EFFECT OF ORGANIC AMENDMENTS AND CARBENDAZIM ON NODULATION AND CONTROLLING OF FOOT AND ROOT ROT OF LENTIL

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This is to certify that thesis entitled, ***"Effect of organic amendments and Carbendazim on nodulation and controlling of Foot and root rot of lentil"*** *submitted to the Faculty of AGRICULTURE, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of* ***MASTER OF SCIENCE (MS) in PLANT PATHOLOGY,*** *embodies the result of a piece of bona fide research work carried out by Registration No.* ***18-09043*** *under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that such help or source of information, as has been availed of During the course of this investigation has duly been acknowledged.

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ABSTRACT

The present study was conducted for the effect of organic amendments and Carbendazim on nodulation and controlling of foot and root rot of lentil in *in-vitro* and *in-vivo* conditions at Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during the period from November 2018 to May 2019. The experiment consists of 8 treatments such as T_0 = Control, T_1 = Poultry manure, T_2 = Cow dung, T_3 = Spent mushroom compost, T_4 = Carbendazim 50WP, T_5 = Spent mushroom compost + poultry manure, T_6 = Spent mushroom compost + cow dung, T_7 = Spent mushroom compost + Carbendazim 50WP. BARI masur 4 variety was used in this experiment. In i*n-vitro* condition, the reduction of mycelium growth was varied from 12.22% to 36.48% and the highest reduction was recorded in T_7. In field experiment, the lowest disease incidence (10.57%) was recorded in T_7 and the highest disease incidence in control (47.30%). The highest percent reduction of disease incidence over control was found in T_7 (77.65%) followed by T_6 (75.58%). In all cases of yield contributing characters such as number of nodules/plant, pods/plant, seeds/pod, 1000-seed weight and grain yield increased by the combination of spent mushroom compost with cow dung. Significantly the highest yield (1486.70 kg/ha) was found under the treatment T_6 followed by T_7 (1425.70 kg/ha) and T_5 (1403.80 kg/ha), respectively. Carbendazim alone and in combination of spent mushroom compost with Carbendazim and cow dung were statistically similar in respect of performance against Foot and root rot of lentil.

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LIST OF SYMBOLS AND ABBREVIATION

ELABORATION	ABBREVIATION
Percentage	%
Number	No.
Etcetera	etc.
That is	i.e.
Namely	viz.
Degree Celsius	°C
At the rate of	@
Centimeter	cm
Milliliter	ml
Gram	g
Kilogram	Kg
Gram per liter	g/l
Meter square	m^2
Spent mushroom compost	SMC
Continued	Cont'd
Pathovar	pv.
Days after sowing	DAS
Days after inoculation	DAI
Species	spp.

LIST OF SYMBOLS AND ABBREVIATION (cont'd)

ELABORATION	ABBREVIATION
Bangladesh Bureau of Statistics	BBS
Hydrogen per oxide	H_2O_2
Sodium chloride	NaCl
Potato dextrose agar	PDA
Randomized Complete Block Design	RCBD
Least significance difference	LSD
Co-efficient of variance	CV
Degree of freedom	df.
Analysis of variance	ANOVA
Journal	J.
Research	Res.
Science	Sci.
Agricultural	Agril
Agriculture	Agric.
Biology	Biol.
Biotechnology	Biotech
And others	et al.
Genetics	Genet
Government	Govt.
Sher-e-Bangla Agricultural University	SAU
International	Int.
Food and Agricultural Organization	FAO
Horticulture	Hort.

CHAPTER I

INTRODUCTION

Lentil (*Lens culinaris*) is one of the oldest and most familiar food legumes under the family Fabaceae. Lentil is an annual indigenous plant from Western Asia and other parts of the world, including North America. Furthermore, this species is now diversified from Hindukush to Afghanistan and Ethiopia to Mediterranean countries in the world (Faris *et al.,* 2013). Being grown for over 8,000 years (Dhuppar *et al*., 2012), lentil is considered as one of the early domesticated species (Cokkizgin and Munqez, 2013). It is one of the principal pulse crops grown in semi-arid region of the world, particularly in India sub-continent and the dry areas of Middle East (Malik, 2005). It is a common stable food in Asian and North African cuisines but the greatest production of lentil nowadays is in Canada.

In Bangladesh pulses constitute an integral part of the daily diet as a direct source of protein for human beings (Sattar *et al*., 1996). Lentil is cultivated during winter (rabi or post rainy season; Nov-Mar.) under rainfed condition in Bangladesh. About 80% of total lentil in the country is grown in greater Faridpur, Kustia, Jashore, Rajshahi and Pabna districts of Bangladesh (BBS, 2012). Lentil is the second most important pulse crop in terms of area and production but ranks the highest in consumer preference and total consumption in Bangladesh. Lentil is grown on about 351930 acres, producing 175384 metric tons of grains during 2018-2019 (BBS, 2020).

The cultivated area and production of lentil is gradually reduced from the last decades. The average yield of lentil per unit area in Bangladesh is lower than that of other lentil growing countries like Syria, Turkey, Canada, USA and Ethiopia (Hossain *et al*., 1999) due to various abiotic and biotic factors. Lentil is purchased mostly from Australia, Nepal, Turkey and Canada. The low yield of lentil is

accompanied with poor management practices, unavailability of quality seeds and specially lack of proper disease management. Diseases play an important role for yield reduction of lentil seed. Lentil is attacked by a wide range of fungal diseases. Lentil productivity is reduced by pathogens through infection and damage to leaves, stems, roots and pods. It also diminished marketability due to discoloration of the seeds. Lentil is attacked by various numbers of seed-borne diseases such as vascular wilt, collar rot, root rot, stem rot, rust, powdery mildew and downy mildew, which are caused by *Fusarium oxysporum* f.sp. *lentis*, *Sclerotium rolfsii*, *Rhizoctonia solani*, *Uromycis fabae*, *Erysiphe polygoni* and *Peronospora lentis,* respectively (Singh and Tripathy, 1999 ; Khare *et al*., 1979).

Foot and root rot caused by *Fusarium oxysporum* and *Sclerotium rolfsii* is considered as the most destructive disease of pulses in almost all legume growing countries of the world (Anonymous, 1986). The fungus *Sclerotium rolfsii* is a facultative saprophyte and can maintain continuity of generation under adverse situation by the formation of sclerotia. The pathogen has an extremely wide host range and produces sclerotia, which can survive in the soil for many years. In Bangladesh about 44% lentil plants are damaged by foot and root rot disease (Anonymous, 1986). It causes seedling death at early stage of growth resulting poor plant stand which ultimately produces very low yield.

Management of foot and root rot of lentil is usually focused on preventive measures as no effective control measures are available yet. Farmers use chemicals for controlling the disease of lentil in Bangladesh. Chemical control still holds a strong performance in combating destructive disease but it is not environment friendly and high costs of inorganic fungicides. This problem can be solved by using the organic soil amendment because of environment friendly, low cost and enhance the soil fertility. It naturally suppresses pathogens in the soil that cause plant damage and decline in yields. Organic amendments, such as animal and green manure, organic wastes, composts and peats, have been proposed to

control diseases of plants caused by soil-borne pathogens (Hoitink and Fahy, 1986). The amount of soil nitrogen in fields under conventional production systems has been negatively correlated with soil microbial components, whereas soil nitrogen in fields under organic production was positively correlated with soil microbial components present in the soil (Gunapala and Scow, 1998). However, it is also found that microbial activity and biomass is higher in fields with organic amendments than fields with conventional fertilizers (Drinkwater *et al.,* 1995). The use of organic soil amendments has been associated with desirable soil properties including higher water holding capacity and CEC and lower bulk density, and can foster beneficial microorganisms (Doran, 1995; Drinkwater *et al*., 1995).

Spent mushroom substrate, poultry manure and cow dung are good source of soil organic amendments. Spent mushroom compost is an organic medium with fungal mycelium that results from the mushroom cultivating process after mushrooms have been harvested. In recent times mushroom production has been increased dramatically in the world as a result a huge amount of mushroom waste is added to the burden especially around the mushroom cultivation area. There was an estimate that about 70,000,000 metric tons of mushroom waste was generated from mushroom production during the year 2007 in the world (Tajbakhsh *et al.,* 2008). It was reported that across the country (Bangladesh) about 60,000–70,000 of spawn packets per day were produced in the year 2008 (Amin, 2008). Per spawn packet contains 500 gm substrate then 30,000–35,000 kg spawn were produced during mushroom production per day in 2008. From which it can be calculated that per day about 15–18 metric tons of mushroom wastes were produced in Bangladesh in the year 2008 because an average 0.5 ton mushroom waste is produced from each ton of spawned compost (Levanon *et al.,* 1994). Spent mushroom substrate when allowed as waste in the environment can serve as environmental pollution source thereby causes nuisance to the environment which

is hazardous. But this hazardous effect can be turned around for fortune when used as substrate compost for growing agricultural crops. It is also known to provide a balanced nitrogen and carbon source for plant growth and to reduce diseases of plant. Spent mushroom substrate has desirable chemical and physical properties and disease suppressing ability of soil-borne pathogens like *Fusarium oxysporum* f.sp. *ciceri* causing wilt of chickpea, root rot of creeping grass and damping off of cultivated crops.

Poultry manure or cow dung compost along with SMS causes complex organic compounds to be slowly oxidized or to break down into simpler forms, which are further transformed into microbial biomass and reduces plant diseases.

Therefore, environment-friendly management of *Sclerotium rolfsii* with organic substances will be very effective until the development of resistance cultivars against *Sclerotium rolfsii* pathogen in Bangladesh. As no work is done for controlling foot and root rot disease by mushroom compost and so this work is a new effort for non-chemical as well as environment friendly management of foot and root rot of lentil in Bangladesh. The experiment was conducted to asses different organic amendments, foliar application of Carbendazim and their combined application with spent mushroom compost for controlling foot and root rot disease and improvement of lentil production.

The present research work was undertaken with the following objectives:-

- To evaluate the effect of organic amendments and Carbendazim for controlling of foot and root rot disease of lentil
- To compare the response of organic amendments, Carbendazim and their combination with spent mushroom compost on growth and yield contributing characters of lentil
- To find out the effect of organic amendments on nodule formation of lentil

CHAPTER II

REVIEW OF LITERATURE

Sclerotium rolfsii causing foot and root rot of lentil is a well known polyphagous, ubiquitous and a non-target pathogen. It is one of the most destructive soils inhabiting pathogen of lentil reported so far. The available literature of work done on foot and root rot disease of lentil and its management strategies have been reviewed in this chapter. The review of literature pertaining to this thesis is presented in the following headings and sub-headings:

2.1 History, Origin, Distribution and Importance of Lentil

According to USDA (2016) and Bednar *et al.* (2001), among 23 pulses, lentil seeds yield the second highest starch percentage of 47.1% and a greater percentage of insoluble dietary fibers.

Dwivedi *et al.* (2014) stated that lentil is known to be a good source of prebiotics and prevent gut-associated diseases.

Cokkizgin and Munqez (2013) stated that lentil has also been rapidly spread to Egypt, central and southern Europe, the Mediterranean basin, Ethiopia, Afghanistan, India, Pakistan, China and later to the new world including Latin America, Mexico chili, Argentina, Colombia and more recently Canada.

Soltan (2013) reported that lentil seeds are an excellent source of iron and several studies have shown that the consumption of cooked lentil in the diet prevents iron deficiency anemia. Iron being a very important mineral especially for adolescents and pregnant women.

Lombardi-Boccia *et al*. (2013) showed that the predominant proteins in lentil seeds are globulin (47% of the total seed proteins) and an adequate quantity of albumin.

According to Risula (2010), flowers of lentil are self-pollinated and first few flowers on the main stem may abort.

Shyam *et al.* (2007) stated that many different names in different parts of the world are used for the crop lentil; lentil (English), adas (Arabic), mercimek (Turkish), messer (Amharic), dahl or daal (Hindi) and hiramame (Japanese) are the most common names.

According to Ryan *et al.* (2007) and Rodriguez *et al.* (2008), several minerals (zinc, copper, manganese, molybdenum, selenium and boron) and vitamins (thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, folate, α, β and γ tocopherols and phylloquinone) have been well presented in lentil seed. Overall, lentil seeds are considered as one of the best dietary sources that has health-promoting effects on various illnesses.

Sandhu and Singh (2007) stated that it is an annual bushy herb with slender stem and having many branches with erect, semi-erect or spreading growth habit.

Harold McGee (2004) stated that the word "lens" for the lentil is of classical Roman/Latin origin: McGee points out that a prominent Roman family took the name "Lentulus", just as the family name "Cicero" was derived from the chickpea, *Cicer arietinum*.

Hanelt (2001) stated that the plant was given the scientific name *Lens culinaris* in 1787 by Medikus, a German botanist and physician.

According to Saskatchewan Pulse Growers (2000), lentil plants are typically short, but can range from 20 to 75cm in height, depending on growing conditions.

Sattar *et al.* (1996) reported that the cultivated lentil *Lens culinaris* was derived from its wild subspecies *L. culinaris* sub sp. *orientalis*, although other species may also have some genes, according to Jonathan Sauer.

Oplinger *et al.* (1990) reported that lentil is now cultivated in most subtropical and also in Northern hemisphere such as Canada and Pacific Northwest regions.

According to Cubero (1984), lentil was first spread to the Nile from the near east, to Central Europe and then to the Indian Subcontinent and the Mediterranean Basin by the end of Bronze Age.

Anonymous (1984) calculated that in addition to their food value lentil also plays an important role in cropping systems because of its ability to fix nitrogen (101 kg/ha/annum) from atmosphere and thereby enrich the soil.

2.2 Importance and Distribution of *Sclerotium rolfsii*

The pathogen causes root rot, wilt stem rot and foot rot diseases on more than 500 species of cultivated and wild plants including almost all the agricultural and horticultural crops (Punja, 1985; Domsch *et al.,* 1980; Farr *et al*., 1989; Cilliers *et al.*, 2000). Mostly *S. rolfsii* diseases have been reported on dicotyledonous hosts but several monocotyledonous species have also been infected by this pathogen (Aycock, 1966; Ciancio & Mukerji, 2007).

Masum Billah and his associates (2017) reported that *Sclerotium rolfsii* is found to be pathogenic on sunflower, moonbeam, betel vine, lentil, sugar beet, tomato, sweet pumpkin also attack the plants like maize, chick pea, apple, cotton, potato, soybean, oat and some ornamentals.

Madhavi *et al.* (2011) observed that chili is recently affected by dry root rot disease caused by *S. rolfsii* (Sacc.) under rain fed conditions at Andhra Pradesh, India.

Ciancio & Mukerji (2007) stated that *Sclerotium rolfsii* diseases have been reported on dicotyledonous hosts but several monocotyledonous species have also been infected by this pathogen.

Anand and Singh (2004) estimated that in peppermint; this pathogen caused about 5 to 20% of crop loss was observed under field condition.

Bag (2004) reported two new hosts (*Phaius flavus* and *Paphiopedilum venustum*) of *S. rolfsii* from India.

Shokes and Gorbet (1998) stated that a positive correlation of root colonization with the population of *Sclerotium roifsii.*

Kulkarni *et al*. (1994) reported the tuber rot or wilt of potato was a major problem in rainy season of transitional belt of Karnataka. Sporadic incidence of fungal wilts caused by either *S. rolfsii* or *Verticillium* sp. was found in Karnataka, North Gujarat and Sapura plateau of India.

According to Khanna and Sharma (1993), the disease was quite serious in Satara region with an average incidence of 5 % wilt and 1-3 % infection of tubar during 1968. It was destructive in stores and up to 1 % tuber may rot every day.

Ingale and Mayee (1986) reported that *S. rolfsii* caused about 25% seedling mortality in the groundnut cultivar JL-24.

Ahmed and Hossain (1985) observed that collar rot, foot and root rot disease caused by *Sclerotium rolfsii* caused considerable damage both in seedling and adult stages of tomato plant and there existed variations in the incidence of the disease in different parts of Bangladesh.

Wangihar *et al.* (1988) reported that an outbreak of collar and root rot was observed on Capsicum in Maharashtra, India during the first week of October, 1985. The disease was most severe on cultivars Jurala and CA960. The causal agent of the disease was identified as *S. (Corticium) rolfsii*.

In Bangladesh, diseases caused by *S. rolfsii* in different crops have been reported by Meah and Khan (1987) and many others.

