calcium carbonate 4 g, agar 15 g, distilled water 800 ml). To induce the formation of sporangia, 5 mm boiled pieces of grass leaf (*Poa annua*) were placed on PCA at 25°C, and after 24 h. were transferred on to a petri dish in a shallow layer of sterile water or pond water

under fluorescence illumination. Sexual organs were studied on HAS (extract of 20 g ground hemp seeds, agar 15g, distilled water 1l.) containing 30 mg β- sitosterol.

Al-Sheikh and Abdelzaher (2012) conducted morphological studies of *P. aphanidermatum* in grass blade culture. In that, autoclaved grass blades were placed on 2% Water Agar (WA) inoculated with each isolate. After incubation for 2 days at 25°C, colonized grass blades were transferred to autoclaved distilled water and were incubated for 1-14 days at 20°C to follow the fungal development.

2.2.2. Cultural Studies of the Pathogen*, Pythium aphanidermatum*

The mycelium of *Pythium* species is colourless, sometimes lustrous, occasionally slightly yellowish- due to abundant oospores or hyphal swelling or grayish–lilac (Van der Plaats-Niterink, 1981). Production of aerial mycelium is dependent on the medium used. On corn meal agar and potato- carrot agar most species do not produce aerial mycelium whereas on oat meal agar several species produce profuse aerial mycelium (Shalini, 2006).

2.3. PATHOGENICITY TEST

Feng *et al*. (1999) reported that the fescue-wheat bran mix applied at the high level (100 ml/0.9-m^2 plots) produced greater blight ratings in perennial ryegrass caused by *P. aphanidermatum* than did the low level (50 ml/0.9-m^2 plot). The fescue-wheat bran also was generally more effective than either rate of the rye-barley mix in blighting turf. Covering enhanced blighting, when compared to the uncovered control, by raising the relative humidity. Covering plots with black plastic following inoculation resulted in greater blight ratings than did covering with either clear plastic or the geothermal blanket.

According to Adandonon *et al*. (2004) inoculated millet seeds were added to pasteurized sandy loam soil at the rate of 17 g inoculum/kg soil. The control treatments consisted of pasteurized soil to which 17 g sterile millet seeds were added and pasteurized soil without millet seed. Seeds of a local cowpea cultivar susceptible to damping-off and stem rot were surface-sterilized and then planted in pots at four seeds per pot. Pots were kept in the greenhouse at temperatures varying from 21–28 ±C.

EI-Mohamedy and El-Mougy (2009) carried out an experiment in autoclaved clay loamy soil artificially infested with the tested fungal isolates. Fungal mass production used for soil infestation was obtained by growing the tested isolates on sand-barley medium (1:1). Soils were infested individually at a ratio of 5% (w:w) with tested pathogenic fungal cultures and mixed thoroughly to ensure equal distribution of fungal inoculum, then filled in plastic pots (25 cm dia.) and irrigated every second day for 1 week before sowing. Surface sterilized chinese cabbage seeds of either cultivars (using 3% sodium hypochlorite for 5 min, then picked up and air-dried) were planted.

In another inoculation study conducted, Suleiman (2010) watered and left the potted soil for 24 h. before planting three disease-free seeds of cowpea in each pot. At the emergence of the seedlings, mycelial and sporangial suspension of a seven day-old culture with an average density of about 3×10^4 sporangia per cm^3 of *Pythium* was applied by drenching. The seedlings were covered with a large polythene bag to provide a humid environment and to prevent entry of other pathogens.

Al-Sheikh and Abdelzaher (2012) evaluated pathogenicity of *Pythium* spp. in pots. For inoculum preparation 5 grams of grass blade leaf segments (0.5x1 cm) and 2 g glucose were moistened by adding distilled water (10 ml) each in 250 ml flask. After autoclaving, each flask was inoculated with three disks (7 mm dia.) of agar inoculated with growing margins of *Pythium* spp. obtained from CMA culture medium. The inoculated conical flasks were held at 25°C for 10 days. Healthy and diseased seedlings were counted at regular intervals until the development of two true leaves in non-inoculated control. Pre-emergence damping-off was determined as the difference in emergence between healthy control soil and diseased soil.

2.4. SYMPTOMATOLOGY

Onuorah (1973) observed that in older cowpea plants water-soaked, pale green lesion girdling the stem appears at a point a little above soil level. This may extend upwards to about the fifth node but rarely to the growing tip. The leaf petioles at the nodes as well as the basal portions of some side shoots are also

invaded and closer to the ground, the necrosis may be dark-brown in colour, patchy and dry especially if the wet weather is dry. The upward extension of the water-soaked slimy condition is not so rapid. The soft and slimy cortex peels off readily. In hot weather, a copious growth of white cottony mycelium of the fungus girdles the stem at the first node or a little above. If the affected plants are pulled out at this stage, it could be observed that the roots have also been killed by the fungus.

According to Williams and Ayanaba (1975) wet stem rot of cowpea caused by *Pythium aphanidermatum* is characterised by a grey-green water- soaked girdle of the stem extending from soil level up to and sometimes including the lower portions of the lower branches. Infected plants quickly wilt and die.

Symptoms of cowpea root rot consisted of wilting when leaves were still green and shrinking of the stem at or near the soil surface or slightly above. Advanced infection caused stunting of the affected plant, chlorosis, drooping, premature shedding and withering of leaves and death of the plant (Suleiman, 2010).

2.5. MEDIA FOR CULTURING

Neelamegam (1992) reported that PDA promoted the maximum growth of *Pythium indicum* causing damping-off in tomato. Shalini (2006) found the maximum radial growth in oatmeal agar (90.00 mm) which was on par with V-8 juice agar (89.33 mm). This was followed by corn meal agar (83.00 mm) and potato dextrose agar (82.67 mm). Oospore production was observed in oat meal agar, corn meal agar, potato carrot agar, V-8 juice agar and potato dextrose agar.

In vitro experiments by Muthukumar and Eswaran (2008) showed that maximum mycelial growth and dry weight were observed on potato dextrose agar / broth in both solid and liquid media followed by corn meal agar.

Suleiman *et al*. (2011) found that the mycelium of the fungus grew satisfactorily on the entire agar media used. Potato Dextrose Agar (PDA) was found to be the best medium in terms of mycelial extension, mycelial density and sporangial production compared to Malt extract and Czapek-Dox agar. The best

mycelial growth of *P. aphanidermatum* was observed on potato dextrose agar (PDA).

2.6 PHYSIOLOGICAL CHARACTERISTICS

2.6.1. Effect of pH

Littrell and Mc Carter (1970) found out that the optimum pH for the growth of *P. aphanidermatum* was 7.5. The recovery of *P. aphanidermatum* during isolation was seen more at pH 6 (Lumsden *et al*., 1975). Sundarraj (2000) reported that the maximum mycelial dry weight of *P. aphanidermatum* in tomato was observed in pH 7.0 followed by 7.5.

Muthukumar and Eswaran (2008) found out that both acidic and alkaline extremes were found to be deleterious to the growth of the *P. aphanidermatum* and maximum mycelial dry weight in pH 7.0 followed by 7.5 and 6.5. Maheswari and Sirchabai (2011) reported that maximum growth of the *P. aphanidermatum* was noticed in pH 6.

2.6.2. Effect of Temperature

Muthusamy (1972) reported that the growth of *P. aphanidermatum* was maximum only at 28^0C, whereas Lumsden *et al.* (1975) observed optimum growth at 35^0C. Thiruvudainambi (1993) observed that 28^0C was found to be optimum for growth of the *P. aphanidermatum*. The *in vitro* experiments by Muthukumar and Eswaran (2008) showed that the optimum temperature for the mycelial growth of *P. aphanidermatum* on Czapek Dox medium was found to be 28 ^{O}C both in solid and liquid media. The growth of the pathogen was in the decreasing order in both solid and liquid media when temperature was increased from 32-36^0C.

Suleiman *et al.* (2011) found out that the optimum temperature for the mycelial growth of the *P. aphanidermatum* was 30^oC and slow growth at 25^oC and the effect of temperature on the sporangia production of *P. aphanidermatum* showed that sporangia production and germination was best at 30^oC. Sporangia production was poor at 25^oC and 35^oC. There was no sporangia production at 20^oC and 40^oC.

The *in vitro* experiments by Al-Sheikh and Abdelzaher (2012) showed that *P. aphanidermatum* showed temperature maxima higher than 45°C. The optimum temperature range for all isolates was between (30 and 35°C). The minimum temperature supporting mycelial growth was around 10°C. According to Teymoori *et al.* (2012) *Pythium deliense* and *P. aphanidermatum* had the same daily growth rates on CMA, 30 mm at 25 °C. The cardinal temperatures of *P. aphanidermatum* were similar to those of *P. deliense* but Van der Plaats-Niterink (1981) pointed out that optimum temperature for these species were 35-40 and 30 °C respectively.

2.6.3. Effect of Light Intensities

Gul *et al.* (2005) observed that continuous exposure to light for 24 h. per day produced maximum colony growth and continuous exposure to darkness reduced the colony diameter of *Phytophthora syringae* significantly.

Exposure to continuous light for 72 h. resulted in 86.08mm colony growth whereas exposure to 12 h. light and 12 h. darkness resulted in 43.58 mm colony growth after 72 h. similarly exposure to continuous darkness for 72 h. resulted in only 26.50 mm colony growth of *Phytophthora cactorum* (Faisal *et al*., 2005).

2.7. STUDIES ON THE EPIDEMIOLOGY OF THE DISEASE

2.7.1. Survival of the Pathogen

Stanghellini and Nigh (1972) reported that oospores from infected oat root segments buried at 5 and 20 cm in a fallow field soil germinated after burial for 16 months. Soil temperatures at 5 cm ranged from -4 ^{0}C to 54 ^{0}C. In the laboratory tests, oospores survived for 13 months in saturated and air-dried soil at 40 ^{0}C but did not survive for more than a month in saturated soil at 40 ^{0}C.

P. aphanidermatum survive as oospore in soil, infected host tissue and organic debris; as a parasite of asymptomatic plants and in field soils and weed hosts in cultivated and uncultivated areas (Stanghellini and Nigh, 1972; Burr and Stanghellini, 1973; Stanghellini, 1974)

The survival study conducted by Shokes and McCarter (1979) showed that, *P. aphanidermatum* in infested ryegrass seed and as oospores was recoverable for 185 days after submersion in a pond.

Hall *et al.* (1980) found out that oospores of *P. aphanidermatum* appear to be important as overwintering inoculum in turf grass. Survival propagules overwinter in associated with host debris of numerous plant species (Muse *et al*., 1974). Apparently, propagule survival in dead nodes, stem material and roots were more consistent than in smaller particulate organic debris and soil.

Pythium ultimum and *P. aphanidermatum* survive as oospores in turf grass root zones and thatch, and as vegetative mycelium in turf grass leaves and roots. When environmental conditions are not conducive for disease development, survival as sporangia, zoospores and mycelium is considered to be short-lived. Under favourable conditions for disease development mycelium can resume growth and infect a grass plant in 1-2 hours (Allen *et al*., 2004).

P. aphanidermatum was recovered from rhizome rot affected ginger samples up to 40 weeks and 42nd weeks in unsterilized soil and sterilized soil respectively (Shalini, 2006).

2.7.2. Effect of Weather Parameters

Kuo and Hsieh (1991) reported that the diseases caused by *P. aphanidermatum* were most serious during the summer when average daily temperatures reached 23-32 ^{0}C.

Rizvi and Yang (1996) reported that damping off caused by *P. ultimum* and *P. sylvaticum* was favoured by cool and wet soil but *P. aphanidermatum* appears in middle to late summer when temperature are high.

Kucharek and Mitchell (2000) reported that *P. myriotylum* and *P. aphanidermatum* abound in Florida because they are adapted to high soil temperature. Growth of these two species can occur from 40 or 50^{0}F to 105^{0}F, but the optimum temperatures for their growth and infection of plants range between 86 and 98^{0} F.

Pythium blight of turf grass can occur during periods of cool (13 - 18°C / 55 - 64°F), wet weather, but the disease is most severe during periods of hot (30 - 35°C / 86 - 95°F), humid, rainy or cloudy weather. *P. aphanidermatum* is most actively pathogenic when temperatures are between 30° and 35°C (86 - 95°F) and night temperatures remain above 21°C (70°F). The highest frequency of infection

occurs during periods of high relative humidity (>90%) and prolonged leaf wetness (Allen *et al*., 2004).

Ho (2009) confirmed that *P. aphanidermatum* was the most predominant pathogen during the warm summer, with an air temperature higher than 24°C.

Suleiman *et al*. (2011) concluded that the severity of the disease, sporangial production and germination of *P. aphanidermatum* was found to be greatly enhanced by high relative humidity, rainfall and high temperature. This may account for the high incidence and severity recorded for the disease in warm moist periods of the growth seasons.

2.7.3. Host Range

Rahimian and Banihashemi (1979) reported *P. aphanidermatum* as causal agent of cucurbit root rot in the Fars province of Iran for the first time. Saha *et al*. (2008) reported chilly damping off caused by *P. aphanidermatum* from India and Muthukumar *et al*. (2011) reported post emergence damping off chilly from India.

Suleiman (2010) reported that *P. aphanidermatum* was recovered from the roots of sorrel (*Corchorus olitorius*) and tomato (*Lycopersicon esculentum*), with similar symptoms noticed as in cowpea plants. These plants were confirmed as collateral hosts of the pathogen.

2.8. ISOLATION AND IDENTIFICATION OF RHIZOSPHERE ANTAGONISTS

Microorganisms indigenous to the rhizosphere are ideal for biological control, since the rhizosphere provides a first-line defence for roots against attacks by plant pathogens

Trichoderma koningii Rifai showed 72.59 % mycelial inhibition whereas *Trichoderma viride* Pers. showed 67.40 % mycelial inbition against *P. aphanidermatum* isolated from ginger rhizome rot (Shalini, 2006).

Trichoderma species were isolated from chilli rhizosphere (TVC_3) was found to be highly effective in inhibiting the mycelial growth of *Pythium aphanidermatum* and increased the plant growth (Muthukumar *et al*., 2011)

Native antagonistic microorganisms were isolated from the rhizosphere of damping-off affected tobacco nurseries and screened *in vitro* for the antagonistic

action against *Pythium aphanidermatum* causing damping off disease in tobacco by dual culture and cell free culture filtrate techniques. *Aspergillus niger, A. flavus, Trichoderma viride* and *Pseudomonas fluorescens* were found to be potential antagonists against *P. aphanidermatum* (Subhashini and Padmaja, 2012). Patil *et al*. (2012) has observed the broad spectrum inhibition of *Trichoderma* spp. isolated from tomato rhizosphere soil against *P. aphanidermatum* causing root rot of tomato.

2.9. *IN VITRO* MANAGEMENT STUDIES

2.9.1. Biocontrol Agents

P. aphanidermatum isolated from ginger rhizome rot, maximum inhibition of mycelial growth was noticed in *T. harzianum* (Sirsi isolate) (77.77%), which was on par with *T. harzianum* (Dharwad isolate) (76.40 %) and the bacterial bio agent *P. fluorescens* (Sirsi isolate) showed 63.46 % mycelial inhibition (Shalini, 2006).

Nwaga *et al*. (2007) observed that the percentage inhibition by different *Pseudomonas* strains against *Pythium* spp. depend on the bacterial concentration and type of strain used.

Serfling *et al*. (2007) observed that, in greenhouse experiments, symptom severity of a wheat leaf (*Blumeria graminis* f.sp. *tritici*), stem base (*Pseudocercosporella herpotrichoides*), and root (*Fusarium culmorum*) pathogen was reduced significantly when it was inoculated with *Piriformospora indica.* However, in field experiments, symptoms caused by the leaf pathogen did not differ in *Piriformospora indica* colonized compared with control plants.

Sivakumar *et al.* (2012) found that *P. fluorescens* strain Pf 51 exhibited highest inhibition (42.5%) against *P. vexans.* Patil *et al*. (2012) reported that the percentage mycelial inhibition of isolated *Pythium* spp. by *Trichoderma* was ranging from 27.78-69.23%.

2.9.2. Organic Preparations

2.9.2.1. Panchagavya

The preparation, ‘Panchagavyam’ contains cow dung -7 kg and cow ghee-1 kg which are mixed in a clean container thoroughly both in morning and evening

hours and kept aside for 3 days. After 3 days, cow urine -10 litres and water - 10 litres are added. The mixture is kept for 15 days with regular mixing both in morning and evening hours. After 15 days, add cow milk – 3 litres, cow curd - 2 litres, tender coconut water - 3 litres, jaggery - 3 kg and well ripened poovan banana - 12 nos. Panchagavyam can be prepared in a wide mouthed mud pot or concrete tank or plastic can (KAU, 2009).

Anuja Raj (2010) observed that application of organic growth promoters such as Panchgavyam, Dashagavya and EM solution reduced foliar blight of amaranthus caused by *Rhizoctonia solani* when compared to inoculated control, panchagavyam application caused suppression of disease by 47.29%.

Maha Pancha Gavya (MPG), a concoction made from five cow products was tested for its toxicity against *Pythium aphanidermatum* (Edson) Fitz. and its antagonists at 5, 10, 25, and 50% concentration in *in vitro* to find out if it can be used in integration for the control of damping-off in tomato in nursery beds. MPG was very effective inihibiting the growth of *P. aphanidermatum* (Kumar *et al*., 2010).

Among the samples from Panchagavya, the 1000 µl dilution alone showed 100% antifungal activity. Additionally, the remaining dilutions (500, 100 µl) showed moderate antifungal activity. But at no antifungal activity at lower dilution (10 µl) were observed (Joseph and Sankarganesh, 2011).

Adhao (2013) observed that Panchagavya suppressed the growth of *Fusarium oxysporum*. Superior antifungal activity of Panchagavyam was recorded in the plate receiving 4 percentage w/v panchagavyam treatment as compared to control.

2.9.2.2. Fish Amino Acid

The spraying of fish amino acid at weekly interval could reduce leaf spot disease and leaf feeder attack in amaranthus in farmer field. (KAU, 2014)

2.9.3. Chemical Assay

Huang *et al.* (1994) reported that metalaxyl Plus and metalaxyl MZ completely inhibited the mycelial growth of the tested *Pythium* species on water agar when used at 50 and 5 ppm respectively, and also inhibited the production

and movement of zoospores from naturally infected roots at 1 and 0.5 ppm, respectively.

Wheeler *et al.* (2005) reported that the sensitivity of *Pythium* spp. to azoxystrobin was much lower than to mefenoxam. However, some fungi use an alternative respiratory pathway that can interfere with the activity of azoxystrobin in a petri dish assay.

Boughalleb *et al.* (2006) reported that Ridomil Gold MZ and Curvax M suppressed the *in vitro* development of *Phytophthora cactorum* at high doses (100 and 1000 µg per ml) whereas Melody Duo reduced mycelial growth at all tested doses.

The *in vitro* bioassay of fungicides by Suleiman (2011) showed Ridomil as the most effective compound at low concentrations for the complete inhibition of mycelial growth at 50 ppm, whereas benlate and mancozeb completely inhibited the linear growth of the fungus and inhibiting sporangial formation at 150 ppm and 200 ppm active ingredient respectively.

Mihajlovic *et al*. (2013) reported that the tested *Pythium* sp. isolate causing pepper damping off was capable to grow well at 0.006 mg/l azoxystrobin concentration, but it was severely inhibited at 0.0125 mg/l and higher concentrations. The *P. aphanidermatum* isolate also showed a high susceptibility to metalaxyl (EC50=1.27 mg/l). Its hyphal growth was severely inhibited at 1.0 mg/l or higher concentrations.

2.10. *IN VIVO* MANAGEMENT STUDIES

2.10.1. Studies on Biological Control

Field experiment by Sreeramulu *et al*. (1998) showed that the dual inoculation of *G. fasciculatum* and *T. harzianum* was more effective in controlling damping off and black shank disease of tobacco than the individual inoculation, and resulted in better germination count and improved plant growth parameters.

Geetha *et al.* (2000) reported that *Pseudomonas fluorescens* controlled *Pythium* rot and also induced the growth of sugarcane seedlings when it was mixed with potting mixture.

Activated formulation of *Trichoderma harzianum* at 10% resulted in the highest population of *T. harzianum* and showed the least disease incidence of damping off in tomato (Anandh and Prakasam, 2001). Jayasekhar *et al*. (2001) reported that *T. harzanium* + neem cake application and rhizome dip in 0.1% metalaxyl exhibited effectiveness in the reduction of ginger rhizome rot caused by *P. aphanidermatum*, and cost: benefit ratio was highest (1:2.85) with *T. harzianum* + neem cake application followed by 0.1% metalaxyl (1:2.71). *T. harzianu*m alone or in combition with Akomin were found to be an effective antagonist to manage the rhizome rot disease of small cardamom caused by *Pythium vexan* (Vijayan and Thomas, 2002).

The commercial biological control material, TurfMate™, containing *T. harzianum*, has been registered as a preventive agent for the control of Pythium blight on turf grass caused by *P. aphanidermatum* (Allen *et al*., 2004).

Sharma and Sain (2005) reported that *T. harzianum* and *Aspergillus niger* formulation consistently reduced the incidence of *Pythium* damping off of cauliflower and increased the vigour index.

Waller *et al*. (2005) found that *Piriformospora indica* infested barley plants are more resistant to root diseases. *Fusarium* root infection caused a 12-fold decrease in root and shoot fresh weight of 4-week-old plants, compared with control plants, which were infested with neither *P. indica* nor *F. culmorum*. In the presence of *P. indica*, this devastating effect of *F. culmorum* infection was strongly diminished.

