MANAGEMENT OF ALTERNARIA LEAF SPOT OF CABBAGE THROUGH SELECTED FUNGICIDES, SPENT MUSHROOM SUBSTRATE AND THEIR COMBINATION

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MANAGEMENT OF ALTERNARIA LEAF SPOT OF CABBAGE (*BRASSICA OLERACEAE*) THROUGH THE COMBINATION OF SPENT MUSHROOM SUBSTRACTE WITH SOME SELECTED FUNGICIDES

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CERTIFICATE

This is to certify that dissertation entitled ***"MANAGEMENT OF ALTERNARIA LEAF SPOT OF CABBAGE (BRASSICA OLERACEAE) THROUGH THE COMBINATION OF SPENT MUSHROOM SUBSTRACTE WITH SOME SELECTED FUNGICIDES"*** *submitted to the* ***Faculty of Agriculture,*** *Sher-e-Bangla Agricultural University (SAU), Dhaka in partial fulfillment of the requirements for the degree of* ***MASTER OF SCIENCE IN PLANT PATHOLOGY,*** *embodies the result of a piece of bona fide research work carried out by* ***REAJ RAHMAN PATWARY, Registration no. 20-11124*** *under my supervision and guidance. No part of the dissertation 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.

Dated: June, 2022
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DEDICATED
TO
MY BELOVED
PARENTS

LIST OF ABBREVIATIONS AND ACRONYMS

Sl. No.	Abbreviation	Full meaning
1.	@	At the rate
2.	℃	Degree Centigrade
3.	µm	Micrometer
4.	AEZ	Agro-Ecological Zone
5.	ANOVA	Analysis of variance
6.	BARI	Bangladesh Agricultural Research Institute
7.	BARC	Bangladesh Agricultural Research Council
8.	BBS	Bangladesh Bureau of Statistics
9.	C	Carbon
10.	CD	Cow dung
11.	cm	Centimeter
12.	C:N	Carbon Nitrogen ratio
13.	DAT	Days after transplanting
14.	EC	Emulsifiable concentrate
15.	*et al.*	And others
16.	ft	Foot
17.	FYM	Farm yard manure
18.	*g*	Gram
19.	ha	Hectare
20.	HEA	Host extract agar
21.	Kg	Kilogram
22.	L	Liter
23.	lb	Pound
24.	LSD	Least significant difference
25.	m	Meter
26.	ml	Milliliter
27.	MoP	Muriate of potash
28.	N	Nitrogen
29.	SMS	Spent mushroom substrate
30.	P	Phosphorus

31.	p	Probability
32.	PDA	Potato dextrose agar
33.	PDI	Percent disease index
34.	pH	Hydrogen potential
35.	RBD	Randomized block design
36.	SAU	Sher-e-Bangla Agricultural University
37.	SC	Soluble concentrate
38.	SMC	Sheet molding compounds
39.	SMS	Spent mushroom substrate
40.	SMW	Spent mushroom waste
41.	t	Ton
42.	TSP	Triple super phosphate
43.	V/V	Volume/volume
44.	SMC	Spent mushroom compost
45.	WG	Wettable granular
46.	WP	Wettable powder
47.	w/w	Weight/weight

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BY
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ABSTRACT

An field experiment was conducted in the experimental field of Sher-e-Bangla Agricultural University for the management of Alternaria leaf spot of cabbage (*Brassica oleraceae*) through the combination of spent mushroom substrate and some selected fungicides during Rabi season (November, 2021 to March, 2022). The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. The experimental treatments were T_0: Control (No treatment); T_1: Spent mushroom substrate (SMS) @ 15 t ha^{-1}; T_2: Rovral 50 WP @ 2.0 ml/L of water; T_3: Kurenox 50 WG @ 2.5 g/L of water; T_4: Knowin 50 WP @ 20 g/L of water; T_5: Mancer 75 WP @ 2g/L of water; T_6: Spent mushroom substrate @ 15 t ha^{-1} + Rovral 50 WP @ 2.0 ml/L of water; T_7: Spent mushroom substrate @ 15 t ha^{-1} + Mancer 75 WP @ 2g/L of water; T_8: Spent mushroom substrate @ 15 t ha^{-1} + Kurenox 50 WG @ 2.5 g/L of water; T_9: Spent mushroom substrate @ 15 t ha^{-1} + Knowin 50 WP @ 20 g/L of water. Treatment T_6 combined with soil amendment with spent mushroom substate followed by spraying with Rovral 50 wp (0.2%) at 7 days interval showed the best performances reducing leaf infection, plant infection head infection by 52.19 %, 47.49% and 100% respectively while the leaf infection, plant infection head infection respectively were 33.86%, 48.66% and 0.0% respectively. Treatment T_6 reduces the severity of plant by 35.97% while the severity index was recorded 19.94%. Treatment T_6 also yielded the highest plant height (21.79cm), diameter of cabbage head (21.38 cm), weight of cabbage head (2.26kg plant) increasing the cabbage yield by 58.34% over control. As per the performances of the treatments against the disease, the efficacy were ranked as $T_6 < T_9 < T_2 < T_8 < T_4 < T_3 < T_5 < T_1$ as control (T_0).

CHAPTER I

INTRODUCTION

Cabbage (*Brassica oleracea* var. *capitata* L.) is a popular green leafy vegetable of the family Brassicaceae. It is an herbaceous, biennial, dicotyledonous flowering plant distinguished by a short stem crowned with a mass of leaves, typically green but in some varieties red or purple, while mature form a characteristic compact, globular cluster (cabbage head). Cabbage is a great source of vitamin C, with a moisture content of 60.6%. It also contains vitamin B complex, potassium, and calcium (Haque KMF, 2006). Cabbage ranks second in terms of production and area among all vegetables grown in Bangladesh. It is grown on an 18 thousand hectares area with a total production of 312 thousand tons (BBS, 2017).

Several biotic and abiotic stress effects in Bangladesh on its production. Within the biotic stress, Alternaria leaf spot is a common disease incited by several species of *Alternaria.* In cabbage it is caused mainly by two species i.e. *Alternaria brassicae* (Berk.) Sacc. and *A. brassicicola* (Schweintiz) Wiltshire. Symptoms may first develop on young plants in seedbeds, where leaf spots, stunting or damping off may occur. Leaf spots incited by *A. brassicicola* appears as small, dark colored spots which spread rapidly to form circular lesions up to 1 cm in diameter (Singh et. al. 1999). Alternating light and dark concentric rings give the spots the appearance of target spot; a yellow halo may surround the lesion. The spot incited by large-spore form, *A. brassicae* shows much common symtoms compared to *A. brassicicola* and tend to remain larger and lighter in colour. The fungus is primarily seed-borne, but can also come from crop residue. Spores are spread by wind, water splash, human, agricultural tools and equipments. They can also survive in susceptible weeds or perennial crops (Mamgain *et al*., 2013). Under *in vitro* conditions sporulation of *A. brassicae* occurs at optimum temperature of 18-24° C and *A. brassicicola* at 20-30° C. Yield losses due to *Alternaria* infection have been reported to be 10-70% in India (Choudhary *et al*., 2018) and 32-57% in Nepal (Saharan *et. al*., 2016). The disease infect foliage causing extensive damage to tissue involved in photosynthesis and hence result in yield loss.

One of the most effective measures to control the disease caused by *Alternaria* is through application of fungicides. Since the pathogen is air-borne, foliar sprays are most effective for managing the disease. Many systemic and non-systemic fungicides with different concentration have been tested against *A. brassicicola and A. brassicae.* Mancozeb, Thiram, Bavistin, Iprodione, Chlorothalonil, Azoxystrobin, Propineb, Copper oxychloride, etc. have been proved effective for managing pathogen in different concentration in many of the researches. Foliar spray of Dithane M-45 (0.25 %), Kavach (0.1 %) or Foltof (0.25%) at 10 days interval controls *Alternaria* leaf spot on cabbage (Neeraj and Verma, 2010). Propiconazole, Tebuconazole, Hexaconazole, Copper Oxychloride and Mancozeb are found effective against *Alternaria* leaf spot in vitro condition (Kiran *et al.*, 2018). However indiscriminate use of higher dose of chemical fungicides affect environment and human health but also increase input cost (Ahmad and Ashraf, 2016). Therefore, development of effective control measures on diseases caused by harmful organisms is of vital significance for increasing crop productivity and improving the quality.

The management of diseases using chemicals is not safer to environment due to residual problem. Sometimes the pathogens develop new resistant strains. Increasing concern regarding food safety and environmental pollution has generated an interest in eco-friendly practices like soil amendment and application of biocontrol agents to manage the plant diseases.

Spent mushroom substrate (SMS) is often discarded as waste after mushroom harvest in many countries (Hanafi *et al.*, 2018). For every 1 kg of mushrooms produced about 5 kg of SMS remains (Zisopoulos *et al.*, 2016). Therefore, SMS management has become a growing challenge for the mushroom production industry. Consequently, finding environmentally and economically sustainable solutions for this organic waste is highly desirable. Due to its physical properties and nutrient content, SMS has great potential to be employed in the agricultural and horticultural sectors, as well as contribute to reduce the use of non-renewable resources (García-Delgado *et al.*, 2016). The SMS contains nutrients which could be used for the growth of plants. SMS also contains a rich microbiota that can ensure the balance of phytosanitary requirements of crop plants, and

the possibility that some species of microorganisms may induce resistance in pre-cultivated seedlings in this substrate (Siqueira *et al.*, 2011). The advantages of using SMS as a soil fertilizer over chemical fertilizer is that SMS provides slow-release nutrients that will not burn crops upon application. The SMS generally have ideal moisture content. The organic matter content varies from 40 to 60 percent. This range is favourable for plant growth.

During vegetative growth and fruiting of mushrooms, the growing mycelium excretes out different types of compounds such as organic matter, bioactive compounds and lignocellulose degrading enzymes, which after several rounds of mushroom morphogenesis and harvesting can contribute in making SMS as an effective bio-agent against soilborne pathogens causing diseases in plants (Zhao *et al.*, 2017). Moreover, the actinomycetes, bacteria and fungi inhabiting the compost not only play role in its further decomposition but also exert antagonism to the normal pathogens surviving and multiplying in the soil ecosystem. In case of cabbage, a new container medium formulated by using spent forest mushroom compost has been found to support growth of cabbage seedlings and suppress damping off disease caused by *Rhizoctonia solani* (Huang and Huang 2000). The field application of SMS along with fungicide application results in suppression of the fungus pathogen of varied plant hosts. Therefore, by keeping the above facts in mind the present investigation was undertaken with the following objectives:-

i. To isolate, identify and perform the pathogenicity test
ii. To estimate the disease incidence and of *Alternaria* leaf spot of cabbage
iii. To determine the effectiveness of selective fungicide and spent mushroom substrate to manage the disease.

CHAPTER II

REVIEW OF LITERATURE

Many fungal and bacterial diseases of cabbage have been reported from major cabbage growing regions of the world. Among fungal diseases, *Alternaria* leaf spot of cabbage caused by *A. brassicae* is the most common and serious disease. Much research work has been done and reported on different aspects for the management of the disease. The literature so far available pertaining to the present study entitled "Management of alternaria leaf spot of cabbage (*Brassica oleraceae*) through the combination of spent mushroom substrate and some selected fungicides" is being presented under the following headings:

2.1. Occurrence and distribution

Mason (1928) first reported Alternaria from Pusa (Bihar) on a herbarium material of sarson (Brassica sp.) in India.

Azevedo *et al.* (2000) surveyed the intensity of black rot and Alternaria leaf spot of cabbage in Agreste of Pernambuco and determined sample size for disease quantification and reported that the severity of Alternaria leaf spot ranged between 0 to 3.46 per cent in 1997 and between 0 to 9.14 per cent in 1998.

In a survey carried out by Peruch *et al.* (2006) during November 2001 to February 2002 in Pernambuco and Santa Catarina to evaluate the intensity of Alternaria black spot caused by *Alternaria brassicicola* and or *A. brassicae*, it was revealed that there was no significant differences in the severity of the disease in both the states. (average severity in Pernambuco 1.42% and Santa Catarina 0.72%).

Chauhan *et al.* (2009) carried out a survey of disease caused by genus Alternaria on plants of Brassicae family and reported that different species of Alternaria infect different hosts in the family.

Rop *et al.* (2009) studied the establishment of the pathogenic *Alternaria* spp. affecting brassicas in Kenya and determined the distribution and incidence of the disease in

farmers' fields. For this purpose a field survey was carried out in 13 selected districts in Kenya with varying agro-ecological zones. They observed that out of 89 farms surveyed, cabbage was cultivated in 52 farms and the incidence of black spot (upto 30.30%) incited by *Alternaria brassicicola* was rendered in 28 farms.

In an another survey carried out by Dillard and Smart (2013) in New York state, it was found that the disease incidence of Alternaria leaf spot of cabbage was low in the months of June – August and it gradually increased from September- November.

2.2. Causal organisms

Much of the past research has been devoted in distinguishing the two species i.e., *A. brassicae* and *A. brassicicola* on the basis of their morphology, pathogencity and symptom expression. Later the taxonomic confusion between *A. brassicicola* and *A. brassicae* was distinguished by Wiltshire (1947) who concluded that absence of conidial beak in the former distinguishes it from the latter. Besides *A. brassicicola* forms darker lesions in contrast to pale brown lesions by *A. brassicae* and produce sooty black colony, while *A. brassicae* forms white mycelial growth on potato-dextrose agar. Comparative morphological characteristics of *A. brassicae* and *A. brassicicola* revealed that septate mycelium of *Alternaria brassicae* was brown in colour, whereas, in *A. brassicicola*, it was greenish grey and becomes dark olive on ageing. The length of *A. brassicicola* spores measured 45-55 µm which was almost half the spore length of *A. brassicae* i.e., 98-114 µm Changstri and Weber (1963); Rotem (1994) and Koul, (1996) reported that colonies of *A. brassicicola* were usually dark blackish brown, conidiophores singly or in groups, straight or flexuous, pale to mid olivaceous brown and measured 34-76 x 6-10 µm with one or several conidial scars. Conidia were often in chains and measured 40-62 x 11-20 µm in size. Whereas, the hyphae of *A. brassicae* were septate, hyaline to mild olive grey, conidiophores were mild pale to olive grey and measured 29-236 µm in length and 8-17 µm in width, conidia obclavate, pale to olive grey and measured 63-172 x 10-21 µm in size. *A. brassicae* isolates from diverse agro-climatic zones of India has been studied. The morphological characteristics of each isolate such as length, breadth, number

of septa, beak length and beak septa etc. were recorded from 15 days old culture (Mehta *et al.* 2003).