Aycock (1966) stated that *Sclerotium rolfsii* is widely distributed in tropics, subtropics and in warmer parts of temperate zone of the world especially the Southern United States, Central and South America, West Indies, Southern European countries bordering the Mediterranean, Africa, India, Japan, Philippines and Hawaii.

The first confirmed report of losses due to the *Sclerotium rolfsii* pathogen in USA was made by Rolfs (1892) on tomato plants (*Lycopersicon esculentum* M.) in Florida. The first report of S. rolfsii from Pakistan was made by Ahmed *et al.,* (1984) who isolated *Sclerotium rolfsii* from maize (*Zea mays* L.). Subsequent reports were made from oat (*Avena sativa* L.) and mash bean (*Vigna mungo* L.) Hepper) by Shahzad & Ghaffar (1995), lentil (*Lens culinaris* L. Medic.) by Iqbal *et al.* (1995), apple (*Malus sylvestris* L.) by Jahangir *et al.* (1995), and seeds of sugarbeet (*Beta vulgaris* L.) by Ruqia (2001).

Shaw and Ajrekar (1915) isolated the fungus from rotted potatoes and identified as *Rhizoctonia destruens* Tassi. Later, Saccardo (1911) named the fungus as *S. rolfsii*.

2.3 Characterization of *Sclerotium rolfsii*

Paparu *et al.* (2020) worked with 348 isolates of *Sclerotium rolfsii* found that all isolates formed white cultures with fluffy, fibrous, or compact mycelia and fluffy mycelia were most common (84.7%), followed by thin fibrous mycelia (9.2%) and compact mycelia (6.1%). There were differences in growth rate among the agro

ecological zones. Isolates formed small round or large irregular shaped) sclerotia that were either light or dark brown. Of the 348 isolates, 69.6% formed light-brown sclerotia, while 30.4% had dark-brown sclerotia, and the number of sclerotia varied among the agro ecological zones.

Sahana *et al*. (2017) observed that among the media viz., PDA, Kirchoff's agar, SDA, Hunsen's agar and tomato leaf extract showed pure white colonies, where as Richard's agar and nutrient agar showed dull white coloured colony. They also found that on oat meal agar cottony white coloured colony was observed. With regard to growth pattern of *S. rolfsii* they also showed compact growth on PDA and oat meal agar was recorded and on Kirchoff's agar and Hunsen's agar, it showed filamentous growth, on SDA and tomato leaf extract it was fluffy and on nutrient agar colony growth was cloudy type. In the similar experiment they also found that on most of the media mycelial margin was smooth except SDA and Kirchoff's agar which showed filamentous and serrated margin respectively.

Narayan *et al*. (2017) stated that colonies of all isolates were white to pale olive buff in color on PDA media. They also reported that during the early stage of sclerotial growth, the color was white to pale light brown; however, the color changed to cinnamon brown or dresden brown over time.

Parvin *et al.* (2016) stated that all isolates produced cottony white mycelia and regular shaped colony and in most isolates mycelial growth was fluffy. They also showed that dense fluffy rings were produced in isolates S1, S2 and scattered fluffy growth pattern was observed in isolates S3, S4 and S8 and mycelial growth was embedded and compact in isolates S5 and S7.

Punja (1988) showed that the large number of sclerotia produced by *S. rolfsii* and their ability to persist in the soil for several years, as well as the profuse growth rate of the fungus make it well suited facultative parasite and a pathogen of major importance throughout the world.

Willetts (1971) reported that sclerotia are asexual, usually spherical bodies consisting of pseudoparenchymatous aggregations of hyphae, and in most cases, they are resistant to unfavorable environmental conditions.

Aycock (1966) found that sclerotia of *S. rolfsii* survived from 2 months to 7 years in field soil depending on experimental conditions.

2.4 Significance of foot and root rot disease of lentil

Chang *et al*. (2014) reported that loss in yield in inoculated trials of foot and root rot have been as high as 69% in lentil.

Agrios (2005) stated that soil-borne plant pathogens causing root rot disease are among the limiting factors in plant production all over the world. He also found that these pathogens cause economic yield losses on faba bean (*Vicia fabae* L.), Lentil (*Lens culinaris*) and pea (*Pisum sativum* L.) and control is rather difficult.

Begum (2003) stated that various diseases may cause 30-40% yield loss in lentil. She also reported that foot and root rot of lentil disease may cause 100% seedling mortality in monoculture under conductive weather conditions for disease development.

Similarly, Khalequzzaman (2003) observed that with a gradual increase in inoculums levels of *Sclerotium rolfsii* pathogen in soil in lentil field, plant growth, nodulation and yield per plant reduced gradually.

Iqbal *et al*. (1995) made the first report of *sclerotium rolfsii* on lentil (*Lens culinaris*) in Pakistan.

Hwang *et al.* (1994) found that severe root rot drastically reduces the number of roots available for symbiotic nodulation, so nodulation decreases as fusarium root rot severity increases and reduced nodulation can slow plant development and reduce seed yield because of limited nitrogen fixation. They also found that the

outbreak of lentil root rot in eastern Alberta resulted in up to 70% reduction in plant stand in one field during 1991.

Alexander and Stewart (1994) found that the relatively large sclerotia of *S. rolfsii* and *Sclerotinia sclerotiorum* survived longer than the smaller sclerotia of Sclerotinia minor and *Sclerotium cepivorum.*

Dey *et al.* (1993) reported that foot and root rot of lentil caused by *Fusarium oxysporum* and *Sclerotium rolfsii* are common disease in Bangladesh. *Sclerotium rolfsii* Sacc., [teleomorph: *Athelia rolfsii* (Curzi) Tu & Kimbrough] is an important soil-borne pathogen that is very common in tropical, subtropical and other warm temperate regions of the world.

TeKrony and Egli (1991) observed that lentil crops can compensate for unthrifty seedlings, so substantial losses in plant population can occur before yield potential is reduced.

Lin and Cook (1977) reported that in North America, root rot caused by *F. avenaceum (Fr.) Sacc*. was first reported on lentil in eastern Washington in 1973.

2.5 Favorable Climatic conditions for Foot and root rot disease development

Al-Askar *et al.* (2013) found that sclerotial diseases caused by *Sclerotium ro*lfsii occur primarily in warm climates, especially at high temperatures.

Mollah (2012) found that 29^0C and 85% RH, the disease incidence and severity of foot and root rot of betel vine was the highest and it was the lowest when the temperature laid around 18.7^0C and the RH laid around 75%.

Epidemiological studies of Anonymous (2006) and Maiti and Sen (1982) were reported that the maximum temperature, maximum relative humidity and rainfall played an important role in the development of the foot and root rot disease.

Yorinori (1994) reported that humid weather is conducive to sclerotial germination and mycelial growth. Consequently the diseases caused by the fungus are more serious in tropical and subtropical regions than in temperate regions.

Farr *et al.* (1989) found that fungus *Sclerotium roifsii* attacks all plant parts in the contact with the soil under favorable environmental conditions including 4 stems, roots, and fruits.

Smith *et al.* (1989) observed that survival of *Sclerotium roifsii* decreased when depth of burial was greater than 2.5 cm, and that survival decreased in proportion to depth of burial.

Matti and Sen (1988) reported little difference in the proportion of viable sclerotia of *Sclerotium roifsii* recovered under a range of controlled temperature regimes (0 to 40 °C) or under moderate to low soil water holding capacity. It was found that only 11% of sclerotia survived on the soil surface, whereas 94% survived at 10-cm soil depth, after alternating 7-day cycles of wetting and drying over 8 weeks and the relative absence of soil drying at 10-cm depth might account for greater survival of Sclerotia of *Sclerotium roifsii* than at the soil surface.

Punja and Jenkins (1984) stated that cycles of drying and wetting, as well as cycles of freezing and thawing, may decrease survival of sclerotia of *S. rolfsii*. They attributed this trend in part to increasing gravitational pressure at greater depths, which may enhance substrate leakage from sclerotia.

Lingaraju (1977) reported that the saprophytic activity of the *Sclerotium rolfsii* was more at 10% soil moisture and the fungus did not survive when the moisture of soil was raised to 50% and above.

2.6 Symptomatology of Foot and root rot disease

Njambere and Chen (2011) stated that infected foot and root rot young seedlings show damping-off symptoms and plants infected at advanced stage gradually turn pale, droop and dry.

Kulkarni *et al.* (1995) stated that the pathogen *Sclerotium rolfsii* damaged stem, root or tuber and infected the stem and produced dark brown lesion at collar region causing wilt and ultimately plants get dried. Brownish sclerotia like mustard seed are developed at later stages on the root and collar region of the infected plants.

Dastur (1935) gave a well accepted description of the symptom of foot rot disease of paper vine. He stated in *Sclerotium rolfsii* induced foot rot, wet rot associated with wilting of vines is simple and fine young roots are infected first in the diseased plants. The leaves and shoots of infected plants turn yellow wither and ultimately dry out to a pale brown color were reported. As a result black lesion develops causing necrosis of the plant cells. The mycelium invades the stem and rots the affected portions of paper vine plant. So the plant become wilt and finally dies. He also found that abundant white mycelium and small light brown sclerotia were formed on the rotted plants. As results in a diseased plant, the whole underground portion gets more or less completely rotten.

Dwivedi *et al*. (1982) and Bose *et al*. (2003) recorded that the root rot symptoms of the infected plants are many and varied. They also stated that infected plants exhibited symptoms of yellowing and drying of the leaves and affected plants showed discoloration of roots and complete decaying of tap and lateral root system. The root/ bark of infected plants can be easily peeled off, with extensive sloughing and shredding of affected bark. Finally infected plants showed the death of entire plants.

Aycock (1966) stated that the soil borne pathogens *Fusarium oxysporum* and *Sclerotium rolfsii* commonly occurs in the tropics and sub-tropics of the world

causing foot and root rot disease of many crops. It causes seedling death at early growth stage resulting very poor plant stand which ultimately produces very low yield.

2.7 Pathogenicity Studies of *Sclerotium rolfsii*

Jahan *et al*. (2016) investgate a survey of crop on foot and root rot disease of *Piper betle* in Kushtia. She and her associates observed young stems were found more prone to attack than the old ones. Pathogenicity test showed *Sclerotium rolfsii* produced characteristic symptoms on and proved to be the causal pathogen of the disease.

Kashem *et al*. (2011) observed that the highest foot and root rot incidence and the lowest plant stand of lentil in pathogenicity test were recorded in the pots where soil inoculation with the isolate FBg-1 of *F. oxysporum* was done. The highest colony forming unit (4.04 x 106 /g) of *T. harzianum* (TG-2) was recorded in chickpea bran

Meah (2007) tested the pathogenicity of 10 isolates of *Sclerotium rolfsii* on eggplant (var. Dohazari) and he found that all the isolates of *S. rolfsii* significantly affected the seed germination, pre-emergence death, damping off, foot rot and plant stand.

Singh and Thapliyal (1998) stated inoculums density levels of 2.5 to 10 g per kg soil significantly increased the emergence rot which was ranged from 36.70 to 90% in seed and seedling rot of soybean caused by *S. rolfsii*.

Harlapur (1988) estimated that 2 % inoculums were essential for infection. But, maximum infection (100%) was noticed in inoculums level of more than 4 % in foot rot disease of wheat.

Siddaramaiah and Chandrappa (1988) proved the pathogenicity of *S. rolfsii* on cardamom in pot culture studies by inoculating 25 days old sclerotial cultures

which was grown on sand corn meal medium and observed the symptoms a week after inoculation of pathogen.

Thammasa *et al.* (1982) made an investigation on the Pathogenicity of *S. rolfsii*, and reported that the pathogen could infect its host cotton severely and disease severity in average was 84%. The pathogen caused pre and post emergence damping off symptoms of cotton seedlings.

Datar and Bindu (*1974*) showed the pathogenicity of *S. rolfsii* on sunflower by soil inoculation method under glasshouse condition. The inoculums was prepared by growing the fungus on sterilized maize bran medium and mixed with the sterilized soil one week before sowing of sunflower. They reported that typical symptoms were produced within a week of inoculation in the field.

Sengupta and Das (1970) studied the cross inoculation of *S. rolfsii* isolates from groundnut, wheat, potato, guava and bengal gram. They estimated that bengal gram was the most susceptible host against *S. rolfsii*.

Kilpatrick and Merkle (1967) stated the effect of different levels of *S. rolfsii* inoculums on foot rot of wheat and found that, 0.5 and 1.0 % inoculums was superior to 3, 5 and 10 %. However, considerable amount of infection was recorded in 2% inoculums and 100 % disease in 6 % and above inoculums level.

2.8 Incidence and severity of *Sclerotium rolfsii*

Khalequzzaman (2016) found that the lowest disease incidence of foot and root rot of lentil (21.67%) was obtained from when seed treatment with Provax 200 (2.5 g/kg seed) which was followed by seed treatment with *Trichoderma harzianum* compost (1:5) by 26.67% and apparently healthy looking seeds (27.33%), and the highest incidence (41.50%) was obtained from untreated control.

Dey *et al*. (1993) stated that it was quite evident that foot and root rot of lentil caused by *Fusarium oxysporum* and *Sclerotium rolfsii* had immense impact on germination, disease incidence, seedling mortality and yield.

Mollah (2012) observed that foot and root rot of betel vine in Satkhira district, highest disease incidence were found in August (12.50% to 32.50%) and lowest disease incidence was found in December (0% to 8.33%) in 2010. He also reported that the highest disease incidence was found in August (18.75% to 50%) and the lowest disease incidence were found in December (0% to 2.08%) in 2011.

Khan (1996) reported the incidence and severity of collar rot of sunflower in 15 varieties which were grown at 3 agro-ecological zones (AEZ) of Bangladesh. He found that young plants were more susceptible to collar rot. He also observed incidence of the disease was minimum in Rabi season and a higher percentage of plants were killed in Kharif-I season.

Mengistu and Negussie (1994) used different fungicides were showed that the tested fungicides significantly decreased incidence of root rot of lentil and increased yield. Among these fungicides highest performance was found with Secure 600wg (0.2%) (Fenamidone+Mancozeb) against incidence of foot and root rot.

Kulkarni *et al.* (1994) while studying the most susceptible growth stage of groundnut to *S. rolfsii*, maximum mortality was recorded in 15 days old plants and the least mortality in 105 days old plants of groundnut.

Dey *et al.* (1993) stated that it was quite evident that foot and root rot of lentil caused by *Fusarium oxysporum* and *Sclerotium rolfsii* had immense impact on germination, disease incidence, seedling mortality and yield.

Palakshappa (1986) surveyed the incidence of *S. rolfsii* on *Piper betle* L. in different areas of Karnataka state during 1984-85. He recorded 35 to 39% disease incidence.

2.9 Effect of Spent mushroom compost on growth, yield and disease of plants

Orluchukwu *et al.* (2018) reported that spent mushroom substrate (SMS) which gave the second highest yield of okra but was significantly different from control which had the lowest yield. At 10 WAP there was no significant difference in all treatments and SMS had the highest yield while control had the lowest yield.

Adedeji and Aduramigba Modupe (2016) observed that a high inhibition of mycelium growth of *F. oxysporum* f. sp. *lycopersici* was of tomato recorded for all the tested concentrations of unsterilized spent mushroom compost, with 0.05, 0.10 and 0.15g/ml concentrations significantly higher than the lowest concentration (0.01g/ml), however, for sterilized spent mushroom compost, only the highest concentration (0.15g/ml) significantly inhibited the mycelia growth, the lower concentrations of 0.01 and 0.05g/ml rather stimulated the growth of the pathogen.

Prabu *et al.* (2014) reported that the spent mushroom compost has positive influence on cowpea plant morphological parameters tested, plants were tested on three phases in 15 day intervals, on the 45th day the number of compound leaves, leaf length and area increased from control (7.5 ± 1.0, 10.1 ± 2.0 and 10.1 ± 2.0) to pot soil with 250 g spent mushroom compost (9.6 ± 2.10, 12.69 ± 2.5 and 12.5 ± 1.16) and pot soil with 500g spent mushroom compost (8.7 ± 2.10, 11.49 ± 1.16 and 11.4 ± 1.4). Similar trend was observed by them in number and length of root, root nodule and shoot of cowpea. They observed significant increase in almost all the parameters in Treatment-I (pot soil with 250g SMC) than in Treatment-II (pot soil with 500g SMC) as compared to control.