P. fluorescens strain isolated from tomato rhizosphere was capable of producing high levels of chitinase, β-1,3-glucanase, cellulase, fungitoxic metabolites and siderophores and a potential biocontrol of *P. aphanidermatum* under *in vitro* and *in vivo* conditions (Jayaraj *et al*., 2007).

Zamanizadeh *et al*. (2011) found that *T. harzianum* strain T969 significantly reduced seedling infection at a rate of 82% when applied into soil medium at a concentration of 107 conidia ml/L.

Rabiey *et al.* (2013) suggest that *P. indica* can protect wheat seedlings from Fusarium crown rot damage and reduce inoculums return to the soil.

2.10.2. Chemical Control

Figueiredo and Lellis (1980) reported that Kocide 6F [copper hydroxide] and Gafex [copper oxychloride] gave the best control over black pod rot disease of cocoa caused by *Phytophthora palmivora.* Copper oxychloride 0.3% drench alone, recorded lower seedling mortality than control treatment for controlling seedling damping off in tomato by *P. aphanidermatum* (Gunasekaran *et al*., 1994).

Lin *et al*. (2002) found that metalaxyl MZ was effective against the root rot of pea seedlings grown under soil less situation, when applied at 50 and 300 ppm concentration.

Dithane M-45+Vapour Guard, followed by Kocide 101 and Dithane M-45 were the most effective treatments against flower rots caused by *Pythium aphanidermatum* and other fungal pathogens (Hilal *et al*., 2003).

In ginger rhizome rot caused by *P. aphanidermatum* application of 0.3% Ridomil MZ resulted in the lowest incidence of the disease. Under field conditions, application of Ridomil MZ resulted in the highest seed germination (96.50%) and yield (250.25 q/ha), and lowest disease incidence (5%) (Singh *et al*., 2004).

The best protection against Ranunculus basal rot caused by *Pythium* sp. was obtained using azoxystrobin and good results were obtained also with metalaxyl-M (Rapetti *et al*., 2006). Boughalleb *et al* (2006) reported that i*n vivo* Melody Duo was the less effective fungicide for controlling Phytophthora disease in apple. Melody Duo (5.5% iprovalicarb + 61.25% propineb) and Ridomil (8.0% metalaxyl + 64.0% mancozeb) were highly inhibitory to the *P. capsici* causing foot rot of black pepper (Shashidhara *et al*., 2008). Singh (2008) found that application of three sprays of Ridomil MZ at 10-day intervals was the most effective in controlling both early and late blights of potato. New fungicides Antracol (Propineb) and different rates of Melody Duo (5.5% iprovalicarb + 61.25% propineb) served as promising alternatives to existing fungicides such as Ridomil MZ (8.0% metalaxyl + 64.0% mancozeb).

Ridomil MZ(metalaxyl + mancozeb)) @ 0.2% (corm dip for 1 h. followed by three sprays) was found most effective in controlling Phytophthora colocasia blight (57.9%) followed by Aliette 80 WP (52.0%), Melody Duo (iprovalicarb 55% + propineb 61.25%) (50.9%) and blitox 50(copper oxychloride) (42.5%) (corm dip for 1 h. followed by three sprays) with corresponding increase in yield (Singh, 2009).

Palakshappa *et al.* (2010) reported that during kharif 2006 the treatment viz Metalaxyl M+Mancozeb (4+64) WP @ 1.20% was found effective against chilly damping off caused by *Pythium* sp. and recorded 20.80 % seedling mortality. However the same treatment was found on par with 0.3 and 0.6 per cent and recorded 30.62 and 24.62 % seedling mortality respectively Where as in kharif 2007 the treatment metalaxyl M+mancozeb (4+64) at 1.2 % was significantly superior over lower concentrations (24.85%) and was found on par with Ridomil MZ at 0.3 % and reduced percentage seedling mortality to an extent of 27.20 %.

In vivo chemical assay by Yadav and Joshi (2012) showed that Ridomil MZ (8.0% metalaxyl + 64.0% mancozeb) 72 WP at 100, 200 and 300 µg ml-1 and copper oxychloride at 2000, 3000 and 4000 µg ml-1 concentrations were found effective and significantly superior in inhibiting mycelial growth as well as dry mycelial weight of *P. aphanidermatum* causing damping off in Bidi tobacco.

Sivakumar *et al.* (2012) reported that application of copper oxychloride and carbendazim resulted in 68.0% reduction of cardamom rhizome rot caused by (*Pythium vexans, Fusarium oxysporum and Rhizoctonia solani*).

According to Mihajlovic *et al.* (2013) the efficacy of the fungicides Quadris (azoxystrobin) and Ridomil gold (metalaxyl M) 480 WP were 57.5% and 75.0%, respectively in a greenhouse assay for pepper damping off caused by *P. aphanidermatum*.

2.10.3. Organic Amendments

Mani and Marimuthu (1994) found that decomposed coirpith gave comparable results with *Trichoderma hamatum* and *T. viride* giving 16.3 and 11.5% post emergence damping off in *Capsicum annum* caused by *Pythium aphanidermatum* compared with 75% in the untreated inoculated controls, in a

series of pot experiments. Decomposed coir pith alone and in combination with *T. harzianum* gave the best survival of plants 60 days after sowing.

Amaresh *et al.* (2004) reported that neem cake and farm yard manure application resulted in higher germination of ginger rhizome compared to the control. Neem cake amendment resulted in the lowest disease incidence (22.92%) of rhizome rot caused by *P. aphanidermatum* and highest yield (32.91 q/ha) followed by application of FYM.

Mature, vermicomposted animal manure gave the best result in reducing root rot disease coco yam caused by *Pythium myriotylum* incidence. The lowest disease severity was obtained when the soil was amended with the mature, composted or vermicomposted, animal manure (Artavia *et al*., 2010).

Materials and methods

3. MATERIALS AND METHODS

The present study on the 'Integrated management of Pythium stem rot of vegetable cowpea (*Vigna unguiculata* sub.sp. *sesquipedalis* (L.) Verdcourt) was conducted during the period 2012-2014 at the Department of Plant Pathology, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala. The materials used and methods followed are described below.

3.1. ISOLATION OF THE PATHOGEN

Cowpea plants exhibiting stem rot symptoms were collected from cowpea growing areas of Thiruvananthapuram district were brought to the laboratory and isolation was carried out to obtain pure cultures of the pathogen. Isolation from the stem and root portion were separately done. Isolation was done by following Suleiman (2010), cutting small pieces of stem and roots from the advancing margin of lesions which were then immersed in 0.1 % mercuric chloride for 30 sec, washed three times in sterile distilled water, and blotted dry before being placed on PDA (Potato Dextrose Agar). The mycelium emerging from of the tissues was sub-cultured to another petri plate containing sterile PDA incubated in room temperature (28 ± 1^0C).

3.2. PATHOGENICITY TEST

The pathogenicity of the fungal isolates from disease affected cowpea plants were proven following Koch's postulates and the most virulent pathogen isolate was selected for further study. The surface sterilised (0.1% mercuric chloride) viable cowpea seeds of susceptible variety (Vellayani Jyothika) were sown in sterilized soil taken in eight paper cups of 8 cm dia. and eight seeds were sown per cup. The cups were maintained under laboratory conditions. After sowing the seeds, the soil in each cup was inoculated with a mixture of the mycelia, sporangia and oospores in a sterile water suspension from a seven day-old cultures of the eight pathogen isolates (Suleiman, 2010). One non- inoculated cup was kept as control with seeds without inoculum. The cups were labelled and covered with polythene covers. The observations on seed germination and infection were taken one week after inoculation.

For proving the pathogenicity in mature plants (one month old plants), the selected most virulent pathogen isolate from seedling assay (P2) was inoculated in the soil. For that, the seeds of susceptible cowpea variety Vellayani Jyothika was sown in the pots having potting mixture (sand : soil : cowdung) (1:1:1). Two seeds were sown in each pot. The fertilizer dose was given as per the recommendation given in the package of practices of KAU (2011). After one month the inoculation of the selected pathogen was done by following four methods, the details of which are given below

1) The pots were inoculated with the sterile water suspension containing mycelial, sporangial and oospores from a seven day-old culture of the pathogen isolate grown in PDA medium (Suleiman, 2010).
2) Another method followed was EI-Mohamedy and El-Mougy (2009) method with slight modification. Fungal mass multiplication used for soil infestation was done by growing the tested isolates on sand-oats medium (1:1) and soils were inoculated individually with the selected culture P2 and mixed thoroughly to ensure equal distribution of fungal inoculum in plastic pots (25 cm-dia.) and irrigated every day.
3) The third method was done by following Al-Sheikh and Abdelzaher (2012) method with slight modification. Five grams of grass blade leaf segments (2cm) of *Cynodon dactylon* sterilized by boiling in tap water for 10 min and 2g glucose were taken in a sterilized petri plates containing 20 ml sterile water. Then each Petri plates were inoculated with the aerial mycelium of *Pythium sp.* obtained from three days old culture. Kept the Petri plate undisturbed for four days till the oospore formation and inoculated the contents of one Petri dish per plant at the root portion of the healthy host plants.
4) In the fourth method, the fungal mycelium taken from four day old culture was inoculated on the basal stem portion by making a small slit with sterile blade and was covered with moistened cotton and finally with polypropylene paper around the stem to maintain moisture.

In all the above cases the plants were covered with polythene covers to provide sufficient moisture to promote infection.

3.3. IDENTIFICATION OF THE PATHOGEN

The most virulent *Pythium* spp. isolate selected from the pathogenicity test was identified based on the cultural characteristics and morphological features as per the guidelines described by Van Der Plaats-Niterink (1981).

3.3.1. Cultural Characteristics

Cultural characteristics of the selected *Pythium* sp. was studied based on the nature of pattern of aerial mycelium produced and radial mycelial growth by the *Pythium* sp. on CMA at 25^0C as per the method Al-Sheikh and Abdelzaher (2012). In order to study the cultural characteristics of selected *Pythium* sp., 5 mm mycelial disc cut from three days old culture of selected *Pythium* sp. was inoculated at the centre of CMA plates and the plates were incubated at 25^0C. The nature of pattern of aerial mycelium and radial mycelial growth were observed 24 h. after inoculation.

3.3.2. Morphological Characteristics

In order to have a detailed study on morphological characteristics of selected *Pythium* sp., morphological features keys of *Pythium* spp. identification was followed (Van Der Plaats-Niterink (1981).

(1) Oogonia – ornamentation, size, color, shape, wall thickness.

(2) Sporangia – size, shape, presence/absence.

(3) Antheridia – shape, monoclinous /diclinous, number, size.

(4) Chlamydospores – presence/absence, wall thickness.

(5) Hyphae – diameter, ornamentation.

The morphological characteristics of *Pythium* were studied by adopting the method described by Al-Sheikh and Abdelzaher (2012), for growing the hyphae of the fungus, autoclaved grass blades (*Cynodon dactylon*) of size 1-2 cm were placed on corn meal agar (CMA) inoculated with selected *Pythium* isolate. After incubation for 2 days at 25°C, colonized grass blades were transferred to autoclaved water and were incubated for 3-4 days at 28±1°C for fungal development. Then, microscopic slides were prepared after staining with

lactophenol cotton blue (Appendix II) and were observed under Motic BA 210 compound microscope (Motic image plus (version 2.0 ML)) and the morphological characteristics were studied.

3.4. EVALUATION OF NUTRIENT MEDIA FOR CULTURING OF THE PATHOGEN

The selected *Pythium* sp. was used for evaluating the suitable medium for culturing of the pathogen. Studies were done under both solid and liquid culture.

3.4.1 Growth on Different Solid Media

The growth of the pathogen, *Pythium* sp. was compared on different solid media. Different solid media used were Potato Sucrose Agar (PSA), Potato Dextrose Agar (PDA), Oat Meal Agar (OMA), Corn Meal Agar (CMA), Carrot Agar (CA), Malt Extract Agar (MEA), Czapek Dox agar (CDA) (Appendix 1).

20 ml of each medium was poured into sterile Petri dishes. Culture disc of 5 mm was taken from three days old culture of the pathogen and placed at the centre of the Petri dishes. Three replications were maintained for each treatment. The plates were incubated at 28 ± 1^0C. Observation on colony growth was taken when complete growth was reached in any one of the media tested.

3.4.2. Growth on Different Liquid Media

The liquid media used were Potato Sucrose Broth (PSB), Potato Dextrose Broth (PDB), Oat Meal Broth (OMB), Corn Meal Broth (CMB), Carrot Broth (CB), Malt Extract Broth (MEB) and Czapek-Dox Broth (CDB). Hundred ml of broth was poured in 250 ml conical flasks and sterilized by autoclaving at 1.1 Kg/cm^2 for 20 min. Four mycelial discs of 5mm dia., cut out from three days old culture of the pathogen were inoculated in liquid medium and flasks were incubated at 28 ± 1^0C for ten days. Mycelium was filtered through Whatman No.1 filter paper and dried in the oven at 60^0C and weighed on the next day onwards till concurrent results were obtained.

3.5. PHYSIOLOGICAL CHARACTERISTICS

The selected *Pythium* sp. isolate was used to study the physiological characteristics of the pathogen.

3.5.1. Effect of Temperature

The pathogen (P2 isolate) was grown in different temperature levels so as to determine the optimum temperature for culturing of the pathogen as described by Suleiman *et al*. (2011). For this, mycelial discs of five mm size were inoculated at the centre of the solidified PSA plates and the plates were incubated at different temperature levels *viz.*, 4, 20, 25, 35 and 40^0C and also at room temperature (28.5^0C). Three replications were maintained for each temperature level. The colony diameter of the pathogen grown under different temperature levels was recorded at 24 h. after incubation.

The pathogen was also grown in potato sucrose broth at different levels of temperature. Hundred ml of PSB was poured in 250 ml conical flask and sterilized by autoclaving. Mycelial discs of 5 mm dia. cut from three days old culture of the pathogen were inoculated in liquid medium and flask were incubated at different temperature levels *viz.*, 4, 20, 25, 35 and 40^0C and also at room temperature (28.5^0C). Three replications were maintained for each temperature level. The mycelium was filtered through Whatman No.1 filter paper and dried in the oven at 60^0C and weighed on the next day onwards till the weight remained constant.

3.5.2 Effect of pH

In order to study the effect of pH on mycelia growth of *Pythium*, the method followed by Muthukumar and Eswaran (2008) was adopted.

For determination of the optimum pH levels for culturing the pathogen *Pythium* sp., PSA media was prepared, the pH was adjusted to 6, 7 and 8 by a pH meter by adding 0.1 *N* alkali (NaOH) or acid (HCl), and sterilized by autoclaving. The media were melted and poured into petri plates at the rate of 20 ml per plate and inoculated with 5 mm mycelial disc of the pathogen at the centre and incubated at 28 ± 1^0C for 24 h. Five replications were maintained for each pH values. The diameter of the colonies of the pathogen was recorded 24 h. after incubation.

The pathogen was also grown in potato sucrose broth in different levels of pH. PSB media was prepared, the pH was adjusted to 4.5, 5.5, 6.5, 7.5, 8.5 and 9.5

by a pH meter by adding 0.1 *N* alkali (NaOH) or acid (HCl) and 100 ml of PSB was poured in 250 ml conical flask and sterilized. Four mycelial discs of 5 mm dia. cut out from three days old culture of the pathogen were inoculated in liquid medium and flask were incubated at 28 ± 1^0C for ten days. Three replications were maintained for each pH level. Mycelium was filtered through Whatman No.1 filter paper and dried in the oven at 60^0C and weighed on the next day onwards till there was no weight change.

3.5.3. Effect of Light

The pathogen was subjected to different light conditions following the method adopted by Gul *et al.* (2005) so as to determine the optimum light conditions for the growth of the pathogen. Different periods of exposure to light and darkness were tried; 24 h. fluorescent light (500 lux), 24 h. darkness and ambient light conditions (400 lux during day time and darkness during night time). The pathogen inoculated on PSA plates were incubated at above mentioned light conditions and the radial growth in terms of colony diameter was compared 24 h. after incubation to find out the optimum light requirement by the pathogen.

The pathogen was also grown in Potato Sucrose Broth in different light conditions. Hundred ml of PSB was poured in 250 ml conical flask and sterilized by autoclaving at 1.1 kg/cm^2 for 20 min. Four mycelial discs of 5 mm dia., cut from three days old culture of the pathogen were inoculated in liquid medium and flask were incubated at different light conditions i.e., 24 h. fluorescent light (500 lux), 24 h. darkness and ambient light conditions (400 lux during day time and darkness during night time) for ten days. Four replications were maintained for each light condition and the mycelium was filtered through Whatman filter paper No.1 and dried in the oven at 60^0C and weighed on the next day onwards till concurrent results were obtained.

3.6. STUDIES ON THE EPIDEMIOLOGY OF THE DISEASE

3.6.1. Survival of the Pathogen *Pythium aphanidermatum* in Crop Debris

In vitro studies were conducted on the survival of the pathogen in the plant debris by the method of Shalini (2006). The infected stem portions and soil

collected from field were brought to laboratory and buried the infected stem portion in collected field soil taken in a plastic pot. Weekly isolation was carried out from stored buried infected stem portion on PSA medium.

The nature of survival of the pathogen on crop debris was studied by following the method adopted by Hall *et al*. (1980). For that, small bits of infected stem portion stored for survival study was cut out and ground by using mortar and pestle. The ground content was collected in cavity slide by adding small amount of water. The cavity slide was kept inside the moist chamber for one day. Then it was transferred into microscopic slides, stained with lactophenol cotton blue and observed under the microscope for the survival structures.

3.6.2. Correlation Studies on the Influence of Various Weather Parameters on the Incidence of Stem Rot of Vegetable Cowpea

The weather parameters prevailing in the field during disease incidence were collected from College of Agriculture, Vellayani, Thiruvananthapuram. The various parameters studied include maximum temperature, minimum temperature, soil temperature at 20 cm depth, relative humidity (morning), relative humidity (evening), total rainfall were correlated with the disease incidence using statistical correlation method.

3.6.3. Study on the Host Range of the pathogen in Other Crops Grown in Vegetable Field and Common Weeds.

Survey was conducted in different stem rot disease endemic areas to study the host range of the pathogen in other vegetable crops grown in those areas. The plant showing similar stem rot and wilting symptoms were collected in paper covers and brought to the laboratory for isolation as described in the 3.1.

In vitro inoculation was conducted to study the host range of the pathogen in common weeds. For this, the common weeds seen in the disease affected cowpea field were up rooted without damaging the roots along with soil and collected in separate polythene bags. The weeds collected were *Amaranthus viridis, Cynodon dactylon, Cyperus rotundus, Cleome rutidospermum, Brachiaria miliformis and Panicum repens*. The collected weeds were potted separately in small pots and filled with potting mixture. After establishing the growth, the pots were inoculated

with mycelial, sporangial and oospore suspension. Un inoculated control of each weed was also maintained in pots.

3.7. ISOLATION AND TESTING OF RHIZOSPHERE FUNGAL ANTAGONISTS

3.7.1. Isolation of Fungal Antagonists from Rhizosphere Region of Cowpea Plant

The mycoflora of the rhizosphere of the disease free cowpea plants among stem rot infected cowpea plants in the field was studied. The dilution plate technique described by Johnson and Curl (1972) was followed for the isolation of fungal antagonists from the rhizosphere region of cowpea. Rhizosphere soil along with roots were collected from the disease free plants in the stem rot infected cowpea fields. One gram of rhizosphere soil was transferred to 99 ml sterile distilled water in 250 ml conical flasks and shaken for 20 min in a rotary shaker. From this, the 10^{-4} dilution was prepared.

One ml of 10^{-4} was plated using pour plate method on Martin's Rose Bengal Agar medium (Appendix 1). The plates were incubated at room temperature for three to four days. After the incubation fungal colonies were examined and transferred to plates and subsequently purified by hyphal tip method. The purified cultures of the predominant fungi were then stored under refrigerated conditions for identification and subsequent studies for antagonism.

3.7.2. *In Vitro* Screening for Evaluating the Antagonistic Efficacy of the Fungal Isolates Against *P. aphanidermatum* in Dual Culture.

The fungal isolates obtained by serial dilution techniques were primarly evaluated for their antagonistic potential against *P. aphanidermatum* by cross culture method followed by Jubina (1997) inoculating three fungal isolates at the three corners of a Petri plate at a distance of 3.5 cm from the centre of the 9 cm Petri plate. The pathogen being a very fast grower it was inoculated on the centre of the Petri plates one day after the inoculation of isolated fungal antagonist.

Mycelial discs of 5 mm dia. taken from the actively growing margins of three days old culture of the seven isolated antagonists were inoculated on PSA

plates as described above and the plates were incubated at room temperature. Three replications were maintained for each treatment.

On the second day after incubation 5 mm mycelial discs cut out from the three days old culture of *P. aphanidermatum* were placed on the centre of the culture plates, maintaining 3.5 cm distance between the discs of the pathogen and the fungal isolates. The procedure was repeated in all plates. Control plates were also maintained with only *P. aphanidermatum.* The plates were incubated in room temperature. Observations were recorded on radial growth, over growth and inhibition zone.