2.3. Isolation and Pathogenicity

Valvi *et al.,* (2019) (b) isolated *Alternaria brassicae* from infected leaves of cauliflower on potato dextrose agar medium. The pathogenicity of the isolated fungus was proved by inoculating healthy seedlings of cauliflower.

Gunda *et al.,* (2018) collected cabbage leaves infected with *Alternaria* spp. during the survey. The sterilized infected cabbage small leaves pieces were placed on PDA medium in petri dishes. These plates were incubated at room temperature (27±40C) for proper development of the pathogen and also proved its pathogenicity.

Patwari, (2017) isolated *Alternaria brassicae* from leaf spot/blight infected cabbage leaf samples on potato dextrose agar (PDA) medium and proved its pathogenicity by inoculating one month old seedlings of cabbage.

Gaikawad, (2013) isolated *Alternaria brassicae* from leaf spot/blight infected cabbage leaf samples on potato dextrose agar (PDA) medium and proved its pathogenicity by inoculating one month old seedlings of Cabbage with the spore-cum-mycelial suspension (2×10^5).

Giri *et al.* (2013) used five different methods for inoculation of *Alternaria* on cabbage and reported the spore suspension drop method was the best method for inoculation and also proved the pathogenicity by detached leaves technique.

Deep and Sharma (2012) isolated *Alternaria* species including 16 *Alternaria brassicae* and six *Alternaria brassicicola* from infected Cauliflower leaf samples. These cultures were purified and maintained on potato dextrose agar (PDA) media at 25^0C.

Reshu and Khan (2012) isolated *Alternaria brassicae* and *A. brassicicola* from diseased leaves of Mustard and multiplied on potato dextrose agar (PDA) medium in petri plates.

Mishra *et al.* (2009) isolated *Alternaria brassicae* from diseased leaves of Cauliflower. This pathogen was isolated on potato dextrose agar medium on the basis of colony character and spore morphology and identified.

2.4. Identification

Giri *et al.*, (2013) examined the conidia of *Alternaria brassicae*. The conidia were muriform, beaked, bottle shaped, and measuring 92.3-102.5 µm long and 10.5-20.5 µm wide with 6-10 or even more transverse septa and a few or none longitudinal septa. The longitudinal septa, when found, were only in the middle cells. The conidium was developed from a bud formed by the apical cell of conidiophore. The main body of the conidium was oblong with its formal end protruding the terminal cell tapered into a beak.

Chand and Chandra. (2014) examined the conidia of *A. brassicicola*. The conidia were muriform, olivaceous brown colour with nonexistent beaks. Conidial length and width varied from 13-120 µm and 6-16µm respectively. The shape of the conidia was cylindrical to obclavate with cross and longitudinal septa. Isolates showed light black and grey white colours in Host Extract Agar media.

Pramila *et al.*, (2014) observed 10 isolates of *A. brassicae* for morphological, cultural, pathogenic and molecular variation. Colour of colonies ranged between of white to light brown. Conidial length and width varied from 105- 135 µm and 10-20 µm respectively. Horizontal septa varied from 6.9-9.4. Colony was circular in shape.

Singh *et al.*, (2015) collected ten isolates of *Alternaria brassicae* from different geographical region in India. They studied the morphological, physiological and cultural variation. The length and width of conidiophores varied from 36.4-36.8 µm and 4.73-6.58 µm respectively. Conidiophore septation varied from 4.53-6.25 in different isolates of *A. brassicae*. Conidial length and width varied from 104.02- 142.47 µm, 11.62-16.95 µm respectively. Beak length varied from 43.35-70.57 µm, transverse and longitudinal septa varied from 6-8.3 µm and 0.25-2.75 µm respectively.

Saha *et al.*, (2016) collected 23 isolates of *A. brassicae* from different cultivars in Uttar Pradesh. They characterized them for cultural, morphological, pathogenic and molecular

variations. They reported that colony colour was white, dark brown to light brown and pinkish white. The maximum length of conidia ranged from 150-122 μm with 8-9 transverse and 2 vertical septation.

Yadav *et al.,* (2016) isolated *A. brassicae* from four different locations. They identified these isolates on the basis of morphological and cultural characteristics. Colonies of all the isolates were moderately fast growing, amphigenous, ashy gray, fluffy, circular. Conidia were obclavate to muriform, ovate, elongated, singly on conidiophore, sometimes in short acropetal chain. Highest average conidial size (140×20.7 μm and 138.6×22.9 μm) with 8.2 and 6.8 average transverse septa and 2.0 and 1.2 average longitudinal septa respectively. Highest average beak length of 54.2 μm was recorded. The maximum mean radial growth of fungus was at temperatures of 25^0C.

2.5. Symptomatology

Valvi *et al.* (2019b) described the symptoms appeared initially as small, circular, dark, yellow spots on the lower leaves. Later on, these spots enlarged into circular areas with concentric rings and possibly surrounded by yellow halo. Later on, these spots enlarged into gray to black lesions of 0.5 to 1 cm diameter. As the disease progressed, the lesions attended target board pattern due to formation of many concentric rings with wavy margin. Centres were coated with sooty black spore masses and later it drops out, producing shot holes.

Meena *et al.* (2010) described the symptoms of the Alternaria disease which were characterized by formation of spots on leaves, stem and branches. *A. brassicae* and *A. brassicicola* can affect the host species at all stages including the seed. On seedlings, symptoms included dark stem lesions immediately after germination that can result in damping-off or stunted seedlings. Symptoms were first visible on lower leaves with appearance of black points, which later enlarged to develop into prominent, round, concentric spots of various sizes. With progress, the disease appeared on middle and upper leaves with smaller sized spots, when defoliation of lower leaves occurs.

Upadhyaya *et al.* (2008) reported that the pathogen attack initially the leaves show minute, light brown punctuation in the centre. Leaf spots were mostly light brown in colour and later turn dark brown. In the later stage, central portion become scarious.

Kucharek (2000) reported that leaf spot was the most common symptom of Alternaria disease. Petioles (leaf stems) displayed elongate dark spots when infected. Brown to black spots on leaf blades varied in size. A yellow halo around brown lesions on the leaf margin is more indicative of Alternaria leaf spot. Spotting of broccoli heads and cauliflower curds appeared as brown to black discoloration due to the severe disease incidence in leaf phase.

Ellis (1968) studied the symptoms caused by *Alternaria brassicae* on leaves forming circular, zonate, light brown to grayish or dark brown spots measuring less than 0.5 to 12 mm diameter in size. Sometimes, coalescing of the leaf spots occurs and the entire leaf area affected. On the midrib of the leaves the spots were oblong or linear, sunken. On the heads of cauliflower and broccoli black spots are formed.

2.6. Effect of fungicides

Godika and Pathak (2002) tested the efficacy of Mancozeb (0.2%), Antracol (0.2%), Ridomil MZ (0.25%), Bayleton (0.05%) and Copper oxychloride (0.3%) in controlling blight disease (*A. brassicae*) and white rust (*Albugo candida*) in India. All treatments resulted in lower disease severity and higher crop yield as compared to the control. Antracol spraying resulted in the lowest Alternaria blight severity, whereas Ridomil MZ resulted in the lowest white rust severity.

Kumar and Singh (2003) determined the efficacy of Captan, Iprodione, Ridomil MZ, Zineb, Ziram and Blitox-50 at recommended rates against Alternaria blight (caused by *A. brassicae*) infecting radish. The fungicides were sprayed immediately after the appearance of initial symptoms and 10 and 20 days later. All fungicides significantly reduced disease incidence. The best control of the disease was obtained by spraying plants with Iprodione, followed by Mancozeb and Ridomil-MZ.

The fungicides such as Rovral 50 WP (0.20%), Dithane M-45 (0.25%), Ridomil (0.10%), Bavistin (0.25%) and Knowin (0.25%) were evaluated for the management of Alternaria blight (*A. brassicae* and *A. brassicicola*) of cauliflower by Khoda *et al.* (2003) The fungicides were applied as foliar sprays for 4 times at 10 days interval starting from the development of curd. The maximum reduction in severity of stalk rot and pod blight and the highest increase in seed yield over control was achieved with Rovral followed by Dithane M-45.

Sidlauskiene *et al.* (2003) studied the effect of different methods of controlling Alternaria leaf spot in cabbage. The treatments included Benzothiadiazole (Bion 50 WG) at 0.05 kg/ha, Azoxystrobin (Amistar 250 SC) at 0.8-1 L/ha, Tolyfluanid (Euparen 50 WG) at 1.5 kg/ha and copper hydroxide (Champion 50 WP) at 2.5 kg/ha. Azoxystrobin was the most effective fungicides as it reduced disease incidence to the tune of 88 to 93 per cent.

Field efficacy of Ridomil MZ, Antracol and Copper oxychloride was tested against Alternaria blight of mustard (Yadav 2003). Ridomil MZ was the best fungicide not only in controlling the disease but also increasing the yield.

Narain *et al.* (2006) evaluated the efficacy of fungicides, Carbendazim (0.1%), Mancozeb (0.2%), Chlorothalonil (0.2%), Roko (0.2%) and Aminocel (0.1%) against Alternaria leaf spot (*A. brassicae*) on broccoli. Among them Mancozeb was the most effective fungicide.

Prasad and Lallu (2006) conducted a field trial on efficacy of different spraying combinations of three fungicides against Alternaria blight of mustard. Comparative analysis of various spraying schedules revealed that first spray of Carbendazim (0.1%) + Mancozeb (0.2%) followed by two sprays of Mancozeb (0.2%) at early date of sowing (October) was the best combination in reducing the disease severity on leaves.

Khan *et al.* (2007) used three systemic fungicides; Topsin-M (70%WP), Ridomil MZ and Bavistin alone and in combination with four non-systemic fungicides such as Captaf (Captan 50WP), Indofil M-45 (Mancozeb 75WP), Indofil Z-78 (Zineb 75WP) and Thiram (Thiram 75WP) both in vitro and in vivo for their effectiveness to manage

Alternaria blight of rapeseed-mustard caused by *Alternaria brassicae*. All fungicides significantly reduced the severity of the disease but Ridomil MZ was the most effective.

Singh *et al.* (2008) used four fungicides, namely Iprodione (0.2%), Mancozeb (0.2%), Ridomil MZ (0.25%) and Apron S.T. (0.5%), as seed treatment, were evaluated against *A. brassicae*, causing Alternaria blight of mustard. All the four fungicides were significantly superior in reducing the disease intensity over the untreated control. Iprodione was found to be the most effective fungicide in controlling the disease and increasing the yield.

Arunakumara *et al.* (2010) studied the fungicidal management of early blight of tomato caused by *Alternaria solani* and reported that maximum disease control with 28.5 PDI was recorded in Propiconazole (0.1%) followed by 35.33% in Pyraclostrobin (0.2%). Benomyl was the least effective fungicide.

Gaikawad (2013) evaluated four fungicides viz., Propiconazole (25EC), Hexaconazole (5EC), Mancozeb (75WP) and Copper oxychloride (50WP) for the management of Alternaria blight/leaf spot (*A. brassicae*) of cabbage under greenhouse conditions (in vivo). Among the fungicides tested, Mancozeb recorded the least disesase incidence (15.30%). It was followed by Propiconazole (18.36%), Hexaconazole (19.52%) and Copper oxychloride (20.65%).

2.7. Effect of spent mushroom substrate

2.7.1. Spent mushroom substrate for disease management

Spent Mushroom Substrate (SMS) often referred to as, Mushroom Compost is the growing medium that results from the mushroom growing process. Mushroom Compost is made from agricultural materials, such as hay, straw, straw horse bedding, poultry litter, cottonseed meal, cocoa shells and gypsum. Spent mushroom substrate is rich with many potential antagonists. Scanning of literatures showed that many researchers have exploited the potential effect of SMS in disease control.

Solanke *et al.* (2020) conducted an experimental to see the effect of organic manure against Alternaria leaf spot of cabbage in the plot size 2×2 m^2/ eight treatment is farm yard manure 5 t/ha spent mushroom compost 500 kg/ha, poultry manure 5 t/ha, vermicompost 10 t/ha, neem cake 500 kg/ha, and leaf mold 3 t/ha, goat manure 10 t/ha, along with the control. Observation each treatment was replicated three times data generally by using RBD design on percentage of disease intensity @ 30, 45 and 60 DAT. Observed size of cabbage and head Weight of cabbage and yield. Results that all the organic amendments significantly reduced the incidence of Alternaria leaf spot of cabbage with control whereas among the treatment T_2 (Neem Cake) and T_1 (Spent Mushroom Compost) significantly reduced incidence of Alternaria leaf spot cabbage as compared with other treatments.

Shitole *et al.* (2013) reported the combination of *Trichoderma viride* @ 5 g/kg SMS + *Pseudomonas Jluorescens* @ 5 g/kg SMS where SMS and soil proportion was 75:25 found highly effective with minimum pre emergence (6.82%) and post emergence (9.09%) damping off as against control (36.36 and 50% pre and post emergence damping off) in tomato.

Remya (2012) conducted a pot culture experiment and reported that among the various SMS used, paddy straw SMS of *P. sajor- caju* and isolated antagonists from SMS, were found to be most effective for the management of soil bome diseases of ginger. Roshna (2013) reported that the isolates of *T. hamatum* and *B.subtilis* from pleurotus SMS provided luxuriant growth and was found effective against phytophthora diseases of pepper cuttings.

Singh *et al.* (2012) conducted the phylogenetic study proved that the SMS from different mushrooms harbour diverse microbial population. Out of different SMS, the one from *P. sajor-caju* harbor fungi *(Schizophyllum commune* and *Pezizomycotina* sp.) and bacteria (*Bacillus licheniformis, P. Jluorescens* and *B. pumilus)* with appreciable level of ligninolytic enzymes activities and decolorization potential against textile effluent.

Zhu *et al.* (2012) noticed the antibacterial activity of polysaccharide from SMS against *E. coli.* Phan and Sabaratnam (2012) concluded that SMS was no longer regarded as a waste

but as a renewable resource from the mushroom industry. Not only SMS can be employed in a number of green technology endeavours, three enzymes recovered are potentially useful for the bioremediation of pollutants and other industrial biotechnology purposes. One of them is production of lignocellulosic enzymes such as laccase, xylanase, lignin peroxidase, cellulase and hemicellulase.

Egein *et al.* (2011) carried out a pot culture experiment with two tomato varieties tropimech (TT3) and tomate U82B (TT2), 4-6 weeks old seedlings were transplanted into potted soil amended with spent mushroom substrate at the ratio 1:3, 1:4, 1:5 and 1:6 in plastic pots. Stem diameter, plant height, number of leaves, disease incidence and disease severity were observed against *Fusarium oxysporum.*

Parada *et al.* (2011) reported that plants treated with water extract from SMS or autoclaved water extract against anthracnose in cucumber reduced the disease. According to them water-soluble and heat-stable compounds in the SMS enhance the state of systemic acquired resistance and protect cucumbers from anthracnose.