In the similar study Prabu *et al.,* (2014) observed that the number, length, fresh weight and dry weight of the pods of cowpea was higher in the crop underwent pot soil with 250g SMC than yield corresponding to crops in pot soil with 500g SMC and control.

Subhash (2012) estimated that seed treatment with *Trichoderma viride* @ 4 g/kg seed + *Pseudomonas fluorescens* @ 10 g/kg of seed+ *Rhizobium* sp. @ 25 g/kg seed + PSB @ 25 g/kg seed and addition of 300 g of SMS per 10 kg soil found effective to reduce wilt incidence up to (22.22%) of chickpea at 90 DAS.

Kim *et al.* (2011) reported that spent mushroom substrate SMS is the substrate that remain after the harvesting of mushroom and it is source of minerals and have nutritional characteristics for crop cultivaton.

Kadiri and Mustapha (2010) reported that SMS mixed with loamy soil produced significantly greater plant height, stem girth, numbers of leaves and the total leaf area of cowpea and tomato.

Outsan *et al.* (2007) observed that the electrical conductivity of the spent mushroom substrate was 3.0 ds/m. means of easily quantifying salinity in the salt affected soils.

Rosario *et al.* (2001) stated that spent mushroom substrate was organic waste resulting from commercial mushroom production and major ingredients of SMS was composted straw, hay, peat, horse manure also nutrient like potassium salt, Ammonium sulphate, super phosphate and 66 percent of water.

Williams *et al.* (2001) reported that spent mushroom substrate promotes faster crop growth establishment, improved crop density and yield, increased rooting and less need for fertilizer and irrigation for cultivation.

Mongxin *et al.* (2001) found that the pH, electrical conductivity and acid neutralizing capacity were 6.6, 9.0, and 21 to 66 dsm1.of spent mushroom substrate, respectively.

Bansal and Gupta (2000) stated that utilization spent of *Agaricus bisporus* and bio agents can control *Fusarium oxysporum* causing wilt of fenugreek through their effectiveness and also reduced many diseases of other cultivated plants.

Stewart *et al.* (1998) stated that spent mushroom substrate has been reported to be a useful soil amendment for improving the physical and chemical conditions of the soil and provide nutrients for 4 consecutive vegetable crops (sweetcorn, cabbage, potato, cabbage).

Nelson and Crafts (1996) reported that spent mushroom substrate is discarded as waste in numbers of cases but it also may be used as soil amendment or in agricultural, horticultural and environmental amelioration and also used for disease management like damping of chili, tomato plants.

2.10 Effect of poultry manure on growth, yield and disease of plants

Faruk and Khatun (2020) concluded that integration of poultry manure with chemical fungicide Provax 200 WP or soil amendment with Tricho-composts is the best treatment for management of seedling diseases and increasing plant growth and yield of chickpea followed by soil amendment with poultry manure alone, seed treatment with Provax 200 WP and *T. harzianum* spore suspension.

Aktar *et al.* (2019) observed that seed and straw yields of lentil were increased due to application of cowdung or poultry manure along or with inorganic fertilizers over the control treatment. The highest number of nodules (12.7, 26.6, 35.1 and 30.0, respectively) was always recorded in T_4 (Inorganic +1.5 t ha-1 poultry manure) treatment and the lowest number of nodules noted in T_6 (control) treatment.

Siddique *et al*. (2018) showed that poultry manure and biocontrol-agent are effective against *Sclerotium rolfsii* causing foot and root rot disease of eggplant.

Mohbe *et al.* (2018) observed that poultry manure (T_8) showed, best performance in initial plant height at 15 DAS, pod formation 8.0 and 17.53 at 45 and 60 DAS respectively, highest nodule formation at 60 DAS 27.27, maximum yield per plant 9.31 gram and maximum seed yield per plot 1368gram of green gram.

Isitekhale *et al.* (2013) found that the application of 4 to 6 ton ha^{-1} of poultry manure and its residual effects appeared to be good for tomato production. Isitekhale and his asociates also stated that the application of fertilizer alone proved less effective when compared to poultry manure alone. However, combined application of NPK fertilizer and poultry manure had shown to be more effective.

Melero- Vara *et al*. (2011) stated that previous amendment of fod-infested soil with poultry manure increased Fusarium wilt disease control over soil solarization alone, improved carnation yield and quality of carnation and also increased plant vigor, thus providing a satisfactory alternative to methyl bromide.

Aba *et al.* (2011) stated that 10 t/ha of poultry manure per annum gave the best yield attributes in both clones of plantain, indicating a similar nutrient demand pattern.

2.11 Effect of cow dung on growth, yield and disease of plants

Zaman *et al*. (2017) sated that cow dung application have been found to be increased the total N, available P, exchangeable K, Ca, Mg, available S, Zn and B contents in soils and biomass yield of stevia over the control significantly with the increased levels of CD up to its highest level in both soils.

Gudugi (2013) stated that cow dung significantly increased the growth and yield of plants over the control. He also observed that, use of cow dung at the rate of 15 to 20 t ha-1 will significantly improve the performance of Okra comparable to use of inorganic fertilizer.

Hannan *et al.* (2012) reported that combined use of cowdung, BINA-biofertilizer, and BAU-biofungicide showed a profound effect in reducing root rot disease and in increasing plant growth parameters of lentil under field conditions.

Tanimu *et al.* (2007) concluded that, the use of cow dung has significant effect on the growth and yield performance of vegetable maize and the application of 15 t/ha using the Bataji local variety of vegetable maize has produced the overall best result in almost all the parameters including the final yield.

Solaiman and Rabbani (2006) stated that cow dung, at the rate of 5t/ha, in combination with half the recommended dose of inorganic nutrients appeared to be the best combination of fertilizer and natural nutrients which provided the maximum benefit of tomato to cultivator.

Basak *et al.* (2002) showed that complete suppression of mycelial growth of *S. sclerotiorum* of cucumber may be possible if different herbal plant extracts are added with fresh cow urine and cow dung before application.

Basak and Lee (2001) proved that cow urine and cow dung had some effectiveness in sup-pression of conidial germination and mycelial growth of *F. oxysporum* f. sp. *cucumerinum* causing Fusarium wilt of cucumber plants.

2.12 Chemical control of *Sclerotium rolfsii*

Siddique *et al.* (2018) stated the chemical fungicide Bavistin 50 WP (Carbendazim) showed the best effects amongat the treatments in reducing foot and root rot disease and increasing the fruit yield in eggplant.

Khalequzzaman (2016) reported that that seed treatment with Provax 200 (2.5 g/kg seed) showed better performance followed by seed treatment with *Trichoderma harzianum* compost (1:5) and apparently healthy looking seeds to control foot and root rot and increase yield of lentil.

Shahiduzzaman (2015) used Bavistin 50WP, Provax 200, Trichoderma compost, Neem leaves extract, Garlic clove extract and Allamanda leaf extract. He found that the reduction of disease severity of lentil under Bavistin 50WP and

Trichoderma compost was identical. He also stated that the maximum and significant disease reduction was achieved with Provax 200 compared to other treatments.

Hoque *et al.* (2014) worked with Rovral 50 wp (0.2%), Sceure 600wg (0.2%), Bavistin 70 wp (0.2%), Captan 50 wp (0.2%) and Control. He and associates stated that spraying the fungicides in the experiment which promotes yield promoting characters and increased yield and thus considerably reduced foot rot diseases of lentil.

Parvin (2013) worked with Foot and root rot disease of betel caused *by Sclerotium rolfsii* found and found that the lowest percent foot area diseased of stem were recorded in case of Bavistin (0.71%) treated plot at 120 DAT followed by Topgan (0.94%) and the highest percent foot area diseased of stem (2.87%) were recorded in case of untreated control condition while garlic clove extract and *Trichoderma harzianum* treated plot showed (1.69%) and (1.60%) percent foot area diseased of stem respectively at 120 DAT.

Rondon *et al*. (1995) conducted experiment with Copper Oxychloride, Vinclozolin (as Ronilan), Iprodione (as Rovral), Metalaxyl (as Ridomyl), Chlorothalanil (as Daconil), PCNB [Quintozene], Captan, Benomyl, Carboxin + Thiram and Thiabendazole at five concentrations against the growth and sclerotia formation of *Sclerotium rolfsii*. Carboxin + Thiram, Copper Oxychloride and Quintozene were found to be most effective for chickpea, both in inhibiting mycelia growth and scierotia formation at low concentration.

Shahid *et al*. (1990) used ten fungicides in vitro test and found Ridomil [Metalaxyl] was the most effective in inhibiting mycelia growth and sclerotial production of *Sclerotium rolfsii causing* collar rot disease of lentil. He and his associates stated, Benlate [Benamyl] and Metalaxyl inhibited germination of

sclerotia most effectively and Metalaxyl and Benomyl at 500 ppm applied as seed treatment and soil drench, respectively.

Dutta (1975) stated that soil application of fungicides such Bavistin (0.5- 0.7%), Brassicol (0.1%), three times at 20 days interval has been effective in controlling foot and tuber rot caused by *Sclerotium rolfsii* of tuberose.

CHAPTER III

MATERIALS AND METHODS

The experiment was conducted to evaluate the effect of organic amendments and Carbendazim on nodulation, growth and yields contributing characters and management of foot and root rot disease of lentil. The details of the materials and methods i.e. experimental period, location, soil and materials that were used, experimental treatment and design, growing of crops, data collection and analysis procedure that followed for the conduction of this experiment has been presented under the following headings and sub-headings:

3.1 Experimental site

The experiment was conducted in the Central Farm, net house and laboratory of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka- 1207. Geographically the experimental area was located at $23^0$41'N latitude and $90^0$22'E longitudes at the elevation of 8.6 m above the sea level.

3.2 Experimental period

The experiment was carried out from November 2019 to March 2020.

3.3 Soil Characteristics

The experimental plot was a medium high land belonging to the Modhupur tract under the Agro Ecological Zone (AEZ) 28 (Appendix I). The soil texture was silty loam, non-calcarious, dark grey soil of Tejgaon soil series with a pH 6.7. The characteristics of soil are given in Appendix II.

3.4 Weather Condition

The experiment was carried out during the period from last week of November 2019 to March 2020. The average temperature, precipitation and relative humidity of those months are given below:

Table 1. Average weather conditions in Dhaka, Bangladesh in November 2019- March 2020

Season and year	Average Temperature (°C)	Average Precipitation (mm)	Average Humidity (%)
Winter, 2019	20.5	16.67	72

Source: Metrological Department of Bangladesh

3.5 Treatments

There were eight treatments in the experiment and each treatment was replicated 3 times. Treatments were included different organic manure, Carbendazim and spent mushroom substrate compost along with their combination.

T_o = Control

T_1 = Poultry manure

T_2 = Cow dung

T_3 = Spent Mushroom Substrate Compost (SMC)

T_4 = Carbendazim (Bavistin 50 WP)

T_5 = Spent mushroom compost + Poultry manure

T_6 = Spent mushroom compost + Cow dung

T_7 = Spent mushroom compost + Carbendazim (Bavistin 50 WP)

3.6 Experiment conducted

For fulfilling the objectives of the study, the research was split into the experiments.

- Application of different treatments for foot and root rot disease management at field condition.
- Isolation and identification of fungal pathogen in the laboratory and pathogenicity test in net house.
- Bio-assay of organic amendments and Carbendazim by poison food technique.

3.7 Application of different treatments for foot and root rot disease management at field condition

3.7.1 Variety

BARI Masur-4 variety was used for this experiment. Seeds of BARI masur-4 were collected from local market.

3.7.2 Design of the experiment

The experiment was carried out in Randomized Complete Block Design (RCBD) with three replications.

3.7.3 Land preparation

A piece of medium high land with well drainage system was selected for lentil cultivation. The experimental field was first ploughed on 10 November 2019. The land was ploughed thoroughly with a power tiller and laddering was done to obtain a desirable tilt. The clods of the land were hammered to make the soil into small pieces. Weeds, stubbles and crop residues were cleaned. The final ploughing and land preparation was done on 15 November 2019.

3.7.4 Field layout

The layout was done as per experimental design on 20 November 2019. The field was divided into three blocks each of which representing a replication. Each block then divided into 8 unit plots. The unit plot size was 2.0 m × 1.75 m. Two adjacent block to block distances was 1.0 m; plot to plot distance was 0.5 m (Appendix III). The treatments were distributed randomly among the unit plots of each block. Total field size was 18 m × 9 m (Figure 1).

Figure 1. Experimental layout of the field

3.7.5 Collection of Spent mushroom compost, Poultry manure, Cow dung and Carbendazim

The organic amendments poultry manure and cow dung was collected from Farm of Sher-e Bangla Agricultural University and spent mushroom compost was collected from Biotech Savar, Dhaka. One fungicide namely Bavistin 50 WP was collected from local market of Dhaka (Plate 1).

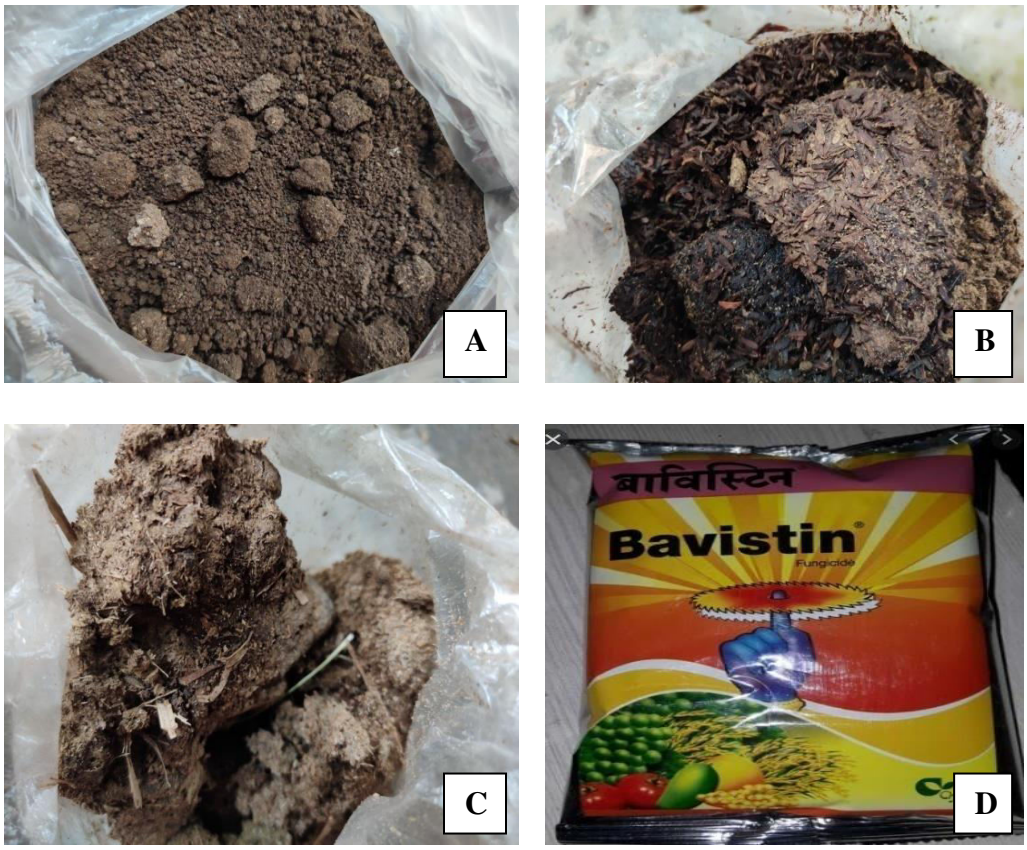


Plate 1. Treatments used in the field experiment; A. Spent Mushroom Compost; B. Poultry manure; C. Cow dung; D. Carbendazim (Bavistin 50WP)

3.7.6 Application of Spent mushroom compost, Poultry manure, Cow dung in the field soil

The organic amendments such as poultry manure, cow dung and spent mushroom compost were used as soil amendment 5.25 kg/ plot before three days of seed sowing. The organic manures were mixed thoroughly with soil (Figure 2).

The following amount of organic manures was used which are shown in table 2.