The fungal isolates that showed maximum antagonistic action towards the pathogen were selected for final screening by dual culture technique by Skidmore and Dickinson (1976) for confirmation. For that, mycelial discs of 5mm diameter taken from the actively growing margins of three days old cultures of the three selected isolated antagonists were inoculated on PSA plates at a distance of 2.5 cm from the centre of the plates and the plates were incubated at room temperature. Three replications were maintained for each treatment.

On the second day after incubation five mm mycelial discs cut from the three days old culture of the pathogen *P. aphanidermatum* were placed opposite to the colony of the fungal antagonist at a distance of 2.5 cm from the centre of the 9 cm dia. culture plate, maintaining five cm distance between the discs of the pathogen and the antagonist. The procedure was repeated in all plates. Control plates were also maintained with only *P. aphanidermatum.* The plates were incubated in room temperature. Observations were recorded on radial growth, over growth and inhibition zone.

Percentage inhibition of the pathogen over control was calculated by adopting the formula (Fokkema, 1976),

$$I\ (\%) = R1\text{-}R2 / R1 \times 100$$

I – percentage growth inhibition

R1 – Growth of pathogen in control

R2 – Growth of pathogen in treatment

The most efficient fungal antagonists isolates from dual culture experiment was selected for morphological characterization and used for evaluating the efficiency of disease suppression during *in vitro* seedling assay.

3.7.3. Identification and Morphological Characterization of Selected Fungal Antagonists

Three fungal antagonists showing good *in vitro* mycelial inhibition of *P. aphanidermatum* were selected for morphological identification. For the morphological characterization of selected fungal antagonists, single spore isolation technique for fungal purification and slide culture technique (Riddell, 1950) for morphological identification was followed.

3.7.3.a. Single Spore Isolation

Single spore isolation technique described by Dhingra and Sinclair (1985) was used for the purification of *Trichoderma* sp. For that the spore suspension was prepared by inoculating small piece of mycelium with conidia into a test tube containing sterile distilled water and shook vigorously. After that, one loopfull of spore suspension was streaked on Water Agar (WA) in a zig-zag manner. The inoculated WA plates were incubated at room temperature for 24 h. After that, well isolated colonies germinating from single conidia were sub cultured to new PDA plates. The inoculated plates were incubated at room temperature for 5 days and used as inoculum for further identification studies.

3.7.3.a. Slide Culture Technique (Riddell, 1950)

Petri plates containing a filter paper, two pieces of glass rods, two coverslips and one microscopic slide were autoclaved. Using a sterile blade, 5 mm wide blocks of plain agar medium were cut and placed two 5mm agar block at two sides of the slide by using an inoculation needle. The four corners of the agar block were then inoculated with fungal antagonist's mycelium and placed the coverslips over the agar block. The slide culture units were incubated at room temperature after moistening the filter paper in the unit. The observation was taken 48 h. after inoculation.

3.8. *IN VITRO* ASSAY OF DIFFERENT AGENTS

3.8.1. *In Vitro* Evaluation of Bio Control Agents and Fungal Endophyte against *P. aphanidermatum.*

The fungal endophyte *Piriformospora indica* and KAU released biocontrol agents *Pseudomonas fluorescens* (MTCC 5694) and *Trichoderma* sp. (MTCC 5693) were evaluated against *P. aphanidermatum* under *in vitro* conditions.

Mycelial discs of 5 mm dia. taken from the actively growing margins of seven days old cultures of the *P. indica* and three days old culture of *Trichoderma* sp. were inoculated on separate PSA plates at a distance of 2.5 cm from the centre of the nine cm plates and the plates were incubated at room temperature.

On the third day after incubation five mm mycelial discs cut out from the three days old culture of the pathogen *P. aphanidermatum* were placed opposite to the colony of the *P. indica* (Varma *et al*., 2013) and *Trichoderma* sp. plated on separate plates at a distance of 2.5 cm from the centre of the 9 cm dia. culture plate, maintaining five cm distance between the discs of the pathogen and the antagonist.

In the case of *P. fluorescens* (KAU culture), 5 mm mycelial discs cut out from the three days old culture of the pathogen *P. aphanidermatum* were placed on the centre of the PSA plates and two streaks were done with bacterial biocontrol agent *Pseudomonas fluorescens* on the both side perpendicular to the disc 2.5 cm apart as described by Aneja (2003).

Four replications were maintained for all treatments. The all plates were incubated in room temperature. Observations were recorded on radial growth, over growth and inhibition zone.

3.8.2 *In Vitro* Evaluation of Organic Preparations against *P. aphanidermatum*

3.8.2.a. Preparation of Panchagavyam

Panchagavyam was preparaed by following the steps described in the organic POP, KAU (2009). Cow dung (7 kg) and cow ghee (1 kg) were mixed in a clean plastic bucket thoroughly both in morning and evening hours and keep aside for three days. After three days, cow urine (10 litres) and water (10 litres) were

added. The mixture was kept for 15 days with regular mixing both in morning and evening hours. After 15 days, 3 litres of cow milk, cow curd (2 litres), tender coconut water (3 litres), jaggery (3 kg) and well ripened poovan banana (12 nos.) were added in to this. Then kept the bucket under shade by covering with mosquito proof net and stirred the content twice a day at morning and evening. The stock solution was used for disease management studies after 30 days.

3.8.2.b. Preparation of Fish Amino Acid

Fish amino acid was prepared by following the steps described by Weinert *et al*. (2014) with slight modifications, mixing one kilogram of sardine fish (*Sardina pilchardus*) with one kilogram of jaggery in a plastic can and kept the plastic can under shade condition and covered the mouth with paper and tie it with the string. Kept the can undisturbed for 25 days. After 25 days, the content was filtered through muslin cloth and stored in the same can. The filtered content was used in the field by mixing with water (5ml per litre of water).

3.8.2.c. In vitro Evaluation of Organic Preparations

The *in vitro* evaluation of organic preparations i.e. pachagavyam and fish amino acid against *P. aphanidermatum* was done by using poisoned food technique (Nene and Thapliyal, 1993). The concentrations tested were 2.5%, 5% and 10% for both the organic preparations.

In order to study this, 47.5 ml, 45 ml and 40 ml distilled water in three separate 250 ml conical flask and 50 ml double strength PSA medium in another three separate 250 ml conical flask were taken and sterilized by autoclaving at 1.1 Kg/cm^2 for 20 min. The organic preparations were filtered initially with Whatman No.1 filter paper and finally filter was sterilized by passing through bacterial filter (0.22 µm) before adding to the media. Added desired amount of filter sterilized organic preparations in to the desired amount of sterile distilled water and then mixed with 50 ml molten double strength PSA to get desired concentration. After that, 20 ml of the amended medium was poured in to sterilized petri plate under aseptic conditions in laminar air flow inoculation chamber and allowed to solidify. The same procedure was repeated for all concentrations for both organic preparatons.

Each plate was inoculated in the centre with 5 mm mycelial disc cut from the three days old *P. aphanidermatum* culture individually under aseptic conditions and incubated at room temperature.

Unamended PSA plates inoculated with *P. aphanidermatum* served as checks. Radial growth of the test isolates was recorded after 24 h and 48 h of incubation. Percentage inhibition of growth over control was calculated using the formula (Vincent, 1927).

$$I = \frac{C - T}{C} \quad X\ 100$$

Where,

I = percentage inhibition.

C = growth of *P. aphanidermatum* in unamended medium.

T = growth of *P. aphanidermatum* in amended medium.

3.8.3. *In Vitro* Evaluation of Chemical Fungicides against *P. aphanidermatum*

The *in vitro* chemical fungicides evaluation of *P. aphanidermatum* was done by using Poisoned food technique (Nene and Thapliyal, 1993). Five commercially available fungicides (Table 1) were used for the same.

In order to study this, 50 ml distilled water in three separate 250 ml conical flask and 50 ml double strength PSA medium in another three separate 250 ml conical flask were taken and sterilized by autoclaving at 1.1 Kg/cm^2 for 20 min. Added desired concentration of fungicide in to the 50 ml sterile distilled water and then mixed with 50 ml molten double strength PSA to get desired concentration. There after 20 ml of the poisoned medium was poured in to sterilized petriplate (9.0 cm dia.) under aseptic conditions in Laminar Air flow inoculation chamber and allowed to solidify. The same procedure was repeated for all fungicides.

Each plate was inoculated in the centre with 5 mm dia. disc cut out from three days old test *P. aphanidermatum* culture individually under aseptic conditions and incubated at room temperature.

Unamended PSA plates inoculated with *P. aphanidermatum* served as checks. Radial growth of the test isolates was recorded after 24 h and 48 h of

incubation. Percentage inhibition of growth over control was calculated using the formula mentioned under *3.8.2.c.*

3.8.4. *In Vitro* Evaluation of Different Agents for the Control of Disease on Seedlings.

The selected fungal antagonists, the fungal endophyte *P. indica,* organic amendments such as neemcake, vermicompost, coirpith compost and spent mushroom subtrate (SMS). The organic preparations such as panchagavyam and fish amino acid, the KAU released biocontrol cultures of *P. fluorescens and Trichoderma* sp. and five commercially available fungicides such as metalaxyl MZ, azoxystrobin, iprovalicarb + propineb, cuprous hydroxide and copper oxychloride were assessed for the management of the disease on seedlings at the recommended concentration (Suleiman, 2010).

In order to conduct the seedling assay, the paper cups were filled with sterilized potting mixture soil autoclaved at 1.1 kg/cm^2 for 120 min. Four seeds were sown per cup and maintain three replication cups for each treatment. The soil of each cups were inoculated with mycelial, sporangial and oospores water suspension of a seven day-old culture of *P. aphanidermatum*. The cups were then inoculated with mycelial and spore suspension of seven days old culture of fungal antagonists and fungal endophyte *P. indica*. In the case of organic amendments such as neemcake, vermicompost, coirpith compost and spent mushroom substrate (SMS), the upper layer of the soil in the cups were mixed with each amendments separately and after that the seeds were sown followed by the inoculation of pathogen done.

The remaining treatments like organic preparations such as panchagavyam and fish amino acid, the KAU released biocontrol cultures of *P. fluorescens* (MTCC 5694) *and Trichoderma* sp. (MTCC 5693) and five commercially available fungicides such as metalaxyl MZ, azoxystrobin, iprovalicarb + propineb, copper hydroxide and copper oxychloride were applied directly in to the cups immediately after the inoculation of pathogen at their field dose. Also, maintained inoculated control cups by inoculating cups with pathogen without any

treatments and un inoculated cups without inoculation of pathogen and any treatments for the comparison (Table 2).

The observation for percentage seedling mortality were taken 10 days after inoculation. The formula used for the calculation of percentage seedling mortality given below (Suleiman, 2010),

$$\text{Percentage seedling mortality} = \frac{\text{No. of seedlings dead}}{\text{Total no. of seedlings per cup}} \times 100$$

3.9. FIELD EVALUATION OF SELECTED AGENTS

The promising treatments selected from the *in vitro* seedling assay, such as two organic preparations like fish amino acid and panchagavyam; two talc based formulation of biocontrol agents such as *Pseudomonas fluorescens* and *Trichoderma* sp.*;* five chemical fungicides such as metalaxyl MZ, azoxystrobin, iprovalicarb + propineb, copper hydroxide and copper oxychloride were tested in the field during July 2013 at College of Agriculture, Vellayani to screen the different agents selected from the seedling assay.

The susceptible cowpea variety Vellayani Jyothika was used for the field level evaluation of the selected agents. The seeds were sown in the pro-trays filled with potting mixture consisting of vermicompost and coirpith in 1:1 ratio. The seedlings were transplanted on the field 10 days after sowing. Four plants were maintained in one pits (block) and 2 m x 2 m spacing were maintained between pits. The design followed was RBD and five blocks were maintained. The treatments given as soil drenching alone and soil drenching as well as foliar spray that were given in Table 3. The treatments were given from the 2^{nd} week onwards after transplanting and once in fifteen days interval up to harvest.

Daily observations were made and recorded the disease occurrence in the field. The Percentage Disease Incidence were calculated by using the formula given below

$$\text{Percentage Disease Incidence} = \frac{\text{No. of plants dead}}{\text{Total no. of plants}} \times 100$$

Based on the above formula the Percentage Disease Incidence was recorded. Biometric observations such as number of leaves, plant weight, plant height, root length, root weight and yield were also recorded.

Table 1. Different concentrations of fungicides tested against *P. aphanidermatum* (*in vitro*)

Sl. No.	Fungicides	Concentrations (%)
1	Metalaxyl MZ (4% metalaxyl + 64% mancozeb) 68%WP	0.1
		0.2
		0.3
2.	Azoxystrobin 23%SC	0.1
		0.15
		0.2
3.	Iprovalicarb 5.5% + Propineb 61.25%	0.1
		0.2
		0.3
4.	Copper hydroxide 77%WP	0.1
		0.2
		0.3
5.	Copper oxychloride 50%WP	0.1
		0.2
		0.3

Table 2. Different agents tested in the *in vitro* seedling assay

Sl. No.	Treatments	Concentration
1.	**Organic amendments**	
	Vermicompost	5g/cup
	Coirpith compost	5g/cup
	Neem cake	5g/cup
	Spent mushroom substrate (SMS)	5g/cup
2.	**Fungal antagonist**	
	Trichoderma koningii (T-1)	
	Trichoderma viride (T-2)	
	Pencillium citrinum	
3.	**Fungal endophyte** (*Piriformospora indica)*	
4.	**Organic preparations**	
	Panchagavyam	5%
	Fish amino acid	5%
5.	**Biocontrol agents**	
	Talc based formulation of *Pseudomonas fluorescens*	2%
	Talc based formulation of *Trichoderma* sp.	2%
6.	**Chemical Fungicides**	
	Metalaxyl MZ (4% metalaxyl + 64% mancozeb) 68%WP (1700 g a.i/ 1000 L)	0.2%
	Azoxystrobin 23%SC (125 g a.i/500L)	0.15%

(Contd..)

Table 2. continued

	Copper hydroxide 77%WP (625 g a.i/ 500 L)	0.2%
	Copper oxychloride 50%WP (1000g a.i/500 L)	0.2%
7.	Control (un inoculated)	
8.	Control (inoculated)	

Table 3. Organic preparations, Biocontrol agents and Chemical Fungicides tested against stem rot of cowpea

Sl. No.	Treatments	Method of application	Recommeded Dosage	Concentration (%)
T1	Fish amino acid	Soil drenching + foliar spray	-	5
T2	Panchagavyam	Soil drenching + foliar spray	-	5
T3	Talc based formulation of *P. fluorescens*	Soil drenching + foliar spray	20g/L	2
T4	Talc based formulation of *Trichoderma* sp.	Soil drenching + foliar spray	20g/L	2
T5	Metalaxyl MZ 68%WP (4% metalaxyl + 64% mancozeb)	Soil drenching + foliar spray	(1700 g a.i/ 1000 L)	0.2
T6	Azoxystrobin 23%SC	Soil drenching + foliar spray	(125 g a.i / 500L)	0.15
T7	Iprovalicarb 5.5% + Propineb 61.25%	Soil drenching + foliar spray	(1500g a.i / 500L)	0.2
T8	Copper hydroxide 77% WP	Soil drenching	(1000g a.i/ 750 L)	0.2
T9	Copper oxychloride 50%WP	Soil drenching	(1000g a.i/ 500 L)	0.2
T10	Control (without any treatment)	-		-

Results

4. RESULTS

The present study on the 'Integrated management of Pythium stem rot of vegetable cowpea (*Vigna unguiculata* sub.sp. *sesquipedalis* (L.) Verdcourt) was conducted during the period 2012-2014 at the Department of Plant Pathology, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala. The results obtained from the laboratory and field experiments are summarized below:

4.1. ISOLATION OF THE PATHOGEN

The pathogen causing the stem rot disease in vegetable cowpea, *was* isolated from the infected stem showing typical symptoms. The samples were collected from different cowpea growing areas of Thiruvananthapuram district during the surveys and the isolation was done following standard procedures mentioned under materials and methods. Repeated isolations from the diseased portions of the stems and roots collected from different locations yielded *Pythium* spp. (Edson) Fitzp. on PDA.

Eight isolates were obtained from different locations (Table 4). The isolates were serially numbered from P1 to P8 and were used for pathogenicity study for selecting the most virulent isolate of the pathogen for further studies. The colony morphology and growth pattern of collected isolates were described in Table 5 (Plate 1).

4.1.1. Symptomatology of the Pythium Stem Rot of Vegetable Cowpea

The symptoms include wilting of the entire leaves followed by wet rotting, initial appearance of water soaked lesions at basal stem portion (10 cm above ground) spreading to aerial stem, lower branches and roots (Plate 2). The plants collapsed one or two days after the symptom expression. During summer months, instead of wet rot, dry rot symptoms appeared with red coloured margins on the basal stem portion (Plate 3(3a)). The rotting also began from the aerial stem portion near to the branches instead of basal stem portion, and in this case the regions towards root portion are not affected whereas the shoot portion are affected by wilting symptoms and plant succumbs to the disease (Plate 3(3b)). White cottony mycelial growth of pathogen could be seen at the early morning at

Table 4: Details of isolates collected from different locations of Thiruvananthapuram district

Sl.No.	Isolates	Locations
1.	P1	College of Agriculture, Vellayani , Thiruvananthapuram
2.	P2	College of Agriculture, Vellayani , Thiruvananthapuram
3.	P3	Karamana
4.	P4	Venganoor
5.	P5	Kalliyoor
6.	P6	Pothencode
7.	P7	Athiyanoor
8.	P8	Pallichal

Table 5. Colony morphology of eight *Pythium* spp. isolates

Isolates	Mycelial morphology (PDA medium)	Shape of the sporangium	Radial growth at 24 h after inoculation (29.5^{O}C) (cm)
P1	White cottony mycelium	Lobed sporangium	5.1
P2	-do-	-do-	7.0
P3	-do-	-do-	5.8
P4	-do-	-do-	5.0
P5	-do-	-do-	4.6
P6	-do-	-do-	4.5
P7	-do-	-do-	4.3
P8	-do-	-do-	4.2

Plate 1. *Pythium* spp. isolates collected from different locations

A.

B.

C.

D.

Plate 2. Typical symptoms of Pythium stem rot of cowpea
A) Wilting of entire leaves B) Stem rot symptom C) Progress of symptom on the stem of affected plant D) Rotting portion spread to root system

Plate 3.(3a & 3b) Variation in the symptom expression in summer months in the field

Mycelial growth on inner part of the affected stem

Plate 4. White cottony out growth of pathogen on affected stem at high relative humidity condition

infected stem portion during periods of high relative humidity(Plate4).The stages of infection extend from three weeks after planting up to the harvesting stage (Plate5).

4.2. PROVING OF PATHOGENICTY

All the eight isolates tested i.e., P1to P8, showed varying levels of seedling mortality with both pre and post emergence damping off. Among this, isolate P2 showed high virulence and caused hundred percentage seedling mortality in pathogenicity test compared to all other isolates (Table 6, Plate 6). Therefore the isolate P2 was rated as the most virulent pathogen isolate and selected for further studies. Lobed sporangia (Plate 7) and oospores (Plate 8) of the pathogen were observed under microscope when the infected root hairs were crushed and stained.Pathogenicity test with pathogen isolate P2 in mature plants showed that none of the inoculation methods (Plate 9(9a), Plate 9(9b)) showed disease symptoms in mature plants.

4.3. IDENTIFICATION OF THE PATHOGEN

The virulent pathogen isolate P2 was identified based on its cultural and morphological characteristics.

4.3.1. Cultural Characteristics

The isolate P2 was cultured on Corn Meal Agar (CMA) for characterization, to study its cultural characters. The pathogen was found to be a fast grower i.e., its radial growth was 50 mm after 24 h. of inoculation at 25^0C and colony had cottony aerial mycelium without any special pattern (Plate 10(10a)).

4.3.2 Morphological Characteristics

The dimensions of the main hyphae ranges from 3.1 to 6.8 µm (av. 4.23 µm) wide. Sporangia consisting of terminal complexes of swollen hyphal branches of varying length and up to 24.7 µm wide (Plate 10(10d)). Oogonia terminal, globose, smooth, 18-21.6 µm (av. 19.7 µm) dia. (Plate 10(10c)). Antheridia mostly intercalary, broadly sac-shaped, 9.4-13.6 µm (av. 10.9 µm) long and 7.5-10.4 µm (av. 8.78 µm) wide, one per oogonium, monoclinous or diclinous (Plate 10(10c)); oospores aplerotic, 14.1-19.5 µm (av. 16.4 µm) dia. (Plate 10(10b)), wall 1-2 µm (1.85 µm) thick and chlamydospores were absent.