Ntougias *et al.* (2008) conducted pot experiment to assess suppersiveness against soil-borne and foliar pathogens of tomato with spent mushroom substrate and peat at 1:3 w/w ratios. The effect of SMS on disease suppression (damping off caused by *P. aphamdermatum)* and plant growth promotion in tomato was also reported by Sanam and Gokulapalan (2010). Qiao *et al.* (2011) reported that SMS hydrolysates could be useful for the cultivation of microorganisms and the production of high value compounds such as nisin and lactic acid.

According to Davis *et al.* (2005) Mushroom compost (spent mushroom substrate, SMS) exhibits suppressive characteristics against various fungi, as well as against plant diseases caused by fungi. In addition, mushroom compost has physical and chemical characteristics that make it ideal for blending with landscape mulch to enhance growth of horticultural plants.

According to Ntougias *et al.* (2004), the bacterial diversity in SMC is greatly affected by the origin of the initial material, its thermal pasteurization treatment and the potential

unintended colonization of the mushroom substrate during the cultivation process. SMC contain Gram-positive bacteria, associated with the genera *Bacillus*, *Paenibacillus*, *Exiguobacterium, Staphylococcus, Desemzia, Camobacterium, Brevibacterium, Arthrobacter* and *Microbacterium* of the bacterial divisions *Firmicutes* and *Actinobacteria.*

Cronin *et al.* (1996) noticed clarified water extracts of slurries of spent mushroom substrate (SMS) inhibited conidial germination of the apple scab pathogen *Venturia inaequalis* up to 98% by *in vitro* method and the extracts produced from sterile SMS were virtually ineffective compared with those from non-sterile SMS.

Kleyn and Wetzler (1981) found the most common microflora associated with SMS are bacterial isolate like *Bacillus licheniformis,* actinomycete isolates *viz., Streptomyces diastaticus* and *Thermoactinomyces vulgaris.* Other actinomycete isolates included *Streptomyces albus, Streptomyces griseus, Thermoactinomyces thalpophilis, Thermomonospora chromogena,* and *Thermomonospora fusca,* and the fungal isolates like *Aspergillus fumigatus* and *Humicola grisea* var. *thermoidea,* Other fungal isolates included *Aspergillus jlavus, Aspergillus nidulans, Aspergillu sterreus, Aspergillus versicolor group, Chrysosporium luteum, Mucor spp., Nigrospora* spp., *Oidiodendron* spp., *Paecilomyces* spp., *Penicilliumchrysogenum, Penicillum expansum,Trichoderma viride,* and *Trichurus* spp.

2.7.2. SMS as soil amendment

Roy *et al.* (2015) reported that SMC which also contains simpler form of protein-rich component formed through modification of agricultural materials by the fungus after few cycles of cultivation can be used as very good soil conditioners for the cultivation of fruits, vegetables flower and foliage crops.

Ashrafi *et al.* (2014) observed mushroom, the fruiting body of macro fungi, as an important food item with its high nutritive and medicinal values. Mushroom cultivation has consistently been increasing and producing huge amount of mushroom waste that is

spent mushroom substrate every year. About five kilograms of fresh compost are needed to produce one kilogram of mushrooms.

According to Sendi *et al.* (2013) spent mushroom waste (SMW), otherwise known as spent mushroom substrate (SMS) contains nutrients which could be used for the growth of plants. SMS is generally nontoxic to plants and therefore, could be employed as soil amendment for different crops. The SMW is claimed to be a source of humus formation, and humus provides plant micronutrients, improves soil water holding capacity, soil aeration, and helps maintain soil structure.

Fidanza *et al.* (2012) reported that fresh mushroom compost had an average pH of 6.6, with an average carbon-nitrogen ratio of 13:1. Organic matter content averaged 25.86% (wet weight), 146.73 lb/yard3 (wet volume) or 60.97% (dry weight). For the primary macronutrients, average total nitrogen content averaged 1.12% (wet weight), 6.40 lb/yard3 (wet volume) or 2.65% (dry weight), phosphorus measured 0.29% (wet weight), 1.67 lb/yard3 (wet volume) or 0.69% (dry weight), and potassium was 1.04% (wet weight), 5.89 lb/yard3 (wet volume) or 2.44% (dry weight). Average soluble salt content was 13.30 mmho/cm (wet weight basis).

Medina *et al.* (2012) concluded that the soil application of spent mushroom substrates improved soil fertility, since soil organic C and N, available P and phosphatase activity were increased significantly by the organic fertilization with these substrates, this soil fertility improvement being higher with T_1 treatment(50 % V/V of agaricus and pleurotus) . Also, the addition of the spent mushroom substrate did not alter the soil salinity or the pH value. However, low N mineralization of these materials, N immobilization, was contributing to low N losses. These results suggested that the organic N mineralization of spent mushroom substrate was predominantly determined by nutrient demand of the crop.

According to Wei *et al.* (2011) the appropriate timing for spent mushroom substrate additions to the soil should be approximately one month prior to the planting, since after this period N immobilization was not observed in the alkaline soil of this experiment.

Eudoxie and Alexander (2011) used fine SMS as a media for growing tomato seedlings. The greater plant height and seedling vigour was attained with five week after sowing. The SMSF treatment naturally supplied all the essential nutrients required for proper growth and development. Available levels of the macronutrients were much greater in the SMS than that applied as a standard fertilizer supplement for nursery cultivation.

Bindhu (2010) reported that as compared to soil the organic carbon content of spent mushroom substrates was high. She also reported that the spent mushroom substrate registered a pH range of 6.9 to 7.2 with average calcium content and more accumulation of nitrogen and calcium in fruits, phosphorus in roots and potassium in stem and leaves of tomato.

Kadiri and Mustapha (2010) concluded that spent mushroom substrate, which presently has no economic value, is strongly recommended for use as a soil conditioner in order to enhance the yield of vegetables.

Dar *et al.* (2009) observed supplying plant nutrients through the integrated use • of Agaricus -SMC (WB- SMC) of a narrow C:N ratio and 90 kg fertilizer N ha^{-l} gave an advantage over the use of Oyster-SMC (OY- SMC) and/or fertilizer N. The OY-SMC, with a wider C:N ratio, reduced N recovery and agronomic N efficiency but its incorporation with fertilizer N can increase yield, recovery efficiency and agronomic efficiency compared with application of OY-SMC alone. The use of WB- SMC in rice cropping enhanced C sequestration but the effect due to OY-SMC with a wider C:N ratio compared to WB-SMC with a narrow C:N ratio was higher because of low mineralization and high immobilization of its C.

Polat *et al.* (2009) noticed that all of the spent mushroom compost treatments resulted in higher yield than control treatment. The highest total fruit yield was obtained at 40 ton ha-1 and it was followed by 80 and 20 ton ha^{-1} SMC applications ($p<0.05$). When the effects of SMC application on fruit width were investigated, the highest values were found at 80 kg ha^{-1} SMC application ($p<0.05$). Based on the study it was concluded that as an organic material source and amendment of greenhouse soil application of at least 6

months kept SMC was very effective and beneficiary for cucumber production, quality and recycling the spent mushroom compost.

Medina *et al.* (2009) used spent mushroom substrate (SMS) of *Agaricus bisporus* (SMS-AB) and *Pleurotns ostreatus* (SMS-PO) in different ratios for growing tomato, courgette and pepper. For the growth of tomato all the substrates were found suitable, while for pepper and courgette all SMS-AB based substrates and the media containing low dose of SMS-PO and SMS-50 were found to be adequate for the growth. The media with SMS-PO presented lower pH and electrical conductivity values, but their low total water holding capacity could have limited the retention of nutrients from fertirrigating system of nurseries.

Castro *et al.* (2008) reported the use of a substrate composed of two parts soil with high organic matter content and one part oyster mushroom spent substrate (obtained after two crops of *Pleurotus* cultivation on a sunflower seed hulls based substrate) resulted in improvement of the growth and nutritional status of common sage plants *Salvia officinalis* cultivated in pots. This improvement can be due to the PSS contribution to higher air porosity and content of certain essential mineral nutrients to the substrate. It can also be suggested that washing out excess of salts present in the PSS, which demands time and labor, is not necessary for the cultivation of plants tolerant/resistant to drought or salinity.

Trygve *et al.* (2007) used *Agaricus* spent mushroom substrate (SMS) in turf industry in the northeastern United States for soil improvement. When tilled into soil at high rates, some turf grass managers claim that SMS inhibits turf seed germination. Incorporation of high rates of SMS represents a potential problem for turf grass establishment.

Suess and Curtis (2006) observed Value-Added Strategies for Spent Mushroom Substrate as it contains a diverse range of soil microorganisms. This was proven by its disease suppressing properties and its effectiveness in bioremediation. The biological properties of SMS enhance its marketability as a soil conditioner. Addition of microorganisms to soil would enhance and accelerate regular soil processes such as nutrient mobilization and aggregate formation. SMS might have variable chemical and physical properties due

to variability of ingredients and processing; however, it is generally regarded as a neutral soil amendment. SMS will neither add a great amount of the macronutrients like nitrogen, phosphorous and potassium (NPK) to the soil nor will it tie up nutrients. The PH of SMS is around 7 which is suitable for most crops as all essential nutrients are available to plants at this PH. SMS has the potential to play an important role as a biological disease suppressant. SMS is rich in microorganisms, such as disease fighting bacteria and fungus. It naturally suppresses pathogens in the soil that cause plant damage and decline in yields.

Chong (2005) noticed that the spent material is rich in certain nutrients and has physical properties, such as aeration, porosity and water retention capacity. Spent mushroom compost can be used as a soil or potting mix supplement to grow crops including fruit, vegetables, corn *(Zea mays),* and potted foliage and greenhouse flowers.

According to Guo and Chorover (2004) weathering of piled material in the field is a popular method to treat spent mushroom substrate of Agaricus (SMS) before reuse. During the weathering process, rainfall and snowmelt pass through SMS piles and a large amount of solutes is released in the leachate.

Polat *et al.* (2004) reported statistically significant differences among different levels of spent mushroom compost applications, and two and four tons/ha gave the best result in terms of total and marketable yield in lettuce.

Iwase *et al.* (2000) observed that spent compost of *Volvariella volvacea* on addition to soil increased the yield of tomatoes seven folds and the yields of soybean, lettuce and radish two fold each. Furthermore, they observed that addition of *Agaricus* spent compost to the soil produced greater yields of cabbage, cauliflower, beans and celery compared to addition of poultry manure to soil.

CHAPTER III

MATERIALS AND METHODS

The materials and methods used in the experiment were organized in this chapter, which includes a brief overview of the experimental location, soil, climate, land preparation, experimental design, treatments, soil and plant sample collection, cultural operations and analytical methods. The detailed methodology is given below

3.1. Experimentation site description

3.1.1. Location and Period

The research was carried out during the rabi season at the Sher-e-Bangla Agricultural University Farm, Sher-e-Bangla Nagar, Dhaka-1207 from November 2021 to March 2022. It is located at latitude 90.2^0N and longitude 23.5^0E. The precise location of the experimental site is depicted on a map (Appendix -I)

3.1.2. Soil

According to Bangladesh soil classification, the soil of the experimental field is under the Tejgaon series of AEZ No. 28, Madhupur Tract and was classified as Shallow Red Brown Terrace Soils. A composite sample was prepared prior to the experiment by collecting dirt from various locations across the field at depths ranging from 0 to 15 cm. Before testing for physical and chemical properties, the soil was air-dried, crushed, and sieved through a 2 mm sieve. Appendix II describes some of the soil's early physical and chemical characteristics.

3.1.3. Climate

The climate of the experimental site is subtropical with three distinct seasons: the monsoon season from November to February, the pre-monsoon period or hot season from March to April and the monsoon season from May to October.

3.2. Planting material

The seeds of selected cabbage variety Hybrid Bandha kopi (F1) were collected from Bangladesh Agricultural Development Corporation (BADC), Gabtoli, Dhaka.

3.3. Experimental design

The experiment was laid out in Randomized complete block design (RCBD) with single factor and three replications. Total 30 unit plots were used in the experiment with 10 treatments.

3.4. Experimental treatments

There was single factor in this experiment namely management of alternaria leaf spot of cabbage through the combination of selected fungicide with spent mushroom substrate as mentioned below:

T_0: Control (No treatment applied)

T_1: Spent mushroom substrate (SMS) @ 15 t ha^{-1}

T_2: Rovral 50 WP @ 2.0 ml/L of water

T_3: Kurenox 50 WG @ 2.5 g/L of water

T_4: Knowin 50 WP @ 20 g/L of water

T_5: Mancer 75 WP @ 2g/L of water

T_6: Spent mushroom substrate @ 15 t ha^{-1} + Rovral 50 WP @ 2.0 ml/L of water

T_7: Spent mushroom substrate @ 15 t ha^{-1} + Mancer 75 WP @ 2g/L of water

T_8: Spent mushroom substrate @ 15 t ha^{-1} + Kurenox 50 WG @ 2.5 g/L of water

T_9: Spent mushroom substrate @ 15 t ha^{-1} + Knowin 50 WP @ 20 g/L of water

Spent mushroom substrate is the leftover after different flushes of mushrooms have been harvested. Among the fungicides used in the experiment, Rovral is of Ipridione group; Kurenox is of Copper oxide group; Knowin is of Carbendazim group and Mancer is of Carbendazim+Mancozeb group.

3.5. Seedbed preparation and seed sowing

The Seedbeds each of size of 2.5 × 1.5 ft^2 was prepared in Department of Plant Pathology for raising seedlings of cabbage. Seedbed containing a mixture of equal proportion well decomposed cow-dung and loam soil. Before sowing, the germination test of seeds was done and on an average 80% germination was found. Seeds were then sown on the 28th October, 2021. The seed was sown@ 500 gm /ha in the Seedbed bed, covered with thin layer of soil. According to agricultural package and practice sowing was done at 15 cm row to row and 10 cm plant to plant distance. Irrigation was given at all critical growth stages. These raised beds were irrigated whenever required with the help of sprayer.

3.6. Field preparation

The soil was well prepared and good tilth ensured for commercial crop production. The target land was divided into 30 equal plots (2.5 × 1.5 m^2) with plot to plot distance of 0.50 m and block to block distance was 0.75 m. The land of the experimental field was ploughed with a power tiller. Later on, the land was ploughed three times followed by laddering to obtain desirable tilth. The corners of the land were spaded and larger clods were broken into smaller pieces. After ploughing and laddering, all the stubbles and uprooted weeds were removed and then the land was ready. The field layout and design of the experiments were followed immediately after land preparation.