Table 2. Organic amendments dose used in the experimental plot

Fertilizer name	Applied dose (Kg/ 3.5 m^2 plot)	Recommended dose* (Kg/ha.)
Poultry manure	5.25	15000
Cow dung	5.25	15000
Spent mushroom compost	5.25	15000
Spent mushroom compost + Poultry manure (1:1)	2.625 + 2.625	15000
Spent mushroom compost + cow dung (1:1)	2.625 + 2.625	15000

Figure 2. Application of different organic amendments in the field

3.7.7 Seed sowing

Selected healthy and disease free seeds were planted in the experimental field. Lentil seeds were sown in rows at a rate of 30 kg ha^{-1} on 25 November 2019 following a continuous seeding method. The row to row distance was 30 cm while the seeding depth was 2-3 cm.

3.7.8 Intercultural Operation

3.7.8.1 Thinning

Seeds started germination of three Days after sowing (DAS). Thinning was done two times; first thinning was done at 8 DAS and second was done at 15 DAS to maintain optimum plant population in each plot.

3.7.8.2 Irrigation

Irrigation was done at 10-15 days interval. But additionally supplementary irrigation was provided as per treatment before flowering of lentil plants.

3.7.8.3 Weeding

Weeding was done four times in the experimental period at 20 days after sowing, 40 days after sowing, 55 days after sowing and 70 days after sowing.

3.7.8.4 Spraying of fungicide

Fungicide Bavistin 50 WP@ 0.2% solutions was prepared separately by taking requisite amount of fungicide dose. 400 ml fungicide was sprayed at T_4 and 200 ml was sprayed at T_7. Spraying was done at 15 days interval for 3 times by hand sprayer. Spraying was done at 30 DAS, 45 DAS and 60 DAS. Precautions were taken to avoid drifting of spray materials from plant to neighboring plants.

3.7.9 Crop sampling and data collection

During the growing period the plots of lentils were inspected regularly to record the foot and root rot disease and to measure different agronomic parameter. Dead plants were removed from the field after counting and infected plants were collected to identify foot and root rot pathogens. Plants of half square meter from each treatment were randomly selected and marked with sample stricks and following data were recorded.

3.7.9.1 Plant height

The plant height was measured at 40 days, 65 days after seed germination and harvest with a meter scale from the ground level to the top of the plants.

3.7.9.2 Number of branches per plant

The number of branches per plant was counted at 40 days, 65 days and harvest from 5 selected plants. The average number of branches per plant was calculated.

3.7.9.3 Number of nodules per plant

The total number of nodules of selected plants from each plot was counted at 40 days, 65 days and 90 days after sowing and data were recorded as the average of 5 plants selected at random from the inner rows of each plot.

3.7.9.4 Number of pods per plant

The total number of pods of selected plants from each plot was counted at 40 days, 65 days and harvest and data were recorded as the average of 5 plants selected at random from the inner rows of each plot.

3.7.9.5 Number of seeds per pod

The number of seeds of 5 selected pods from each plot was counted and data were recorded and average number of seed per pod was calculated.

3.7.9.6 Weight of 1000 seeds

One thousand cleaned, dried lentil seeds were counted from each harvest sample and weighed by using a digital electric balance and weight was expressed in gram.

3.7.9.7 Seed weight per plant

Dried lentil seeds of 5 selected plants were counted from each harvest sample and weighed by using a digital electric balance and weight was expressed in gram.

3.7.9.8 Stover yield per plant

Sun dried stover of 5 selected plants was weighted and weight was expressed in gram.

3.7.9.9 Seed yield

The seeds collected from (2.0 × 1.75) square meter of each plot were sun dried properly. The weight of seeds was taken and converted the yield in kg per hectare.

3.7.9.10 Stover yield

The stover collected from (2.0 × 1.75) square meter of each plot was sun dried properly. The weight of seeds was taken and converted the yield in kg per hectare.

3.7.9.11 Estimation of harvest index of lentils

For calculation of Harvest Index dried grain and straw were weighed by using a digital electric balance.

Harvest index of lentil was calculated by the following formula:

$$\text{Harvest index (\%)} = \frac{\text{Economic yield}}{\text{Biological yield}} \times 100$$

Where

Economic yield = grain yield

Biological yield = grain and straw yield

3.7.9.12 Assessment of disease incidence of foot and root rot

For estimation of disease incidence every plant was counted and also counted the infected plants within observation area of each treatment and then expressed in percentage.

Disease incidence data was calculated by following formula (Nutter *et al.,* 2006; Agrios, 2005; Kranz, 1988; James, 1974; Large, 1966):

$$\text{Disease incidence (\%)} = \frac{\text{Number of diseased plants}}{\text{Number of total plant observed}} \times 100$$

Reduction of disease incidence over control was calculated by using the following formula (Abbott, 1925):

$$\text{Disease Incidence Reduction over Control (\%)} = \frac{C - T}{C} \times 100$$

Where,

C = Percent disease incidence on control plot

T = Percent disease incidence on treatment plot

3.7.10 Harvesting

Harvesting was done 7 March 2020 when leaves began to fall, stem and pod turn brown or straw in colour and seeds were hard and rattle with 15% moisture inside.

3.7.11 Threshing and storage

Lentils were allowed to dry for 4 days on threshing floor and threshed by manually. The clean seed was sun dried for 3 days to bring their moisture content at 9-10%. The seed was safely stored in appropriate bins to protect them from bruchids.

3.8 Isolation and Identification of fungal pathogen in the laboratory and pathogenicity test in net house

3.8.1 Collection of diseased specimens

Diseased roots of lentil were collected from the experimental field of Sher-e-Bangla Agricultural University (SAU), Sher-e- Bangla Nagar, Dhaka- 1207. Collected root samples were put in polyethylene bags immediately after collection to protect them from drying. Then the samples were preserved in refrigerator at 4 °C for future use.

3.8.2 Sterilization of Materials and Equipments

Liquid materials, such as PDA media and distilled water were sterilized in an autoclave following the method (Hazra, 1988) at 121 °C under 15 pound per square inch (PSI) for 30 min. For surface sterilization, 0.1 % sodium hypochlorite (NaOCl) was used for infected plant material and rectified spirit was used for sterilization other equipment's like inoculation-needles, forceps, inoculation chamber, hands etc.

3.8.3 Isolation of Causal Organism (*Sclerotium rolfsii*) by Tissue Planting Method

3.8.3.1 Moist blotter method

The infected roots of lentil were cut into several pieces by scissors and placed on the moist filter paper (Whatman no.1). Three pieces of filter paper were moistened by dipping in sterile water were placed in each of the petridish. The petridishes with the diseased specimens were incubated at 22±2 °C under 12/12 alternating cycles of NUV and darkness in the incubation room of the Pathology Laboratory of Sher-e-Bangla Agricultural University (SAU) for 5-6 days.

3.8.3.2 Preparation of Potato Dextrose Agar (PDA) media

Two hundred grams of cleaned, washed and peeled potato tubers were chopped into small pieces. Later chopped pieces of potato were boiled in distilled water and the extract was collected by filtering through muslin cloth. Dextrose (20 gm) and agar (20 gm) were dissolved in the potato extract and the volume was made up to 1000 ml by adding distilled water. Known quantity of PDA medium was dispensed into number of conical flasks. The flask was plugged with non absorbent cotton and finally wrapped with brown paper. The flasks containing dispensed medium was sterilized at 121 °C temperature with 15 PSI pressure for about 30 minutes. Then the medium was acidified by adding of lactic acid (1:10) in medium. The medium was dispensed aseptically into sterile glass Petri-dishes inside the inoculating chamber and allowed to cool down to solidify.

3.8.3.3 Isolation, identification, multiplication and confirmation of *Sclerotium rolfsii*

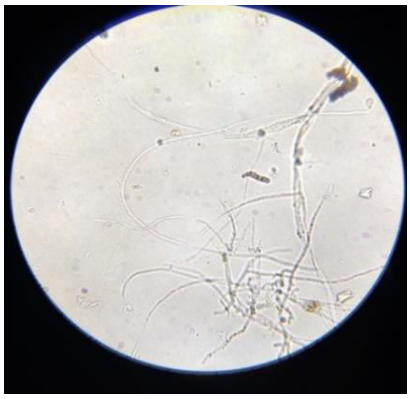
The seedlings of lentil showing typical symptoms of foot and root rot were collected from university fields. The fresh collected diseased seedlings of lentil were thoroughly washed under running tap water to remove surface soil, sand particles and other contaminants. Then infected portion of roots of the lentil seedlings were cut into small pieces (5 mm). The cut portion of roots was surface sterilized with 1% clorox for 2-3 minutes, and rinsed with sterilized water for 3 times and dried on sterile filter paper. Samples were placed in moist blotter then mycelia were found after seeing it in compound microscope then samples were placed on PDA media in 9 cm petridishes and incubated at 25± 1 °C. During incubation root pieces were examined daily to investigate fungal growth. After incubation, the inoculated plates were observed to identify the causal organism of disease.

Pure culture of the isolates were prepared following hyphal tip methods (Tuite, 1969; Mian, 1995) and subsequently transferred to fresh PDA slants in petridishes. Petridishes and test tube slants containing pure culture of *Sclerotium rolfsii* were stored at 4 °C. After 20-30 days mustard seed like brown sclerotia were formed (Plate 2).

Foot and root infected root of lentil

Infected root segment on moist blotter

Pure culture on PDA media



Mycelium of *Sclerotium rolfsii* under compound microscope

Sclerotia produced on PDA media

Plate 2. Flow chart of isolation, identification and culture of *Sclerotium rolfsii* on PDA media

3.8.4 Net house experiment (Pathogenicity Test)

3.8.4.1 Soil collection

Soil was collected from the experimental fields of Sher-e-Bangla Agricultural University, Dhaka-1207.

3.8.4.2 Sterilization of soil

Soil was dripped with 40% formalin solution @ 200 ml/cft soil and kept covered with polyethylene sheets for 2-3 days. Then the soil was uncovered and pulverized enough and kept for 2-3 days to release the gas of formalin.

3.8.4.3 Preparations of pots

Sterilized soil was dispensed at the rate of desired amount per pot. Then the pots were arranged.

3.8.4.4 Seed sowing in pots

Disinfected viable seeds of lentil susceptible to foot and root rot variety BARI Masur-4 was sown in pots with 10 seeds per pot in the net house. Watering was continued till the seedlings were established.

3.8.4.5 Pathogenicity tests for *Sclerotium rolfsii*

The pathogenicity test of the isolate was done for confirmation of the pathogenicity of *Sclerotium rolfsii* isolate. The pathogenicity of *Sclerotium rolfsii* isolate was confirmed by Koch's postulates using the method of Chevalier *et al.* (1991). The plants were inoculated after germination, at the age of 21 days after germination and 25 ml of mycelial solution (two plates with fungal colonies) into 150 ml of distilled sterile water applied in each pot to the soil near the stem. The mycelial solution was sprayed on to the seedlings until runoff while water was used for spraying the control treatment. Inoculated pots were covered with polythene bags. Periodical observations were made for the development of

symptoms on the plants after inoculation. Experiments were done with three replications. Infected yellow to dark brown lentil seedlings were observed after 7th day of inoculation. After 7 day of inoculation, 86.66% disease incidence was recorded in *Sclerotium rolfsii* inoculated lentil plants; whereas the disease was not developed in un inoculated lentil pot. The *Sclerotium rolfsii* isolates were re-isolated from infected plants part and compared with original culture and thus Koch's postulates were proved. The re-isolation revealed that the isolated fungi from diseased lentil seedlings were found to be identical with those used for artificial inoculation (Plate 3).

Plate 3. Pathogenicity tests of *Sclerotium rolfsii*; A. Pure culture of *Sclerotium rolfsii,* B. Healthy seedlings of lentil C. Seedlings covered with polythene sheet after artificial inoculation with *Sclerotium rolfsii*, D. Foot and root rot infected seedling 7 days after inoculation with *Sclerotium rolfsii*

3.9 Bio-assay of organic amendments and Carbendazim by poison food technique

Poisoned food technique was used to test the effect of spent mushroom compost, poultry manure, cow dung and Bavistin 50WP and their combination with SMC on the growth of *Sclerotium rolfsii in-vitro* under laboratory conditions.

3.9.1 Preparation of the aqueous extracts of SMC, poultry manure and cow dung

From SMC, poultry manure and cow dung, 0.15g of each was suspended in 100 ml of sterile distilled water to obtain 15% concentrations. Each suspension was agitated by shaking very well to obtain even particle distribution. The extracts of organic manures were sterilized by autoclaving at 121^{0}C for 15 minutes (Plate 4).

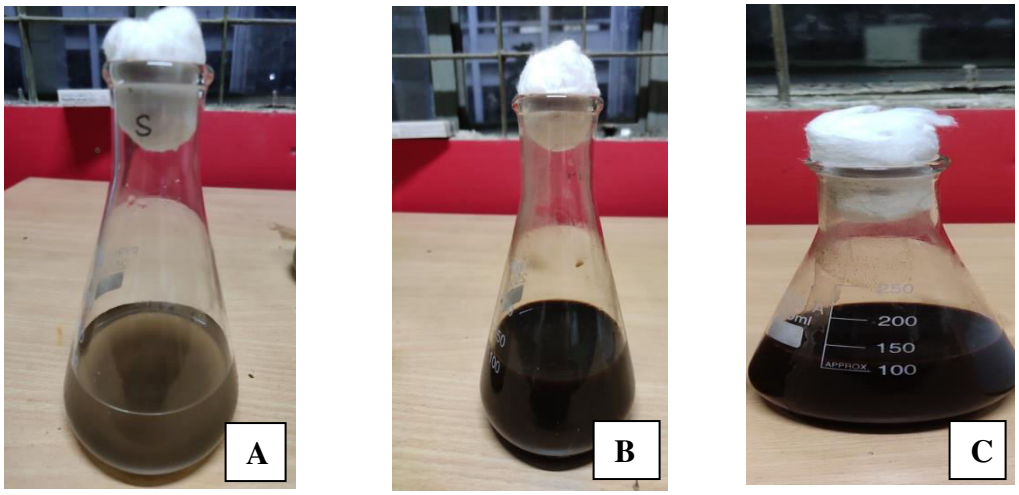


Plate 4. Preparation of the aqueous extracts of different treatments; A. Spent mushroom compost; B. Poultry manure; C. Cow dung

3.9.2 Preparation of the fungicide solution

To prepare 0.2% Bavistin 50WP solution, two (2) grams of Bavistin was mixed with one liter of cool sterilized water (Figure 3).

Figure 3. Preparation of 0.2% Bavistin 50WP solution

3.9.3 Poisoned food technique

At first one ml each of the portions of the SMC, poultry manure, cowdung suspension and Carbendazim 50WP solution was poured into sterile petridishes. Combined plates had 0.5 ml of the each portion and then Ten ml of cool molten, PDA was aseptically poured into each petridish and rotated gently to ensure even dispersion. Control plates had 1 ml of sterile distilled water mixed with cool PDA. Then the plates were left to solidify. Inoculation was done by placing a 5 mm diameter mycelia disc taken from the edge of a fresh culture (5-6 days old) of *Sclerotium rolfsii* in the centre of the plates with 3 replications. All plates were incubated at 25±2°C in incubation chamber. Data were collected on mycelia growth and recorded from 1 day after inoculation till when control plates were fully covered with test pathogen's growth.

3.9.4 Measurement of radial growth (cm) and determination of percent inhibition

Radial growth (cm) of *Sclerotium rolfsii* in petridishes was recorded from first day to fifth days after incubation. The radial growth (cm) of mycelium of each plate

was measured by taking average of the two diameters taken for each colony and then these plates were kept for 30 days for sclerotia formation (Figure 4).

Percent inhibition in mycelia growth was determined using the formula below:

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

I = percentage inhibition of mycelia growth

C = radial growth of pathogen in control

T = radial growth of pathogen in treated plate.

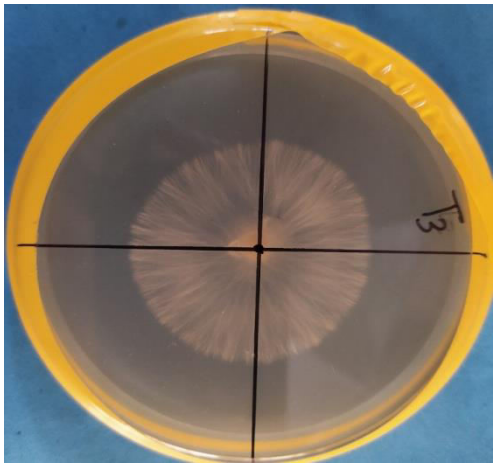


Figure 4. Measurement of radial growth of *Sclerotium rolfsii* on PDA

3.10 Statistical Analysis

The data obtained for different characters were statistically analyzed to observe the significant differences among different treatments. The recorded data were analyzed by using computer based software MSTAT-C. The significance of the difference among the means values was estimated by the Least Significant Difference (LSD) test at 5% level of probability.