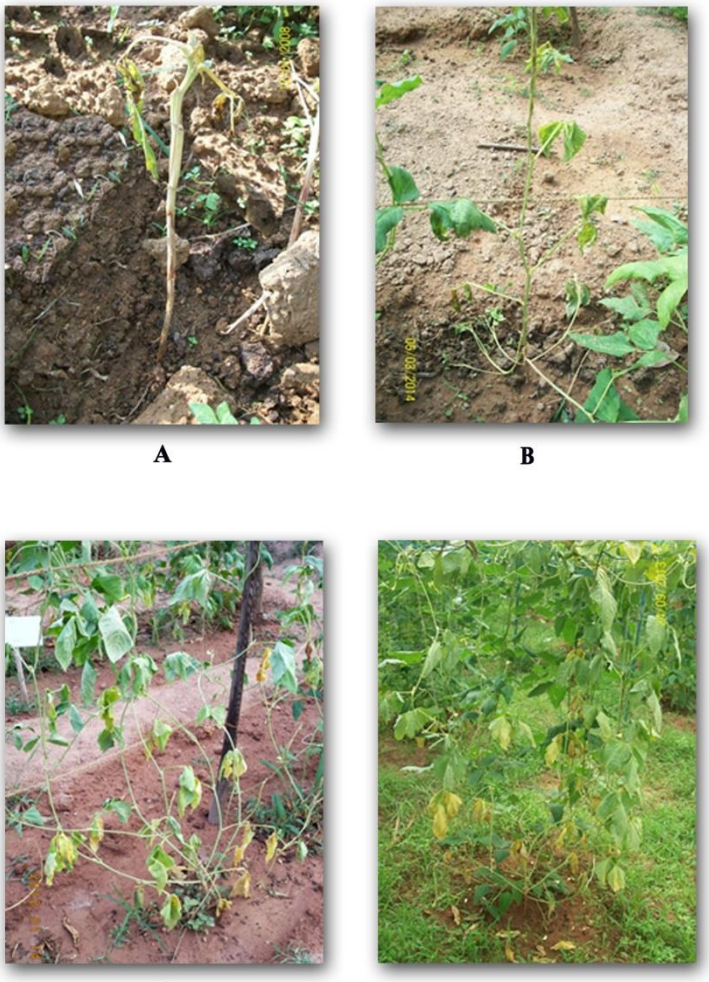


Plate 5. Expression of field symptoms of Pythium stem rot at different crop stages
A) Three weeks after planting B) One month after planting
C) Before flowering stage D) Flowering stage

Table 6. Seedling mortality by different *Pythium* isolates

Isolates	Seedling mortality (%)*
P1	50
P2	100
P3	50
P4	87.5
P5	62
P6	75
P7	75
P8	12.5

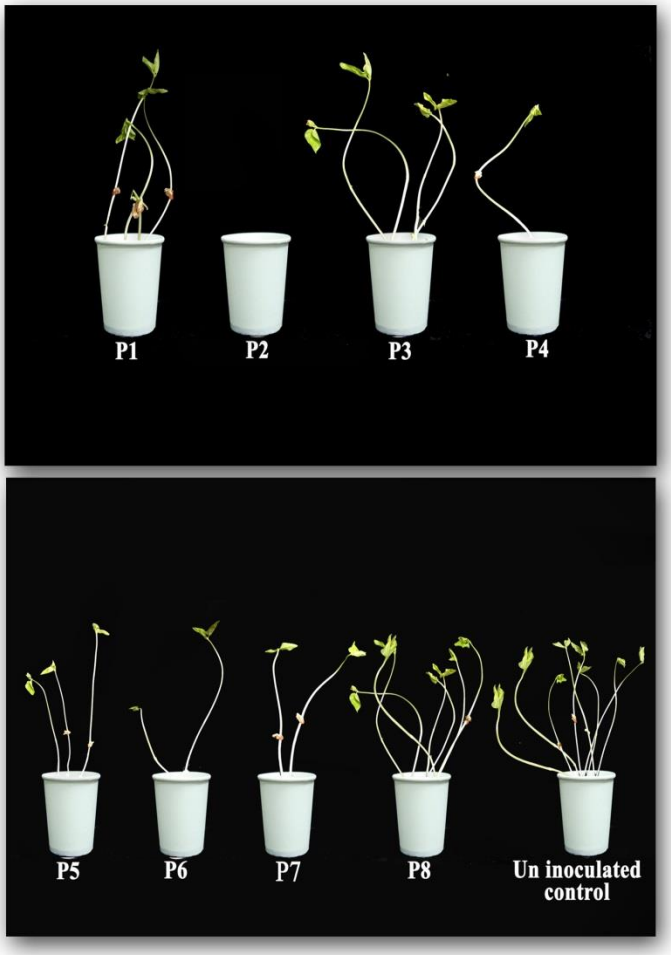


Plate 6. Pathogenicity test with eight isolates

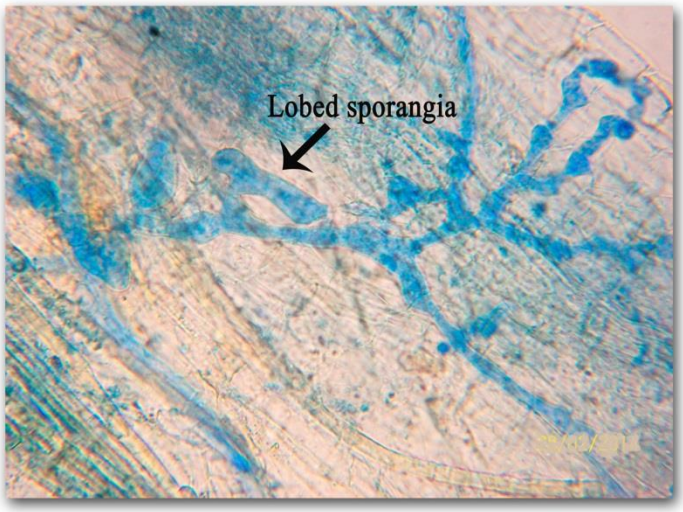


Plate 7. Microscopic picture of lobed sporangium of *P. aphanidermatum* in the infected tissue (400X)

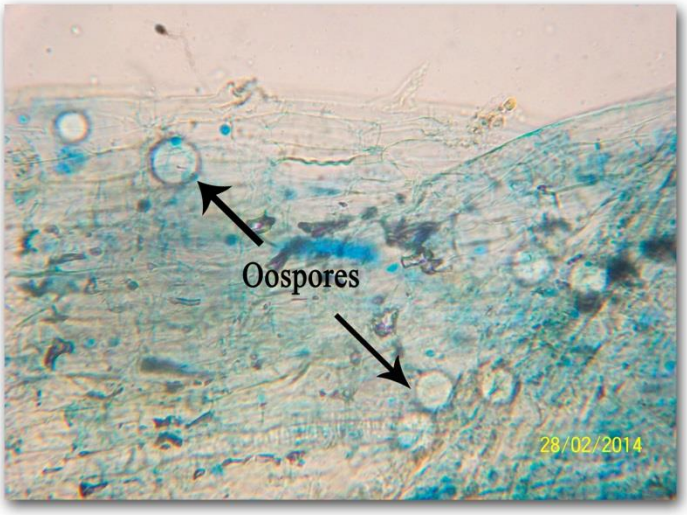
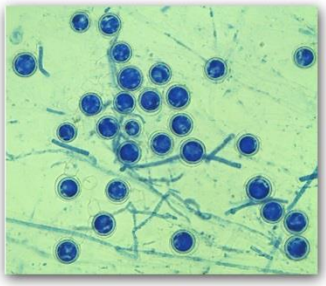


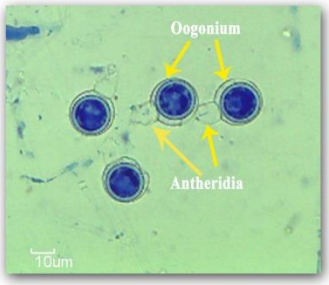
Plate 8. Microscopic picture of oospores of *P. aphanidermatum* in the infected tissue (400X)

Plate 9. (9a & 9b) Inoculation methods used in mature plants

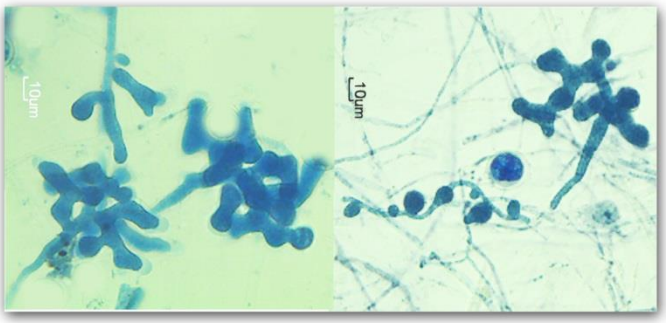
10a. Growth of *P. aphanidermatum* on CMA at 25 ^{0}C at 24 hrs after inoculation



10b. Oospores (100X)



10c. Antheridia and Oogonia (400X)



10d. Lobed sporangium (400X)

Plate 10. (10a, 10b, 10c & 10d) Cultural and morphological characters of *P. aphanidermatum*

4.3.3. Identification

Based on the above cultural and morphological studies, it was found that, the cultural and morphological characters of the isolate P2 were similar to characteristics of *Pythium aphanidermatum* described by Van der Plaats-Niterink (1981). Therefore, the pathogen causing stem rot disease of vegetable cowpea was identified as *Pythium aphanidermatum* (Edson) Fitzp (Plate 11).

4.4. EVALUATION OF NUTRIENT MEDIA FOR CULTURING OF THE PATHOGEN

The cultural characteristics of the pathogen in different culture media were studied with the isolate P2 and the best medium for culturing of the pathogen was determined. Studies were done under both solid and broth culture conditions.

4.4.1 Growth on Different Solid Media

The growth of the pathogen, *Pythium aphanidermatum* (P2 isolate) was compared on different solid media. The result showed that 24 h after inoculation, full growth was obtained in PSA medium (radial growth of 9.0 cm) which was significantly superior from all other media, followed by PDA (8.6 cm). The pathogen was found to have reduced growth in CDA (4 cm) (Table 7, Plate 12).

4.4.2 Growth on Different Liquid Media

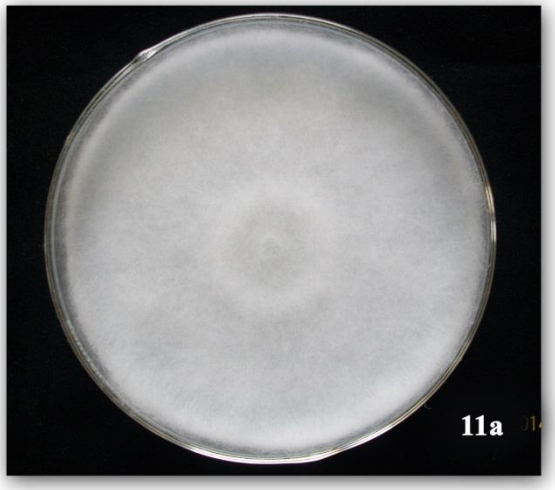
The results of the study revealed that maximum dry weight of mycelial mat was produced on PSB which was on par with that obtained on PDB. The least weight of mycelial mat was obtained on CDA (Table 8, Plate 13).

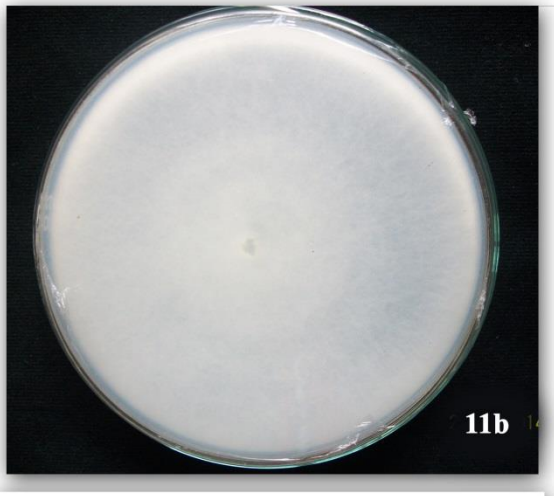
4.5. PHYSIOLOGICAL CHARACTERISTICS

4.5.1. Effect of Temperature

The pathogen (P2 isolate) was grown at different temperature levels *viz*., 4, 20, 25, 35 and 40^0C and also at room temperature (28.5^0C) on PSA so as to determine the optimum temperature for culturing of the pathogen. The radial growth of the pathogen was found to be maximum at 40^0C (9 cm) which was on par with growth at 35^0C (8.93 cm) followed by room temperature (8.5 cm) after 24 h. of inoculation (Table 9, Plate 14).

The pathogen was also grown in Potato Sucrose Broth at different levels of temperature as mentioned above except at 4^0C. The result of the experiment





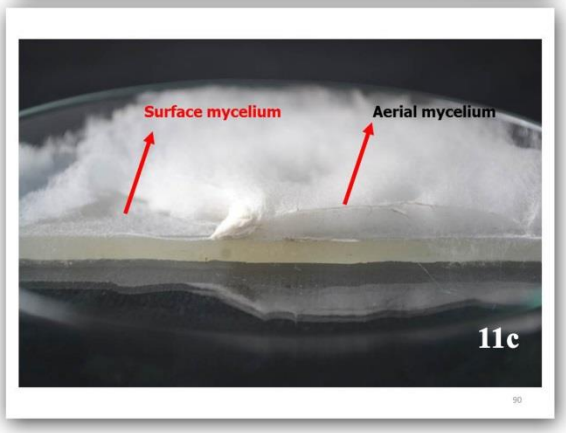


Plate 11. (11a, 11b &11c) Typical colony of *P. aphanidermatum* on PSA

Table 7. Colony diameter of *P. aphanidermatum* in different solid media

Sl.No.	Treatments	Mycelial growth of *P. aphanidermatum* (cm)*
1.	PDA	8.60[b]
2.	PSA	9.00[a]
3.	OMA	8.43[c]
4.	CA	7.60[d]
5.	CMA	8.43[c]
6.	MEA	6.60[e]
7.	CDA	4.00[f]
	SE(±)	5.19
	CD (0.05)	0.15

*Mean of four replications
Observations taken 24h after inoculation.
Figures followed by the same letter do not differ significantly according to one way ANOVA at P=0.05
PSA : Potato Sucrose Agar PDA : Potato Dextose Agar
OMA : Oat Meal Agar CA : Carrot Agar
CMA : Corn Meal Agar MEA : Malt Extract Agar
CDA : Czapek-Dox Agar

Table 8. Mycelial dry weight of *P. aphanidermatum* in different liquid media

Sl.No.	Treatments	Mycelial dry weight (mg) at 10 DAI*
1.	PDB	267.76[a]
2.	PSB	274.00[a]
3.	OMB	152.86[b]
4.	CB	76.16[c]
5.	CMB	61.13[d]
6.	MEB	160.05[b]
7.	CDB	24.50[e]
	SE(±)	4.37
	CD (0.05)	13.26

*Mean of four replications

Figures followed by the same letter do not differ significantly according to one way ANOVA at P=0.05

PSA : Potato Sucrose Agar PDA : Potato Dextose Agar
OMA : Oat Meal Agar CA : Carrot Agar
CMA : Corn Meal Agar MEA : Malt Extract Agar
CDA : Czapek-Dox Agar

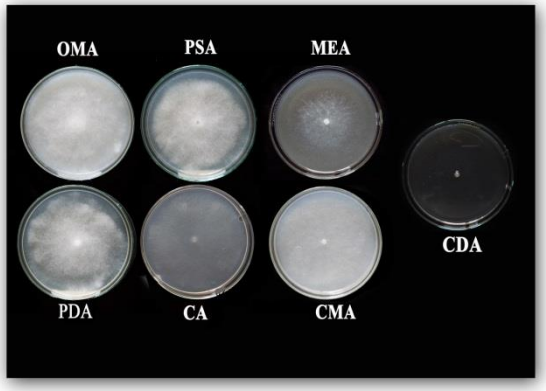


Plate 12. Growth of *P. aphanidermatum* on different solid media

Plate 13. Growth of *P. aphanidermatum* on different liquid media

showed that maximum dry weight of the mycelial mat was 328.12 mg at 35^0C which was on par with mycelial dry weight at room temperature (325.97 mg) after 10 days of inoculation (Table 10, Plate 15).

4.5.2. Effect of pH

The pathogen was grown at three acidic (4, 5 and 6) and one alkaline (8) pH conditions on PSA for determining of the optimum pH levels for culturing the pathogen. Upon comparison of growth 24 h after inoculation, it was observed that the pathogen was found to give maximum radial growth at pH 6 (8.92 cm) which was on par with the growth obtained in pH 7 (8.77 cm) and pH 8 (8.70 cm) (Table 11, Plate 16).

The pathogen was also grown in Potato Sucrose Broth in different levels of pH *viz.*, 4.5, 5.5, 6.5, 7.5, 8.5 and 9.5. The maximum dry weight of mycelial mat was observed at pH 5.5 (258.46 mg) which was significantly different from all other treatments, and followed by dry weight at pH 4.5 (215.66 mg) and pH 6.5 (208.33 mg). The least mycelial mat dry weight was observed at pH 9.5(185.83 mg).Thus, the most ideal pH for the growth of the pathogen was 5.5 (Table 12, Plate 17).

4.5.3. Effect of Light

The pathogen was subjected to three light conditions *viz.,* 24 h. light, 24 h. darkness and the ambient light conditions. At 10 DAI, growth in terms of the colony diameter on PSA was compared (Table 13, Plate 18). The result of the experiment revealed that the pathogen produced maximum radial growth at 24 h. darkness (8.94 cm) which was significantly different from the other two conditions of light, there was no significant difference between the growth at 24 h. light and ambient light condition (8.43 cm).

The pathogen was also grown in Potato Sucrose Broth at different light conditions as above. The result revealed that there was no significant difference between the dry weight of mycelial mat at different light conditions (Table 14, Plate 19).

4.6. STUDIES ON THE EPIDEMIOLOGY OF THE DISEASE

4.6.1. Survival of the Pathogen *P. aphanidermatum* in Crop debris

Table 9. Growth of *P. aphanidermatum* under different temperature condition in solid media.

Sl. No.	Treatments	Colony diameter (cm)*
1.	4^0C	0.06^e
2.	20^0C	3.43^d
3.	25^0C	6.90^c
4.	35^0C	8.93^a
5.	40^0C	9.00^a
6.	Room temperature (28.5^0C)	8.50^b
	SE(±)	0.12
	CD(0.05)	0.39

*Mean of four replications

Observations taken 24h after inoculation.

Figures followed by the same letter do not differ significantly according to one way ANOVA at P=0.05

Table 10. Mycelial dry weight of *P. aphanidermatum* on different temperature conditions in liquid media.

Sl. No.	Treatments	Mycelial dry weight (mg) at 10 DAI*
1.	20^0C	216.07[d]
2.	25^0C	264.25[c]
3.	35^0C	328.12[a]
4.	40^0C	287.45[b]
5.	Room temperature (28.5^0C)	325.97[a]
	SE(±)	4.81
	CD(0.05)	14.50

*Mean of four replications

Figures followed by the same letter do not differ significantly according to one way ANOVA at P=0.05

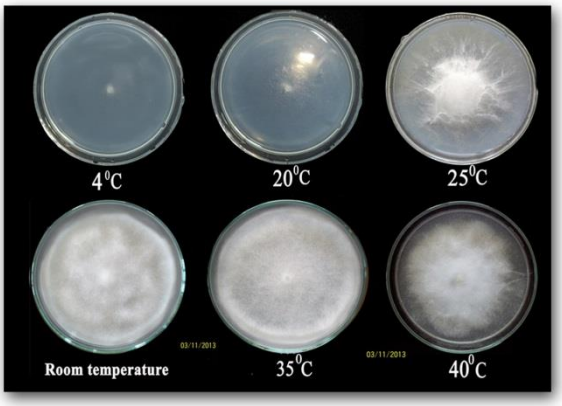


Plate 14. Growth of *P. aphanidermatum* on different temperature condition on PSA

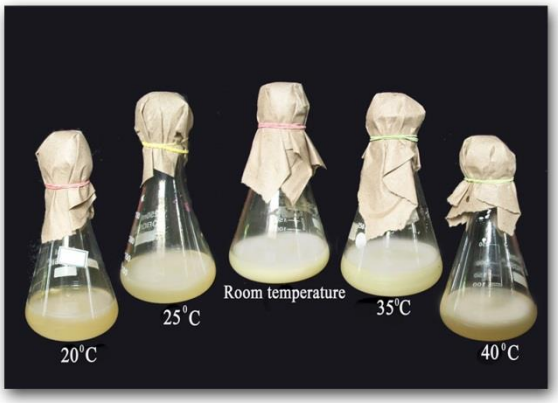


Plate 15. Growth of *P. aphanidermatum* on different temperature conditions on PSB

Table 11. Growth of *P. aphanidermatum* under different pH conditions

Sl. No.	Treatments	Colony diameter (cm)*
1.	pH 4	8.07[b]
2.	pH 5	8.30[b]
3.	pH 6	8.93[a]
4.	pH 7	8.70[a]
5.	pH 8	8.70[a]
	SE(±)	0.081
	CD(0.05)	0.24

*Mean of four replications

Observations taken 24h after inoculation.

Figures followed by the same letter do not differ significantly according to one way ANOVA at P=0.05

Table 12. Mycelial dry weight of *P. aphanidermatum* under different pH conditions in liquid media.