3.7. Manure and fertilizer

Recommended fertilizers were applied at the rate of 300 kg urea, 250 kg triple super phosphate (TSP) and 250 kg muriate of potash (MoP) per hectare used as source of nitrogen, phosphorus and potassium, respectively. Moreover, well-decomposed cow-dung (CD) was also applied at the rate of 5 ton/ha to the field at the time of land preparation (BARI, 2019).

3.8. Spent mushroom substrate application

Spent mushroom substrate was incorporated in the soil to selected experimental unit plot @ 15t/ha. Thus each unit plot (375 m2) received 5.625 kg substate.

3.9. Seedling transplanting

The 30 days old healthy and uniform sized seedlings from the nursery bed was transplanted on November 28, 2021 in the main field. Each plot contained 10 seedlings of cabbage with 2 rows followed by 60 cm and 40 cm row to row and plant to plant distance, respectively.

3.10. Intercultural operation

After raising seedlings, various intercultural operations such as gap filling, weeding, earthing up, irrigation, pest and disease control were accomplished for better growth and development of the cabbage seedlings.

3.10.1. Gap filling

The transplanted seedlings in the experimental plot were kept under careful observation. Very few seedlings were damaged after transplanting and such seedling were replaced by new seedlings from the same stock. Replacement was done with healthy seedling having a boll of earth which was also planted on the same date by the side of the unit plot. The transplants were given shading and watering for 7 days for their proper establishment.

3.10.2. Weeding

The hand weeding was done at 15, 30, 45 and 60 DAT to keep the plots free from weeds.

3.10.3. Earthing up

Earthing up was done at 20 and 60 DAT on both sides of rows by taking the soil from the space between the rows by a small spade.

3.10.4. Irrigation

Light watering was given by a watering can at every morning and afternoon after transplanting. Following transplanting it was continued for a week for rapid and well establishment of the transplanted seedlings. Beside this a routine irrigation was given at 3 days intervals.

3.10.5. Plant protection measures

Plant protection measures from different pathogens were taken according to par different treatment requirement.

3.11. Harvesting

Harvesting of the cabbage was not possible on a certain or particular date because the head initiation as well as head maturation period of plants were not similar. Only the compact marketable heads were harvested with fleshy stalk by using sharp knife. Before harvesting of the cabbage head, compactness of the head was tested by pressing with thumbs.

3.12. Method of recording observations

For recording disease incidence of Alternaria leaf spot five plants per treatment per replication were randomly selected and tagged. The initial observations were recorded before first spray and final observations were recorded 15 days after the last spray on the basis of development of spots and lesions by considering the whole leaf area hundred percent.

3.13. Collection of different data

Data were collected on the following parameters:

i. Disease incidence of plant
ii. Plant height (cm)
iii. Number of leaves plant^{-1}
iv. Disease incidence of leaves
v. Disease severity index
vi. Days required for head formation
vii. Diameter of head (cm)
viii. Percent head infection
ix. Days required for head maturity

x. Head weight with unfolded leaves (kg plant^{-1})

xi. Fresh wt of head (kg plant^{-1})

xii. Yield / plot (kg/m)

xiii. Yield (t ha^{-1})

3.14. Data recoding procedure

3.14.1. Disease incidence of plant

Number of infested plant plot^{-1} was counted from total number of plants from each plot at 15 days interval starting from 30 days after transplanting (DAT) and continued up to 60 DAT and their average was recorded.

3.14.2. Plant height (cm)

Plant height was measured from five randomly selected plants using meter scale in centimeter from the ground level to the tip of the longest leaf at 15 days interval starting from 30 days after transplanting (DAT) and continued up to 60 DAT and their mean value was calculated.

3.14.3. Number of leaves plant^{-1}

Number of leaves per plant was counted from five randomly selected plants at 15 days interval starting from 30 days after transplanting (DAT) and continued up to 60 DAT and their average was recorded.

3.14.4. Disease incidence of leaves

The Disease incidence of leaves average was calculated after counting the total number of leaves plant^{-1} from the 5 selected plant samples.

3.14.5. Disease severity index

Five infected plants were selected randomly from each plot for scoring. Three sprays were applied at an interval of 10 days. The first spraying was done at the first appearance

of disease symptom. Disease data were recorded before every spray. The disease intensity was recorded in 0-5 rating scale (Conn. *et al.* 1990) as described below.

0 = No infection

1 = < 5% leaf area infection

2 = 6-10% leaf area infection

3 = 11-25% leaf area infection

4 = 26-50% leaf area infection

5 = >50% above leaf area infection

Percent disease index (PDI) was calculated using the recorded data according to (Sultana *et. al*., 2018)

$$PDI = \frac{\text{Sum of total ratting} \times 100}{\text{Total number of observations} \times \text{Highest grade in the scale}}$$

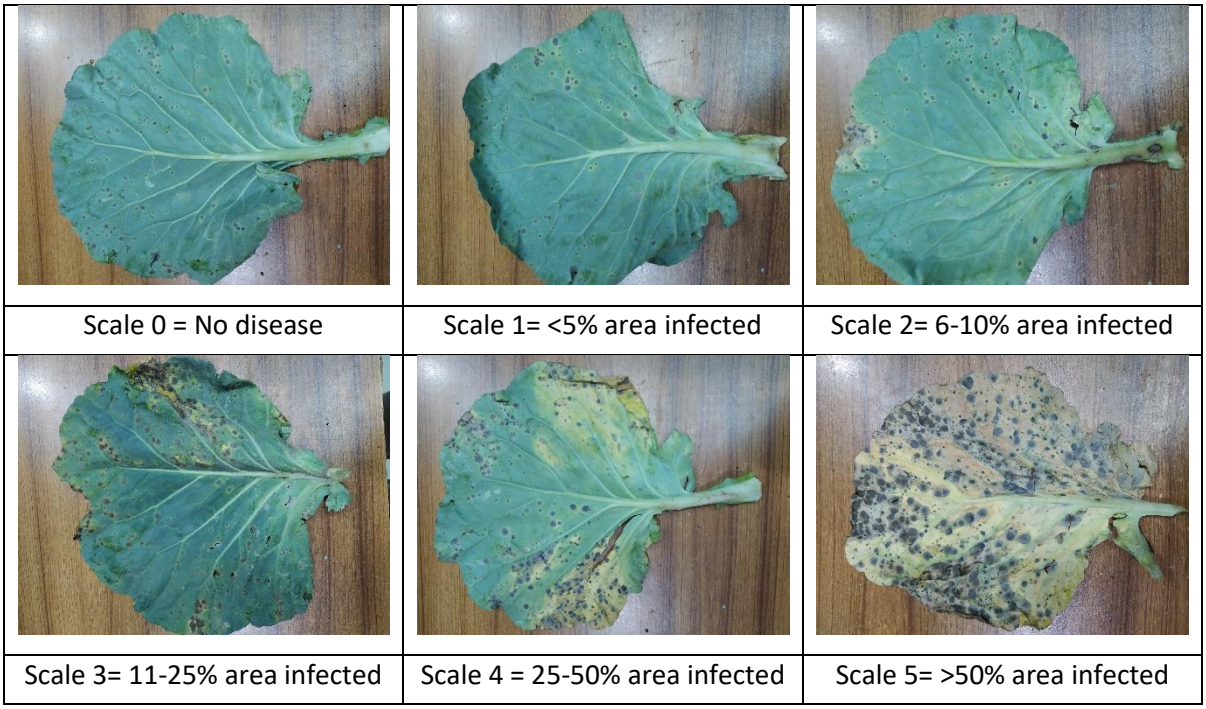

Plate 1. Photographs of 0-5 rating scale showing leaf infected area

3.14.6. Days to head formation

Each plant of the experiment plot was kept under close observation to count days to 1st head formation. Total number of days from the date of transplanting to the 1st head formation was recorded.

3.14.7. Diameter of head (cm)

The heads from sample plants were sectioned vertically at the middle position with a sharp knife. The diameter of the head was measured in centimeter (cm) with a meter scale as the horizontal distance from one side to another side of the widest part of the sectioned head and mean value was recorded.

3.14.8. Percent head infection

Percent head infection determine by following formula

$$\text{Percent head infection} = \frac{\text{Infected head area (cm)} \times 100}{\text{Total head diameter}}$$

3.14.9. Days to head maturity

Maturity is based on head compactness. A compact head can be only slightly compressed with moderate hand pressure. A very loose head is immature, and a very firm or hard head is mature. Days to head maturity occurred between 60-70 DAT.

3.14.10. Head weight with unfolded leaves (kg plant^{-1})

The head of the cabbage with unfolded leaves were harvested from selected plants from each unit plot and then weighted by a weighing machine and recorded the weight of Head weight with unfolded leaves per plant.

3.14.11. Fresh weight of head (kg plant^{-1})

After harvest of head from selected plants from each unit plot the unfolded leaves were removed from the head and weighted by a weighing machine and recorded the weight of head as fresh weight of head per plant.

3.14.12. Yield plot^{-1} (kg)

Yield per plot was recorded by multiplying average yield weight of head per plant with total number of plants per plot and expressed in kilogram.

3.14.13. Yield (t ha^{-1})

The yield per hectare was measured by converted yield per plot into yield per hectare and expressed in ton.

3.15. Materials required in the laboratory Test

The cabbage leaves showing typical symptoms of Alternaria leaf spot disease were collected from Agronomy Field, Sher-e-Bangla Agricultural University Farm, Dhaka. The infected aerial parts of the diseased samples were carefully placed in paper bags, properly tagged and brought to laboratory for further studies.

The common laboratory medium Potato Dextrose Agar (PDA) was used for isolation of causal organism from the diseased leaf sample. The PDA was also used for the *in vitro* evaluation of bio-agents and fungicides against the causal organism.

All the chemicals used in different experiments were of analytical grade. All the chemicals used for experiment were obtained from the Department of Plant Pathology.

Standard Borosil brand glassware *viz*., Petri plates, test tubes, conical flasks, beakers, measuring cylinders, pipette, glass rods, glass slides, spirit jars etc. were used for laboratory work.

Common laboratory equipment's such as Autoclave, Laminar air flow cabinet, BOD incubator, Refrigerator, Research microscope, Electronic weighing balance were used for experimental purpose.

3.16. Isolation of causal organism

3.16.1.Visual observations of diseased symptoms

Visual observations of disease symptoms were recorded in the field to study the development of the disease in a plant population under natural conditions. Symptoms were first visible on lower leaves with appearance of brown points, which later enlarged to develop into prominent, round, concentric spots of various sizes (Meena *et. al*.,2010).

Plate 2. Visiting field for the appearance of visual symptoms of the disease

3.16.2. Isolation

Fresh samples of diseased leaves showing typical symptoms were washed gently with running tap water to remove extraneous material. The infected leaves were cut into small beats containing half infected and half healthy portion. These bits were disinfected with 0.1% Sodium hypochlorite and 70% Ethanol for 30 seconds and then washed serially 3 times in sterilized distilled water to remove the traces of surface disinfectants. Then these bits were placed on sterilized blotter paper for removing excess surface water and then transferred aseptically sterilized Petri plates containing sterilized, solidified PDA medium with the help of sterilized forceps. The Petri plates were kept in BOD incubator for incubation at 25±2 ºC till the fungal mycelium fully covered the surface of the medium. Bits with well-developed fungal growth without contamination were placed on another sterilized, solidified PDA medium containing Petri plates for maintaining pure culture. The pure culture fungal growth was transferred to PDA slants and maintained as stock culture for further studies. (Thilagam *et al.*, 2017).

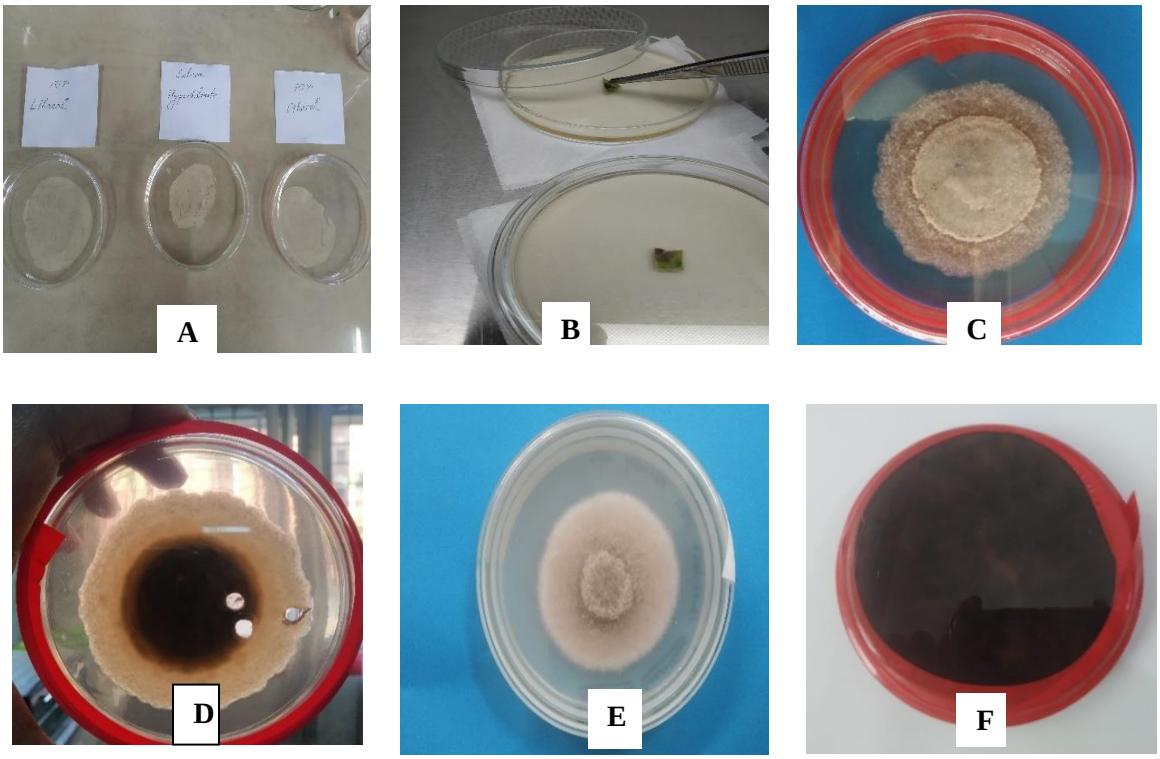

Plate 3. Isolation and purification of causal organism. (A) Surface sterilization of leaf samples(B) Placicing leaf cutting in PDA media; (C) Incubation of the samples from leaves ; (D) Scooping of hypha ; (E) Mycelial growth on PDA; (F) Pure culture of the pathogen.