CHAPTER IV

RESULTS

4.1 Symptoms of foot and root rot disease of lentil

The characteristics of foot and root rot symptoms were observed in the experimental field of SAU under natural conditions. The foot and root rot disease of lentil affects mainly the roots, leading to poor emerge of seedlings, stunted growth of plants and reduced yield. Symptoms include sunken lesions and brown or discoloration on roots, shrinking root system and root decay. As a result the plants became light brown to red and finally plants dry up and die (Plate 5). Plant infected during late stages of development of lentil plants show stunted growth. Opportunistic pathogens colonize and feed on decaying tissue of lentil plants, which makes the symptoms worse. Brownish mustard seed sclerotia like are developed at later stages on the root and collar region of the infected plants (Plate 6). In the field the foot and root rot often occurs in patches and may expand if conditions are favorable for the pathogens.

Plate 5. Foot and root rot symptoms showing in standing plants (A&B)

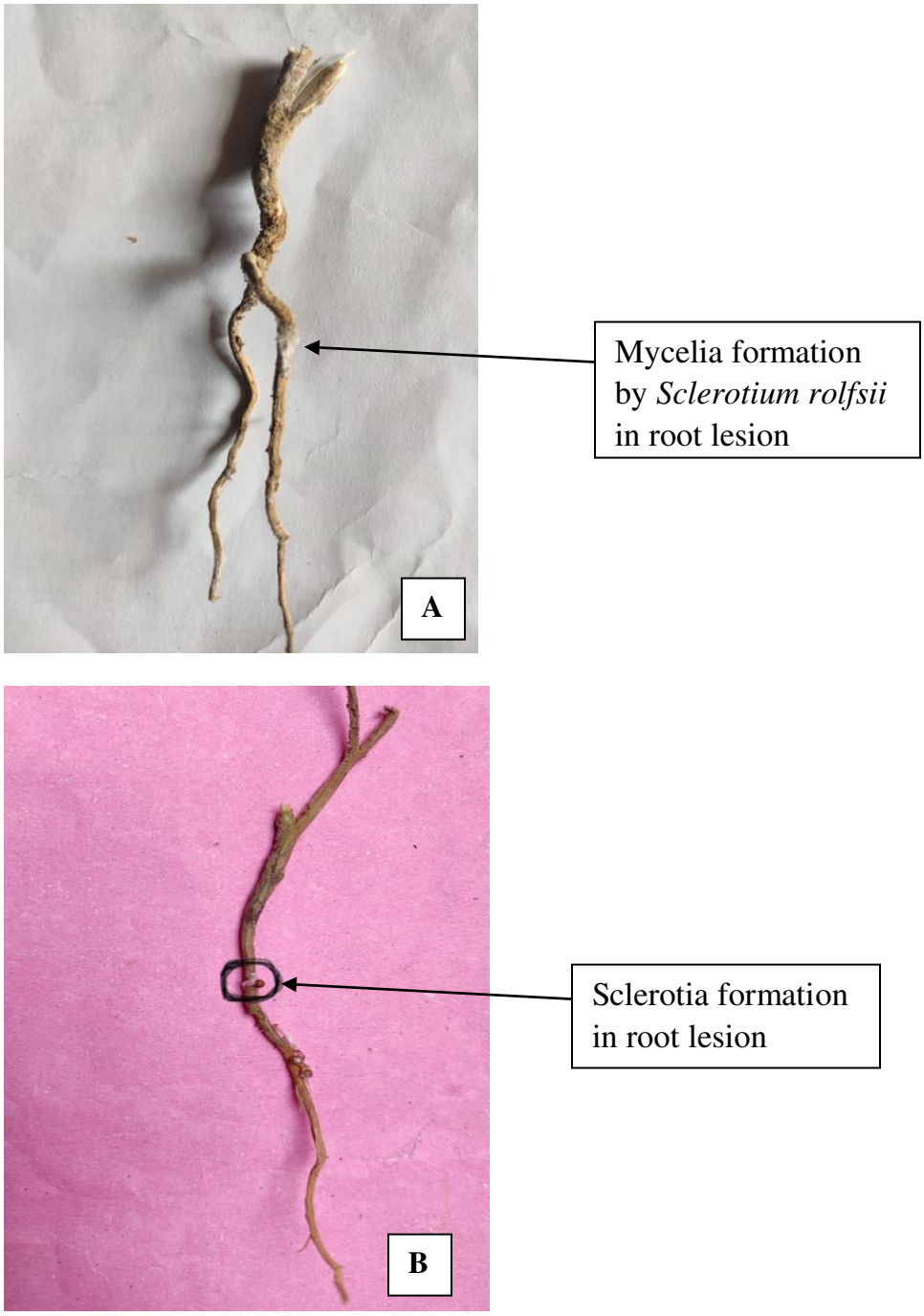


Plate 6. Mycelia and sclerotia formation by *Sclerotium rolfsii* in the infected root lesion; A. Mycelium formation by *Sclerotium rolfsii* and B. Sclerotia formation in root lesion

4.2 *In-Vitro* efficacy of organic amendments, Carbendazim and their combination with spent mushroom compost in poisoned food technique

In-vitro condition efficacy of spent mushroom substrate compost (SMC), Bavistin 50WP, cow dung, poultry manure and their combination with SMC on radial mycelial growth of *Sclerotium rolfsii* was significant (Table 3, Plate 7) where all treatments reduced the radial mycelial growth of the fungus. The lowest radial mycelial growth (0.93 cm, 1.4 cm, 2. 43 cm, 3.37 cm and 5.71 cm) was recorded in case of T_7 at 1 day, 2 days, 3 days, 4 days and 5 days after inoculation, respectively and the highest radial mycelial growth (2.26 cm, 3.16 cm, 5.00 cm, 6.15 cm and 9.00 cm) was recorded in T_0 at 1 day, 2days, 3days, 4 days and 5 days after inoculation on PDA media. Radial mycelial growth for all the tested organic soil amendments, Bavistin 50WP and combinations with SMC ranged from 5.71 cm to 9.00 cm recorded after inoculation of 5 days. At 5DAI, the lowest radial mycelial growth was found in T_7 which was statistically similar to T_4 and T_6. The highest radial mycelium growth was recorded in untreated control preceded by T_2 (7.9 cm), T_1 (7.8 cm), T_3 (7.4 cm), T_5 (6.91 cm), T_6 (6.1 cm) and T_4 (6.1 cm) and T_7 (5.71 cm) at 5 days after inoculation. The highest percent inhibition (36.48%) was recorded in T_7 statistically identical to T_6 (32.22%) and T_4 (32.22%) at 5 days after inoculation. No growth inhibition was found in control plate and full colonization of mycelium was observed within 5 days after inoculation.

Table 3. *In-vitro* efficacy of organic amendments, Carbendazim and their combination with spent mushroom compost against *Sclerotium rolfsii*

Treatments	Radial mycelial growth (cm) of *Sclerotium rolfsii*					% Inhibition of mycelial growth (5 DAI)
	1 DAI	2 DAI	3 DAI	4 DAI	5 DAI	
T_0	2.26 A	3.16 A	5 A	6.15 A	9 A	0.00
T_1	1.56 B	1.94 B	3.08 BC	4.31 B	7.8 BC	13.33
T_2	1.3 BC	2.03 B	3.33 B	4.35 B	7.9 B	12.22
T_3	1.16 CD	1.63 B	2.9 C	3.71 C	7.4 C	17.78
T_4	1.13 CD	1.71 B	2.6 DE	3.61 CD	6.1 E	32.22
T_5	1.33 BC	1.81 B	2.86 CD	3.66 CD	6.91 D	23.14
T_6	1.13 CD	1.66 B	2.46 E	3.51 DE	6.1 E	32.22
T_7	0.93 D	1.4 B	2.43 E	3.37 E	5.71 E	36.48
LSD (0.05)	0.14	0.4	0.12	0.08	0.21	2.34
CV (%)	12.42	25.71	5.07	2.55	3.63	13.74

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly by LSD at 0.05 level of probability. CV = Coefficient of varience

DAI = Days after inoculation

T_0 = Control, T_1 = Poultry manure, T_2 = Cow dung, T_3 = Spent mushroom compost (SMC), T_4 = Carbendazim (Bavistin 50 WP), T_5 = Spent mushroom compost + Poultry manure, T_6 = Spent mushroom compost + Cow dung and T_7 = Spent mushroom compost + Carbendazim (Bavistin 50 WP)

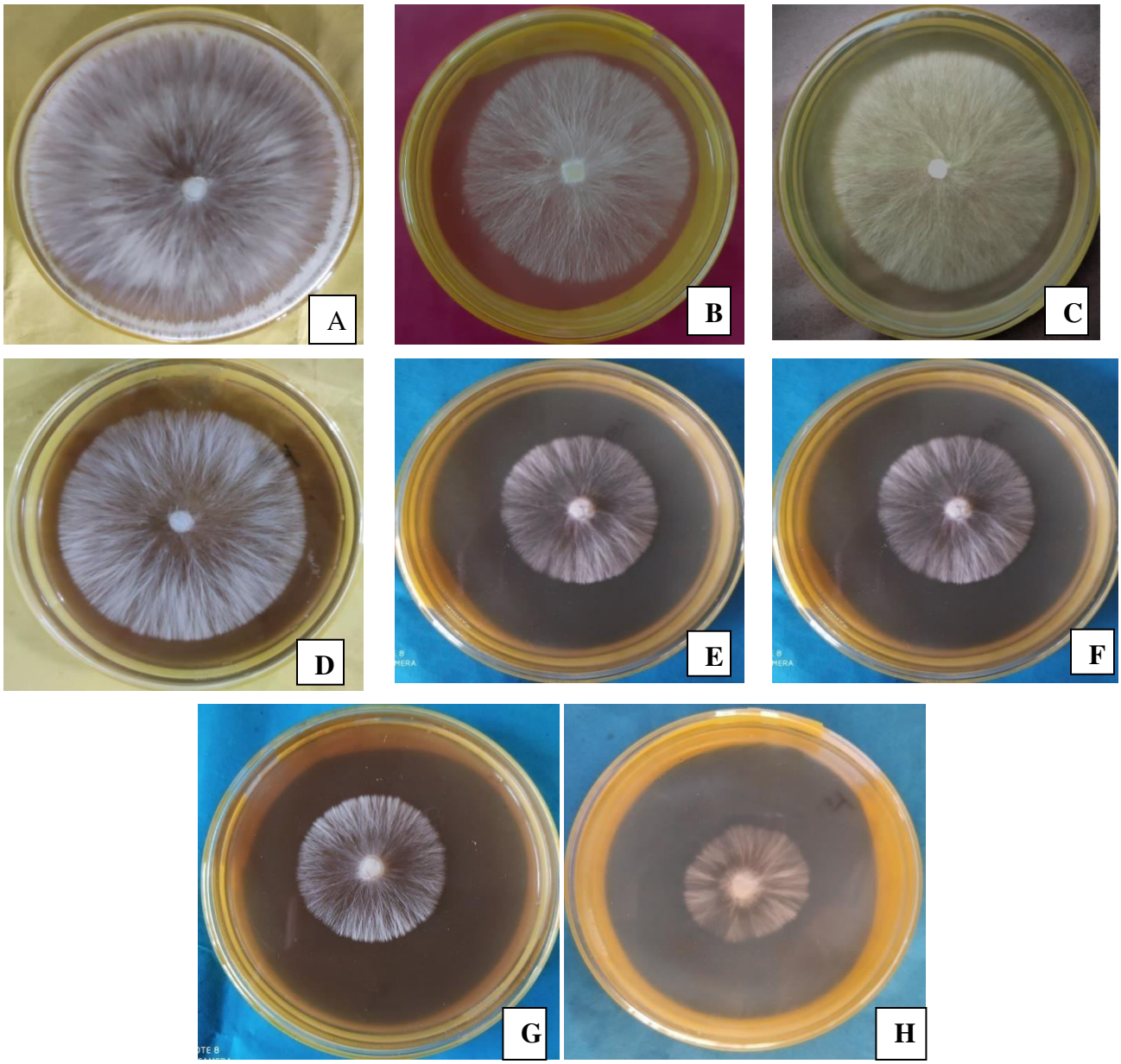


Plate 7. Radial mycelial growth of *Sclerotium rolfsii* under A. Control, B. Poultry manure, C. Cow dung, D. Spent mushroom compost, E. Carbendazim 50 WP, F. Spent mushroom compost + Poultry manure, G. Spent mushroom compost + Cow dung and H. Spent mushroom compost + Carbendazim 50 WP at 5 days after inoculation

4.3 Relationship between radial mycelial growth and percent inhibition of mycelial growth of *Sclerotium rolfsii*

Correlation between radial mycelial growth and percent inhibition of mycelial growth of *Sclerotium rolfsii* at 5 days after inoculation was presented in Figure 5. Correlation study was done to establish the relationship between radial mycelial growth and % inhibition of mycelial growth of *Sclerotium rolfsii* at 5 days after inoculation at different treatments and negative correlation was observed. It was evident that the equation y = - 11.09x + 99.87 gave a good fit to the data and the co-efficient of determination (R^2 = 1) fitted regression line had a significant regression co-efficient at 5 DAI of *Sclerotium rolfsii*. It showed that radial mycelia growth at 5 DAI was strongly as well as negatively correlated with percent inhibition of mycelial growth of *Sclerotium rolfsii*.

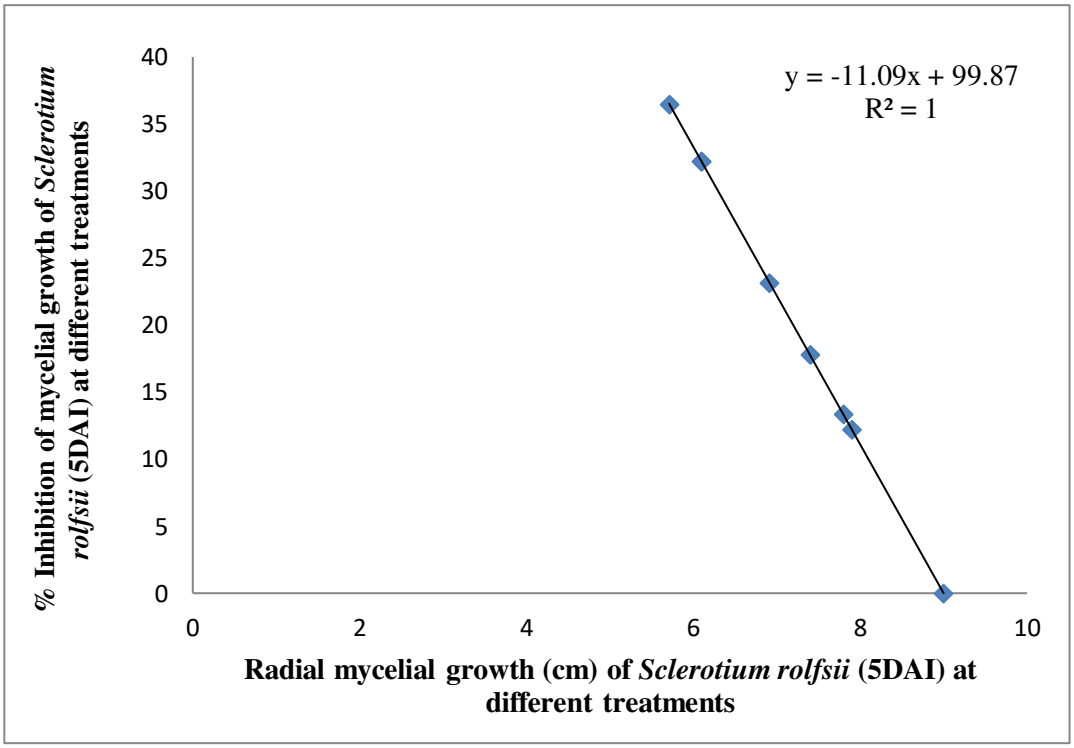


Figure 5. Relationship between radial mycelial growth and percent inhibition of mycelial growth of *Sclerotium rolfsii* at 5DAI

4.4 Effect of organic amendments, Carbendazim and their combination with spent mushroom compost on percent disease incidence of foot and root rot disease of lentil

Data recorded on percent disease incidence of foot and root rot disease of lentil was affected by the application of spent mushroom compost, poultry manure, cow dung, Bavistin 50WP and their combinations with SMC (Table 4). At 40 DAS**,** the disease incidence was varied from 0.98% to 2.98% and the lowest disease incidence was observed in T_6 that was statistically similar to T_3, T_4, T_5, T_6 and T_7. In case of application of poultry manure (T_1) and cow dung (T_2), the plant disease incidence was 8.09% and 8.10%, respectively which were statistically and significantly similar. Again, plant disease incidence in case of SMC (3.6%) alone and SMC combined with poultry manure (4.5%) was also statistically similar. The highest disease incidence was observed in T_0.