Sl. No.	Treatments	Mycelial dry weight (mg) at 10 DAI*
1.	pH 4.5	215.66[b]
2.	pH 5.5	258.46[a]
3.	pH 6.5	208.33[b]
4.	pH 7.5	201.30[c]
5.	pH 8.5	195.46[c]
6.	pH 9.5	185.83[d]
	SE(±)	2.95
	CD(0.05)	9.09

*Mean of four replications

Figures followed by the same letter do not differ significantly according to one way ANOVA at P=0.05

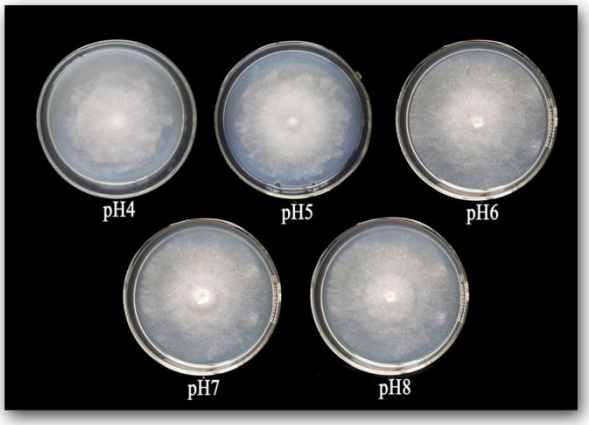


Plate 16. Growth of *P. aphanidermatum* on different pH range on PSA

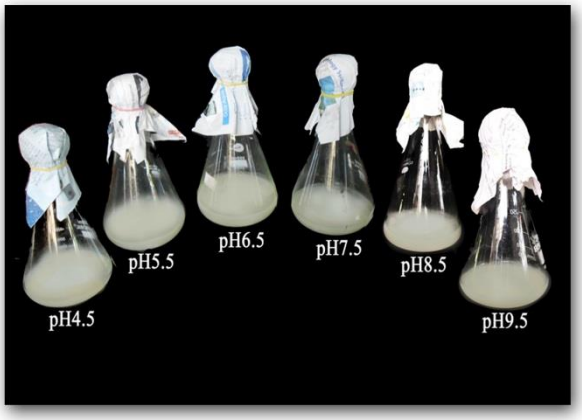


Plate 17. Growth of *P. aphanidermatum* on different pH range on PSB

Table 13. Growth of *P. aphanidermatum* under different light conditions

Sl. No.	Treatments	Colony diameter (cm)*
1.	24 h Light (500 lux)	8.61[b]
2.	Ambient Light (400 lux at day time + Darkness at night time)	8.43[b]
3.	24 h Darkness	8.94[a]
	SE	0.01
	CD(0.05)	0.05

*Mean of four replications

Observations taken 24h after inoculation.

Figures followed by the same letter do not differ significantly according to one way ANOVA at P=0.05

Table 14. Mycelial dry weight of *P. aphanidermatum* under different light conditions in liquid media

Sl. No.	Treatments	Mycelial dry weight (mg) at 10 DAI*
1.	24 h Light (500 lux)	179.20[a]
2.	Ambient Light (400 lux at day time + Darkness at night time)	179.92[a]
3.	24 h Darkness	168.04[a]
	SE	-
	CD(0.05)	NS

*Mean of four replications

Figures followed by the same letter do not differ significantly according to one way ANOVA at P=0.05

Plate 18. Growth of *P. aphanidermatum* under different light condition on PSA

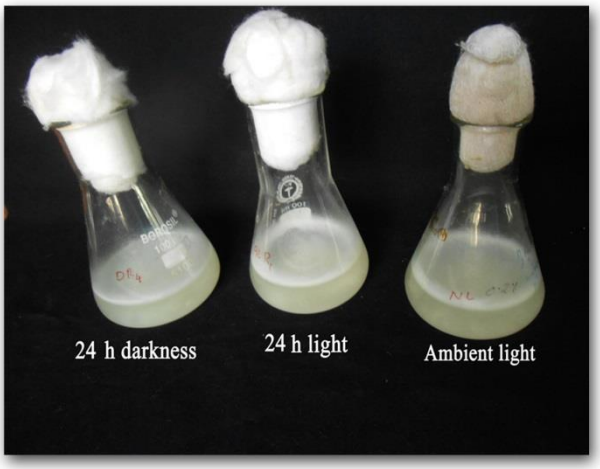


Plate 19. Growth of *P. aphanidermatum* under different light conditions in PSB

The survival of *P. aphanidermatum* on crop debris was studied under *in vitro* conditions. *P. aphanidermatum* was recovered from the buried sample up to 15 weeks (Table 15). After 15 weeks, no recovery of the pathogen was observed on the PSA after isolation from the buried infected stem portions.

The nature of survival of the pathogen on crop debris was studied by observing the survival structures of the pathogen in the crop debris. The microscopic study revealed that oospores are the main survival propagules that help in the survival of *P. aphanidermatum* on crop debris (Plate 20).

4.6.2. Correlation Studies on the Influence of Various Weather Parameters on the Incidence of Stem Rot of Vegetable Cowpea.

The weekly weather parameters prevailing in the field were correlated with the percentage disease incidence so as to study the influence of weather parameters on the disease incidence of stem rot of vegetable cowpea. The various parameters studied were minimum temperature, maximum temperature, soil temperature at 20 cm depth, relative humidity (morning), relative humidity (evening) and total rainfall.

The correlation coefficients are presented in Table 16. The results revealed that percentage disease incidence of stem rot disease was significantly negatively correlated with the maximum temperature and soil temperature at 20 cm depth from two weeks to harvesting stage of the crop. Therefore, it can be inferred that as the maximum temperature and soil temperature at 20 cm depth increases the disease incidence will decrease.

4.6.3. Studies on the Host Range of the Pathogen in Other Crops Grown in Vegetable Field and Common Weeds.

Attempts were made to isolate *P. aphanidermatum* from vegetable plants, other than cowpea, growing in and around the area where infection was detected and exhibiting stem rot or damping off symptoms. *P. aphanidermatum* was isolated from stem of seedling and mature snake gourd (*Trichosanthes anguina*) (Plate 21(21a)), seedlings of chilli (*Capsicum annuum*) (Plate 21(21b)), tomato (*Lycopersicon esculentum*) (Plate 21(21c)) and Red amaranthus (*Amaranthus*

Table 15. Survival of the pathogen Pythium aphanidermatum in crop debris		
Sl.No.	**Week**	**Recovery of *P. aphanidermatum***
1.	1	+
2.	2	+
3.	3	+
4.	4	+
5.	5	+
6.	6	+
7.	7	+
8.	8	+
9.	9	+
10.	10	+
11.	11	+
12.	12	+
13.	13	+
14.	14	+
15.	15	+
16.	16	-

+ Positive - Negative

Table 16. Correlation of Percentage disease incidence with the weekly weather parametrs from 2 weeks to harvesting stage.

Parameters	1	2	3	4	5	6
PDI	-0.37111*	-0.14686	-0.51526**	0.251339	0.198685	0.050819

1. Maximum Temperature
2. Minimum Temparature
3. Soil Temperature at 20 cm depth
4. Relative humidity (Morning)
5. Relative humidity (Evening)
6. Total Rainfall

*Correlation coefficient significant at 0.05 - 0.312

**Correlation coefficient significant at 0.01 - 0.403

tricolor) (Plate 21(21d)) with similar rotting symptom without any lesions. Thus, these plants were confirmed as collateral hosts of the pathogen.

The host range of *P. aphanidermatum* on different common weeds growing in the cowpea field was studied by artificial inoculation studies. None of the weeds artificially inoculated were found to take up the infection (Table 17).

4.7. ISOLATION AND TESTING OF RHIZOSPHERE FUNGAL ANTAGONISTS

4.7.1. Isolation and Identification of Fungal Antagonists from Rhizosphere Region of Cowpea Plant

Different rhizosphere fungi were isolated from the rhizosphere of the disease-free cowpea plants among the stem rot infected cowpea plants in the field. The details of fungal antagonists isolated are given in Table18 (Plate 22).

4.7.2. *In Vitro* Screening for Evaluating the Antagonistic Efficacy of the Fungal Isolates Against *P. aphanidermatum* in Dual Culture.

In the primary evaluation out of seven fungal isolates, three fungal isolates i.e., one *Penicillium* sp. and two *Trichoderma* sp. showed antagonistic action against *P. aphanidermatum* (Plate 23). For further confirmation, the three fungal isolates were screened again by dual culture technique on PSA. In that, *Penicillium* sp. gave a inhibition of 44% of the mycelial growth of the pathogen (Plate 24) whereas the two *Trichoderma* sp. i.e T-1 (Plate 25) and T-2 (Plate 26) showed mycelial inhibition of 42% and 47% respectively with overgrowth type of antagonistic action against the pathogen.

Hence, the *Penicillium* sp. and two *Trichoderma* sp. were selected for evaluating the efficiency of disease suppression in *in vitro* seedling assay and were morphologically characterised for species level identification.

Based on the morphological studies, the details are given in the Table 19 the isolate T-1 was identified to be *Trichoderma koningii* (Plate 27), isolate T-2 was *Trichoderma viride* (Plate 28) and *Penicillium* sp. was *Penicillium citrinum* (Plate 29).

21a. Snake gourd (*Trichosanthes anguina*)

Stem rot symptom on infected snake gourd stem

21b. Chilly (*Capsicum annuum*)

21c. Red amaranthus (*Amaranthus tricolor*)

21d. Tomato (*Lycopersicon esculentum*)

Plate 21. (21a, 21b, 21c & 21d) Host range of *P. aphanidermatum* on different vegetable crops

Table 17. Response of common weeds of cowpea field to artificial inoculation of *P. aphanidermatum*

Sl No.	Name of the weeds	Disease reaction
1.	*Amaranthus viridis*	-
2.	*Cynodon dactylon*	-
3.	*Cyperus rotundus*	-
4.	*Cleome rutidospermum*	-
5.	*Brachiaria miliformis*	-
6.	*Panicum repens*	-
+ Positive - Negative		

Table 18. List of predominant fungal isolates from the rhizosphere of cowpea plants

Sl. No.	Name of fungal isolates	No. of isolates
1.	*Aspergillus* sp.	3
2.	*Penicillium* sp.	2
3.	*Trichoderma* sp.	2

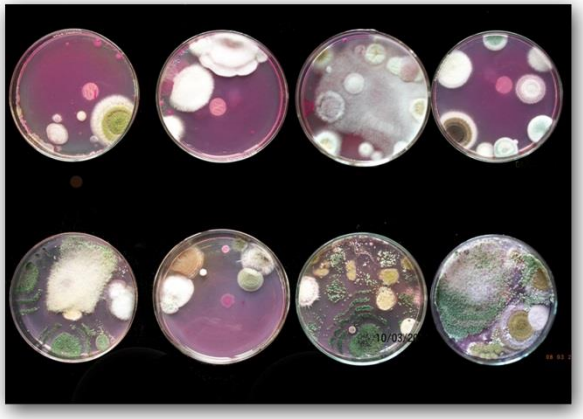


Plate 22. Fungal colonies from soil on RBA medium

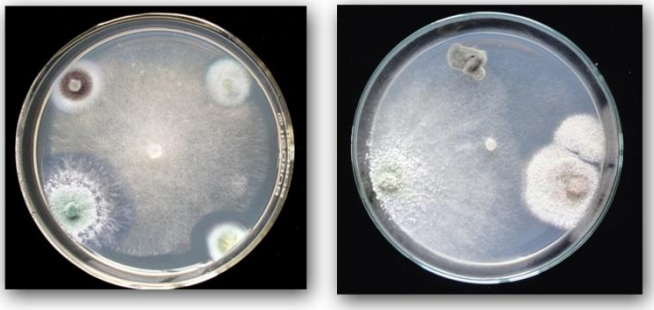


Plate 23. Primary evaluation of isolated fungi for their antagonism towards *P. aphanidermatum*

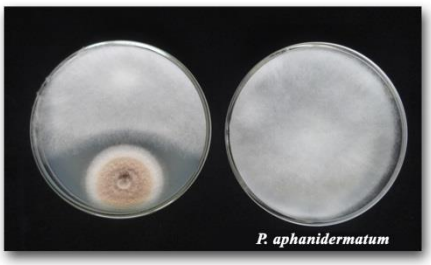


Plate 24. ***In vitro*** **antagonism of** ***Penicillium citrinum*** **against** ***P. aphanidermatum***

Plate 25. ***In vitro*** **antagonism of** ***Trichoderma koningii*** **(T-1) against** ***P. aphanidermatum***

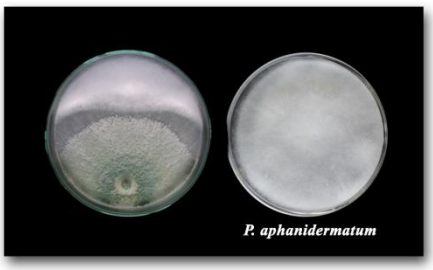
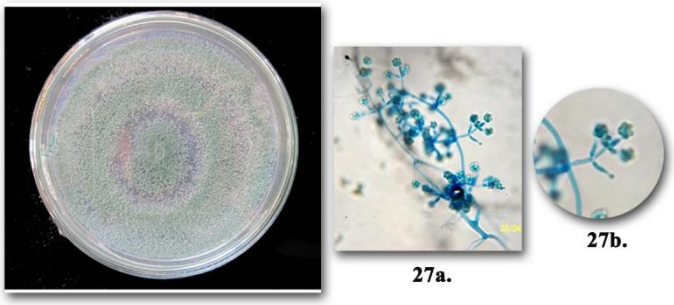


Plate 26. ***In vitro*** **antagonism of** ***Trichoderma viride*** **(T-2) against** ***P. aphanidermatum***

Table 19. Identification of fungal antagonists

Fungal antagonists	**Colony characters**	**Dimensions**
Trichoderma koningii (T-1)	Greenish white to dark green colony, conidiophores are much branched	Phialides – 7.2 – 14.4 µm (av. 9 µm) length
Trichoderma viride (T-2)	Fat growing, white initially and later greenish white mycelium.	Phialides – 7.2 – 25.2 µm (av. 14.58 µm) length
Penicillium citrinum	Yellow to brown colony, conidiophores terminating in a vertical of metulae, phialides ampulliform, conidia spheroidal, smooth.	Phialides – 7 µm length

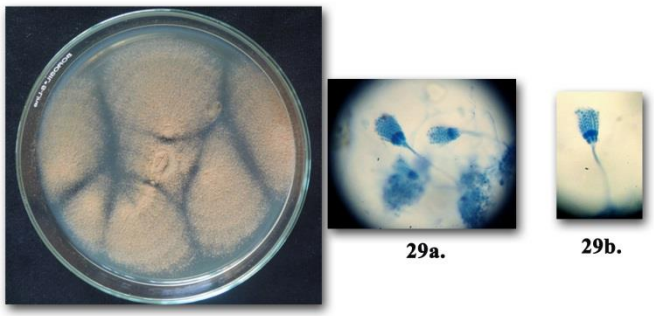


27a. 27b.

Plate 27. Colony of *Trichoderma koningii* : 27a) Conidiophores (400X) 27b) Conidiophores (400X)

28a. 28b.

Plate 28. Colony of *Trichoderma viride* : 28a) Conidiophores (400X) 28b) Conidiophores (400X)



29a. 29b.

Plate 29. Colony of *Penicillium citrinum* : 29a) Conidiophores (400X) 29b) Conidiophores (400X)

4.8. *IN VITRO* ASSAY OF DIFFERENT AGENTS

4.8.1. In *vitro* Evaluation of Bio Control Agents and Fungal Endophyte against *P. aphanidermatum.*

The fungal endophyte *P. indica* and KAU released biocontrol agents *P. fluorescens* and *Trichoderma* sp. were evaluated against *P. aphanidermatum* in *in vitro* condition by dual culture technique in PSA. The results of the study showed that the fungal endophyte *P. indica* (Plate 30) showed no inhibition of pathogen whereas the biocontrol agents *P. fluorescens* (Plate 31) and *Trichoderma* sp. (Plate 32) gave 51% and 38% inhibition of the mycelial growth of the pathogen respectively. *Trichoderma* sp. also showed overgrowth type of antagonistic action against pathogen.

4.8.2. *In Vitro* Evaluation of Organic Preparations against *P. aphanidermatum*

The *in vitro* assay of organic preparations i.e. panchagavyam and fish amino acid against *P. aphanidermatum* was done by using poisoned food technique in PSA. The result of the *in vitro* experiment showed that, panchagavyam gave 100% (Plate 33) inhibition of the growth of the pathogen at all concentrations tested whereas the fish amino acid gave 100% inhibition only at 10% concentration followed by 5% concentration (23%) (Plate 34, Table 20).

4.8.3. *In Vitro* Evaluation of Chemical Fungicides against *P. aphanidermatum*

The *in vitro* chemical management of *P. aphanidermatum* was done by using poisoned food technique in PSA. Five commercially available fungicides were used for the *in vitro* study.

The results of the *in vitro* evaluation of fungicides revealed that at the field concentration, three chemicals viz., copper oxychloride, metalaxyl MZ and copper hydroxide gave 100% inhibition to the growth of the pathogen under *in vitro* culture conditions and the assay differed significantly from other followed by iprovalicarb 5.5% + propineb 61.25% (88.3%) (Table 21, Plate 35). Even at the lowest concentration (0.1%), the fungicides copper oxychloride and metalaxyl MZ gave 100% inhibition of the pathogen growth. The fungicide azoxystrobin was found ineffective in checking the growth of the pathogen *in vitro*.

Plate 30. ***In vitro*** **antagonism of** ***P. indica*** **against** ***P. aphanidermatum***

Plate 31. ***In vitro*** **antagonism of** ***P. fluorescens*** **against** ***P. aphanidermatum***

Plate 32. ***In vitro*** **antagonism of** ***Trichoderma*** **sp. against** ***P. aphanidermatum***

Table 20. *In vitro* evaluation of organic preparations against *P. aphanidermatum*

Organic preparations	**Concentrations (%)**	**Colony diameter of *P. aphanidermatum* (cm)* (% inhibition)**
Panchagavyam	2.5	0 (100%)
	5	0 (100%)
	10	0 (100%)
Fish amino acid	2.5	9 (0%)
	5	6.9 (23.3%)
	10	0 (100%)
* Mean of four replications Observations taken 48h after inoculation.		

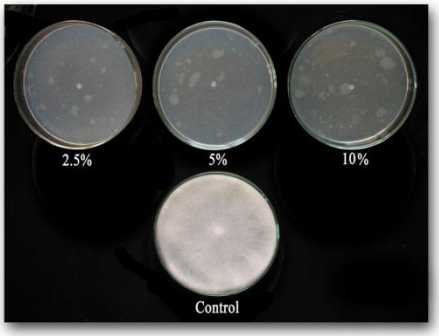


Plate 33. Effect of panchgavyam on radial growth of *P. aphanidermatum*

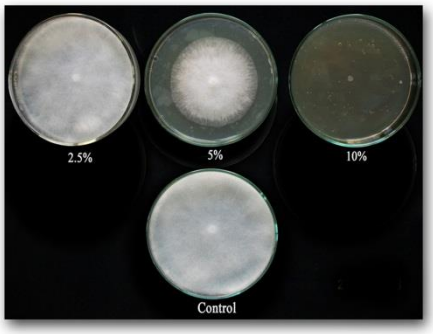


Plate 34. Effect of fish amino acid on radial growth of *P. aphanidermatum*

Table 21. In vitro evaluation of chemical fungicides on the mycelial growth of *P. aphanidermatum*

Fungicides (concentrations)	**Percentage mycelial inhibition of P. aphanidermatum (cm)***		
	C-1 (0.1%)	**C-2 (0.2%)**	**C-3 (0.3%)**
Copper oxychloride (3.0,2.0,1.0 g/L)	100.00(90.00)[a]	100.00(90.00) [a]	100.00(90.00) [a]
Metalaxyl MZ 68%WP (3.0,2.0,1.0 g/L)	100.00(90.00) [a]	100.00(90.00) [a]	100.00(90.00) [a]
Copper hydroxide 77% WP (3.0,2.0,1.0 g/L)	87.99(69.73)[b]	100.00(90.00) [a]	100.00(90.00) [a]
Iprovalicarb 5.5% + Propineb 61.25% (3.0,2.0,1.0 g/L)	80.43(63.75)[c]	88.30(69.98)[b]	53.00(46.75)[b]
Azoxystrobin 23% SC (1.0. 1.5, 2.0 ml/L) (125 g a.i /500L)	**0.1%**	**0.15%**	**0.2%**
	0.00(0.00)[d]	0.00(0.00)[c]	0.00(0.00)[c]
Control	0.00(0.00)[d]	0.00(0.00)[c]	0.00(0.00)[c]
CD(0.05)	(2.56)	(0.87)	(0.77)

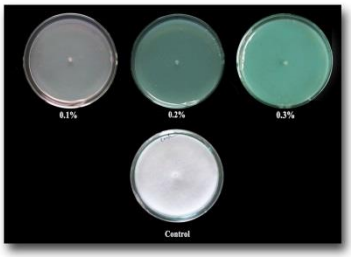
*Mean of four replications

Values in parenthesis are arcsine transformed

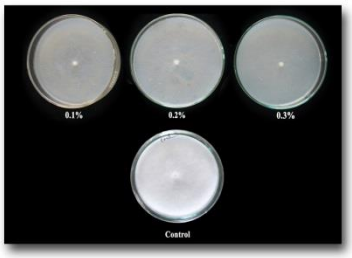
Observations taken 48h after inoculation

Figures followed by the same letter do not differ significantly according to one way ANOVA at P=0.05.

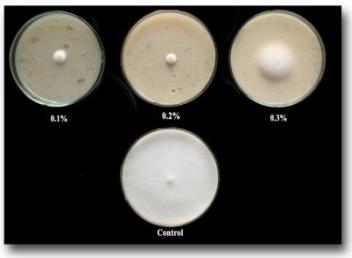
35a.



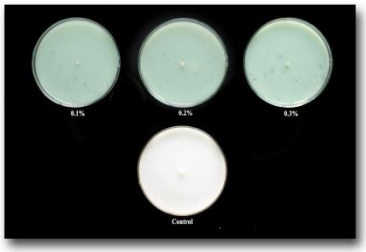
35b.