3.17. Artificial inoculation for pathogenicity test

Seedlings of Hybrid Variety of cabbage planted in the earthen pots containing desired potting mixture. The potting mixture comprising Soil, Sand and FYM in 2:1:1 proportion was autoclaved for three successive days in order to kill the micro flora present if any. Each pot containing one healthy cabbage seedling was maintained and watered regularly. The leaves of test plants were cleaned with sterilized water and allowed to dry. The upper as well as lower surface of leaves were made injured with sand paper for easy penetration of test fungus. Spore cum mycelial suspension of the test pathogen was prepared by

pouring the distilled sterile water in 7-8 days old culture plates. This obtained spore cum mycelial suspension was filtered through muslin cloth and filtrate obtained was suitably diluted with distilled sterile water to get inoculum concentration of 105 spore/ml (Pattanamahakul and Strange, 1999). This spore cum mycelial suspension (10^5 spore/ml) of the test pathogen was used for inoculation by spraying it with an automizer on forty five days old seedlings which are already grown in earthen pots. Sterile water (without inoculum) sprayed on the seedlings grown in earthen pots were maintained as control.

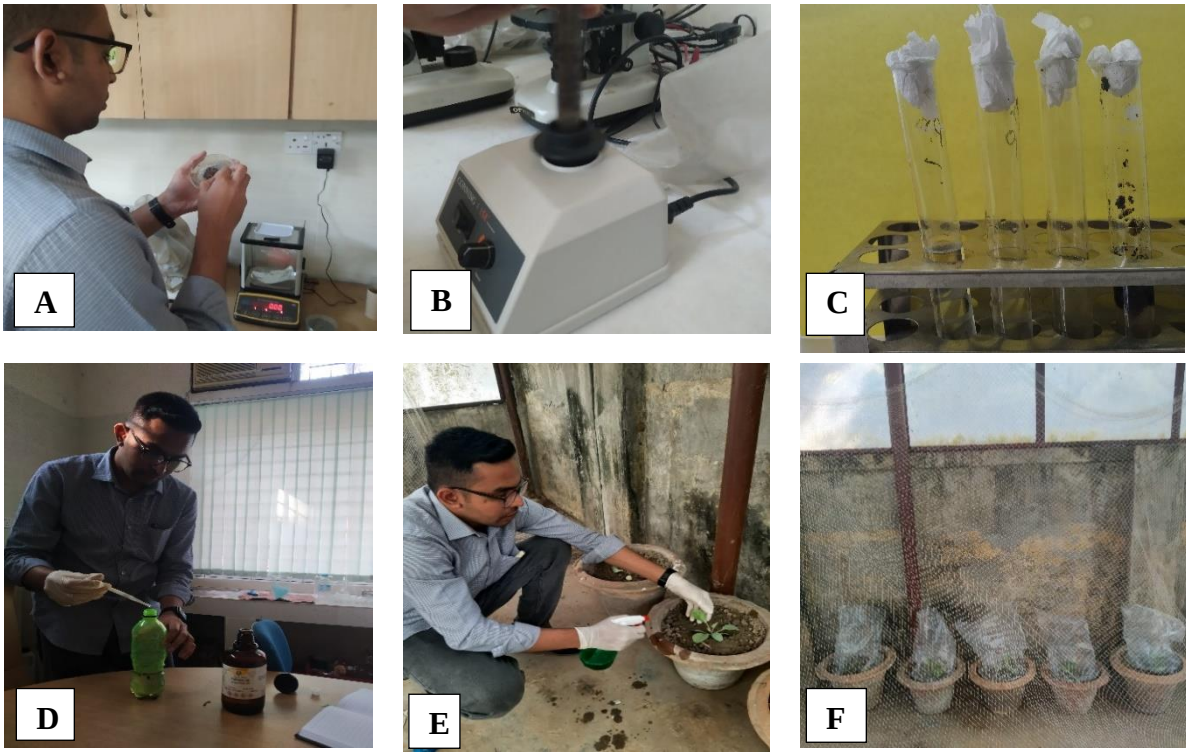

Plate 4. Artificial inoculation steps for pathogenicity test. (A) Preparation of spore suspension; (B) Mixing thoroughly with vortex mixture; (C) Diluted four times; (D) Mixing Tryptan Blue; (E) Spraying with causal orgaism on healthy plants; (F) After spraying, plants were covered with polythene bag and kept in the net house.

3.18. Weekly observations on control plot

The weekly observations on Alternaria leaf spot incidence from control (unsprayed) plot were recorded. The observations of disease incidence were recorded from 11th to 15th meteorological week of 2022.These weekly observations of disease incidence were recorded after first occurrence of disease till 15 days after second spraying. The standard

meteorological week is a seven-day period beginning on Monday and ending on Sunday, commonly used by meteorologists and climatologists for organizing and analyzing weather and climate data.

3.19. Statistical analysis

The collected data were compiled and analyzed statistically using the analysis of variance (ANOVA) technique with the help of a computer package program Statistix 10 software. The significant differences among the treatment means were compared by Least Significant Difference (LSD) at 5% levels of probability (Gomez and Gomez, 1984).

CHAPTER IV

RESULTS AND DISCUSSION

The study was carried out in the experimental firm at Sher-e-Bangla Agricultural University, Dhaka during November 2021 to March 2022. Various data on plant growth, incidence and severity of Alternaria yield under different departments represented in this chapter and discussion in relation to references are presented below:

4.1. Disease Related Data

4.1.1. Disease incidence on leaves (%)

The significant variations were observed among different treatments used for the management practices in terms of leaf infection of cabbage. In terms of disease incidence of leaves, the lowest disease incidence on leaves (33.86%) was found in treatment T_6 which was statistically different from other treatments and followed by T_9 (34.46%), T_7 (34.47%), T_2 (36.12%) and T_4 (37.82%). Conversely, the maximum disease incidence of leaf (70.82%) was found in T_0, which was statistically different from the other treatments and followed by T_1 (52.47%), T_5 (45.31%), T_3 (43.88%) and T_8 (39.73%) (Table 1).

Considering the percent inhibition of leaf disease incidence over control was ranged from 52.19-25.91%. The maximum disease incidence was observed in T_6 followed by T_9 (51.34), T_7 (51.33), T_2 (49.00) and T_4 (46.60); on the other hand, the lowest percentage was observed in T_1 followed by T_5 (36.02), T_3 (38.04) and T_8 (43.90).

From the table it was revealed that among the different treatments, T_6 performed best result in reducing leaf infection caused by Alternaria disease over control (52.19%). Some of researchers studied on Alternaria and its management and their results more or less similar with the present study.

Kumar and Singh (2003) determined the efficacy of Captan, Iprodione, Ridomil MZ, Zineb, Ziram and Blitox-50 at recommended rates against Alternaria blight (caused by *A. brassicae*) infecting radish. The best control of the disease was obtained by spraying plants with Iprodione, followed by Mancozeb and Ridomil-MZ.

Solanke *et al.* (2020) conducted an experimental to see the effect of organic manure against Alternaria leaf spot of cabbage with eight treatment is farm yard manure 5 t/ha spent mushroom compost 500 kg/ha, poultry manure 5 t/ha, vermicompost 10 t/ha, neem cake 500 kg/ha, and leaf mold 3 t/ha, goat manure 10 t/ha, along with the control. Results that all the organic amendments significantly reduced the incidence of Alternaria leaf spot of cabbage with control whereas among the treatments, T_2 (Neem Cake) and T_1 (Spent Mushroom Compost) significantly reduced incidence of Alternaria leaf spot cabbage as compared with other treatments.

Table 1. Effect of treatments on Alternaria leaf spot disease incidence cabbage (Leaf Infection)

Treatments	Disease incidence of leaves (%)	Percent inhibition of leaf disease incidence over control (%)
T_0	70.82 a	0.00
T_1	52.47 b	25.91
T_2	36.12 g	49.00
T_3	43.88 d	38.04
T_4	37.82 f	46.60
T_5	45.31 c	36.02
T_6	33.86 i	52.19
T_7	34.47 h	51.33
T_8	39.73 e	43.90
T_9	34.46 h	51.34
CV(%)	0.12	
$LSD_{(0.05)}$	0.09	

[In a column, numeric value represents the mean of 3 replications; each replication is derived from 5 plants per treatment; in a column means having similar letter(s) are statistically identical at 0.05 level of probability]

[T_0: Control (No treatment); T_1: Spent mushroom substrate (SMS) (@ 15 t ha-1); T_2: Rovral 50 WP; T_3: Kurenox 50 WG; T_4: Knowin 50 WP; T_5: Mancer\ 75 WP; T_6: Spent mushroom substrate + Rovral 50 WP; T_7: Spent mushroom substrate + Mancer 75 WP; T_8: Spent mushroom substrate + Kurenox 50 WG; T_9: Spent mushroom substrate + Knowin 50 WP]

4.1.2. plant Infection (%)

The significant variations were observed among different treatments used for the management practices in terms of disease incidence of plant of cabbage caused by alternaria leaf spot disease. Percent disease incidence of plants, the lowest disease incidence on plants (48.66%) was found in T_6 which was statistically dissimilar from other treatments and followed by T_9 (50.72%), T_7 (51.60%), T_2 (52.30%) and T_4

(59.43%). On the contrary, the maximum disease incidence of plant (92.67%) was found in $T_{0,}$ which was statistically different from the other treatments and followed by T_1 (76.06%), T_5 (70.66%), T_3 (68.71%) and T_8 (61.56%) (Table 2).

Considering the percent inhibition of plant disease incidence over control, the maximum percentage (47.49%) was found in T_6 followed by T_9 (45.27%), T_7 (44.32%), T_2 (43.56%) and T_4 (35.87%). On the other hand, the lowest percentage (17.92%) was found in treatment T_1 followed by T_5 (23.75%), T_3 (25.86%) and T_8 (33.57%).

From the table it was revealed that among the different treatments, T_6 performed best result in reducing plant infection caused by Alternaria disease over control (48.66).

Kumar and Singh (2003) determined the efficacy of Captan, Iprodione, Ridomil MZ, Zineb, Ziram and Blitox-50 at recommended rates against Alternaria blight (caused by *A. brassicae*) infecting radish. The best control of the disease was obtained by spraying plants with Iprodione, followed by Mancozeb and Ridomil-MZ.

Solanke *et al.* (2020) conducted an experimental to see the effect of organic manure against Alternaria leaf spot of cabbage with eight treatment is farm yard manure 5 t/ha spent mushroom compost 500 kg/ha, poultry manure 5 t/ha, vermicompost 10 t/ha, neem cake 500 kg/ha, and leaf mold 3 t/ha, goat manure 10 t/ha, along with the control. Results that all the organic amendments significantly reduced the incidence of Alternaria leaf spot of cabbage with control whereas among the treatments, T_2 (Neem Cake) and T_1 (Spent Mushroom Compost) significantly reduced incidence of Alternaria leaf spot cabbage as compared with other treatments.

The result of the present study is identical with the findings of Solankee et. al. and others

Table 2. Effect of treatments on disease incidence of plant caused by Alternaria leaf spot disease

Treatments	Disease incidence of plant (%)	Percent inhibition of plant disease incidence over control (%)
T_0	92.67 a	0.00
T_1	76.06 b	17.92
T_2	52.30 g	43.56
T_3	68.71 d	25.86
T_4	59.43 f	35.87
T_5	70.66 c	23.75
T_6	48.66 j	47.49
T_7	51.60 h	44.32
T_8	61.56 e	33.57
T_9	50.72 i	45.27
CV(%)	0.11	
$LSD_{(0.05)}$	0.12	

[In a column, numeric value represents the mean of 3 replications; each replication is derived from 5 plants per treatment; in a column means having similar letter(s) are statistically identical at 0.05 level of probability]

[T_0: Control (No treatment); T_1: Spent mushroom substrate (SMS) (@ 15 t ha-1); T_2: Rovral 50 WP; T_3: Kurenox 50 WG; T_4: Knowin 50 WP; T_5: Mancer\ 75 WP; T_6: Spent mushroom substrate + Rovral 50 WP; T_7: Spent mushroom substrate + Mancer 75 WP; T_8: Spent mushroom substrate + Kurenox 50 WG; T_9: Spent mushroom substrate + Knowin 50 WP]

4.1.3. Disease incidence on head (%) under different tretments

The significant variations were observed among different treatments used for the management practices in terms of percent head infection of cabbage caused by alternaria leaf spot disease. But among the treatments, T_2, T_6, T_7 and T_9 treatments had no head infection caused by alternaria leaf spot. The head infestation was ranged from 11.13-0.3% where the lowest percent was observed in T_4 which was statistically different from others. On the other hand, the maximum percentage was observed in T_0, which was statistically dissimilar from the other treatments and followed by T_1 (5.53%), T_5 (2.67%), T_3 (1.14%) and T_8 (0.93%). On the other hand, (Table 3).

In case of percent inhibition of percent head infection over control, the maximum percent (100.00%) was observed in treatment T_6 followed by T_9 (100.00%), T_7 (100.00%), T_2 (100.00%) and T_4 (97.30%). On the other hand, the lowest percent (50.31) was observed in T_1 followed by T_5 (76.01%), T_3 (89.76%) and T_8 (91.64%) (Table 3).

From the table it was revealed that among the different treatments, T_6 performed best result in reducing head infection caused by Alternaria disease over control (100.00%).

Kumar and Singh (2003) determined the efficacy of Captan, Iprodione, Ridomil MZ, Zineb, Ziram and Blitox-50 at recommended rates against Alternaria blight (caused by *A. brassicae*) infecting radish. The best control of the disease was obtained by spraying plants with Iprodione, followed by Mancozeb and Ridomil-MZ.

Solanke *et al.* (2020) conducted an experimental to see the effect of organic manure against Alternaria leaf spot of cabbage with eight treatment is farm yard manure 5 t/ha spent mushroom compost 500 kg/ha, poultry manure 5 t/ha, vermicompost 10 t/ha, neem cake 500 kg/ha, and leaf mold 3 t/ha, goat manure 10 t/ha, along with the control. Results that all the organic amendments significantly reduced the incidence of Alternaria leaf spot of cabbage with control whereas among the treatments, T_2 (Neem Cake) and T_1 (Spent Mushroom Compost) significantly reduced incidence of Alternaria leaf spot cabbage as compared with other treatments.

The result of the present study was identical with their results as spent mushroom substrate when applied with spraying of Rovral 50 WP increased the yield of cabbage.

Table 3. Effect of treatments on disease incidence of head of cabbage caused by alternaria leaf spot disease.