At 65 DAS, the highest disease incidence was recorded in T_0 (38.16%) and lowest (5.51%) was recorded from T_7 which was statistically identical to T_4 and T_6. T_1 and T_2 also gave the same disease incidences which were statistically similar (Table 4).

At 90 DAS, disease incidence was ranged from 10.57% to 47.30% and combination of SMC with Bavistin 50WP (T_7) gave the lowest disease incidence that was statistically similar to T_4, T_5 and T_6. Statistically identical result was found in case of T_1, T_2 and T_3, respectively. The highest disease incidence was recorded in control treatment. Among all the treatments, SMC with Bavistin 50WP gave the highest percent reduction of disease incidence (77.65%) followed by T_6 (75.58%) and T_4 (74.90%) (Table 4).

Table 4. Effect of organic amendments, Carbendazim and their combination with spent mushroom compost on percent disease incidence of foot and root rot disease of lentil in field condition

Treatments	% Disease incidence			% Reduction of disease incidence over control
	40DAS	65DAS	90DAS	
T_0	26.98 a	38.16 a	47.30 a	-
T_1	8.09 b	19.47 b	21.94 b	53.62
T_2	8.10 b	18.58 b	22.50 b	52.43
T_3	3.6 bc	11.78 c	19.21 b	59.39
T_4	1.83 c	5.73 d	11.87 c	74.90
T_5	4.55 bc	10.80 cd	13.56 c	71.33
T_6	0.98 c	5.81 d	11.55 c	75.58
T_7	2.00 c	5.51 d	10.57 c	77.65
LSD (0.05)	2.81	2.59	2.61	-
CV (%)	49.84	21.96	16.1	-

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly by LSD at 0.05 level of probability.
CV = Co-efficient of variation
DAS = Days after sowing
T_0 = Control, T_1 = Poultry manure, T_2 = Cow dung, T_3= Spent mushroom compost (SMC), T_4 = Carbendazim (Bavistin 50 WP), T_5 = Spent mushroom compost + Poultry manure, T_6 = Spent mushroom compost + Cow dung and T_7 = Spent mushroom compost + Carbendazim (Bavistin 50 WP)

4.5 Plant height and number of branches per plant

In case of plant height and number of branches per plant significant differences were recorded for different treatments compared to control. The highest plant height (13.50 cm) was recorded from T_6 followed by T_7 (12.29 cm) and the lowest plant height (11.74 cm) was recorded from T_0 at 40 DAS. Statistically similar result was found in case of T_1, T_2, T_3 and T_7. At 65 DAS, the highest plant height (36.5 cm) was recorded from treatment T_6 followed by T_7 (36.16 cm), while the lowest plant height (29.46 cm) was recorded from T_0. Statistically identical result was observed in case of T_3 and T_7. On the other hand the plant height of T_1 was statistically similar to T_2 and Bavistin 50WP (T_4) was statistically identical to T_5 (Table 5). At 90 DAS, The highest plant height (39.82 cm) was recorded from treatment T_6 which was statistically different from other treatments followed by T_7 (39.35 cm) and the lowest (35.50 cm) plant height was recorded from T_0 (Control). Statistically and significantly identical result was observed in case of T_2 and T_5 (Table 5).

The number of branches per plant varied from 2.53-4.80, 4.80-6.40 and 6.00-9.80 at 40 DAS, 65 DAS and 90 DAS, respectively. At all different days after sowing, the lowest numbers of branches were recorded from control (T_0). At 40 DAS, the highest number of branches per plant was recorded from T_6 and T_3 which was statistically identical followed by T_7 (4.53). T_5 was statistically similar with T_7. Similarly at 65 DAS, different treatments showed a remarkable significant variation in number of branches per plant. The highest number of branches per plant was recorded from T_6 which was statistically identical to T_2, T_3, T_4 and T_7 followed by treatment T_7 (6.13). At 90 DAS the highest number of branches per plant was recorded from treatment T_6 which was statistically different from other followed by T_7 (8.86). Statistically similar result was found in case of T_2, T_3 and T_4 but significantly different from each other (Table 5).

Table 5. Effect of organic amendments, Carbendazim and their combination with spent mushroom compost on plant height and number of branches/plant in field condition

Treatments	Plant height (cm)			No. of branches/Plant		
	40 DAS	65 DAS	90 DAS	40 DAS	65 DAS	90 DAS
T_0	11.74 c	29.46 d	35.50 e	2.53 d	4.80 b	6.00 d
T_1	12.16 abc	34.16 c	37.26 cde	3.4 c	6.46 a	7.26 cd
T_2	12.64 abc	34.57 c	37.62 bcd	3.73 bc	5.26 ab	8.13 bc
T_3	12.09 abc	36.14 ab	39.04 abc	4.80 a	5.6 ab	8.26 bc
T_4	11.94 bc	35.46 b	36.96 de	4.00 abc	6.00 ab	8.06 bc
T_5	13.43 ab	35.70 b	37.62 bcd	4.33 ab	4.80 b	8.46 abc
T_6	13.50 a	36.5 a	39.82 a	4.80 a	6.40 ab	9.80 a
T_7	12.29 abc	36.16 ab	39.35 ab	4.53 ab	6.13 ab	8.86 ab
LSD (0.05)	0.72	0.33	0.90	0.39	0.76	0.68
CV (%)	7.08	1.2	2.94	11.93	16.38	10.39

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly by LSD at 0.05 level of probability.
CV = Co-efficient of variation
DAS = Days after sowing
T_0 = Control, T_1 = Poultry manure, T_2 = Cow dung, T_3= Spent mushroom compost (SMC), T_4 = Carbendazim (Bavistin 50 WP), T_5 = Spent mushroom compost + Poultry manure, T_6 = Spent mushroom compost + Cow dung and T_7 = Spent mushroom compost + Carbendazim (Bavistin 50 WP)

4. 6 Number of nodules per plant of lentil under different treatments

Different organic amendments, Bavistin 50WP and their combination with SMC showed a remarkable significant variation in number of nodules per plant (Plate 8). The number of nodules per plant was ranged from 0.86 - 4.00, 7.93 – 24.26 and 10.73 to 28.00 at 40 DAS, 65 DAS and 90 DAS, respectively under different treatments. At 40 DAS, the highest number of nodules per plant was recorded from treatment T_6 followed by T_7 which were statistically identical but significantly different and the lowest number of nodules per plant was recorded from T_0. Statistically similar result was found in case of T_2 and T_4. Similarly, T_3 was also statistically identical to T_5 (Table 6). At 65 DAS, the highest number of nodules per plant was recorded from T_6 which was statistically different from other treatments followed by treatment T_7 and the lowest was observed in T_0. Statistically similar but significantly different result was observed in case of T_3 and T_7. The number of nodules per plant in T_1 was also statistically similar to T_2. At 90 DAS, the highest number of nodules per plant was recorded from treatment T_6 followed by T_7 and the lowest was recorded from T_0 (Table 6).

Table 6. Effect of organic amendments, Carbendazim and their combination with spent mushroom compost on number of nodules/ plant of lentil

Treatments	No. of nodules/plant		
	40 DAS	**65 DAS**	**90 DAS**
T_0	0.86 d	7.93 e	10.73 f
T_1	2.60 bc	12.46 d	13.40 ef
T_2	1.86 cd	11.80 d	14.66 e
T_3	3.46 ab	17.40 b	18.80 cd
T_4	1.73 cd	13.53 cd	16.06 de
T_5	3.33 ab	16.46 bc	19.46 bc
T_6	4.00 a	24.26 a	28.00 a
T_7	3.93 a	18.93 b	22.33 b
LSD (0.05)	0.50	1.63	1.51
CV (%)	22.6	12.97	10.34

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly by LSD at 0.05 level of probability.
CV = Co-efficient of variation,
DAS = Days after sowing
T_0 = Control, T_1 = Poultry manure, T_2 = Cow dung, T_3= Spent mushroom compost (SMC), T_4 = Carbendazim (Bavistin 50 WP), T_5 = Spent mushroom compost + Poultry manure, T_6 = Spent mushroom compost + Cow dung and T_7 = Spent mushroom compost + Carbendazim (Bavistin 50 WP)

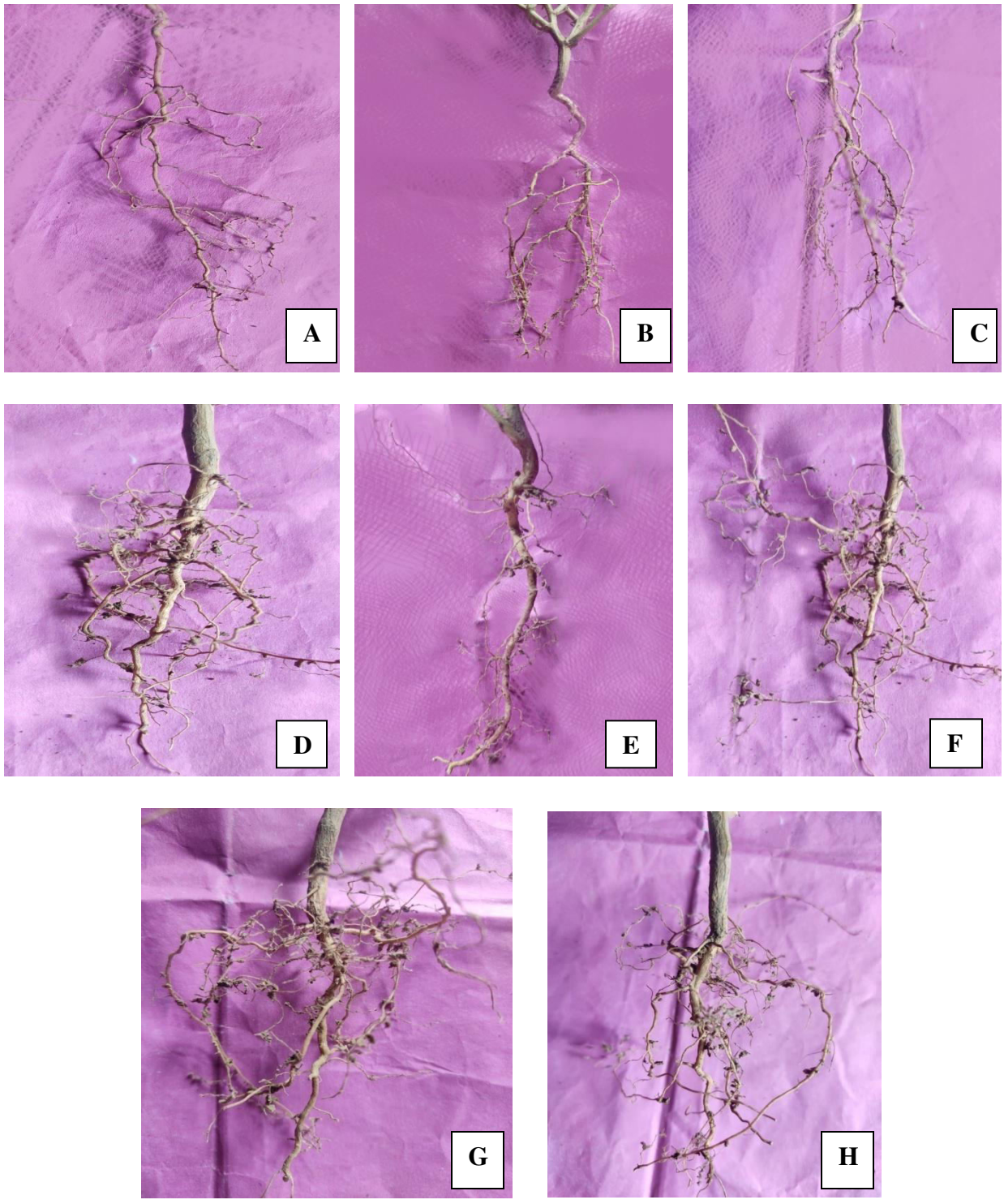


Plate 8. Nodules at root of lentil; A. Control, B. Poultry manure, C. Cow dung, D. Spent mushroom compost, E. Carbendazim 50 WP, F. Spent mushroom compost + Poultry manure, G. Spent mushroom compost + Cow dung and H. Spent mushroom compost + Carbendazim 50 WP

4.7 Yield contributing characters of lentil under different treatments

The number of pods per plant number of seeds pod-^{1} and1000-seed weight varied significantly compared to control. Number of pod per plant was ranged from 46.11 to 123.55 under various treatments of lentil (Table 7). The highest number of pod per plant was recorded from the amended with spent mushroom compost and cow dung treated plot (T_6) which was statistically different from all other treatment and the lowest number of pod per plant was obtained from T_0. Number of pods per plant in T_3, T_5 and T_7 were statistically similar but significantly different. Number of pods per plant under T_1, T_2 and T_3 were also statistically identical.

Number of seeds pod^{-1} was varied 1.44 to 2.00 due to different treatments. The highest number of seeds pod^{-1} was recorded in T_3, T_6 and T_7 which were statistically identical and followed by T_2 and T_5, whereas the lowest number of seeds per pod was obtained from control plot. T_2 and T_5 also gave similar number of seeds pod^{-1} which was statistically and significantly similar with each other (Table 7).

The maximum 1000-seed weight (30.00 g) was recorded in T_6 followed by T_3 and T_5 and T_7 which were statistically and significantly similar while the minimum (16.66 g) was in control. Statistically similar result was also found in case of T_1 and T_2 (Table 7).

Table 7. Effect of organic amendments, Carbendazim and their combination with spent mushroom compost on yield components of lentil

Treatments	No. of pods/plant	No. of seeds /pod	1000 seeds weight (g)
T_0	46.11 d	1.44 d	16.66 c
T_1	68.56 c	1.78 bc	21.66 bc
T_2	70.22 c	1.89 ab	21.66 bc
T_3	97.67 b	2.00 a	26.66 ab
T_4	77.22 c	1.67 c	23.33 abc
T_5	98.89 b	1.89 ab	26.66 ab
T_6	123.55 a	2.00 a	30.00 a
T_7	102.67 b	2.00 a	26.66 ab
LSD (0.05)	6.18	0.10	3.46
CV (%)	8.84	6.7	17.56

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly by LSD at 0.05 level of probability. CV = Co-efficient of variation

T_0 = Control, T_1 = Poultry manure, T_2 = Cow dung, T_3= Spent mushroom compost (SMC), T_4 = Carbendazim (Bavistin 50WP), T_5 = Spent mushroom compost + Poultry manure, T_6 = Spent mushroom compost + Cow dung and T_7 = Spent mushroom compost + Carbendazim (Bavistin 50WP)

4.8 Effect of organic amendments, Carbendazim and their combination with spent mushroom compost on the yields and harvest index of lentil

Seed weight/plant was varied from 2.80 g to 5.56 g due to application of different amendments. The highest seed weight/plant was recorded in T_6 followed by T_7 (5.00 g) and the lowest seed weight/plant (2.80 g) was obtained from control plot. Statistically identical result was found in case of T_3, T_4 and T_7. Again, T_1 and T_2 were also statistically similar to each other (Table 8).

The stover yield plant^{-1} varied from 3.66 g to 7.33 g which was collected after harvesting. The highest stover yield per plant was recorded from T_5 and T_6 which were statistically and significantly similar followed by T_7 (7.00 g) and the lowest was recorded from T_0. The stover yield per plant of T_7 was statistically similar to T_5 and T_6 but significantly different. Statistically identical but numerically different result was observed in case of T_2, T_3 and T_4, respectively (Table 8).