35c.



35d.



35e.

Plate 35. Effect of different levels of different fungicides on radial growth of *P. aphanidermatum* : 35a) metalaxyl MZ 68% 35b) copper hydroxide 77% 35c) azoxystrobin 23% 35d) iprovalicarb 5.5%+propineb 61.25% 35e) copper oxychloride 50%

4.8.4. *In Vitro* Evaluation of Different Agents for the Control of Disease on Seedlings.

The three fungal antagonists obtained during the study, *viz Penicillium citrinum, Trichoderma koningii* and *Trichoderma viride*, the fungal endophyte *Piriformospora indica,* organic amendments neemcake, vermicompost, coirpith compost and spent mushroom substrate (sms) were evaluated for the disease suppression at seedling stage. The organic preparations, panchagavyam and fish amino acid, the KAU released biocontrol cultures of *P. fluorescens* and *Trichoderma* sp. and five commercially available fungicides such as metalaxyl MZ, azoxystrobin, iprovalicarb + propineb, cuprous hydroxide and copper oxychloride were assessed for the management of the disease on seedlings at the recommended concentration.

The results of the experiment revealed that the percentage seedling mortality was lowest for the three fungicide treatments i.e., copper oxychloride (3.01 %)and metalaxyl MZ (3.01 %) and azoxystrobin (11.69 %) which were on par with KAU released biocontrol agent *Trichoderma* sp. (25 %) and were significantly superior over all other treatments followed by the fungicide iprovalicarb + propineb(41.31 %), KAU released biocontrol agent *P. fluorescens* (50 %), fungicide copper hydroxide(58.68 %), organic preparations i.e., panchagavyam (58.68 %) and fish amino acid (58.68 %). The fungal antagonist i.e. T-2 (67.1%), T-1 (82.13%) and *Penicillium citrinum* (75%), fungal endophyte *Piriformospora indica*(88.3%)and organic amendment coirpith (93.3%) gave more than 60% seedling mortality while seedling mortality was 100% in organic amendments such as neemcake, spend mushroom substrate (sms) and vermicompost (Table 22, Plate 36A, Plate 36B, Plate 36C).

From the above observations, the treatments which gave less than 60 % seedling mortality were selected for the field evaluation.

4.9. FIELD EVALUATION OF SELECTED TREATMENTS

The promising treatments selected from the *in vitro* seedling assay, such as two organic preparations, fish amino acid and panchagavyam; two talc based formulation of biocontrol agents, *P. fluorescens* and *Trichoderma* sp. released by

Table 22. *In vitro* evaluation of different bioagents, organic amendments, organic preparations and chemical fungicides for the control of stem rot on seedlings

Sl. No.	Treatments	Percentage seedling mortality (%)*
1.	Vermicompost (5g/cup)	100.00 (90.00)^{gh}
2.	Coirpith compost (5g/cup)	93.39(75.00)^{fgh}
3.	Neem cake (5g/cup)	100.00(90.00)^{h}
4.	Spent mushroom substrate (sms) (5g/cup)	100.00 (90.00)^{h}
5.	*Trichoderma* sp. (T-1)	82.13 (65.00)^{defgh}
6.	*Trichoderma* sp. (T-2)	67.10 (55.00)^{cdefg}
7.	*Penicillium* sp.	75.00 (60.00)^{defg}
8.	Fungal endophyte (*Piriformospora indica)*	88.30 (70.00)^{efgh}
9.	Panchagavyam (5%)	58.68 (50.00)^{cdef}
10.	Fish amino acid (5%)	58.68 (50.00)^{cdef}
11.	Talc based formulation of *Pseudomonas fluorescens* (2%)	50.00 (45.00)^{bcde}
12.	Talc based formulation of *Trichoderma* sp. (2%)	25.00 (30.00)^{abc}
13.	Metalaxyl MZ 68%WP (0.2%) (1700 g a.i/ 1000 L)	3.01 (10.00)^{a}
14.	Azoxystrobin (0.15%) (125 g a.i /500L)	11.69 (20.00)^{ab}

Table 22 continued..

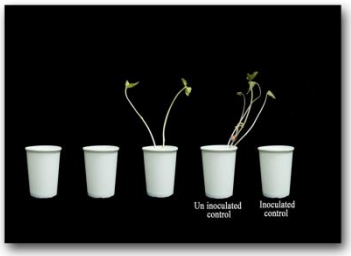
Sl. No.	Treatments	Percentage seedling mortality (%)
15.	Iprovalicarb + Propineb (0.2%) (1500g a.i / 500L)	41.31 (40.00)^{bcd}
16.	Copper hydroxide (0.2%) (1000g a.i/ 750 L)	58. 68 (50.00)^{cdef}
17.	Copper oxychloride (0.2%) (1000g a.i/500 L)	3.01 (10.00)^{a}
18	Control (inoculated)	100.00 (90.00)^{h}
19	Control (un inoculated)	0.00 (0.00)
	SE(±)	9.05
	CD(0.05)	(25.98)

*Mean of four replications

Values in parenthesis are arcsine transformed.

Observations taken 10 days after inoculation.

Figures followed by the same letter do not differ significantly according to one way ANOVA at P=0.05.

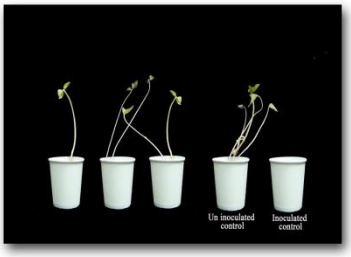


36A(a) Coirpith compost

36A(b) Neem cake

36A(c) Spend mushroom substrate

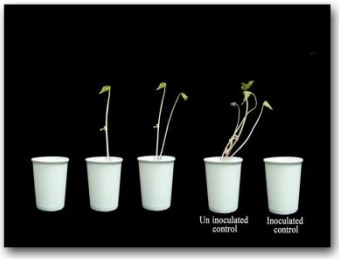
36A(d) Vermi compost



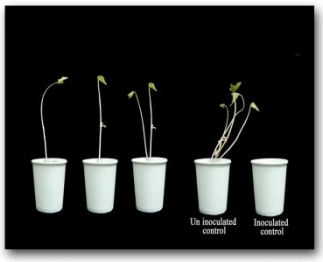
36A(e) Fish amino acid

36A(f) Panchgavyam

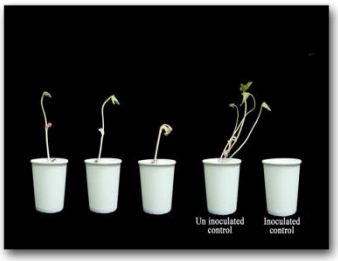
Plate 36 A. (a, b, c, d, e & f) *In vitro* evaluation of organic amendments and organic preparations for the suppression of stem rot of cowpea seedlings



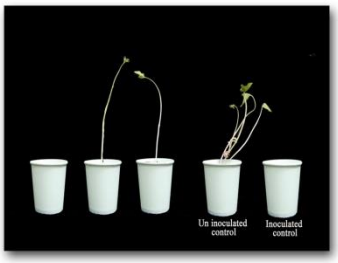
36B(a) Trichoderma koningii (T-1)



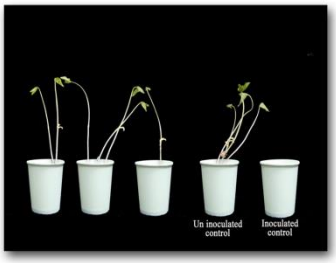
36B(b) *Trichoderma viride* (T-2)



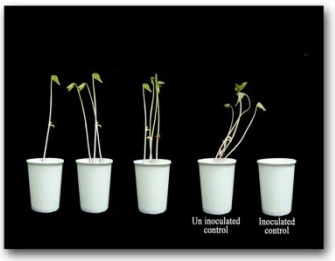
36B(c) *Penicillium citrinum*



36B(d) *Piriformospora indica*



36B(e) *Pseudomonas fluorescens*

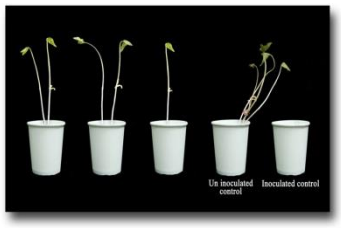


36B(f) *Trichoderma harzianum*

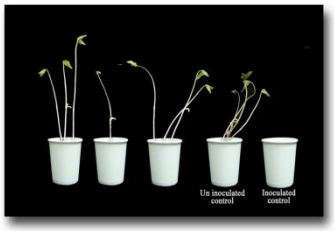
Plate 36 B. (a, b, c, d, e & f) *In vitro* evaluation of fungal antagonists, bio agents and fungal endophyte for the managemnt of stem rot of cowpea seedlings

36C(a) Azoxystrobin

36C(b) Copper oxychloride



36C(c) Copper hydroxide



36C(d) Iprovalicarb+propineb

36C(e) Metalaxyl MZ

Plate 36 C. (a, b, c, d & e) *In vitro* evaluation of fungicides for the control of stem rot of cowpea seedlings

KAU and five chemical fungicides, metalaxyl MZ, azoxystrobin, iprovalicarb + propineb, cuprous hydroxide and copper oxychloride were tested under field conditions (Plate 37).

Results of the field experiment revealed that metalaxyl MZ (4.32 %) and azoxystrobin (4.32%) (Plate 38) followed by fungicide copper oxychloride (9.5 %) and fish amino acid (9.5%) recorded the lowest incidence of the disease in term of Percentage Disease Incidence, which were on par with KAU released bioagents *Trichoderma* sp. (12.84 %) and *P. fluorescens* (16.54 %) followed by panchagavyam (20.61 %) (Table 23). The highest percentage of disease incidence was observed in the untreated control (79.38 %).

On comparison of the average pod yield per plant obtained from each treatments, it was found that the maximum yield was obtained from the treatment azoxystrobin (1003.2 g/ plant) which was on par with all other treatments such as fish amino acid (960.6 g/plant), copper hydroxide (943.26 g/plant), metalaxyl MZ (933.7 g/plant), *P. fluorescens* (916.3 g/plant) and copper oxychloride (914.7 g/plant) (Table 24).

The highest vine length was obtained in the treatment, azoxystrobin (557.75 cm) which was significantly superior to all other treatments (Table 24). Vine length of all other treatment were on par.

When the average plant fresh weight of each treatments were compared; it was found that maximum plant fresh weight was obtained with treatment azoxystrobin (439.25 g) which was on par with all other treatments. A similar trend was noticed with respect to average plant dry weight (Table 24).

Comparison of the average root length and root weight of the respective treatments showed the maximum root length of root was obtained with the treatment azoxystrobin (42.92 cm) (Table 24) which was on par with all other treatments followed by panchagavyam (39.48 cm). In the case of average root weight, it was found that the highest root weight was obtained with the treatment azoxystrobin (40.69 g) which was on par with the all other treatments.

When the average number of leaves per plant of each treatments were compared; it was found that maximum number of leaves were observed with the

Table 23 Field evaluation of selected organic preparations, biocontrol agents and chemical fungicides for management of stem rot of cowpea.

Sl. No.	Treatments	Percentage Disease Incidence (%)*
T1	Fish amino acid	9.55(18.00)[ab]
T2	Panchagavyam	20.61 (27.00)[abc]
T3	Talc based formulation of *Pseudomonas fluorescens*	16.54 (24.00) [abc]
T4	Talc based formulation of *Trichoderma* sp.	12.84 (21.00)[abc]
T5	Metalaxyl MZ 68%WP (1700 g a.i/ 1000 L)	4.32 (12.00) [a]
T6	Azoxystrobin (125 g a.i /500L)	4.32 (12.00)[a]
T7	Iprovalicarb + Propineb (1500 g a.i / 500L)	34.54 (36.00) [bc]
T8	Copper hydroxide 77% WP (1000 g a.i/ 750 L)	39.60 (39.00) [c]
T9	Copper oxychloride 50 WP (1000 g a.i/500 L)	9.55 (12.00)[ab]
T10	Control (without any treatment)	79.38 (63)[d]
	SE(±)	6.61
	CD(0.05)	(18.98)

*Mean of four replications

Values in parenthesis are arcsine transformed.

Observations taken 10 days after inoculation.

Figures followed by the same letter do not differ significantly according to one way ANOVA at P=0.05.

Plate 37. General view of the experimental plot

Plate 38. Field view of cowpea plants treated with azoxystrobin

Table 24. Biometric observations of different treatments of cowpea during field experiment

Sl. No.	Treatments	Pod yield per plant(g)	Vine length (cm)	Plant fresh weight (g)	Plant dry weight (g)	Root length (cm)	Root weight (g)	No. of leaves per plant
T1	Fish amino acid	960.63	477.75[b]	414.25	97.39	41.25	35.65	282 [b]
T2	Talc based formulation of *Pseudomonas fluorescens*	916.39	452.5 [b]	375.25	84.99	40.75	37.37	249.25 [b]
T3	Metalaxyl MZ	933.72	458.75 [b]	365.25	84.91	33.10	35.97	265.00[b]
T4	Azoxystrobin	1003.21	557.75[a]	439.25	101.05	42.92	40.69	374.00[a]
T5	Copper hydroxide 77% WP	943.28	464.25 [b]	420.50	107.80	37.62	35.45	270.25 [b]
T6	Copper oxychloride	914.75	466.00 [b]	424.00	103.84	37.07	35.71	257.25 [b]
T7	Talc based formulation of *Trichoderma harzianum*	923.42	444.75 [b]	367.50	85.67	39.26	36.96	257.00[b]
T8	Iprovalicarb + Propineb	911.36	432.25 [b]	359.50	86.01	37.78	35.45	260.00[b]
T9	Panchagavyam	949.67	457.50 [b]	403.75	91.87	39.48	40.12	289.50[b]
T10	Control (without any treatment)	880.71	469.5 [b]	399.25	98.49	38.20	35.61	262.25 [b]
	CD (0.05)	**NS**	60.81	**NS**	**NS**	**NS**	**NS**	41.78

*Figures followed by same letter do not differ significantly according to two way ANOVA at P=0.05

treatment azoxystrobin (374) (Table 24) which was significantly superior over all other treatments followed by the organic preparations panchagavyam (289) and fish amino acid (282) which was on par with all other treatments.

Discussion

5. DISCUSSION

The present study on the 'Integrated management of Pythium stem rot of vegetable cowpea (*Vigna unguiculata* sub.sp. *sesquipedalis* (L.) Verdcourt) was conducted during the period 2012-2014 at the Department of Plant Pathology, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala. The salient results are discussed here under with the relevant literature available. Williams and Ayanaba (1975) have reported *Pythium aphanidermatum* as a serious pathogen affecting cowpea which is widely cultivated in Nigeria.

Eight isolates of the pathogen, *P. aphanidermatum,* causing the stem rot disease in vegetable cowpea were isolated from different cowpea growing areas of Thiruvanathapuram district. Onuorah (1973) reported the occurrence of *P. aphanidermatum* from Nigeria as the causal organism of cowpea seed decay and severe stem rot. Suleiman (2010) investigated the occurrence, distribution and pathogenicity of *P. aphanidermatum* on cowpea causing root rot from Nigeria.

Pythium stem rot of vegetable cowpea causes wilting of entire leaves followed by wet stem rotting. The initial appearance of water soaked lesions at basal stem portion gradually spreading to aerial stem, lower branches and roots. As a result, the affected plant collapse one or two days after the initial symptom expression. Onuorah (1973) has reported light brown water soaked lesion on the hypocotyls of the affected seedlings causing death of the seedlings, whereas, in the old plants he observed pale green lesion girdling the stem which extend upwards leading to collapse of the entire plant.

As observed in the present study, Onuorah (1973) also observed a copious white cottony growth of mycelium girdling the stem. Williams and Ayanaba (1975) also reported the symptoms of wet stem rot of cowpea from Nigeria as a grey green water soaked girdling of the stem extending from the soil level up to the lower portion of the lower branches and the infected plant quickly wilt and die. Suleiman (2010) has observed wilting of cowpea plants infected with *P. aphanidermatum*, when the leaves are still green with shrinking of the stem at or near the soil surface.

The pathogenicity of *P. aphanidermatum* causing stem rot of vegetable cowpea was proven by inoculating pathogen on germinating seeds resulting in seedling decay and death. Suleiman (2010) also proven pathogenicity *P. aphanidermatum* causing root rot of cowpea by inoculating on potted seedlings by inoculating disease free seeds with pathogen mycelial suspension. In most of the work done the pathogenicity of *P. aphanidermatum* has been proven in seedlings (Adandonon *et al.*, 2004; EI-Mohamedy and El- Mougy, 2009; Al-Sheikh and Abdelzaher, 2012).

The cultural and morphological characters of the isolate P2 used in the study were similar to the characters of *Pythium aphanidermatum* as described by Van Der Plaats-Niterink (1981). Therefore, the pathogen causing stem rot disease of vegetable cowpea was identified as *Pythium aphanidermatum* (Edson) Fitzp. Ali-Shtayeh (1986) has clearly described *P. aphanidermatum* from the soils of West Bank and Gaza Strip, Israel, taking into consideration factors including the nature of hyphae, antheridia, oogonia, oospores, colony morphology etc. which is in consonance with the results of this study. Mostowfizadeh-Ghalamfarsa and Banihashemi (2005) have identified and described *P. aphanidermatum* from the soils of Fars province of Iran after studying the temperature requirement, and other morphological characters of the fungus and the results of this study are in line with their results. Al Sheikh and Abdelzaher (2012) have also studied the occurrence of *Pythium* spp. in Saudi Arabia and have identified 72 isolates of *P. aphanidermatum* causing diseases in maize, potato, tomato and wheat by studying their cultural and morphological characters which also agree with the findings of this study.

In the present study, PSA medium was found to be the best solid medium to support the growth of *P. aphanidermatum* which was significantly superior from all other media, followed by PDA and the fungus exhibited reduced growth on CDA (Fig 1). Suleiman *et al*. (2011) tried the suitability in PDA, Czapek-Dox medium (CDA) and malt extract agar for growing *P. aphanidermatum* and observed that PDA was the best medium for the fungus which is in line with the results of the present study. Similar observation was also reported by Muthukumar

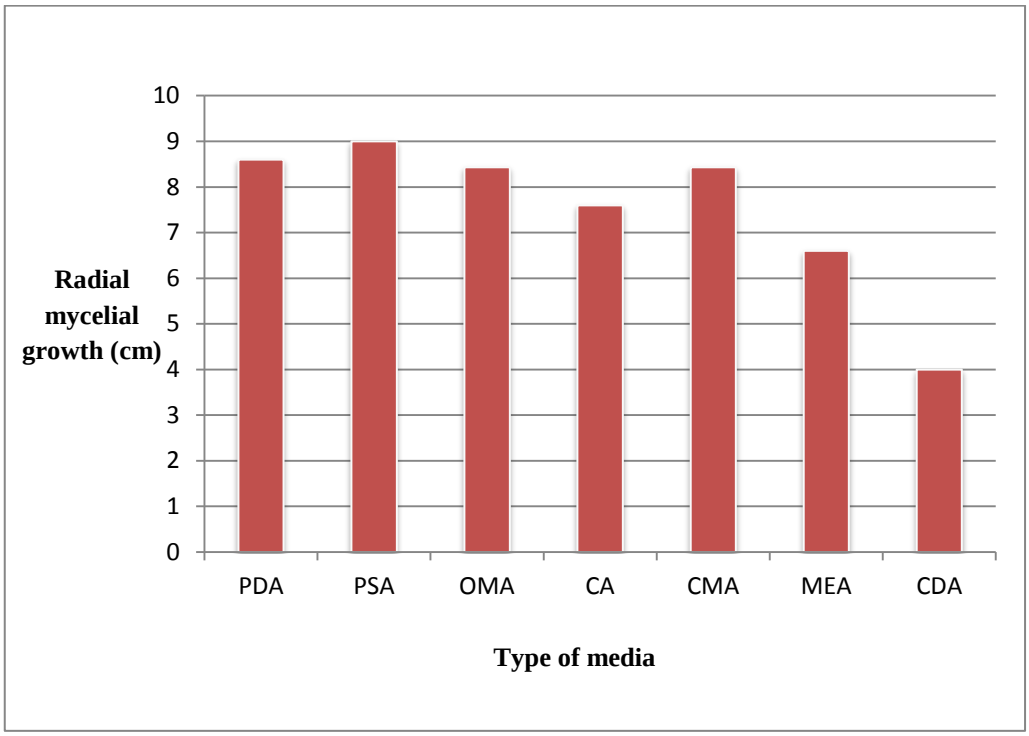


Fig 1. Colony diameter of *P. aphanidermatum* on different solid media 24 h. after inoculation

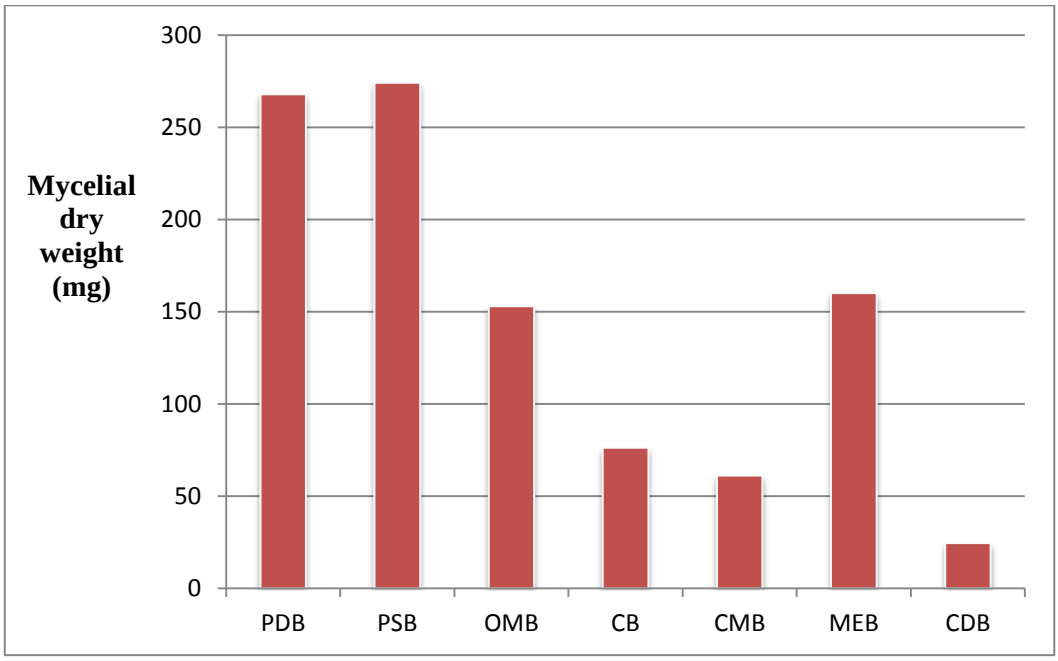


Fig 2. Mycelial dry mat weight of *P. aphanidermatum* on different liquid media 10 days after inoculation

and Eswaran (2008) when they tried suitability on potato dextrose agar, oat meal agar, Richard's agar, Czapek-Dox agar, corn meal agar and yeast extract glucose agar for culturing of *P. aphanidermatum.*

Results of the present study revealed that maximum dry weight of mycelial mat was produced on PSB which was on par with that obtained on PDB while the least weight of mycelial dry mat was obtained on CDB (Fig 2). Muthukumar amd Eswaran (2008) have observed that from among PDB, Oat meal broth, Richards broth, Yeast extract glucose broth and Corn meal broth, PDB recorded the highest mycelial weight as observed in the present study.