Treatments	Percent head infection (%)	Percent inhibition of head over control (%)
T_0	11.13 a	0.00
T_1	5.53 b	50.31
T_2	0.00 g	100.00
T_3	1.14 d	89.76
T_4	0.30 f	97.30
T_5	2.67 c	76.01
T_6	0.00 g	100.00
T_7	0.00 g	100.00
T_8	0.93 e	91.64
T_9	0.00 g	100.00
CV(%)	3.48	
$LSD_{(0.05)}$	0.13	

[In a column, numeric value represents the mean of 3 replications; each replication is derived from 5 plants per treatment; in a column means having similar letter(s) are statistically identical at 0.05 level of probability]

[T_0: Control (No treatment); T_1: Spent mushroom substrate (SMS) (@ 15 t ha-1); T_2: Rovral 50 WP; T_3: Kurenox 50 WG; T_4: Knowin 50 WP; T_5: Mancer\ 75 WP; T_6: Spent mushroom substrate + Rovral 50 WP; T_7: Spent mushroom substrate + Mancer 75 WP; T_8: Spent mushroom substrate + Kurenox 50 WG; T_9: Spent mushroom substrate + Knowin 50 WP]

4.1.4 Disease severity index (%)

The significant variations were observed among different treatments used for the management practices in terms of disease severity of cabbage caused by alternaria leaf spot disease. The lowest percent (19.94%) was found in treatment T_6 which was statistically identical with T_9 (19.98%), T_7 (20.00%), T_2 (20.04%) and followed by T_4 (20.07%) Then again, the maximum percentage (31.14) was found in treatment T_0, which was statistically different from the other treatments and followed by T_1 (26.67%), T_5 (24.44%), T_3 (24.43%) and T_8 (22.25%) (Table 4).

In case of percent inhibition of disease severity over control, the maximum percent (35.97%) was found in treatment T_6 followed by T_9 (35.84%), T_7 (35.77%), T_2 (35.65%) and T_4 (35.55%). Conversely, the lowest percent (14.35%) was found in T_1 followed by T_5 (21.52%), T_3 (21.55%) and T_8 (28.55%) (Table 4).

From the table it was revealed that among the different treatments, T_6 showed the best result in reducing disease severity caused by Alternaria leaf spot over control (35.97%). Some of researchers studied on Alternaria and its management and their results more or less similar with the present study.

Kumar and Singh (2003) determined the efficacy of Captan, Iprodione, Ridomil MZ, Zineb, Ziram and Blitox-50 at recommended rates against Alternaria blight (caused by *A. brassicae*) infecting radish. The best control of the disease was obtained by spraying plants with Iprodione, followed by Mancozeb and Ridomil-MZ.

Solanke *et al.* (2020) conducted an experimental to see the effect of organic manure against Alternaria leaf spot of cabbage with eight treatment is farm yard manure 5 t/ha spent mushroom compost 500 kg/ha, poultry manure 5 t/ha, vermicompost 10 t/ha, neem cake 500 kg/ha, and leaf mold 3 t/ha, goat manure 10 t/ha, along with the control. Results that all the organic amendments significantly reduced the incidence of Alternaria leaf spot of cabbage with control whereas among the treatments, T_2 (Neem Cake) and T_1 (Spent Mushroom Compost) significantly reduced incidence of Alternaria leaf spot cabbage as compared with other treatments.

The finding in respect of disease severity index of the present study was more or less same with their works.

Table 4. Effect of treatments on disease severity of cabbage caused by alternaria leaf spot disease

Treatments	Disease severity index (%)	Percent inhibition of DSI over control (%)
T_0	31.14 a	0.00
T_1	26.67 b	14.35
T_2	20.04 ef	35.65
T_3	24.43 c	21.55
T_4	20.07 e	35.55
T_5	24.44 c	21.52
T_6	19.94 f	35.97
T_7	20.00 ef	35.77
T_8	22.25 d	28.55
T_9	19.98 ef	35.84
CV(%)	0.22	
$LSD_{(0.05)}$	0.09	

[In a column, numeric value represents the mean of 3 replications; each replication is derived from 5 plants per treatment; in a column means having similar letter(s) are statistically identical at 0.05 level of probability]

[T_0: Control (No treatment); T_1: Spent mushroom substrate (SMS) (@ 15 t ha-1); T_2: Rovral 50 WP; T_3: Kurenox 50 WG; T_4: Knowin 50 WP; T_5: Mancer\ 75 WP; T_6: Spent mushroom substrate + Rovral 50 WP; T_7: Spent mushroom substrate + Mancer 75 WP; T_8: Spent mushroom substrate + Kurenox 50 WG; T_9: Spent mushroom substrate + Knowin 50 WP]

4.2. Growth parameters and yield characters

4.2.1. Total number of leaves and Plant height (cm)

The significant variations were observed among different treatments used for the management practices in terms of total number of leaves and plant height of cabbage. The maximum number of leaves (83.83) was found in T_6 which was statistically identical with T_9 (82.77) and followed by T_7 (80.14), T_2 (79.33) and T_4 (76.03). On the other hand, the lowest number of leaves (70.44) was found in T_0 which was statistically identical with T_1 (70.65), T_5 (70.89), T_3 (71.34) and T_8 (72.72) (Table 5).

In case of plant height, the maximum plant height (21.79) was found in treatment T_6 which was statistically similar with T_9 (21.70), T_7 (21.44) and T_2 (20.99) and followed by

T_4 (20.08). On the other hand, the lowest plant height (17.97) was found in T_0 which was statistically similar with T_1 (18.52) and T_5 (18.96) and followed by T_3 (19.34) and T_8 (19.99) (Table 5).

From the table it was revealed that among the different treatments, T_6 showed the best result in reducing disease severity caused by Alternaria leaf spot over control (35.97). Some of researchers studied on Alternaria and its management and their results more or less similar with the present study.

The fungicides such as Rovral 50 WP (0.20%), Dithane M-45 (0.25%), Ridomil (0.10%), Bavistin (0.25%) and Knowin (0.25%) were evaluated for the management of Alternaria blight (*A. brassicae* and *A. brassicicola*) of cauliflower by Khoda *et al.* (2003). Among the the treatments Rovral 50WP showed the best result in case of crop growth.

Polat *et al.* (2004) reported statistically significant differences among different levels of spent mushroom compost applications, and two and four tons/ha gave the best result in terms of total and marketable yield in lettuce.

Table 5. Effect of treatments on total number of leaves and plant height of cabbage.

Treatments	Total number of leaves	Plant height (cm)
T_0	70.44 e	17.97 f
T_1	70.65 e	18.52 ef
T_2	79.33 c	20.99 ab
T_3	71.34 e	19.34 cde
T_4	76.03 d	20.08 bc
T_5	70.89 e	18.96 def
T_6	83.83 a	21.79 a
T_7	80.14 bc	21.44 a
T_8	72.72 e	19.99 bcd
T_9	82.77 ab	21.70 a
CV(%)	2.24	2.86
$LSD_{(0.05)}$	2.92	0.99

[In a column, numeric value represents the mean of 3 replications; each replication is derived from 5 plants per treatment; in a column means having similar letter(s) are statistically identical at 0.05 level of probability]

[T_0: Control (No treatment); T_1: Spent mushroom substrate (SMS) (@ 15 t ha-1); T_2: Rovral 50 WP; T_3: Kurenox 50 WG; T_4: Knowin 50 WP; T_5: Mancer\ 75 WP; T_6: Spent mushroom substrate + Rovral 50 WP; T_7: Spent mushroom substrate + Mancer 75 WP; T_8: Spent mushroom substrate + Kurenox 50 WG; T_9: Spent mushroom substrate + Knowin 50 WP]

4.2.2. Days required for head formation

The significant variations were observed among different treatments used for the management practices in terms of days of head formation of cabbage. The maximum days (17.07) was found in treatment T_0 which was statistically different from others and followed by T_1 (16.67), T_5 (16.63), T_3 (16.60) and T_8 (16.30). On the other hand, the lowest days (15.21) was found in T_6 which was statistically different from others and followed by T_9 (15.43), T_7 (15.80), T_2 (16.06) and T_4 (16.21) (Table 6).

4.2.3. Days required for head maturity

The significant variations were observed among different treatments used for the management practices in terms of days of head maturity of cabbage. The maximum days (68.36 days) was found in treatment T_0 which was statistically different from others and followed by T_1 (66.33), T_5 (65.66), T_3 (65.57) and T_8 (64.51). On the other hand, the lowest days (59.36 days) was found in T_6 which was statistically different from others and followed by T_9 (60.70), T_7 (61.01), T_2 (62.60) and T_4 (63.66) (Table 6).

From the table it was revealed that among the different treatments, T_6 performed best result in reducing the days for head formation and head maturity of cabbage were (15.21 days and 59.36 days, respectively). Some of researchers studied on Alternaria and its management and their results more or less similar with the present study.

Iwase *et al.* (2000) observed that addition of *Agaricus* spent compost to the soil produced greater yields of cabbage, cauliflower, beans and celery compared to addition of poultry manure to soil.

Table 6. Effect of treatments on days of head formation and days of head maturity of cabbage

Treatments	Days required for head formation	Days required for head maturity
T_0	17.07 a	68.36 a
T_1	16.67 b	66.33 b
T_2	16.06 d	62.60 g
T_3	16.60 b	65.57 d
T_4	16.21 c	63.66 f
T_5	16.63 b	65.66 c
T_6	15.21 g	59.36 j
T_7	15.80 e	61.01 h
T_8	16.30 c	64.51 e
T_9	15.43 f	60.70 i
CV(%)	0.43	0.04
$LSD_{(0.05)}$	0.12	0.05

[In a column, numeric value represents the mean of 3 replications; each replication is derived from 5 plants per treatment; in a column means having similar letter(s) are statistically identical at 0.05 level of probability]

[T_0: Control (No treatment); T_1: Spent mushroom substrate (SMS) (@ 15 t ha-1); T_2: Rovral 50 WP; T_3: Kurenox 50 WG; T_4: Knowin 50 WP; T_5: Mancer\ 75 WP; T_6: Spent mushroom substrate + Rovral 50 WP; T_7: Spent mushroom substrate + Mancer 75 WP; T_8: Spent mushroom substrate + Kurenox 50 WG; T_9: Spent mushroom substrate + Knowin 50 WP]

4.2.4. Diameter of cabbage head (cm)

The significant variations were observed among different treatments used for the management practices in terms of diameter of head of cabbage. The diameter was varied from 21.38-16.33cm, the maximum diameter of head was recorded in T_6 which was statistically different from others and followed by T_9 (20.36), T_7 (20.20), T_2 (19.50) and T_4 (18.67) while the lowest diameter of head was recorded in T_0 which was statistically similar with T_1 (16.36) and T_5 (16.43) and followed by T_3 (16.64) and T_8 (17.20) (Table 7).

4.2.5. Head weight with unfolded leaves (kg)

The significant variations were observed among different treatments used for the management practices in terms of head weight with unfolded leaves of cabbage. The maximum weight of unfolded (2.67kg) was found in treatment T_6 followed by T_9 (2.86kg), T_7 (2.81kg), T_2 (2.77kg) and T_4 (2.67kg). On the other hand, the lowest weight

of cabbage with unfolded leaves (2.30kg) was found in T_0 which was statistically similar with T_1 (2.40kg), T_5 (2.72kg), T_3 (2.64kg) and T_8 (2.64kg) (Table 7).

4.2.6. Fresh weight of head (kg/plant)

The significant variations were observed among different treatments used for the management practices in terms of fresh weight of head of cabbage. The maximum weight (2.26kg) was found in treatment T_6 followed by T_9 (2.15kg) and T_7 (2.14kg) which were statistically similar. T_2 (2.07kg) and T_4 (2.04kg) also showed statistically identical result. On the other hand, the lowest weight of cabbage (1.52kg) was found in T_0 which was statistically different from others and followed by T_1 (1.72kg), T_5 (1.84kg), T_3 (1.87kg) and T_8 (2.01kg) (Table 7).

From the table it was revealed that among the different treatments, T_6 performed best result in increasing the diameter of cabbage head, weight of head with unfolded leaves and fresh weight of cabbage head were (21.38 cm, 3.07 kg and 226 kg, respectively).

The findings of the study was more or less similar with the work of Iwase et al. (2000) who observed that addition of Agaricus spent compost to the soil produced greater yields of cabbage, cauliflower, beans and celery compared to addition of poultry manure to soil.

Table 7. Effect of treatments on diameter of head, weight of head with unfolded leaves and fresh weight of head of cabbage

Treatments	Diameter of head (cm)	Head weight with unfolded leaves (kg/plant)	Fresh weight of head(kg/plant)
T_0	16.33 h	2.30 f	1.52 e
T_1	16.36 h	2.40 f	1.72 d
T_2	19.50 d	2.77 bcd	2.07 b
T_3	16.64 g	2.64 e	1.87 cd
T_4	18.67 e	2.67 e	2.04 b
T_5	16.43 h	2.72 cde	1.84 d
T_6	21.38 a	3.07 a	2.26 a
T_7	20.20 c	2.81 bc	2.14 ab
T_8	17.20 f	2.64 e	2.01 bc
T_9	20.36 b	2.86 b	2.15 ab
CV(%)	0.35	2.24	4.55
$LSD_{(0.05)}$	0.11	0.11	0.15

[In a column, numeric value represents the mean of 3 replications; each replication is derived from 5 plants per treatment; in a column means having similar letter(s) are statistically identical at 0.05 level of probability]

[T_0: Control (No treatment); T_1: Spent mushroom substrate (SMS) (@ 15 t ha-1); T_2: Rovral 50 WP; T_3: Kurenox 50 WG; T_4: Knowin 50 WP; T_5: Mancer\ 75 WP; T_6: Spent mushroom substrate + Rovral 50 WP; T_7: Spent mushroom substrate + Mancer 75 WP; T_8: Spent mushroom substrate + Kurenox 50 WG; T_9: Spent mushroom substrate + Knowin 50 WP]

4.2.7. Yield of cabbage (t/ha)

The significant variations were observed among different treatments used for the management practices in terms of yield of cabbage. The yield was ranged from 240.2-151.7 t/ha in the study. The maximum yield was found in T_6 which was statistically different from others and followed by T_9 (215.0 t/ha), T_7 (213.5 t/ha), T_2 (206.7 t/ha) and T_4 (203.2 t/ha). On the other hand, the lowest yield was found in T_0 which was statistically different from others and followed by T_1 (171.5 t/ha), T_5 (183.7 t/ha), T_3 (186.7 t/ha) and T_8 (201.3 t/ha) (Table 7).