Seed yield per hectare ranged from 986.70 to 1486.70 kg under various treatments of lentil. The highest seed yield per hectare was recorded from the T_6 plot which was statistically different from all other treatment followed by T_7 (1425.70 kg). Seed yield of T_7 was statistically identical to T_3 and T_5. The lowest seed yield per hectare was obtained from control plot (Table 8).

The data on stover yield of lentil were statistically analyzed after threshing and drying of stovers of the crop. In case of stover yield, T_6 produced significantly highest stover yield (1917.30 kg ha^{1}) followed by T_3 (1894.70 kg ha^{-1}). The T_0 showed the lowest stover yield (1671.00 kg ha^{-1}) of lentil. Statistically identical result was found in T_3, T_4, T_5 and T_7. The result of stover yield per hectare also showed statistically similar result in T_0 and T_1 (Table 8).

The harvest index was ranged from 37.13% to 43.67%. The highest harvest index was recorded in T_6, whereas lowest was recorded in T_0. The harvest index of T_6 was statistically different from other followed by T_7. The harvest index of T_3, T_4,

T_5 and T_7 were statistically similar. Again, statistically same result was also found in case of T_1 and T_2 (Table 8).

Table 8. Effect of organic amendments, Carbendazim and their combination with spent mushroom compost on the yields and harvest index of lentil

Treatments	Seed weight / plant (g)	Stover yield / plant (g)	Seed yield (kg/ha)	Stover yield (kg/ha)	Harvest Index (%)
T_0	2.80 d	3.66 c	986.70 d	1671.00 c	37.13 c
T_1	3.63 c	5.33 b	1092.40 cd	1685.70 c	39.32 bc
T_2	3.50 c	6.0 ab	1171.40 bcd	1764.30 bc	39.47 bc
T_3	4.70 b	6.66 ab	1386.70 ab	1894.70 ab	42.17 ab
T_4	4.93 ab	6.33 ab	1314.00 abc	1838.30 ab	41.61 ab
T_5	4.96 ab	7.33 a	1403.80 ab	1869.70 ab	42.88 ab
T_6	5.56 a	7.33 a	1486.70 a	1917.30 a	43.67 a
T_7	5.00 ab	7.00 a	1425.70 ab	1871.70 ab	43.13 ab
LSD (0.05)	0.32	0.74	125.56	69.80	2.08
CV (%)	8.84	14.7	11.98	4.71	6.22

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly by LSD at 0.05 level of probability. CV = Co-efficient of variation

T_0 = Control, T_1 = Poultry manure, T_2 = Cow dung, T_3 = Spent mushroom compost (SMC), T_4 = Carbendazim (Bavistin 50 WP), T_5 = Spent mushroom compost + Poultry manure, T_6 = Spent mushroom compost + Cow dung and T_7 = Spent mushroom compost + Carbendazim (Bavistin 50 WP)

4.9 Relationship of number of nodules per plant with number of pods per plant, yield and foot and root rot disease incidence of lentil

Correlation study between numbers of nodules per plant and number of pods per plant of lentil was done and it was revealed positive correlation. It was evident that the equation $y = 4.351x + 7.591$ gave a good fit to the data and the co-efficient of determination ($R^2 = 0.946$) fitted regression line had a significant regression co-efficient at different treatments (Figure 6).

Similarly correlation study was done to establish the relationship between number of nodules per plant and total seed yield of lentil and positive correlation was observed (Figure 7). It was evident that the equation $y = 29.74x + 750.1$ gave a good fit to the data and the co-efficient of determination ($R^2 = 0.828$) fitted regression line had a significant regression co-efficient at different treatments.

On the other hand, correlation study between numbers of nodules/plant and foot and root rot disease incidence of lentil was done and negative correlation was observed (Figure 8). It was evident that the equation $y = -1.594x + 48.39$ gave a good fit to the data and the co-efficient of determination ($R^2 = 0.522$) fitted regression line had a significant regression co-efficient at different treatments.

So, it is revealed that the number of nodules/plant was strongly as well as positively correlated with number of pod/plant and with total yield of lentil but negatively correlated with foot and root rot disease incidence of lentil.

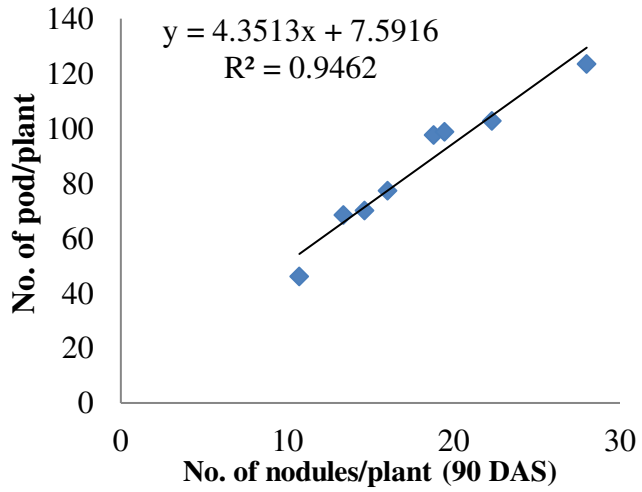


Figure 6. Relationship between number of nodules per plant and number of pod per plant

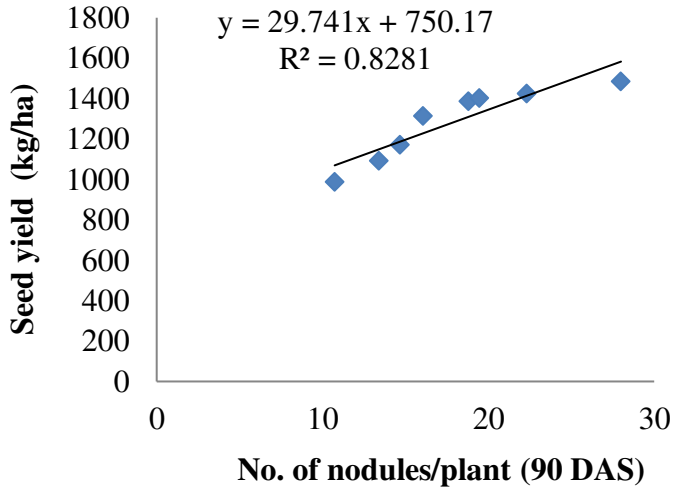


Figure 7. Relationship between number of nodules per plant and yield of lentil

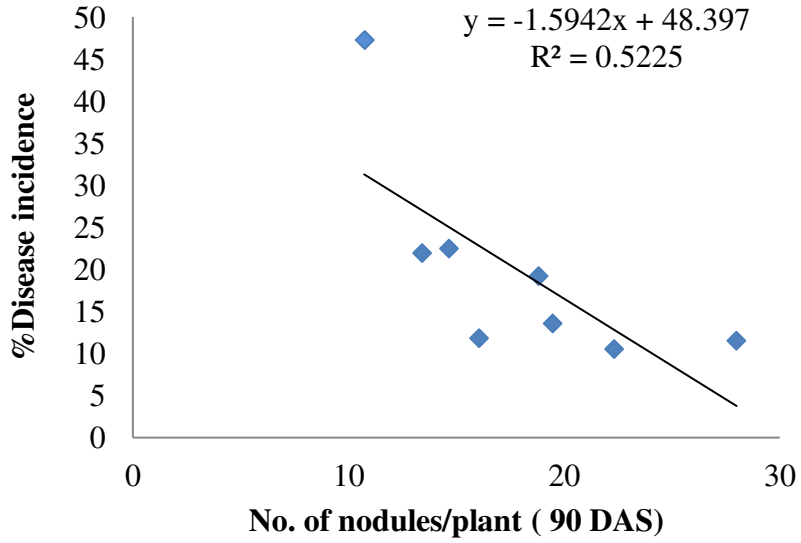


Figure 8. Relationship between number of nodules per plant and foot and root rot disease incidence of lentil

CHAPTER V

DISCUSSION

Effect of different treatments in controlling foot and root rot disease of lentil caused by *Sclerotium rolfsii* was assessed based on the result of *in-vitro* experiment and field experiment. Discussions on laboratory experiment and field experiment of lentil are presented in this chapter.

Laboratory experiment

Spent mushroom substrate compost (SMC) alone or combination with poultry manure, cow dung and Bavistin 50WP significantly inhibited the growth of *Sclerotium rolfsii* in culture media (PDA)**.** It was found that spent mushroom compost extracts alone and combined with other organic amendments such as poultry manure, cow dung and Bavistin 50WP fungicide can be used for combating the economically devastating disease foot and root rot of lentil caused by *Sclerotuim rolfsii.* The present findings were supported by the reports of Adedeji and Aduramigba (2016), where it was observed that sterilized spent mushroom compost (concentration 0.15g/ml) significantly inhibited the mycelia growth of *Fusarium oxysporum* f. sp. *lycopersici*. They also suggested that spent mushroom compost can be used for biopesticides formulation to combat Fusarium wilt of tomato.

The fungicide Bavistin 50WP, organic amendments and their combined assay in the laboratory showed significant effect in reducing radial mycelial growth of *Sclerotium rolfsii.* It was found that Bavistin 50 WP alone and combined with spent mushroom substrate compost inhibited mycelial growth of *Sclerotium rolfsii* 32.22% and 36.48%, respectively in culture media. The present findings were supported by the reports of Rabeya Parvin (2013) where she used different fungicides and biocontrol agents against the growth and sclerotia formation of

Sclerotium rolfsii in *in-vitro* condition. Bavistin 50 WP and Topgan were found to be most effective, both in inhibiting mycelial growth and sclerotia formation at low concentration.

Poultry manure alone and combined with spent mushroom compost reduced radial mycelial growth of *Sclerotium rolfsii* 13.33% and 23.14%, respectively *in-vitro* condition. The present findings were supported by the reports of Hassan *et al.* (2013). They observed that bacteria present in poultry litter extract had an effect in inhibiting the growth of *C. capsici in-vitro* condition.

Results of *in-vitro* test showed that the soil amendment cow dung alone and combined with spent mushroom compost inhibited the radial mycelial growth of *Sclerotium rolfsii* 12.22% and 32.22% which was supported by the findings of Basak *et al.* (2002). It was reported that complete suppression of mycelial growth of *S. sclerotiorum* may be possible if different herbal plant extracts are added with fresh cow urine and cow dung before application.

Field experiment

It was noted that the percent disease incidence was gradually increased with the increase of age of the plant and increasing rate was slower in spent mushroom compost (SMC) with Bavistin 50WP treated plot compared to control. Among all the treatments, Bavistin with organic amendment SMC was the best treatment for reducing percent disease incidence (77.65%) of lentil which was followed by soil amendment with SMC and cowdung (75.58%). It was found that organic amendment SMC with spraying of Bavistin gave the lowest disease incidence that was (10.57%) at 90 DAS. The finding was well supported by Hossain *et al.* (1999). They reported that treatment with Bavistin (0.2%) and Biofertilizer (*Rhizobium*) @ 50 g/kg seed resulted in the best control of seed borne foot and root rot fungi of lentil.

From the result of this finding it was recorded that spent mushroom compost combined with cow dung showed 11.55% disease incidence. The present finding was supported by the reports of Asish *et al.* (2018). They observed that among the nine organic amendments, spent mushroom substrate (SMS) showed 13.89% and 15.28% disease incidence during the winter season 2014-15 and 2015-16, respectively for controlling collar rot disease of tomato caused by *Sclerotium rolfsii*.

The results showed that spent mushroom compost alone or combined with other soil organic amendments such as poultry manure, cow dung and fungicide Bavistin 50WP can be used for controlling foot and root rot disease of lentil caused by *Sclerotuim rolfsii* in the field conditions. The lower disease incidence in anaerobically composted spent mushroom substrate (SMS) treatment is ascribed to the reduction of inoculum density of disease casing organisms (Hofrichter *et al*. 1997; Ahlawat *et al*. 2007b) and higher population of biocontrol agents developed during composting of SMS besides its positive influence on improvement of general vigour of plants. In contrary to the present findings, several researchers had also reported lower incidence of diseases on using SMS as manure in vegetable crops (Harender *et al.,* 1997; Ahlawat *et al.,* 2007b). The lower disease incidence of foot and root rot of lentil is attributed to the enhanced microbial activity in amended soils resulting in reduction in inoculum density of disease causing organisms supported with better plant growth.

In the present study it was observed that spent mushroom compost alone or combined with other organic amendments such as poultry manure, cow dung and fungicide Bavistin 50WP can be used for increasing all growth and yield contributing components of lentil. It was showed that different soil amendment, Bavistin 50WP and their combinations with SMC enhanced plant height significantly. Soil organic amendment spent mushroom compost combined with cow dung gave highest plant height (39.82 cm). The present finding was supported

by the reports of Ugioro *et al.* (2012). They observed that the plant height (cm) and stem girth (cm) of *L. esculentum* and *V.unguiculata* as affected by differently treated spent mushroom substrates and loamy soil at 9 weeks after sowing. At 9 week after sowing, *L. esculentum* grown on unautoclaved oven dried composted spent substrate and loamy soil (UOCSS + loamy soil; 1:9) gave the highest plant height (65.38 cm). Similarly, *V. unguiculata* grown on loamy soil incorporated with unautoclaved oven dried composted spent substrate and loamy soil (UOCSS + loamy soil; 1:9) gave the highest plant height (68.84 cm) compared to the control (53.14 cm).

From the present experiment it was observed that spent mushroom compost combined with cow dung gave maximum number of nodule (28.00), number of branch (9.80) of lentil. The present findings were supported by the reports of Prabu *et al.* (2014). They reported that the spent mushroom compost has positive influence on cowpea plants morphological parameters tested, plants were tested on three phases at 15 days intervals, on the 45th day the number of compound leaves, leaf length and area increased from control (7.5 ± 1.0, 10.1 ± 2.0 and 10.1 ± 2.0) to pot soil with 250 g spent mushroom compost (9.6 ± 2.10, 12.69 ± 2.5 and 12.5 ± 1.16) and pot soil with 500 g spent mushroom compost (8.7 ± 2.10, 11.49 ± 1.16 and 11.4 ± 1.4). Similar trend was observed by them in number and length of root, root nodule and shoot of cowpea.

Considering the number of nodules from the present study, it was revealed that the soil organic amendment SMC with cow dung increased nodulation of lentil and was found 4.00, 24.26 and 28.00, respectively at 40 DAS, 65 DAS and 90 DAS of lentil. The observation in this study of a significant high level of nodulation of lentil was observed at full pod stage of lentil (a possible indication of increased N_2 fixation) which was contradicted with the finding of Zapata *et al.* (1987) that maximum N_2 fixation occurs between the flowering and early pod stages soybean

development when urea was applied as solution (200 ml/m^2) to the nodulating soybean plots.

From the finding of the present study it was also observed that spent mushroom compost combined with cow dung gave the highest number of pods (123.55), seed per plant (2.00), 1000 seed weight (7.33 g), seed weight per plant (5.56 g). The present finding was also supported by the reports of Ugioro *et al.* (2012). They also ovserved that significantly highest pod weight (17.30 g) and seed weight (16.26g) of *V. unguiculata* were obtained when grown on unautoclaved spent mushroom substrates with loamy soil mixture compared to those raised on unautoclaved oven dried loamy soil which had the least pod weight (8.32g) and seed weight (7.60 g).

Yield and yield components of lentil were significantly influenced in treatment where soil was amended with spent mushroom compost alone or combination with other treatments. From the finding the highest stover yield per plant (7.33 g), total seed yield (1486.70 kg/ha), stover yield (1917.30 $kgha^{-1}$) and harvest index (43.67%) were obtained from amended with spent mushroom compost combined with cow dung. Similar results were reported by Ahlawat *et al.* (2011). They found that eighteen month old aerobic and anaerobic composted SMS gave significantly higher pea yield over the other treatments, with highest (1202 g/ m^2) in aerobically composted SMS treatment. The yield of pea increased in aerobic and anaerobic composted SMS treatments was 93.85 % (1202.59 g/m^2) and 72.12 % (1067.78 g/m^2), respectively compared to control (620.37 g/ m^2). They also observed that the quality attributes of fruit viz., pod weight, pod length, number of seeds/pod and seed weight/ pod also followed similar pattern. These findings were also supported by the results of Stewart (1995) where potato yield was increased by the application of spent mushroom substrate.