The radial growth of the pathogen was found to be maximum at 40^0C which was on par with growth at 35^0C followed by the growth at room temperature (8.5 cm) after 24 h of inoculation (Fig 3). A temperature range of 35 – 40^0C has been reported to be conducive for the radial growth of *P. aphanidermatum* (Van Der Plaats-Niterink, 1981).

The pathogen was also grown in Potato Sucrose Broth at different levels of temperature and the results of the experiment showed that maximum dry weight of the mycelial mat was at 35^0C which was on par with mycelial dry weight at room temperature after 10 days of inoculation (Fig 4).

Earlier studies have indicated that a temperature of 28^0C (Muthusamy, 1972; Thiruvudainambi, 1993; Muthukumar amd Eswaran, 2008) and 35^0C (Lumsden *et al*., 1975) were bests suited for the mycelial growth of the fungus whereas Mostowfizadeh-Ghalamfarsa and Banihashemi (2005) and Al- Sheikh and Abdelzaher (2012) have reported that a temperature range between 30-40^0C was the optimum for the mycelial growth of *P. aphanidermatum* from Iran and Saudi Arabia respectively.

The pathogen was found to give maximum radial growth at pH 6 which was on par with the growth obtained in pH 7 and pH 8 (Fig 5). This finding is in line with observations made by earlier workers (Lumsden *et al*., 1975, Gul *et al*., 2005) whereas, Al-Sheik and Abdelzaher (2012) have observed that a pH range of 5-9 was most suited for oospore production of the six isolates of *P. aphanidermatum*.

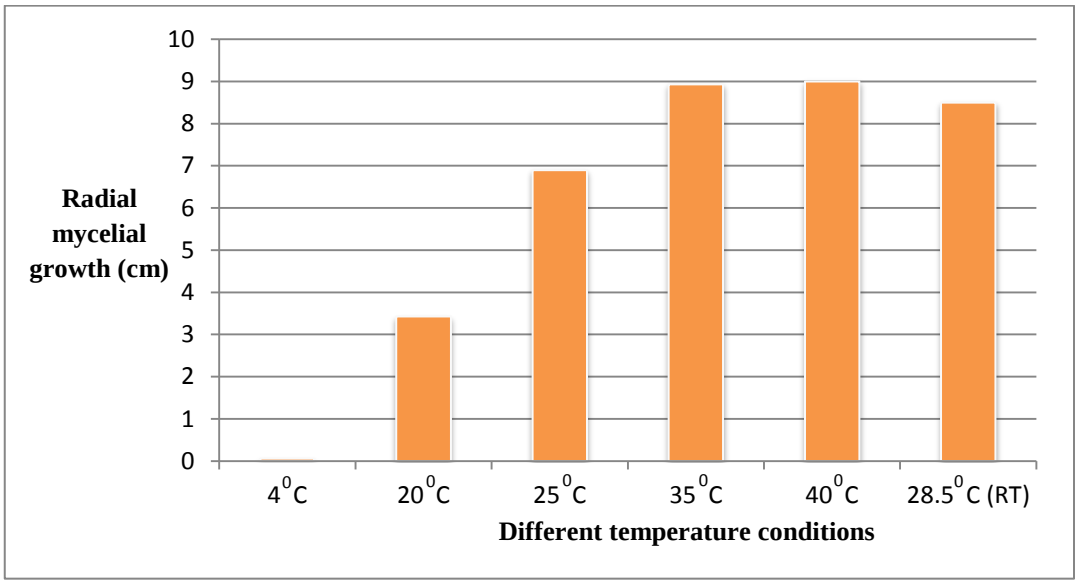


Fig 3. Colony diameter of *P. aphanidermatum* at different temperature conditions in solid media 24 h. after inoculation

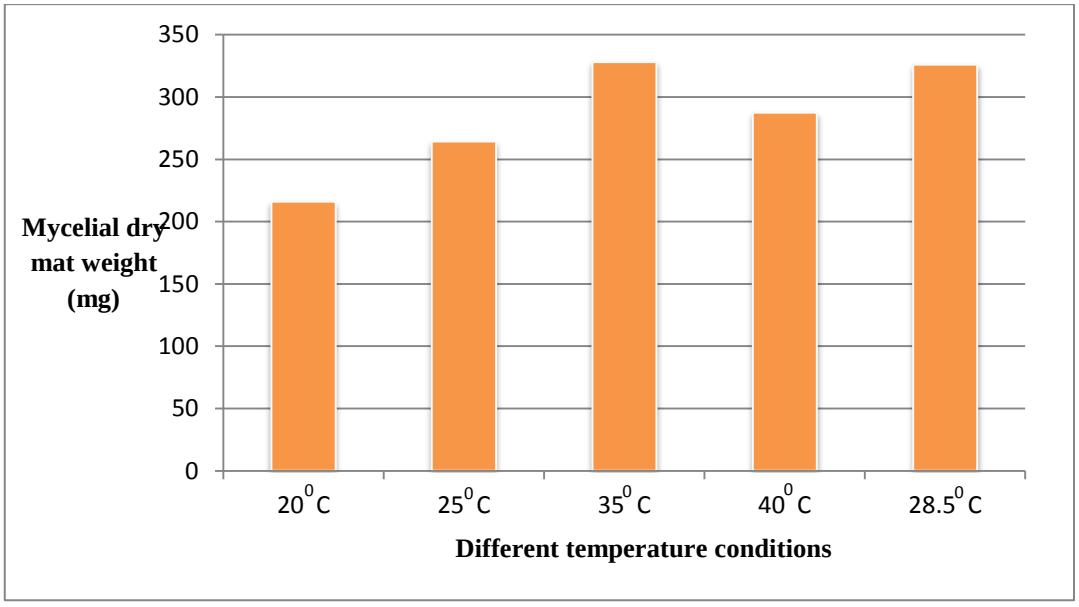


Fig 4. Mycelial dry mat weight of *P. aphanidermatum* at different temperature conditions 10 days after inoculation

The maximum dry weight of mycelial mat was observed at pH 5.5 which was significantly different from all other treatments, and followed by dry weight at pH 4.5 and pH 6.5 (Fig 6). Maheswari and Sirchabai (2011) opined that the optimum pH for the fungus was 6.

The pathogen was found to be showing maximum growth at 24 h in darkness which was significantly different from the other two conditions of light studied (Fig 7, Fig 8). Rizvi and Yang (1996) and Teymoori *et al*. (2012) have reported incubation of plates in darkness to support the growth of *P. aphanidermatum.*

After 15 weeks, no recovery of the pathogen was observed on the PSA after isolation from the buried infected cowpea stem portion. Microscopic studies revealed that oospores are the main survival propagules that help in the survival of *P. aphanidermatum* on crop debris. Hall *et al*. (1980) have also observed that oospores were the important survival structures for the pathogen and that the viability of the fungus lasted for 15 months under temperate conditions on Golf course turfs. This could be due to the variation in the environmental conditions in the tropics and the temperate regions. Burr and Stanghellini (1973) and Allen *et al.* (2004) have also opined that oospores are the main survival structures in the crop debris for *P. aphanidermatum.*

Analysis of the meteorological data revealed that percentage disease incidence of stem rot disease was significantly negatively correlated with the maximum temperature and soil temperature at 20 cm depth from 2 weeks to harvesting stage of the crop. The disease was found to occur in a serious manner when the temperature ranged between 25 and 30^0C.

Kuo and Hsieh (1991) observed that the disease caused by *Pythium* spp. was severe during summer when the temperature ranged between 23-32^0C in China. Ho (2009) has reported the severe incidence of *P. aphanidermatum* when the temperature was higher than 24^0C. Kucharek and Mitchell (2000) have observed the increased incidence of Pythium on vegetables crops in Florida at a temperature range of 30-36^0C. Therefore the temperature regime observed in this study is in consonance with earlier studies.

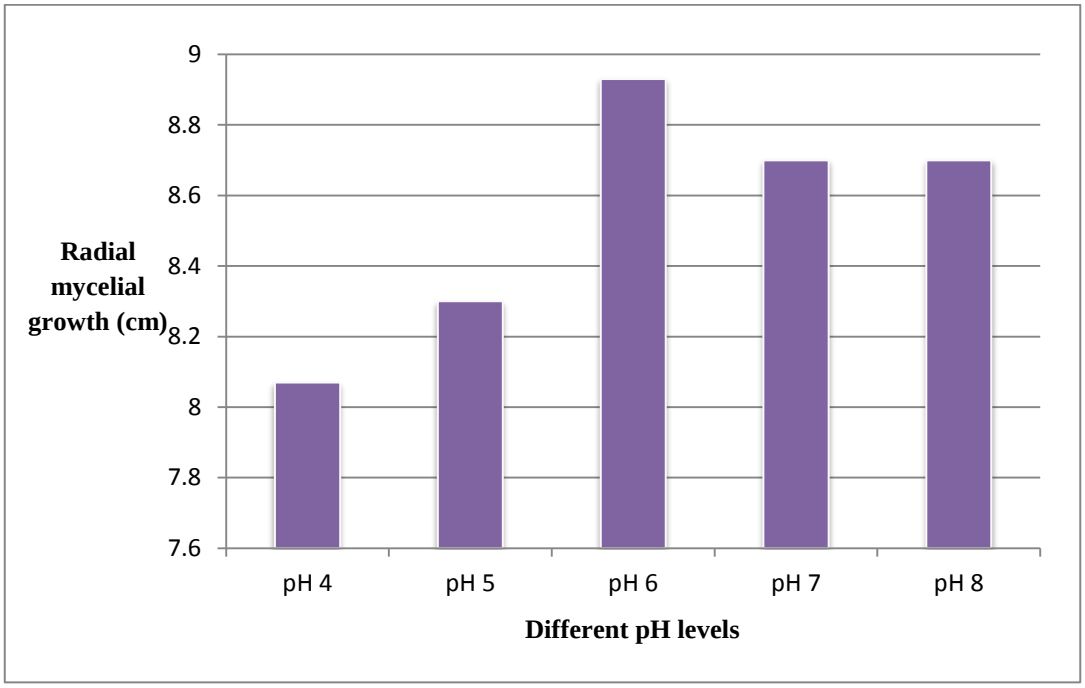


Fig 5. Colony diameter of *P. aphanidermatum* at different pH levels in solid media 24 h. after inoculation

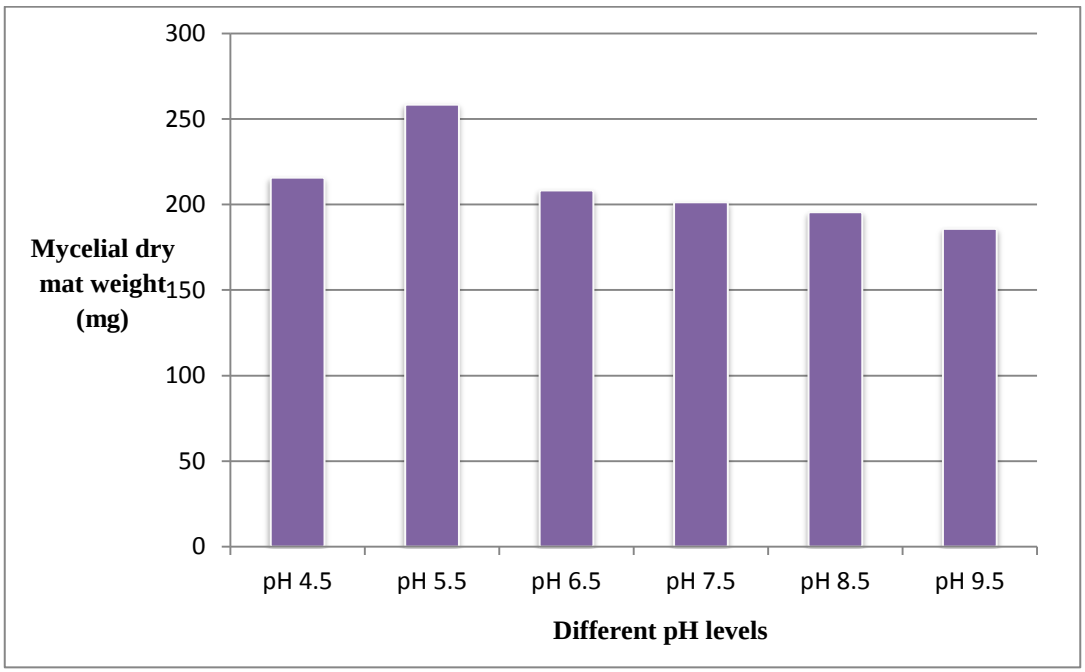


Fig 6. Mycelial dry mat weight of *P. aphanidermatum* at different pH levels at 10 days after inoculation

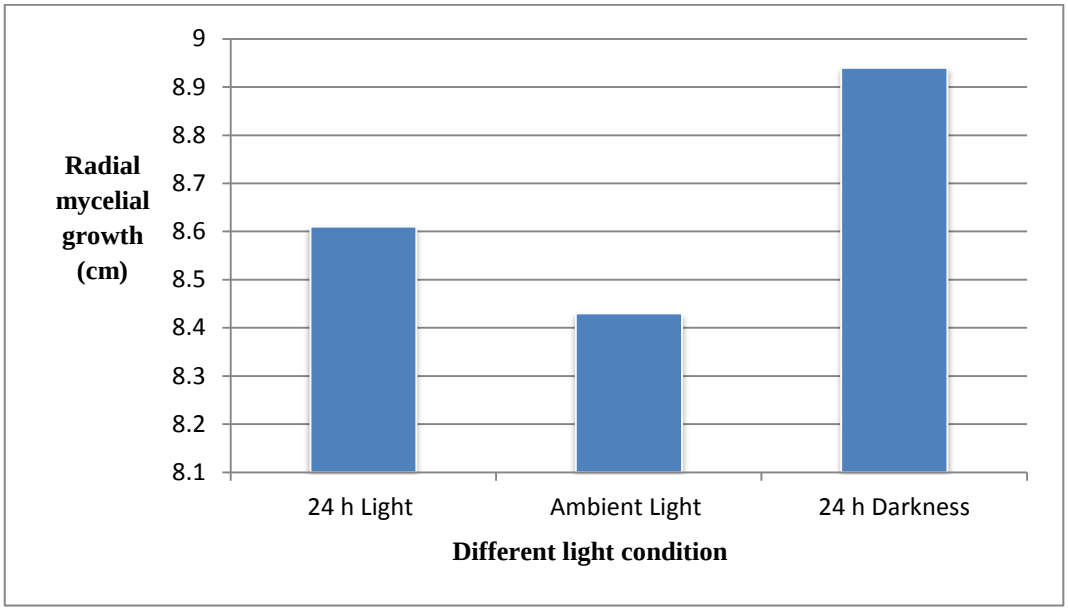


Fig 7. Colony diameter of *P. aphanidermatum* at different light conditions in solid media 24 h. after inoculation

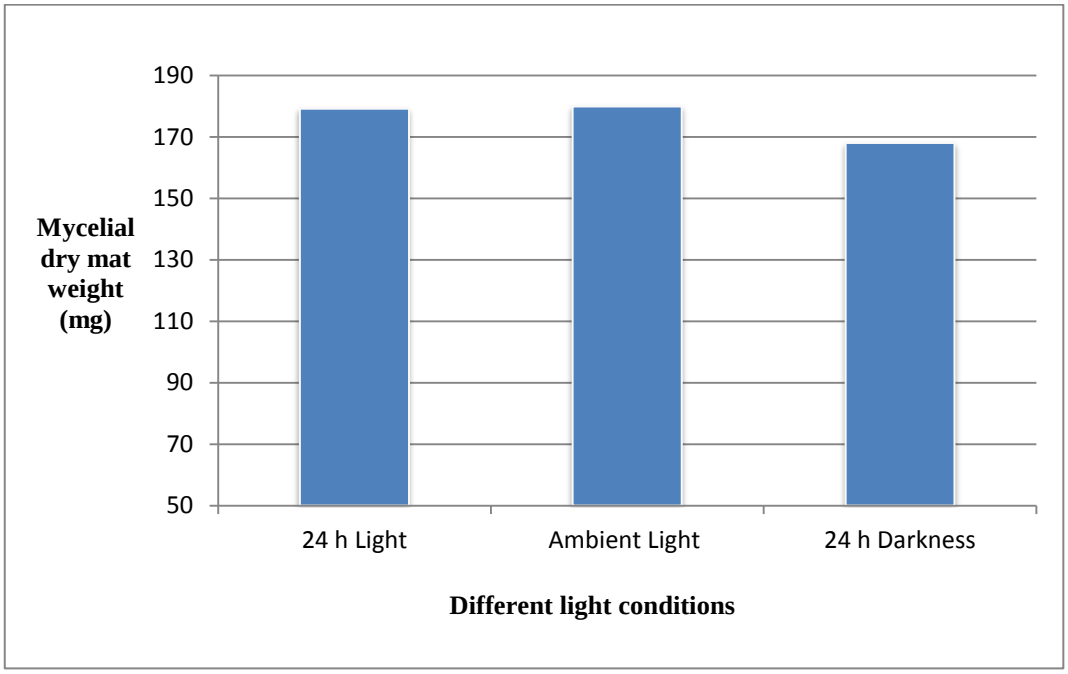


Fig 8. Mycelial dry mat weight of *P. aphanidermatum* at different light conditions 10 days after inoculation

P. aphanidermatum was recovered from stem of seedling and mature snake gourd (*Trichosanthes anguina*), seedlings of chilli (*Capsicum annuum*), tomato (*Lycopersicon esculentum*) and red amaranthus (*Amaranthus tricolor*) with similar rotting symptom without any lesions. Thus, these plants were confirmed as collateral hosts of the pathogen. Rahimian and Banihashemi (1979) have reported the occurrence of *P. aphanidermatum* on cucurbits causing root rot from Iran. Muthukumar *et al*. (2011) reported post emergence damping off of chilli from India and Suleiman (2010) has reported the fungus causing root rot on tomato from Nigeria.

Different rhizosphere fungi were isolated from the rhizosphere of the disease-free cowpea plants among the stem rot infected cowpea plants in the field. Three fungal isolates i.e., *Penicillium citrinum, Trichoderma koningii and T. viride* showed antagonistic action against *P. aphanidermatum*. When these three fungal isolates were screened again by dual culture technique on PSA the *Penicillium citrinum* gave a inhibition of 44% of the mycelial growth of the pathogen and the two *Trichoderma* spp. showed a radial mycelial inhibition of 42% and 47% respectively with overgrowth also against the pathogen. Patil *et al*. (2012) have also observed the broad spectrum inhibition of *Trichoderma* spp. against *P. aphanidermatum.*

The biocontrol agent *Pseudomonas fluorescens* 51% inhibition of the mycelial growth of the pathogen. Nwaga and co-workers (2007) and Sivakumar *et al.* (2012) have also reported the antagonistic activity of *P. fluorescens* against *Pythium* spp.

The *in vitro* assay of organic preparations i.e. pachagavyam and fish amino acid against *P. aphanidermatum* showed that, panchagavyam gave 100% inhibition of the growth of the pathogen at all concentrations tested. Kumar *et al*. (2010) reported that panchagavyam effectively suppressed the growth of *P. aphanidermatum in vitro*.