In case of percent increase of yield (t/ha) over control, the maximum percent (58.34%) was found in treatment T_6 followed by T_9 (41.73%), T_7 (40.74%), T_2 (36.26%) and T_4 (33.95%). On the other hand, the lowest percent (13.05%) was found in T_1 followed by T_5 (21.09%), T_3 (23.07%) and T_8 (32.70%) (Table 7).

From the table it was revealed that among the different treatments, T_6 performed best result in increasing the yield of cabbage over control was (58.34%). The findings of some resarchers showed more or less similar result with the present study.

Kadiri and Mustapha (2010) concluded that spent mushroom substrate, which presently has no economic value, is strongly recommended for use as a soil conditioner in order to enhance the yield of vegetables.

Singh *et al.* (2008) used four fungicides, namely Iprodione (0.2%), Mancozeb (0.2%), Ridomil MZ (0.25%) and Apron S.T. (0.5%), as seed treatment, were evaluated against Iprodione was found to be the most effective fungicide in controlling the disease and increasing the yield.

Table 8: Effect of different treatments on yield of cabbage

Treatments	Gross yield (kg/plot)	Total yield (t/ha)	Percent increase of yield over control
T_0	22.52 a	151.7 g	0.00
T_1	21.50 ab	171.5 f	13.05
T_2	18.67 cd	206.7 c	36.26
T_3	20.67 b	186.7 e	23.07
T_4	20.13 bc	203.2 cd	33.95
T_5	21.35 ab	183.7 e	21.09
T_6	15.17 e	240.2 a	58.34
T_7	18.37 d	213.5 b	40.74
T_8	20.32 b	201.3 d	32.70
T_9	17.15 d	215.0 b	41.73
CV(%)	4.55	7.13	
$LSD_{(0.05)}$	1.53	4.83	

[In a column, numeric value represents the mean of 3 replications; each replication is derived from 5 plants per treatment; in a column means having similar letter(s) are statistically identical at 0.05 level of probability]

[T_0: Control (No treatment); T_1: Spent mushroom substrate (SMS) (@ 15 t ha-1); T_2: Rovral 50 WP; T_3: Kurenox 50 WG; T_4: Knowin 50 WP; T_5: Mancer\ 75 WP; T_6: Spent mushroom substrate + Rovral 50 WP; T_7: Spent mushroom substrate + Mancer 75 WP; T_8: Spent mushroom substrate + Kurenox 50 WG; T_9: Spent mushroom substrate + Knowin 50 WP]

4.3. Relationship between disease incidence and yield of cabbage

4.3.1. Relationship between disease incidence of leaves and yield (t/ha)

Correlation study was done to establish the relationship between the disease incidence of leaves (%) caused by Alternaria leaf spot and yield (t/ha) of cabbage. From the study it was revealed that significant correlation was observed between the incidence of leaves (%) caused by Alternaria leaf spot and yield (t/ha) of cabbage (Figure 1). It was evident from the Figure 1 that the regression equation y = -1.9797x + 282.27 gave a good fit to the data, and the co-efficient of determination (R^2 = 0.8288) showed that, fitted regression line had a significant regression co-efficient. From this regression analysis, it was evident that there was a negative relationship between the incidence of leaves (%) caused by Alternaria leaf spot and yield (t/ha) of cabbage i.e., the yield (t/ha) of cabbage increases with decreases the incidence of leaves (%) caused by Alternaria leaf spot of cabbage.

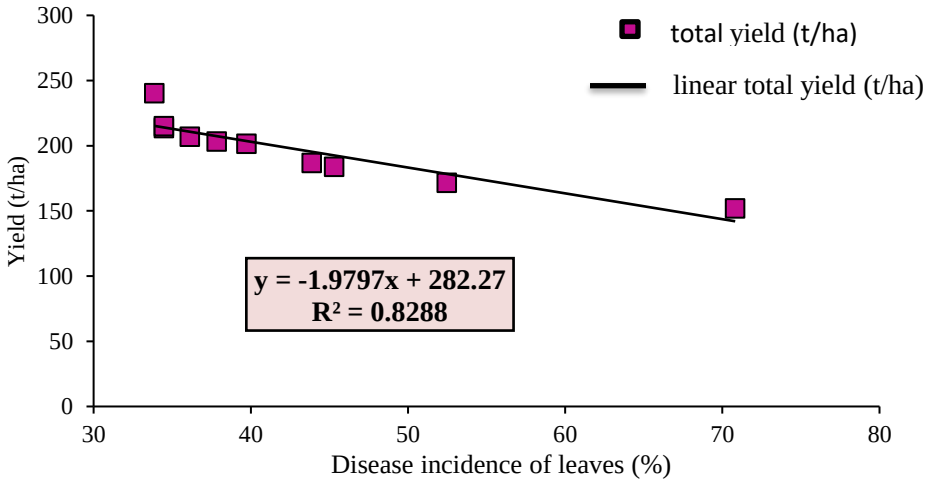

Figure 1. Relationship between disease incidence of leaves (%) caused by Alterneria leaf spot and yield (t/ha) of cabbage

4.3.2. Relationship between disease incidence of plants and yield (t/ha)

Correlation study was done to establish the relationship between the disease incidence of plants (%) caused by Alternaria leaf spot and yield (t/ha) of cabbage. From the study it was revealed that significant correlation was observed between the incidence of plants (%) caused by Alternaria leaf spot and yield (t/ha) of cabbage (Figure 2). It was evident

from the Figure 2 that the regression equation y = -1.7068x + 305.28 gave a good fit to the data, and the co-efficient of determination (R^2 = 0.9145) showed that, fitted regression line had a significant regression co-efficient. From this regression analysis, it was evident that there was a negative relationship between the incidence of plants (%) caused by Alternaria leaf spot and yield (t/ha) of cabbage i.e., the yield (t/ha) of cabbage increases with decreases the incidence of plants (%) caused by Alternaria leaf spot of cabbage.

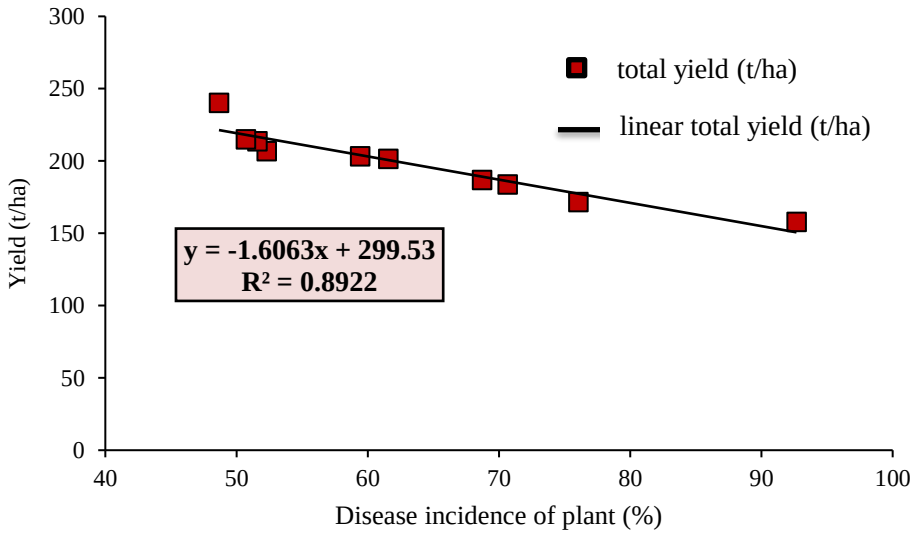

Figure 2. Relationship between disease incidence of plants (%) caused by Alterneria leaf spot and yield (t/ha) of cabbage

4.3.3. Relationship between disease severity index and yield (t/ha)

Correlation study was done to establish the relationship between the disease severity index (%) caused by Alternaria leaf spot and yield (t/ha) of cabbage. From the study it was revealed that significant correlation was observed between the disease severity index (%) caused by Alternaria leaf spot and yield (t/ha) of cabbage (Figure 3). It was evident from the Figure 3 that the regression equation y = -6.0558x + 336 gave a good fit to the data which expressed a negative relationship and the co-efficient of determination (R^2 = 0.846) showed that, fitted regression line had a significant regression co-efficient. The yield (t/ha) of cabbage increases with decreases the disease severity index (%) caused by Alternaria leaf spot of cabbage

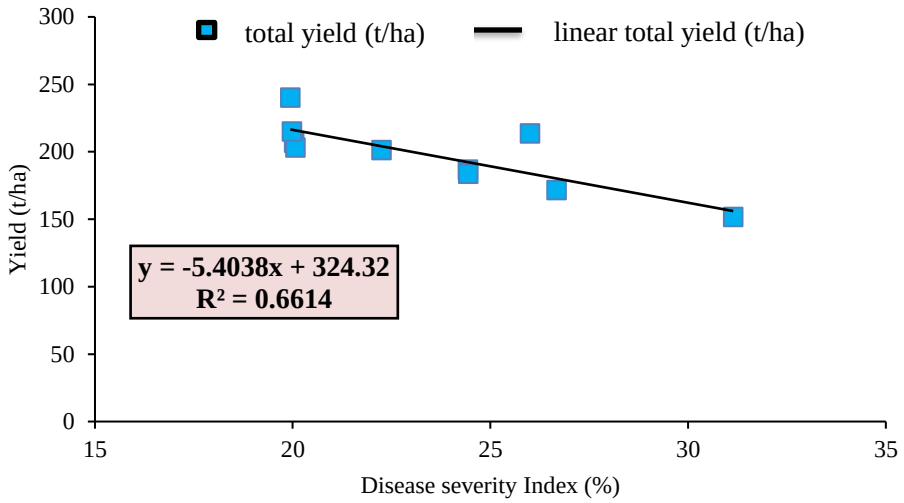

Figure 3. Relationship between disease severity index (%) caused by Alterneria leaf spot and yield (t/ha) of cabbage

4.3.4. Relationship between diameter of cabbage head and yield (t/ha)

Correlation study was done to establish the relationship between the diameter of cabbage head (cm) and yield (t/ha) of cabbage. From the study it was revealed that significant correlation was observed and the regression equation was y = 11.502x – 13.222 gave a good fit to the data, and the co-efficient of determination (R^2 = 0.8042).It was evident that there was a positive relationship between diameter of cabbage head (cm) and yield (t/ha) of cabbage i.e., the yield (t/ha) of cabbage increases with increases diameter of cabbage head (cm).

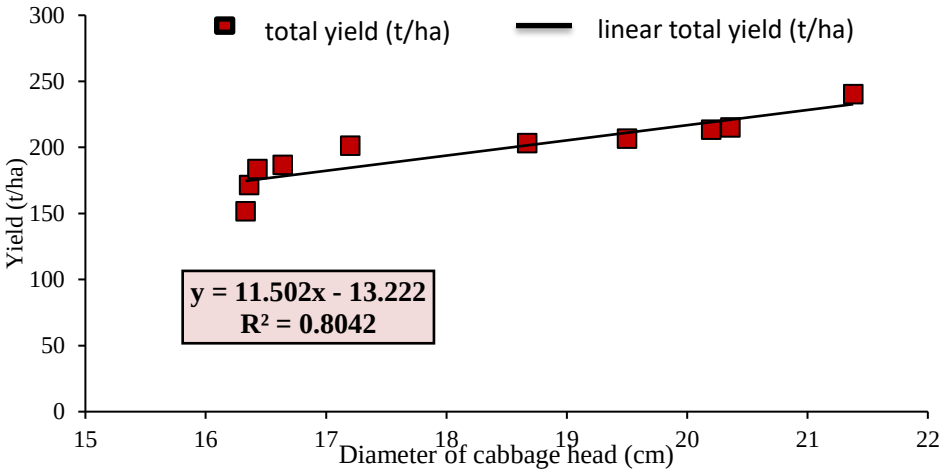

Figure 4. Relationship between diameter of cabbage head (cm) and yield (t/ha) of cabbage

4.4.1. Pathogenicity test

Moist chamber prepared with a wooden frame covered with a muslin cloth was used for incubation of earthen pots (both inoculated and non-inoculated). The humidity (85-90%) was maintained in the chamber by frequently spraying sufficient clean water on the muslin cloth. Earthen pots containing seedlings were watered as and when required till the development of typical disease symptoms i.e. concentric brown rings with yellow halo. Duhan & Suhag (1990) made an pathogenicity test *Alternaria brassicae* and found that the pathogen could infect its host (cauliflower) severely.

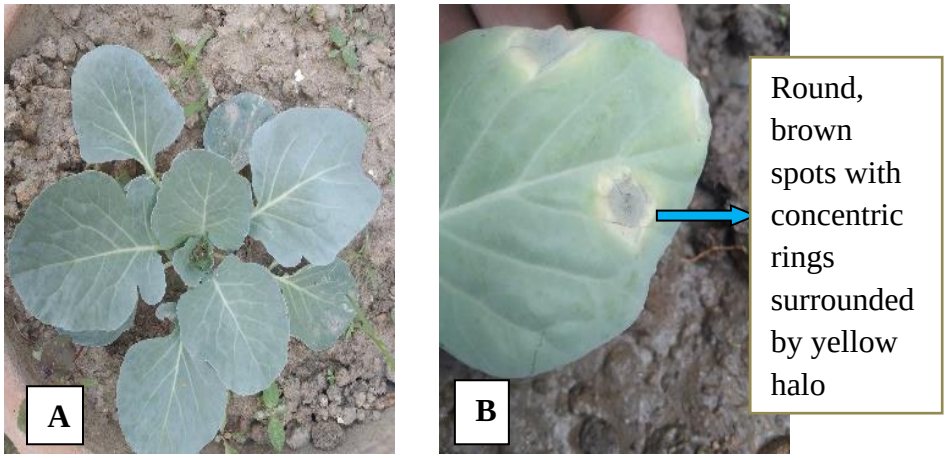

Plate 5. Disease development in the inoculated plant (A) Healthy plant (B) Diseased plant

4.4.2. Reculture

Re-isolation of causal organism was done from the artificially inoculated leaves showing typical symptoms. The fungal growth obtained on PDA medium on re-isolation was compared with the original culture obtained from naturally infected leaves under field conditions.