Similar responses were reported earlier by many investigators (Ahlawat *et al.,* 2006a; Ahlawat *et al.*, 2007a; Ahlawat *et al.,* 2009) in tomato, capsicum and cauliflower by using SMS composted by different methods as manure.

The statistical relationship between total number of nodules at 90 days after sowing and seed yield has been calculated and found positive. This finding was supported by the result of Haque *et al*. (2014). They showed the positive relationship between total number of nodules and grain yield of lentil at 60 days of sowing where the correlation coefficient (r) was 0.6425 at 1% level of probability. So the relationship was positive i.e. increased in number of nodule enhanced the seed yield of lentil. From the present finding it was observed that at 90 DAS, the total number of nodules showed positive relationship with number of pod/plant and yield (kg/ha) of lentil. The finding was supported by Pedersen (2009) that the accelerated nodulation activity at full pod stage was sufficient to maintain a high pod formation and increased grain yield soybean at maturity.

The results indicate that spent mushroom compost influences the plant growth, nodulation, yield and yield components which might be due to the supplementation of nutrients and production of growth promoting substances by spent mushroom compost.

CHAPTER VI

SUMMARY AND CONCLUSION

Foot and root rot of lentil is considered as an important and destructive disease of pulse crops in Bangladesh and also in almost all legume growing countries of the world. It is quite evident that foot and root rot of lentil is caused by *Sclerotium rolfsii* and have immense impact on disease incidence, seedling mortality and yield.

From the result it was observed that all the treatments have significant effect on reducing disease incidence of lentil. All the treatments significantly reduced foot and root rot disease incidence of lentil compared to control.

In *in-vitro* experiment, the highest reduction of mycelium growth (36.48%) was recorded in spent mushroom compost (SMC) combined with Carbendazim 50WP followed by spent mushroom compost combined with cow dung (32.22%) and B Carbendazim 50WP (32.22%), respectively which were statistically similar with each other.

In field condition, application of organic amendments and their combination with SMC showed significant reduction of disease incidence of foot and root rot of lentil. Spent mushroom compost combined with Carbendazim 50WP showed the lowest disease incidence (10.57%) followed by spent mushroom compost combined with cow dung (11.55%) and Carbendazim 50WP (11.87%) at 90 DAS. The highest disease incidence was reported (47.30%) under untreated control.

From this study it is evident that the highest plants height (39.82cm) was observed at combined application of spent mushroom compost with cow dung plots followed by amended with spent mushroom compost combined with Bavistin 50WP (39.35 cm) applied plots while control showed the lower plants height (35.50 cm). Mean number of branches per plant was highest (9.80) in case of

application of spent mushroom compost + cow dung as soil amendments followed by spent mushroom compost + Carbendazim (8.86) and lowest (6.00) from untreated (control). The highest number of nodules per plant (28.00) was recorded at spent mushroom compost + cow dung applied plots followed by mushroom compost + Carbendazim (22.33) and lowest (10.73) in control. Mean pods per plant was highest (123.55) at mushroom compost + cow dung applied plots followed by mushroom compost + Carbendazim (102.67) applied plots and lowest (46.11) at control. Spent mushroom compost alone and combinations with cow dung and Carbendazim 50WP applied plots showed the highest number of seed per pod (2) and the lowest (1.44) at control. The highest seed weight per plant (5.56 g) was recorded in spent mushroom compost + cow dung followed by spent mushroom compost + Carbendazim 50WP (5.00 g) and lowest (2.80 g) in control plots. The highest stover yield per plant (7.33 g) was recorded in both plots treated with spent mushroom compost + cow dung and spent mushroom compost + poultry manure and the lowest (3.66 g) under control. The highest weight of 1000-seeds (30 g) was recorded in the plots treated with mushroom compost + cow dung, whereas the lowest weight (16.66 g) was observed under control treatment. In terms of yield, the highest yield (1486.70 kg/ha.) was achieved by applying spent mushroom + cow dung and the second highest performance was achieved by mushroom compost + Bavistion 50WP (1425.70 kg/ha) and the lowest yield (986.70 kg/ha) was achieved by untreated control. The highest stover yield (1917.30 $kgha^{-1}$) was recorded in treated with spent mushroom compost + cow dung, whereas the lowest stover yield (1671.00 $kgha^{-1}$) was observed under control plots. Spent mushroom compost + cow dung amended plot showed higher harvest index (43.67%) followed by spent mushroom compost + Bavistion 50WP (43.13%) and lowest (37.13%) under control.

Correlation study revealed that negative and significant correlation was observed between the radial mycelial growth and percent inhibition of mycelial growth of

Sclerotium rolfsii. The number of nodules per plant was positively correlated with number of pods/plant and total yield of lentil. The number of nodules per plant was negatively correlated with the disease incidence.

From the results of the experiments, it may be concluded that all organic amendments were more or less effective in reducing foot and root rot disease of lentil. The disease was controlled more effectively through the combination of spent mushroom with Carbendazim. The combination of spent mushroom with cow dung enhanced the plant growth, yield and yield contributing characters of lentil. All organic treatments significantly increased the number of nodules of lentil.

However, further investigation is need to carry out taking more options of soil amendment and fungicides including spent mushroom substrate compost for consecutive year to justify present findings.

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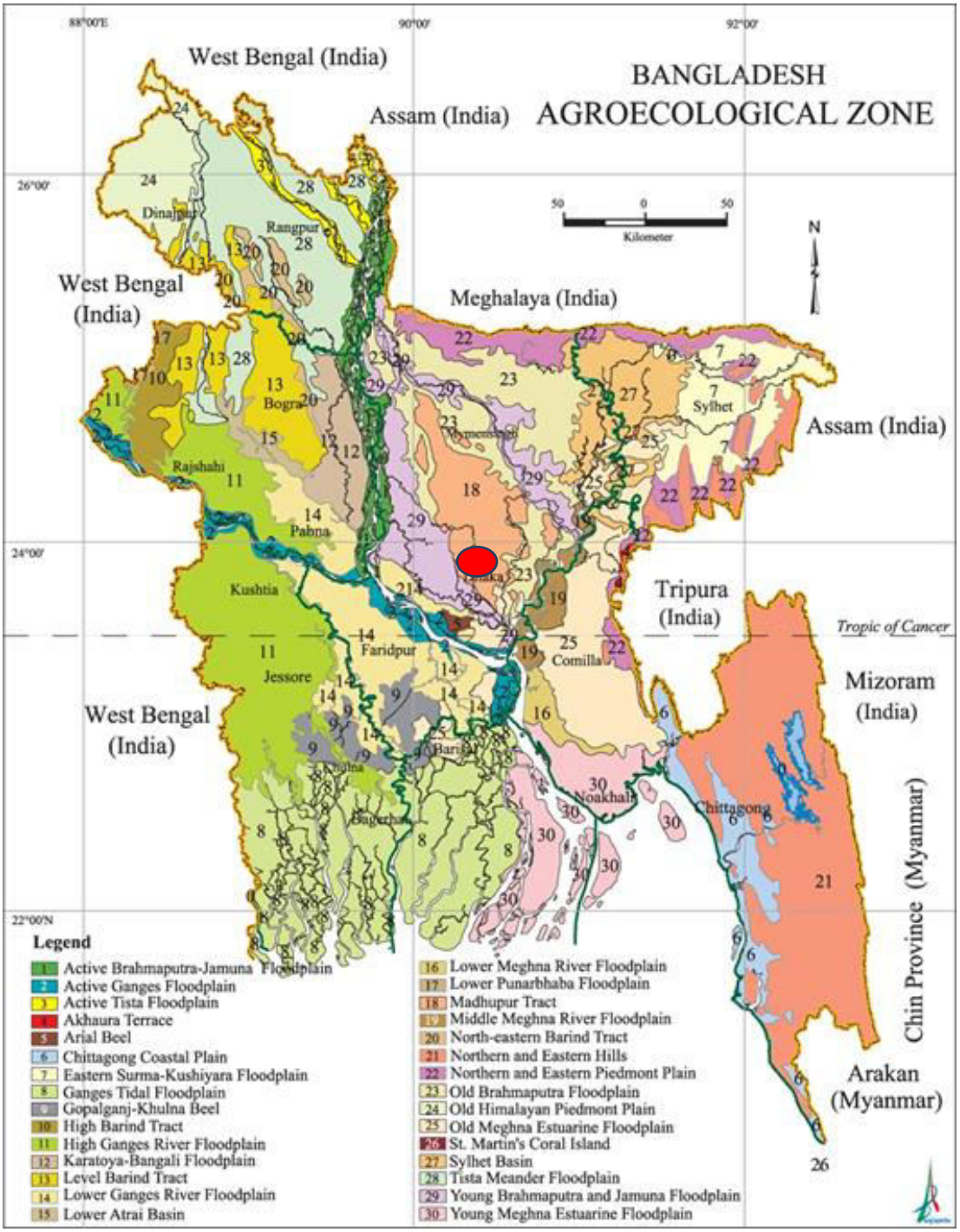
APPENDICES

Appendix I: Map showing the experimental site



Showing the experimental site

Appendix II: Soil characteristics of experimental farm of Sher-e-Bangla Agricultural

University are analyzed by soil Resources Development Institute (SRDI), Farmgate, Dhaka.

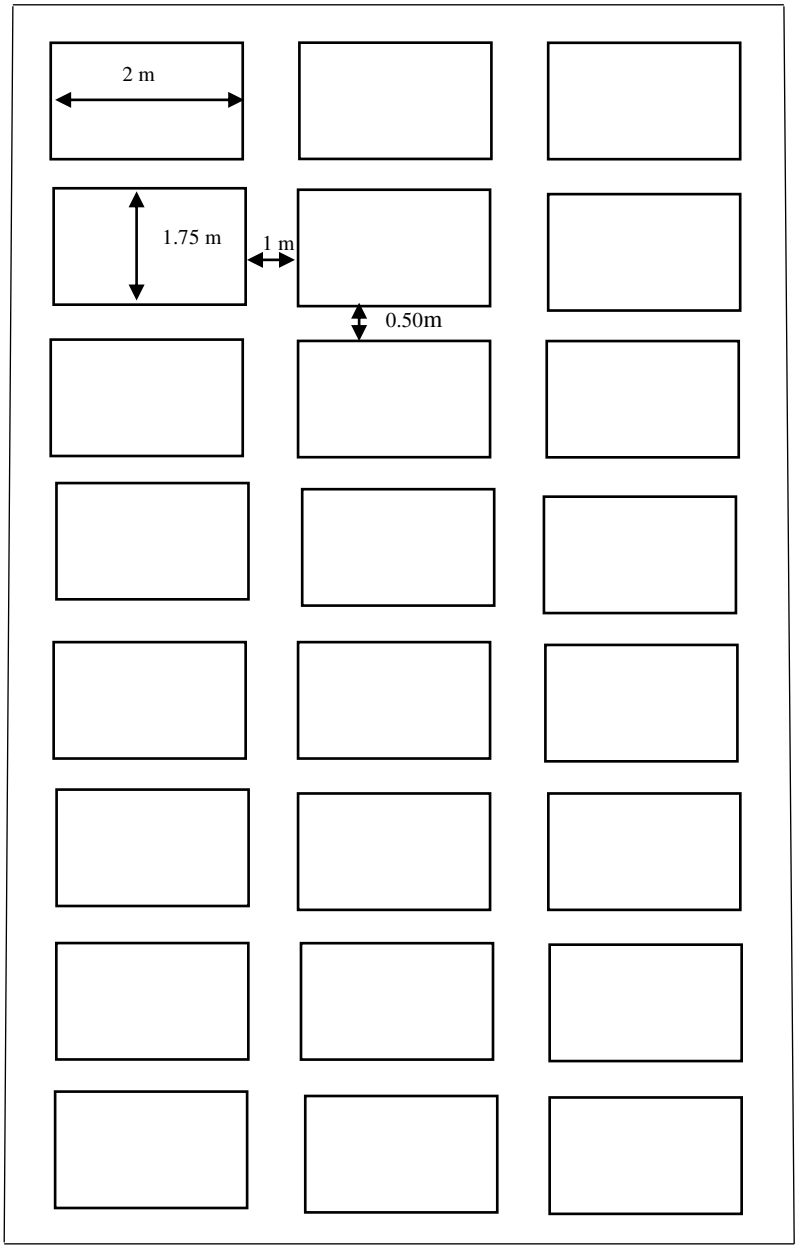
A. Morphological characteristics of the experimental field

Morphological features	**Characteristics**
Location	Farm, SAU, Dhaka
AEZ	Modhupur tract (28)
General soil type	Shallow red brown terrace soil
Land type	High land
Soil series	Tejgaon
Topography	Fairly leveled
Flood level	Above flood level
Drainage	Well drained
Cropping pattern	**N/A**

B. Physical and chemical properties of the initial soil

B. Physical and chemical properties of the initial soil Characteristics	**Value**
Practical size analysis	
Sand (%)	16
Silt (%)	56
Clay (%)	28
Silt + Clay (%)	84
Textural class	Silty clay loam
pH	5.56
Organic matter (%)	1.00
Total N (%)	0.06
Available P (µgm/gm soil)	42.64
Available K (me/100g soil)	0.13

Appendix III: Experimental Field Layout



Legend:

1. Width of the plot	1.75 m
2.length of the plot	2.0 m
3. Space between the block	1.0 m
4. Space between the plot	0.50 m

Appendix IV. ANOVA for radial mycecial growth of *Sclerotium rolfsii* at different days after inoculation

Source of variation	Degrees of freedom	Mean square					% Inhibition of mycelial growth (5 DAI)
		Radial mycelial growth (cm)					
		1 DAI	2 DAI	3 DAI	4 DAI	5 DAI	
Replication	2	0.01	0.12	0.01	0.07	0.06	8.11
Treatments	7	0.51**	0.87*	2.07**	2.46**	3.76**	464.23**
Error	14	0.02	0.24	0.02	0.01	0.06	8.26

**: Significant at 0.01 level of probability; *: Significant at 0.05 level of probability

Appendix V. ANOVA for foot and root rot disease incidence at different days after sowing

Source of variation	Degrees of freedom	Mean square		
		%Disease incidence		
		40DAS	65DAS	90DAS
Replication	2	5.32	3.67	2.69
Treatments	7	222.08**	366.69**	438.61**
Error	14	11.79	10.11	10.17

**: Significant at 0.01 level of probability; ^{ns}: Non-significant

Appendix VI. ANOVA for plant height and number of branches/plant of lentil as influenced by treatments

Source of variation	Degrees of freedom	Mean square					
		Plant height (cm)			No. of branches/Plant		
		40DAS	65DAS	90DAS	40DAS	65DAS	Harvest
Replication	2	0.12	0.25	7.26	0.6	0.17	0.27
Treatments	7	1.32^{ns}	15.72**	6.13**	1.82**	1.35^{ns}	3.74**
Error	14	0.77	0.17	1.24	0.22	0.86	0.7

**: Significant at 0.01 level of probability; ^{ns}: Non-significant

Appendix VII. ANOVA for number of nodules/plant of lentil as influenced by treatments

Source of variation	Degrees of freedom	Mean square		
		Number of nodules/plant		
		40DAS	65DAS	90DAS
Replication	2	3.78	3.18	4.07
Treatments	7	3.94**	75.86**	90.14**
Error	14	0.37	3.96	3.43

**: Significant at 0.01 level of probability; ^{ns}: Non-significant

Appendix VIII. ANOVA for yields components of lentil as influenced by treatments

Source of variation	Degrees of freedom	Mean square		
		No. of pod/plant	No. of seed /pot	1000 seeds weight
Replication	2	482.93	0.04	7.29
Treatments	7	1804.43**	0.11**	52.38*
Error	14	57.23	0.01	18

**: Significant at 0.01 level of probability; *: Significant at 0.05 level of probability

Appendix IX. ANOVA for yields and harvest index of lentil as influenced by treatments

Source of variation	Degrees of freedom	Mean square				
		Seed weight / plant (g)	Stover yield / plant (g)	Seed yield (kg/ha)	Stover yield	Harvest Index (%)
Replication	2	0.06	1.16	36854.7	951.2	11.99
Treatments	7	2.73**	4.56**	96322.8*	27251.3*	17.99*
Error	14	0.15	0.83	23648.8	7308.6	6.53

**: Significant at 0.01 level of probability; *: Significant at 0.05 level of probability