The results of the *in vitro* evaluation of fungicides indicated that at the field concentration, three chemicals viz., copper oxychloride, metalaxyl MZ and copper hydroxide gave 100% inhibition to the growth of the pathogen. Even at the

lowest concentration (0.1%), the fungicides copper oxychloride and metalaxyl MZ gave 100% inhibition of the pathogen growth whereas the fungicide azoxystrobin was found ineffective in checking the growth of the pathogen *in vitro*. Suleiman (2011) has reported the fungicidal property of mancozeb against *P. aphanidermatum* inhibiting the growth of the fungus in vitro at 150ppm and 200ppm active ingredient. Sensitivity of *Pythium* spp. to azoxystrobin was much lower than many other fungicides tested and this has been attributed to the alternative respiratory pathway that can interfere with the activity of the fungicide (Wheeler *et al*., 2005, Stevenson *et al*., 2004).

The percentage seedling mortality was lowest for the three fungicides treatments i.e., copper oxychloride, metalaxyl MZ and azoxystrobin which were on par with KAU released biocontrol agent *Trichoderma* sp. and were significantly superior over all other treatments followed by the fungicide Iprovalicarb + Propineb, KAU released biocontrol agent *P. fluorescens*, fungicide copper hydroxide, organic preparations i.e., panchagavyam and fish amino acid. Boughalleb *et al*. (2006) has reported the efficacy of Ridomil gold MZ 68 in reducing the *in vitro* growth of *Phytophthora cactorum.* Yadav and Joshi (2012) have observed the inhibition of *P. aphanidermatum in vitro* by the fungicides, Ridomil MZ (200, 300 and 400 ig $ml^{-1)}$ and copper oxychloride (2000, 3000 and 4000 ig ml^{-1}).

The promising treatments selected from the *in vitro* seedling assay, fish amino acid and panchagavyam; two talc based formulation of biocontrol agents, *Pseudomonas fluorescens* and *Trichoderma* sp. released by KAU and five chemical fungicides, metalaxyl MZ, azoxystrobin, iprovalicarb + propineb, cuprous hydroxide and copper oxychloride were tested under field conditions.

Metalaxyl MZ (84.61% disease reduction) and azoxystrobin (84.61% disease reduction) followed by copper oxychloride (76.92 % disease reduction) and fish amino acid recorded the lowest incidence of the disease in term of percentage disease incidence, which was on par with KAU released bioagents *Trichoderma* sp. and *P. fluorescens* followed by panchagavyam (Fig 9). Allen *et al.* (2004) have reported the efficacy of *T. harzianum* in controlling Pythium

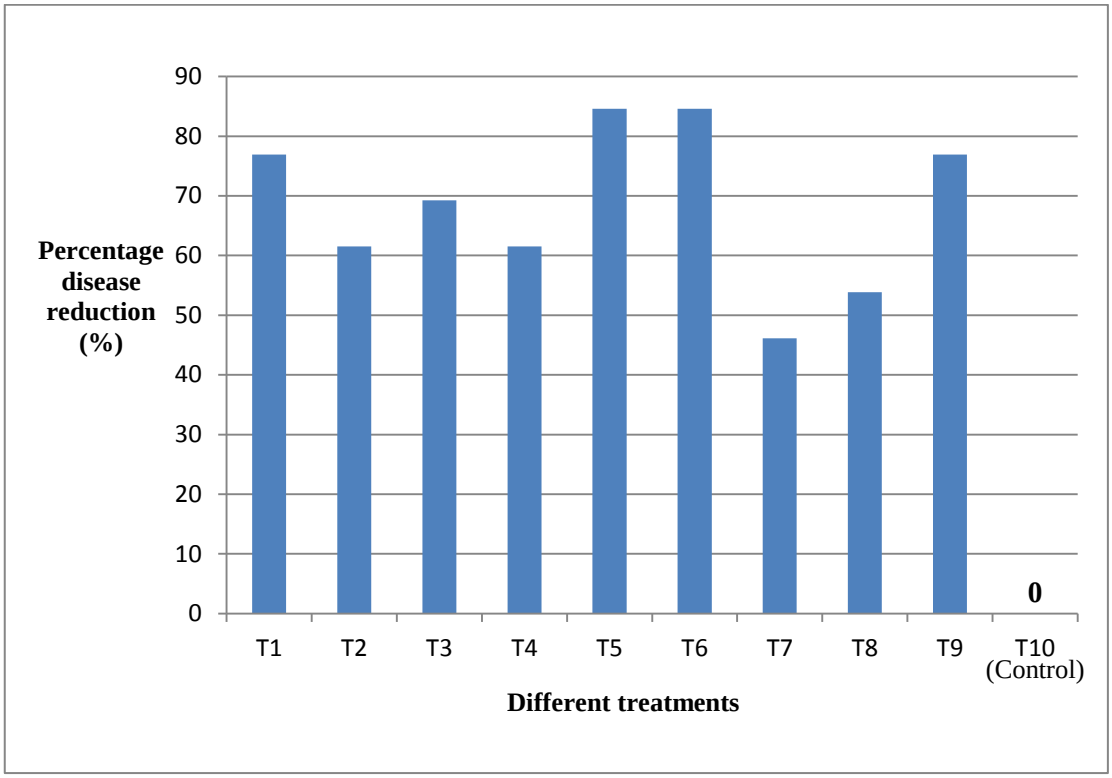


Fig 9. Graph showing the percentage disease suppression of different treatments over control

T1- Fish amino acid

T2- Panchagavyam

T3- Talc based formulation of *Pseudomonas fluorescens*

T4- Talc based formulation of *Trichoderma* sp.

T5- Metalaxyl MZ

T6- Azoxystrobin

T7- Iprovalicarb + Propineb

T8- Copper hydroxide

T9- Copper oxychloride

T10- Control

blight of turfgrass caused by *P. aphanidermatum* and Vijayan and Thomas (2002) have observed efficacy of *T. harzianum* in controlling rhizome rot of small cardamom. El- Mohamedy (2009) have reported efficacy of bioagents i.e., *T. harzianum* and *P. fluorescens* in controlling Pythium rot of chinese cabbage caused by *P. ultimum* in field condition.

Figueiredo and Lellis (1980) have reported efficacy of copper oxychloride in controlling black pod rot of cocoa caused by *Phytophthora palmivora*. Sivakumar *et al*. (2012) have also reported efficacy of copper oxychloride in controlling rhizome rot of small cardamom caused by *P. vexans*.

Singh *et al*. (2004) have reported lowest incidence of rhizome rot of ginger caused by *P. aphanidermatum* when this crop was sprayed with metalaxyl MZ. Rapetti *et al.* (2006) have also reported efficacy of metalaxyl-M and azoxystrobin in controlling basal rot of ranunculus caused by *Pythium* sp. and Efficacy of Ridomil MZ application in controlling both early and late blight of potato caused by *P. infestans* (Singh, 2008) and colocasia blight caused by *P. colocasiae* (Singh, 2009) were reported. Thiessen *et al*. (2014) have observed reduction in pod rot of peanut caused by *Pythium* spp. when it was sprayed with azoxystrobin.

The maximum pod yield was obtained from the treatment azoxystrobin which was on par with all other treatments. The highest vine length was obtained in the treatment, azoxystrobin which was significantly superior to all other treatments. When the average plant fresh weight of each treatments were compared; it was found that maximum plant fresh weight was obtained with treatment azoxystrobin. The average root length and root weight of the treatments showed that longest root length was obtained with the treatment azoxystrobin which was on par with treatment panchagavyam and were significantly different from all other treatments. In the case of average root weight, it was found that the highest root weight was obtained with the treatment azoxystrobin which was on par with all other treatments. When the average number of leaves per plant of each treatments were compared; it was found that maximum number of leaves were observed with the treatment azoxystrobin which was significantly superior over all other treatments.

Bhuvaneswari and Raju (2012) have observed growth-promoting effects of strobilurin fungicides on treated rice plants, apparently by delaying leaf senescence and having water-conserving effects which could be the reason for the increased biometric traits in the present study.

Summary

6. SUMMARY

The present study on the 'Integrated management of Pythium stem rot of vegetable cowpea (*Vigna unguiculata* sub.sp. *sesquipedalis* (L.) Verdcourt) was conducted during the period 2012-2014 at the Department of Plant Pathology, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala.

The pathogen *P. aphanidermatum* causing the stem rot disease in vegetable cowpea, was isolated from the infected stem showing typical symptoms. Eight *Pythium* spp. isolates were obtained from different cowpea growing areas of Thiruvananthapuram district during the surveys. Repeated isolations from the diseased portions of the stems and roots collected from different locations yielded same pathogen on PDA.

The symptomatology of Pythium stem rot disease include, wilting of the entire leaves followed by wet rotting, initial appearance of water soaked lesions at basal stem portion (10 cm above ground) spreading to aerial stem, lower branches and roots. The plants died one or two days after the symptom expression. During summer months symptoms appeared as dry rot with red coloured margins at the basal stem portion. The rotting also began from the aerial stem portion near to the branches instead of basal stem portion, and root portion were not affected whereas the shoot portion would be affected by wilting symptoms and plant succumbs to the disease. White cottony mycelial growth of pathogen could be seen at the early morning at infected stem portion during periods of high relative humidity. The stages of infection extended from three weeks after planting up to the harvesting stage.

In the pathogenicity test, all the eight isolates tested showed varying levels of seedling mortality with both pre and post emergence damping off. Among this, the isolate P2 exhibited the maximum virulence causing hundred percentage seedling mortality and therefore it was selected for further studies.

Morphological and cultural characteristics showed that the pathogen was a fast grower i.e., its radial growth on CMA was 50 mm after 24 h of inoculation at 25^0C and colony had cottony aerial mycelium without any special pattern. The

width of main hyphae of pathogen ranges from 3.1 to 6.8 µm (av.4.23 µm). Sporangia consist of terminal complexes of swollen hyphal branches of varying length and up to 24.7 µm wide. Oogonia terminal, globose, smooth, 18-21.6 µm (av. 19.7 µm) dia. Antheridia mostly intercalary, broadly sac-shaped, 9.4-13.6 µm (av. 10.9 µm) long and 7.5-10.4 µm (av. 8.78µm) wide, one per oogonium, monoclinous or diclinous; oospores aplerotic, 14.1-19.5 µm (av. 16.4 µm) dia., wall 1-2 µm (1.85 µm) thick and chlamydospores were absent. Based on the above cultural and morphological studies, the isolate P2, the pathogen causing stem rot disease of vegetable cowpea was identified as *Pythium aphanidermatum* (Edson) Fitzp.

The radial growth of the pathogen, *P. aphanidermatum* was compared on different solid media and PSA medium was found superior followed by PDA. In case of the media broth, maximum dry weight of mycelial mat was produced on PSB which was statistically on par with that obtained on PDB.

Studies on the optimum conditions for culturing of the pathogen revealed that, the pathogen was found to give maximum growth at 30 to 35^0C range in both solid and liquid medium. In the case of suitable pH, the pH range 6 to 8 showed maximum radial growth in soild media whereas in liquid media pH 5.5 was found to give maximum mycelial dry weight.

Studies on different light conditions for culturing of the pathogen showed that, 24 h. full darkness condition was suitable for maximum radial growth in solid media, whereas, any light conditions were suitable for good mycelial growth in broth.

The survival of *P. aphanidermatum* on crop debris was studied under *in vitro* conditions. *P. aphanidermatum* from the buried sample was detected up to 15 weeks. After 15 weeks, no recovery of the pathogen was observed on the PSA on isolation from the buried infected stem portion. The microscopic study revealed that oospores are the main survival propagules that help in the survival of *P. aphanidermatum* on crop debris.

Correlation studies on the influence of various weather parameters on the incidence of Pythium stem rot disease of vegetable cowpea indicated that percentage disease incidence of stem rot disease had a significantly negative

correlation with the maximum temperature and soil temperature at 20 cm depth from 2 weeks to harvesting stage of the crop.

The host range studies of the *P. aphanidermatum* revealed that, vegetable crops, such as snake gourd, tomato, chilli and red amaranthus were collateral hosts of the pathogen whereas none of the weeds artificially inoculated were found to take up the infection.

Different rhizosphere fungi were isolated from the rhizosphere of the disease-free cowpea plants among the stem rot infected cowpea plants in the field. Out of that seven predominant fungal isolates were selected. Three fungal isolates i.e., two *Trichoderma* spp. and one *Penicillium* sp. which showed antagonistic action against pathogen were further subjected to dual culture technique and selected for seedling assay.

The isolated fungal antagonists were identified based their cultural and morphological characteristics as *Trichoderma koningii, Trichoderma viride* and *Penicillium citrinum.*

The results of the *in vitro* study showed that the fungal endophyte *Piriformospora indica* showed no inhibition of pathogen whereas the biocontrol agents *Pseudomonas fluorescens* and *Trichoderma* sp. (KAU Culture) gave 51% and 38% inhibition of the mycelial growth of the pathogen respectively. *Trichoderma* sp. also showed overgrowth type of antagonistic action against pathogen.

The *in vitro* assay of organic preparations revealed that, panchagavyam gave 100% (Plate 33) inhibition of the growth of the pathogen at all concentrations tested whereas the fish amino acid gave 100% inhibition only at 10% concentration followed by 5% concentration (23%).

The results of the *in vitro* evaluation of fungicides revealed that at the field concentration, three chemicals viz., copper oxychloride, metalaxyl MZ and copper hydroxide gave 100% inhibition to the growth of the pathogen under *in vitro* culture conditions and the assay differed significantly from other treatments followed by iprovalicarb 5.5% + propineb 61.25% (88.3 %) Even at the lowest concentration (0.1 %), the fungicides copper oxychloride and metalaxyl MZ gave

100% inhibition of the pathogen growth. The fungicide azoxystrobin was found ineffective in checking the growth of the pathogen *in vitro*.

The results of the *in vitro* seedling assay experiment revealed that the percentage seedling mortality was lowest for the three fungicides treatments i.e. copper oxychloride (3.01%) and metalaxyl MZ (3.01%) and azoxystrobin (11.69%) which were on par with KAU released biocontrol agent *Trichoderma* sp. (25%) and were significantly superior over all other treatments followed by the fungicide Iprovalicarb + Propineb (41.31%), KAU released biocontrol agent *P. fluorescens* (50%), fungicide copper hydroxide (58.68%), organic preparations i.e. panchagavyam (58.68%) and fish amino acid (58.68%). The fungal antagonist i.e. T-2 (67.1%), T-1 (82.13%) and *Pencillium citrinum* (75%), fungal endophyte *Piriformospora indica* (88.3%)and organic amendment coirpith (93.3%) gave more than 60 % seedling mortality while seedling mortality was 100 % in organic amendments such as neemcake, spend mushroom substrate (sms) and vermicompost.

Results of the field experiment revealed that metalaxyl MZ (4.32%) and azoxystrobin (4.32%) followed by fungicide copper oxychloride (9.5%) and fish amino acid (9.5%) recorded the lowest incidence of the disease in term of percentage dsease incidence, which was on par with KAU released bioagents *Trichoderma* sp. (12.84%) and *P. fluorescens* (16.54%) followed by panchagavyam (20.61%).

The maximum pod yield was obtained from the treatment azoxystrobin (1003.2 g/ plant) which was on par with other treatments such as fish amino acid (960.6 g/plant), copper hydroxide (943.26 g/plant), metalaxyl MZ (933.7 g/plant), *P. fluorescens* (916.3 g/plant) and copper oxychloride (914.7 g/plant).

The highest vine length was obtained in the treatment, azoxystrobin (557.75 cm) which was significantly superior to all other treatments. Vine length of all other treatments were on par. When the average number of leaves per plant of each treatment were compared; it was found that maximum number of leaves were observed with the treatment azoxystrobin (374) which was significantly superior over all other treatments.

When the average plant fresh weight of each treatments were compared; it was found that maximum plant fresh weight was obtained with treatment azoxystrobin (439.25 g) which was on par with all other treatments. A similar trend was noticed with respect to average plant dry weight.

Comparison of the average root length and root weight of the respective treatments showed the maximum root length of root was obtained with the treatment azoxystrobin (42.92 cm) which was on par with all other treatments followed by panchagavyam (39.48 cm). In the case of average root weight, it was found that the highest root weight was obtained with the treatment azoxystrobin (40.69 g) which was on par with the all other treatments.

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Appendices

APPENDIX-I

COMPOSITION OF MEDIA USED

1. Potato Dextrose Agar

Peeled and sliced potatoes - 200 g

Dextrose ($C_6H_{12}O_6$) - 20 g

Agar-agar - 20 g

Distilled water - 1000 ml

Potatoes were boiled in 500 ml of distilled water and the extract was collected by filtering through a muslin cloth. Agar-agar was dissolved separately in 500 ml of distilled water. The potato extract was mixed in the molten agar and 20 g of dextrose was dissolved in to the mixture. The volume was made upto 1000 ml with distilled water and medium was sterilized at 15 psi and 121 ºC for 15 min.

2. Potato Sucrose Agar

Peeled and sliced potatoes - 200 g

Sucrose - 20 g

Agar-agar - 20 g

Distilled water - 1000 ml

Agar-agar - 16 g

Distilled water - 1000 ml

3. Czapek-Dox Agar

$NaNO_3$ - 2 g

K_2HPO_4 - 1 g

$Mg(SO_4).7H_2O$ - 0.5 g

KCl - 0.5 g

$FeSO_4$ - 0.1 g

Sucrose - 30 g

Agar-agar - 20 g

Distilled water - 1000 ml

4. Martin's rose Bengal agar

Dextrose - 10g

Peptone - 5g

KH_2PO_4 - 1g

$MGSO_4.\ 7H_2O$ - 0.5g

Rose bengal - 33mg/l.

Agar-Agar - 20g

Distilled water - 1000ml

5. **Oat Meal Agar**

Oats - 30g

Agar-Agar - 20g

Distilled water - 1000ML

6. **Carrot Agar**

Carrot - 20g

Agar-Agar - 20g

Distilled Water - 1000ml

7. **Corn Meal Agar**

Corn flakes - 60 g

Agar-Agar - 20g

Distilled Water -1000ml

8. Malt extract agar

Malt extract - 20g

Agar-agar - 20g

Distilled water - 1000ml

APPENDIX - II

COMPOSITION OF STAIN USED

1. Lactophenol –Cotton blue

Anhydrous lactophenol	-67.0ml
Distilled water	-20.0ml
Cotton blue	-0.1g

Anhydrous lactophenol prepared by dissolving 20 g phenol in 16 ml lactic acid in 3ml glycerol.

Integrated Management of Pythium Stem Rot of Vegetable Cowpea (*Vigna unguiculata* sub.sp. *sesquipedalis* (L.) Verdcourt)

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Abstract of the thesis

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ABSTRACT

The study entitled ‘Integrated management of Pythium stem rot of vegetable cowpea (Vigna unguiculata sub sp. sesquipedalis (L.) Verdcourt) was conducted during the period 2012-2014 at College of Agriculture, Vellayani, Thiruvanathapuram to investigate the stem rot disease of cowpea caused by Pythium spp. and to evolve an integrated management package for the control of the disease.

Eight pathogen isolates were collected from the different locations of Thiruvanathapuram district. From this the most virulent pathogen isolate was selected for the study. The pathogen isolated was identified as Pythium aphanidermatum based on cultural and morphological studies. PSA was found to be the best solid medium at 35 to 400C at pH 6 to 8 range under full darkness whereas PSB and PDB broths were the best liquid media for supporting the growth of the pathogen at 30 to 350C at pH5.5 under any light conditions.

The survival studies of the pathogen, P. aphanidermatum revealed that oospores were the main survival propagules help for 15 weeks survival on crop debris. Correlation studies on the influence of weather parameters on the disease incidence indicated that the disease was negatively correlated with maximum temperature and soil temperature. The host range study indicated that snake gourd at flowering stage, tomato, chilly and red amaranthus at seedling stage was found to be collateral hosts, none of the weeds artifically inoculated were found to take up the infection.

The biocontrol agents (KAU cultures) Pseudomonas fluorescens and Trichoderma sp. gave moderate inhibition of the mycelial growth of the pathogen. In organic preparations, Panchagavya gave complete inhibition of the growth of the pathogen at all concentrations tested whereas the fish amino acid gave full inhibition only at 10% concentration. In the fungicides in vitro assay, three fungicides copper oxychloride, copper hydroxide and metalaxyl MZ gave good suppression of the pathogen growth at all concentrations.

The results of the in vitro seedling assay experiment revealed that the percentage seedling mortality was lowest for the three fungicides treatments i.e., copper oxychloride, metalaxyl MZ and azoxystrobin and one KAU released biocontrol agent Trichoderma sp.

The result of the field experiment showed as a chemical management, foliar spraying and soil drenching of metalaxyl MZ (0.2%) or azoxystrobin (0.15%) or fish amino acid (5%) or panchgavya (5%) or copper oxychloride (2%) or Pseudomonas fluorescens or Trichoderma sp. @ 2% of the talc based formulation or soil drenching with copper oxychloride (2%) at 2 weeks after planting at 10 days interval could control the disease effectively.

In the case of severe, endemic occurrence of the disease, chemical control by spraying and drenching with metalaxyl MZ (0.2%) or azoxystrobin (0.15%) or soil drenching with copper oxychloride (0.2%) can be recommended (10 days interval). In a condition where organic disease management needed the result of this study indicates the prophylactic drenching of copper oxychloride (0.2%) or spraying and drenching of fish amino acid (5%) or Panchagavya (5%) or the use of spraying of KAU released bioagents Pseudomonas fluorescens or Trichoderma sp.@ 2% two weeks after planting at 10 days interval can be recommended