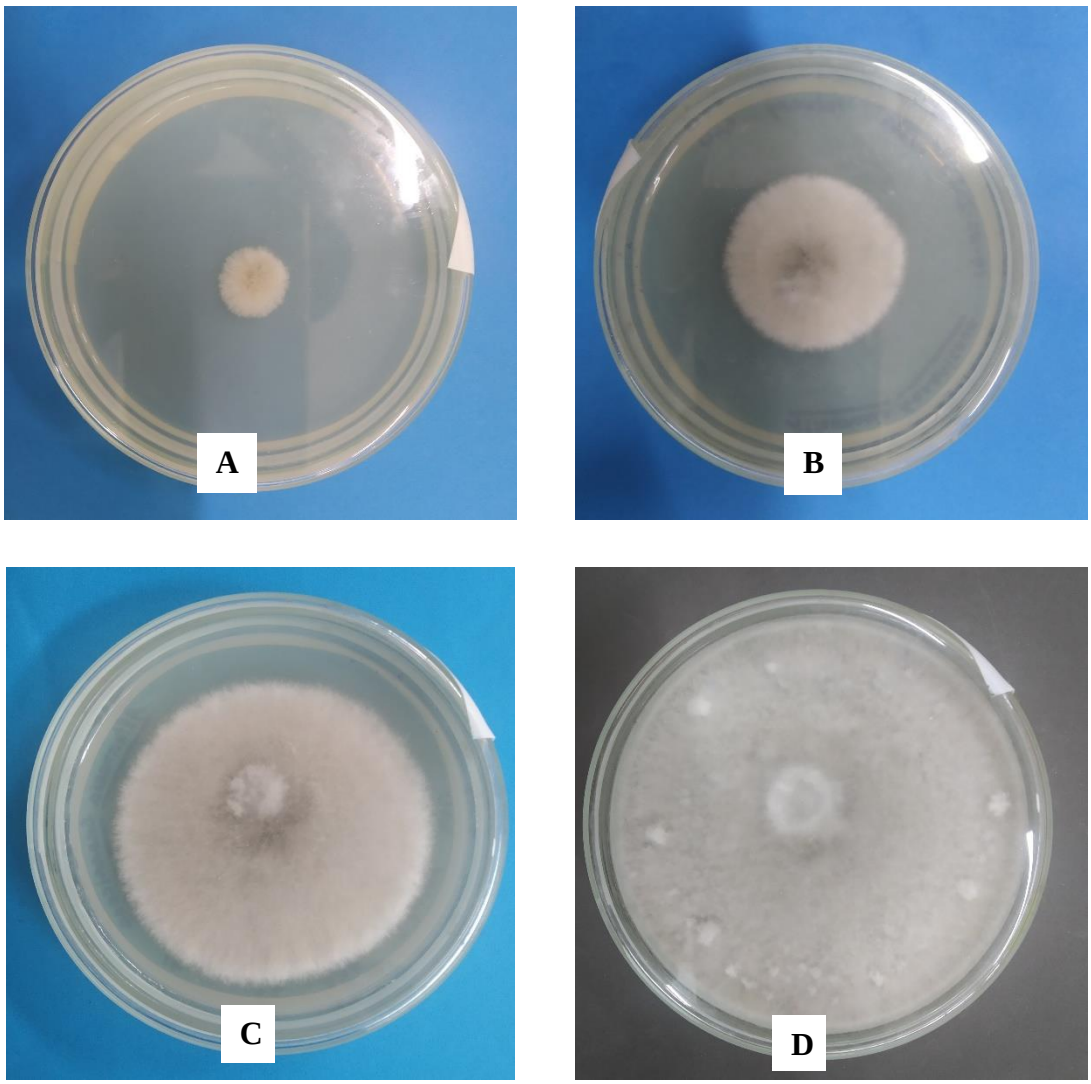

Plate 6. Showing successive radial colony growth of *Alternaria brassicae* ; (A) 3 days old culture; (B) 7 days old culture; (C) 11 days old culture; (D) 20 days old culture.

4.4.3. Identification of the causal organism

The pure culture of re-isolated test pathogen was identified by comparing its morphological and colony characters with the information available in the reviewed literature for fungal identification as well as identification was confirmed by the m observation at the Department of Plant Pathology, SAU.

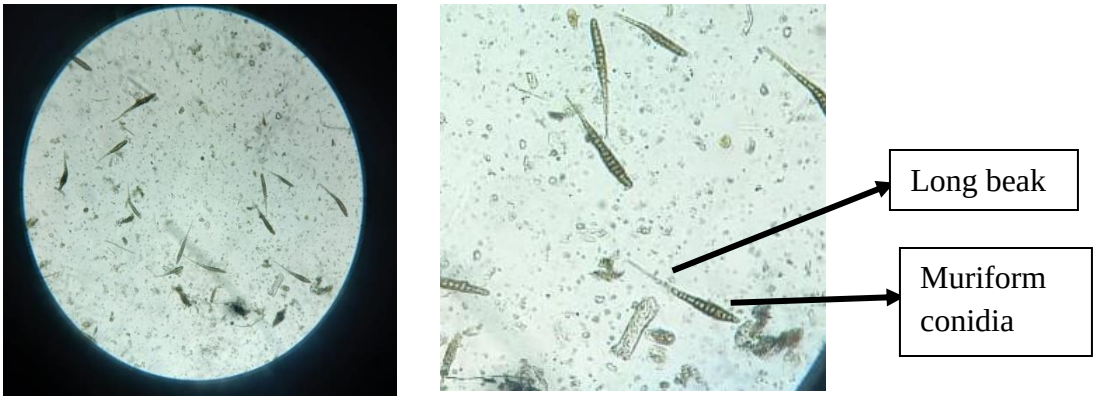

Plate 7. Microscopic view of mycelia and conidia of *Alternaria brassicae*

CHAPTER V

SUMMARY AND CONCLUSION

The present study was conducted at the Sher-e-Bangla Agricultural University central Farm, Dhaka-1207, Bangladesh with nine (09) treatments along with untreated control during November 2021 to March 2022. Seeds were sown in seed bed on 28th October, 2021 then transferred to the main field on 28th November, 2021 in Randomized Complete Block Design (RCBD) with three replications. During this study disease incidence of Alternaria leaf spot was observed and data were collected from the experimental field.

Regarding % of leaf infection, it ranged from 33.86 to 70.82%. Among the treatments, T_1 (Spent mushroom substrate) showed less disease incidence (52.47%) than untreated control (70.82%). Among the fungicides, T_2 (Rovral) showed better performance and T_6 (Spent mushroom substrate + Rovral 50 WP) revealed the best result in reducing % incidence of leaf infection caused by Alterneria *brassicae* over control (52.19%).

In case of reducing disease incidence of plants caused by Alterneria leaf spot disease T_6 (Spent mushroom substrate + Rovral 50 WP) showed the best result 47.49% over control. In terms of plant disease incidence over control, better result 43.56% was found in T_2 (Rovral 50 WP) and the lowest result was found in T_1 (17.92%). Again, the best result was found in case of integrated . T_6 (SMS+ Rovral 50 WP).

Among the different treatments, T_6 (Spent mushroom substrate + Rovral 50 WP) performed best results in reducing disease incidence of head (100%) caused by Alterneria leaf spot over control. (100%)

From this study, it is evident that the lowest disease severity (19.94%) observed when Spent mushroom substrate was used along with spraying of Rovral 50WP . T_6 (Spent mushroom substrate + Rovral 50 WP) . Again, plants showed much inhibition of disease severity index 35.97% over control when spent mushroom substrate was used as soil amendment with Rovral 50 WP in T^6 while reduced T_1 valued 14.35% and T_2 reduced 35.65% disease index individually.

The highest number of leaves (83.83) and highest plant height (21.79)cm were observed in case of T_6 (Spent mushroom substrate+ Rovral 50 WP) and the lowset number of leaves (70.44) and lowest plant height (17.95) were observed in T_0 (control) .

Among the different treatments, T_6 (Spent mushroom substrate + Rovral 50 WP) esulted the best result in reducing the days for head formation and head maturity of cabbage which were 15.21 days and 59.36 days, respectively.

In terms of increasing the diameter of cabbage head, weight of head with unfolded leaves and fresh weight of cabbage head T_6 (Spent mushroom substrate+ Rovral 50 WP) showed the best result which were (21.38 cm), (3.07 kg) and (2.26 kg) respectively.

The study clearly showed that the yield of cabbage is enhanced due to the use of spent mushroom substrate (171.5) over control (151.7). But it was remarkable yield when Rovral 50 WP was incorporated with SMS in T_6 (240.2). The percent increase of yield over control when used T_6 (Spent mushroom substrate+ Rovral 50 WP) was 58.34% whereas it was 13.05% when only SMS was used and 36.26% when Rovral 50 WP was used individually.

From the present study it can be concluded that, Rovral 50 WP (Ipridione group) was effective fungicide among the others fungicides like Kurenox 50 WG (Copper Oxychloride), Knowin 50 WP (Carbendazim) and Mancer 75 WP (Carbendazim + Mencozeb). Beside spraying of Rovral 50 WP along with application of spent mushroom substrate in soil showed the best performance against the Alternaria leaf spot disease of cabbage and decreased the disease incidence and disease severity of cabbage caused by Alternaria leaf spot. Better performance was recorded in respect of number of leaves, plant height, diameter of the cabbage head, days required for head formaton, days required for head maturity, head weight with unfolded leave, fresh weight of head. As well as it can increase the yield of the cabbage.

CHAPTER VI

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CHAPTER VII

APPENDICES

Appendix- I: Map of the SAU farm land.

Appendix-II: Physical and chemical properties of the soil.

CHARACTERISTICS		VALUE
Particle size analysis (%)	% Sand	30
	% Silt	40
	% Clay	30
Consistency		Granular and friable when dry
Soil Textural		Loam to Clay loam
pH		5.6
Bulk Density (g/cc)		1.45
Particle Density (g/cc)		2.53
Organic carbon (%		0.45
Organic matter (%)		0.78
Total N (%)		0.06
Available P (ppm)		20.0
Exchangeable K (meq/100g soil)		0.12

Appendix- III : Enumeration of bacterial and fungal population from the substrate at different stages of cropping

Microorganisms	Population at different stages of cropping		
	*Stage 1	**Stage 2	***Stage 3
Fungal population (×10^5 cfu/g of substrate)	25.33	48.67	55.33
Bacterial population (×10^5 cfu/g of substrate)	55.67	29.33	17.33

*Stage 1 – at the time of spawn running

**Stage 2 – at harvest

***Stage 3 – after harvest

Appendix- IV: Some pictorial representation of the experimental works

Photograph 1: Seed packet used for research work collected from BADC

Photograph 2: Spent mushroom substrate

	
Photograph 3: Packet of Rovral 50 WP	Photograph 4: Packet of Kurenox 50 WG
	
Photograph 5: Packet of Knowin 50 WP	Photograph 6: Packet of Mancer 75 WP
	
Photograph 7: Seed of cabbage	Photograph 8: Seed sowing on seed bed

Photograph 9: Seedlings of cabbage on seed bed

Photograph 10: Measurement of spent mushroom substrate for treatment application

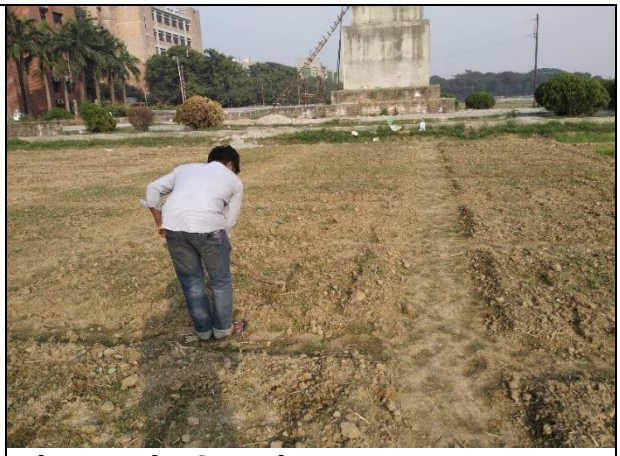
Photograph 11: Spent mushroom substrate applied in the filed

Photograph 12: Transplanting of seedlings on main field

Photograph 13: Main field

Photograph 14: Gap due to death of the transplanted seedling

Photograph 15: Transplanting of seedlings



Photograph 16: Earthing up

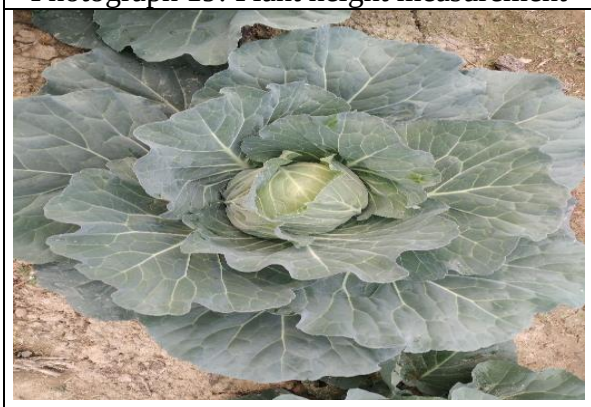
Photograph 17: Watering in the field

Photograph 18: Irrigation to the plants

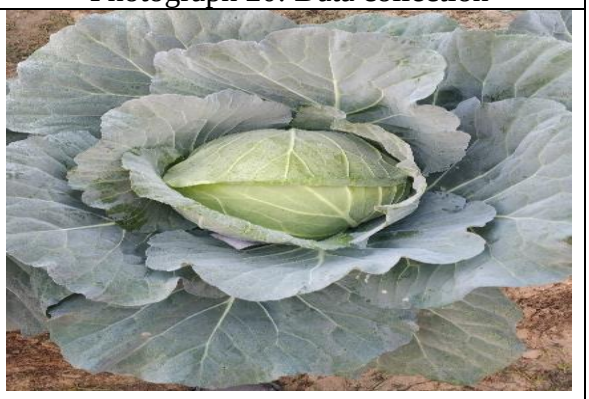
Appendix- V: Some pictorial representation of experiment results

Photograph 19: Plant height measurement

Photograph 20: Data collection

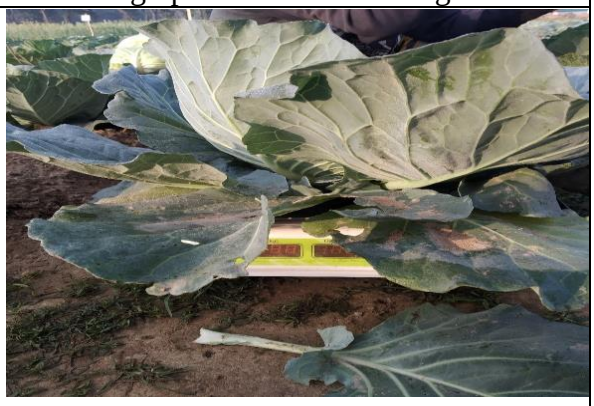


Photograph 21: Cabbage head formation



Photograph 22: Mature cabbage head

Photograph 23: Diameter of cabbage head measurement



Photograph 24: Weighing of Cabbage with unfolded leaves

Photograph 25: Fresh weight of head

Photograph 26: Diseased cabbage head